Cells Tissues Organs

Cells Tissues Organs 2001;168:5–23

Towards Defining the Role of Glycans as Hardware in Information Storage and Transfer: Basic Principles, Experimental Approaches and Recent Progress

D. Solís[a] J. Jiménez-Barbero[b] H. Kaltner[d] A. Romero[c] H.-C. Siebert[d]
C.-W. von der Lieth[e] H.-J. Gabius[d]

[a]Instituto de Química Física Rocasolano, [b]Instituto de Química Orgánica and [c]Centro de Investigaciones Biológicas, Consejo Superior de Investigaciones Científicas, Madrid, Spain; [d]Institut für Physiologische Chemie, Tierärztliche Fakultät, Ludwig-Maximilians-Universität München, and [e]Zentrale Spektroskopie, Deutsches Krebsforschungszentrum Heidelberg, Germany

Key Words
Crystallography · Drug design · Lectin · Molecular modeling · NMR spectroscopy · Sugar code

Abstract
The term 'code' in biological information transfer appears to be tightly and hitherto exclusively connected with the genetic code based on nucleotides and translated into functional activities via proteins. However, the recent appreciation of the enormous coding capacity of oligosaccharide chains of natural glycoconjugates has spurred to give heed to a new concept: versatile glycan assembly by the genetically encoded glycosyltransferases endows cells with a probably not yet fully catalogued array of meaningful messages. Enciphered by sugar receptors such as endogenous lectins the information of code words established by a series of covalently linked monosaccharides as letters for example guides correct intra- and intercellular routing of glycoproteins, modulates cell proliferation or migration and mediates cell adhesion. Evidently, the elucidation of the structural frameworks and the recognition strategies within the operation of the sugar code poses a fascinating conundrum. The far-reaching impact of this recognition mode on the level of cells, tissues and organs has fueled vigorous investigations to probe the subtleties of protein-carbohydrate interactions. This review presents information on the necessarily concerted approach using X-ray crystallography, molecular modeling, nuclear magnetic resonance spectroscopy, thermodynamic analysis and engineered ligands and receptors. This part of the treatise is flanked by exemplarily chosen insights made possible by these techniques.

Abbreviations used in this paper

CRD	carbohydrate recognition domain
NMR	nuclear magnetic resonance
NOE	nuclear Overhauser enhancements
trNOE	transferred nuclear Overhauser enhancements

Introduction

The regular order of events in fertilization, embryogenesis and tissue maturation mirrors the working of an intricate network of molecular interactions apportioned

KARGER
Fax + 41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

1422–6405/01/1682–0005$17.50/0

Accessible online at:
www.karger.com/journals/cto

Dr. Dolores Solís
Instituto de Química Física Rocasolano
Consejo Superior de Investigaciones Científicas
Serrano 119, E–28006 Madrid (Spain)
Fax +34 91 564 2431, E-Mail d.solis@iqfr.csic.es

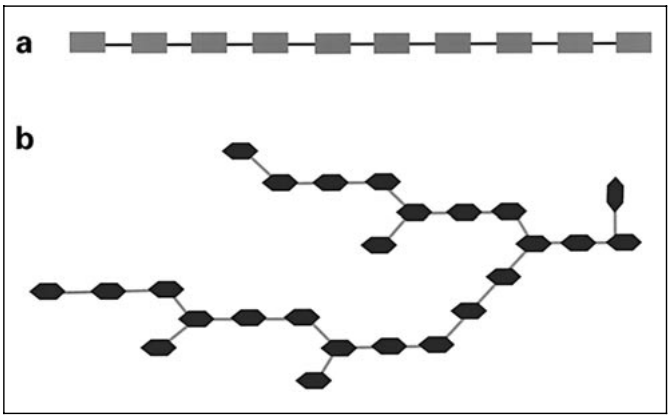

Fig. 1. Illustration of the principal difference between linear code systems of nucleic acid or protein sequences (**a**) and branched oligosaccharides (**b**).

among a panel of adhesion, signaling and effector molecules. Temporal and spatial dynamics in life sciences calls for molecular associations which are specific even during brief encounters. Only a transient nature of contacts, albeit biochemically legitimate, is compatible with cell fusion or migration and serves as a prelude for example to blastocyst implantation. This impressive feat elaborated at the level of the information transfer between receptors and ligands and meticulously monitored histologically [Denker, 1983, 1993] prompts to answer the question concerning its molecular basis.

Naturally, protein-protein interactions come to one's mind to relate biochemical determinants to functions. Coded in the genome and produced by an assembly program governed by the genetic code, these tools not only act as primary mediators but can also be subject of post-translational modifications. These additions to protein structure can enable to reprogram and adapt protein function according to the actual requirements, as seen in the case of reversible phosphorylation. Beyond tuning of an enzymatic activity, e.g. in the classical example of interconversion of glycogen phosphorylase forms a and b, the addition of a phosphate group to a protein scaffold can further establish suitable docking sites via src homology 2 (SH2) domains. Unquestionably, the development of various kinases and phosphatases fulfils important tasks rendering maintenance and even refinement of this system evolutionarily advantageous for the organism.

This argument, i.e. to ascribe functionality to post-translational modifications employing a complex synthetic and regulatory machinery, similarly applies to glycosylation, the covalent attachment of saccharides to protein or lipid carriers. Not only the presence of glycans but also their individual structure is to be given attention to grasp their potential as hardware in dealing with information. Among other factors, the possibility to engage various hydroxyl groups in a glycosidic linkage of two monosaccharides bristling with such functional groups is a prerequisite for oligosaccharides to excel at information storage [Laine, 1997]. As shown in figure 1, this chemical characteristic readily facilitates to introduce branches into a polymer relative to the linear chains of nucleic acids and proteins. Equipped with elegant and highly sensitive techniques to determine glycan sequence [Dwek et al., 1993; Hounsell, 1997; Geyer and Geyer, 1998; Hermentin and Witzel, 1999], it has become feasible to assess how the letters of the monosaccharide alphabet (the third alphabet of life) are connected in individual chains. Based on CarbBank cataloguing the documented variability for protein-linked sugar chains currently already exceeds the number of 1,000 distinct structures considerably [Vliegenthart and Casset, 1998].

In view of the notable fraction of the genome devoted to the production of the large array of enzymes assigned to glycan assembly [Brockhausen and Schachter, 1997; Sears and Wong, 1998; Kapitonov and Yu, 1999] it is reassuring that this investment into carrier modification will not be futile but can lead to functional relevance [Roseman, 1970; Marshall, 1972; Winterburn and Phelps, 1972; Berger et al., 1982; Olden et al., 1982; Sharon, 1984, 1998; Varki, 1993; Cook, 1994; Kopitz, 1997; Rudd and Dwek, 1997; Sharon and Lis, 1997; Mann and Waterman, 1998; Reuter and Gabius, 1999]. Indeed, the growing appreciation of the concept to consider oligosaccharides as information-storing coding units is reflected in the term 'sugar code' [Laine, 1997; Gabius, 2000]. It denotes their importance beyond influencing merely physicochemical properties such as glycoprotein solubility or charge density. Since the complex carbohydrates can bear significance for fertilization and implantation [Denker, 1970a, b; Denker and Aplin, 1990], a further comment on the way of writing sugar words is pertinent. Recalling the possibility for branching (fig. 1), the fact that the structural depiction of each oligosaccharide (code word) would be incomplete by only providing the sequence of the units deserves to be accentuated. Anomeric positioning (α or β) and the linkage points (for the trisaccharide in fig. 2: α2,6 and β1,4, when read from the nonreducing end) are essential to define the primary structure. With the complete information about the sequence at hand, the next question concerns the topology. To delineate features of the third dimension of glycan structure, it is imperative to take

stock of the preferential source for conformational flexibility. It stems from the dihedral angles φ and ψ of the glycosidic bond and ω of the exocyclic hydroxymethyl group (fig. 2). By changing just one parameter, the topological presentation of functional groups of the affected sugar moiety becomes notably different (fig. 2). Also, the energy content of each conformer is expected to be altered. Regarding biomolecular interactions in ligand docking as a process governed by shape, it is now inspiring to provide the readers with insight into how to answer the question on describing the 3-dimensional structure of oligosaccharides without deluging this audience with too many details from biophysical chemistry.

How to Relate Sequence to Shape

Similar to a hiker in a hilly landscape, any glycan molecule can reach different levels of 'height', in this case not above sea level but in terms of energy content of the conformation. When the individual contributions to the potential energy are adequately taken into account by the design of a force field, the conformational space of a saccharide can be drawn as a map [Pérez et al., 1994, 1999; Woods et al., 1995; Kozár and von der Lieth, 1997; von der Lieth et al., 1997, 1998; Woods, 1998; Bush et al., 1999]. Each set of the angles φ and ψ (see fig. 2) defines a spot in the plane, and the respective energy level can then be entered, rendering the comparison to topographic maps obvious (fig. 3). To illustrate what conformers from low- and high-energy levels look like, the right section of figure 3 provides two examples from positions denoted by dots in the maps. Spatial closeness of atom groups can amount to repulsive forces. To avoid them, accommodation into rather extended relaxed conformers is favored. When inspecting the low-energy sections of the maps more closely, it becomes apparent that the disaccharide under scrutiny can adopt two different, energetically equivalent φ-, ψ-combinations within the central valley (fig. 3). Since a dynamic interconversion requires crossing of an energy barrier, computational approaches to simulate the behavior of molecules have been extended to the dimension of time.

The trajectories of changes in torsion angles with time in molecular dynamics calculations reflect the molecular traffic between low-energy positions [von der Lieth et al., 1997, 1998]. They affirm the possibility that a certain carbohydrate sequence can form more than one shape. Alluding to the classical comparison of ligand association with a 'lock and key' mechanism ('Um ein Bild zu gebrauchen, will ich sagen, dass Enzym und Glucosid wie Schloss und Schlüssel zu einander passen müssen, um eine chemische Wirkung auf einander ausüben zu können' [Fischer, 1894]), these results can best be envisioned by viewing carbohydrates with this inherent degree of limited flexibility as a 'bunch of keys' [Hardy, 1997]. This premise, i.e. the rapid fluctuation of torsion angles between two or more combinations, brings about a precarious situation for the interpretation of experimental data. In this field of interest they originate from nuclear magnetic resonance (NMR) investigations exploiting intramolecular magnetization transfer. However, the time scales of internal molecular dynamics and spectroscopic sampling are not equal. In fact, the monitoring covers periods sufficient for cycles of interconversion.

Focusing on proton pairs of carbohydrates as transmitter-receiver configuration in through-space resonance

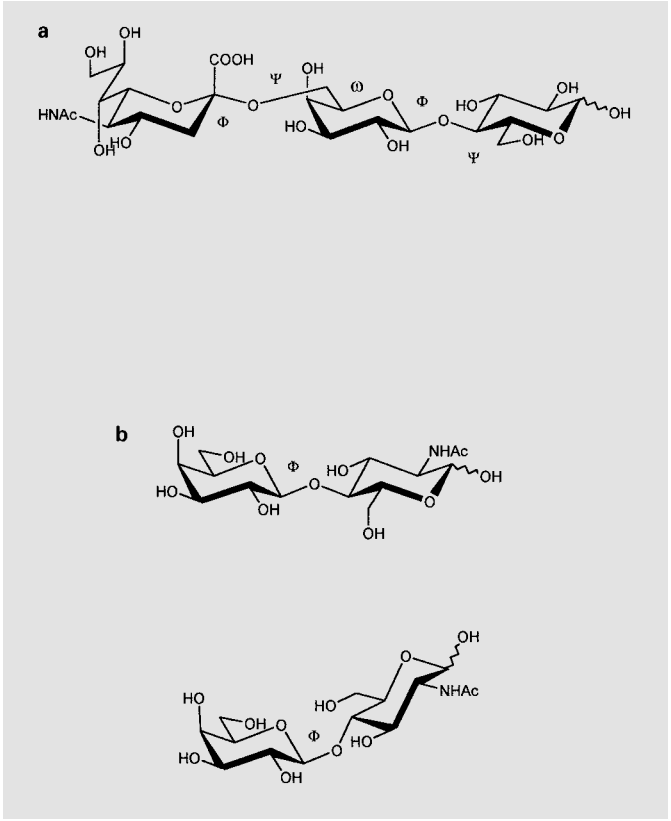

Fig. 2. Schematic representation of the two dihedral torsion angles of glycosidic bonds (φ, ψ) and the additional degree of rotational freedom provided by involvement of the 6′-hydroxymethyl group (ω), using α2,6-sialyllactose as example (**a**). The impact of variation of one torsion angle on the conformation of Galβ1,4GlcNAc is illustrated in **b**.

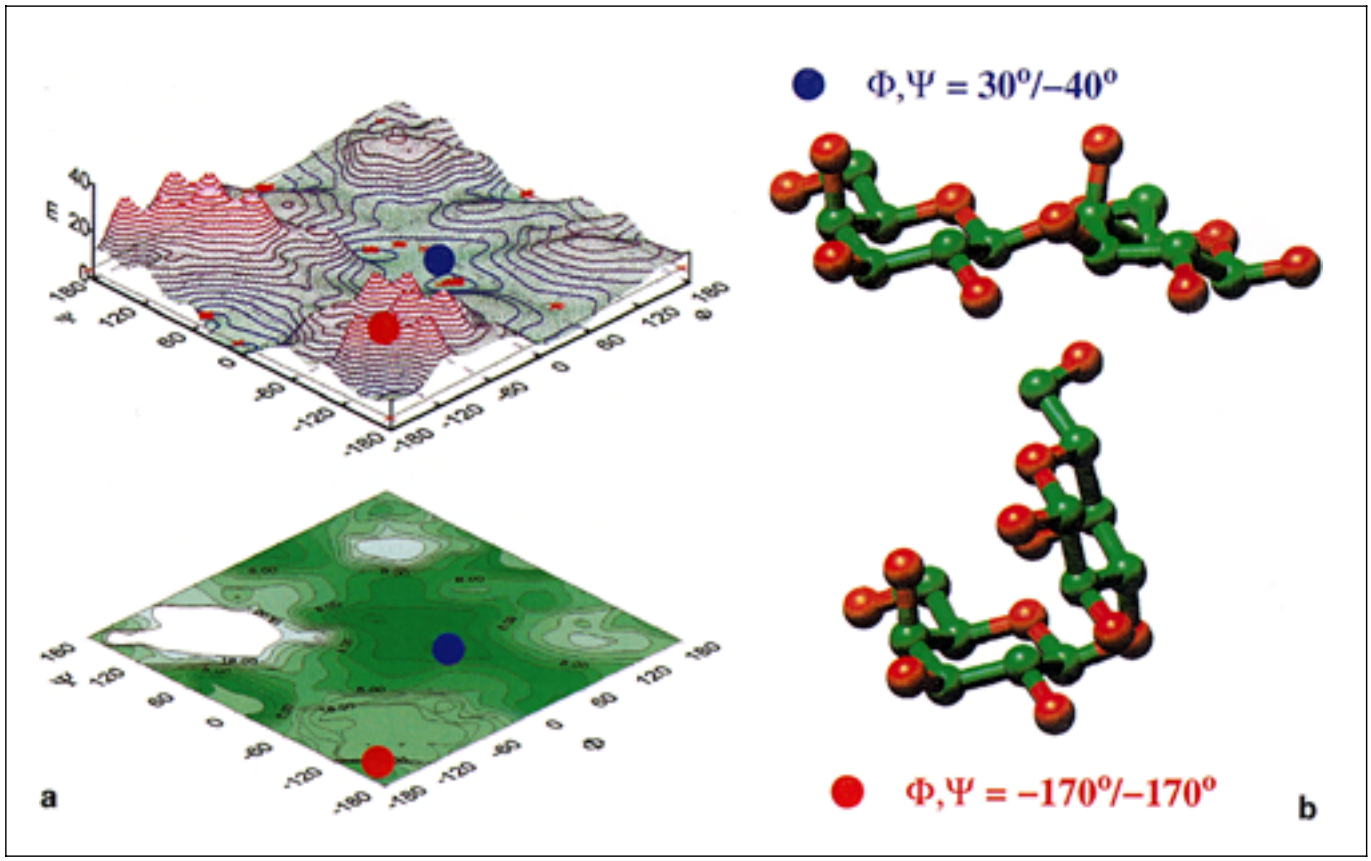

Fig. 3. Illustration of the conformational energy contour map for the φ,ψ-torsion angles in the case of the disaccharide Galβ1,3Gal in 3- and 2-dimensional representation (**a**). Low-energy positions were also calculated by the conformational clustering approach, results being indicated by crosses in the 3-dimensional figure. Depiction of two conformations from high- versus low-energy sections of the maps (symbolized by dots) visualizes the correlation between the relative positioning of monosaccharide units and the energy content of the disaccharide (**b**).

which yield nuclear Overhauser enhancements (NOE) up to a distance of 3.5 Å, the individual conformers will all contribute to signal averaging. In the case of a rigid molecule it does not matter that the time scale for NMR monitoring is rather long, because the lack of conformer interconversion renders the inability to take snapshots of rapid conformational fluctuations without impact. For flexible molecules the NOE spectrum will necessarily reflect time and ensemble averaging, and we owe it to computational approaches to manage weighing of parameters for likely conformers instead of being faced with virtual conformations [Jardetzky, 1980; Cumming and Carver, 1987; Carver, 1991; Pearlman, 1994; Imberty, 1997; Siebert et al., 1997a; Woods, 1998]. An instructive example of how the combination of experimental NMR data and computer-assisted calculations are integrated into pinpointing the presence of two conformations (keys) has been presented previously for a digalactoside [Siebert et al., 1996; von der Lieth et al., 1998]. Its eminent relevance for the delineation of differential conformer selection by nonhomologous lectins will be discussed in the chapter dealing with lectin-ligand interaction.

Terms such as 'keys' or 'sugar code' imply that cells are endowed with decoding devices or 'locks'. Chemically, such sensors are carbohydrate-binding proteins. They are referred to as lectins, unless ligand binding is coupled to enzymatic activity (as in glycosyltransferases or glycosidases) or the protein is an antibody [Barondes, 1988]. Frequently, a carbohydrate recognition domain (CRD) is linked to other modules in mosaic-like proteins [Barondes, 1988; Gabius, 1994, 1997a; Kishore et al., 1997]. Their expression is known in animals since an observation of agglutinating capacity of rattlesnake venom in 1860 [Mitchell, 1860], and especially the last 2 decades

have brought remarkable progress and a refined classification scheme [Sharon and Lis, 1972; Barondes, 1981; Ashwell and Harford, 1982; Lis and Sharon, 1986; Gabius, 1987a, b, 1991, 1997a; Lee, 1992; Weigel, 1992; Drickamer and Taylor, 1993; Powell and Varki, 1995; Kaltner and Stierstorfer, 1998; Sinowatz et al., 1998; Zanetta, 1998; Munday et al., 1999; Probstmeier and Pesheva, 1999; Vasta et al., 1999; for collections of reviews and experimental protocols see Gabius and Gabius, 1993, 1997]. A gratifying aspect of this progress is to discover the cause of diseases in defects of glycosylation tailored for recognition and to devise therapeutic strategies [Brockhausen et al., 1998; Reuter and Gabius, 1999; Schachter, 1999]. It is worth noting in this context that an eel lectin and inhibition of the lectin-dependent agglutination of histo-blood group H(O)-bearing erythrocytes by *L*-fucose have paved the way to define the epitopes of the well-known ABH blood group system as carbohydrates [Watkins and Morgan, 1952; Watkins, 1955, 1999; Krüpe, 1956; Kilpatrick and Green, 1992]. The continued value of lectins as analytical tools notwithstanding, elucidation of functions of these molecules has meanwhile moved into the spotlight. Whether binding is coupled with signaling to translate the message into a cellular activity will be dealt with in the next chapter.

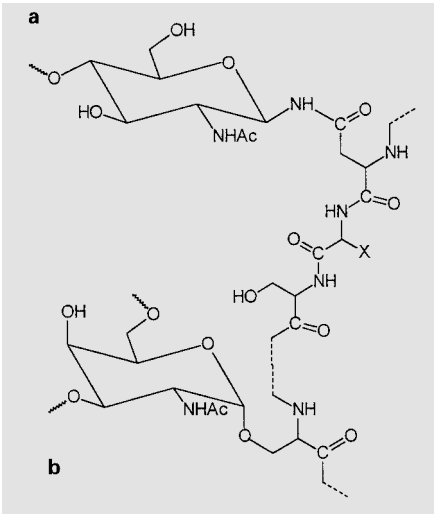

Fig. 4. Illustration of the linkage points for N-glycosylation with the GlcNAcβ1-Asn-X-Ser sequon whose sugar chain is extended at the 4′-position (**a**) and for mucin-type O-glycosylation with the GalNAcα1-Ser(Thr) structure, chain elongation starting at 3′- and 6′-positions (wavy lines, **b**).

Lectins: The Link between Sugar Code and Cellular Response

Presented on proteins primarily as N- and O-glycans (the respective linkage regions are shown in fig. 4) and on glycolipids [Kopitz, 1997; Reuter and Gabius, 1999], the complexity of the carbohydrate chains will encompass structural code words from the sugar alphabet especially at the spatially accessible nonreducing tips of antennae [Varki, 1996; Hooper et al., 1997; Reuter and Gabius, 1999]. Their accessibility contributes to turn them into suitable partners for intermolecular contact. For this purpose, members from currently five different lectin families are engaged [Gabius, 1997a]. In addition to the accommodation of the ligand into the binding pocket or grove the topology of binding site presentation is crucial for triggering ensuing responses. Tools to cross-link molecules, a recurring theme in signaling, and to home in on targets for adhesion or endocytosis, exploiting sugar signals as a postal code for site-specific delivery, present a characteristic architecture in this respect. As shown in figure 5, galectins, C-type and I-type lectins furnish instructive examples for the correlation between design and function. Downstream to the initial contact, a plethora of transduction pathways, currently being disentangled e.g. by use of site-specific inhibitors [Timoshenko et al., 1999a, b], can forward the message of recognitive interplay to effectors [Villalobo and Gabius, 1998]. It is thus not surprising that the characterization of endogenous lectins and their functions has entered the limelight as potential target for drug design [Rüdiger et al., 2000]. For this purpose, synthetic neoglycoproteins offer a means of exploiting the biological significance of lectins in cell biology and medicine [Stowell and Lee, 1980; Aplin and Wriston, 1981; Gabius, 1988, 1997b; Danguy et al., 1991, 1995, 1998; Lee and Lee, 1991, 1994a, b; Gabius and Gabius, 1992; Lee, 1992; Gabius et al., 1993, 1998; Roy, 1996a, b; Biermann et al., 1997; Mammen et al., 1998; André et al., 1999a]. Also, their application in cell biology can be crucial to attribute ligand properties to carbohydrate epitopes which are under strict, so far only phenomenologically detected spatial and/or temporal control. By assaying histo-blood trisaccharide-exposing neoglycoconjugates in cancer research it has for example become apparent that the common monitoring of the carbohydrate display is expanded rationally by 'reverse lectin histochemistry' [Gabius et al., 1993] to underpin prognostic relevance of presence of distinct determinants, for example A-/H-trisaccharide-binding activities in lung cancer [Kayser et al., 1994; Kayser and Gabius, 1999]. Coupled with mas-

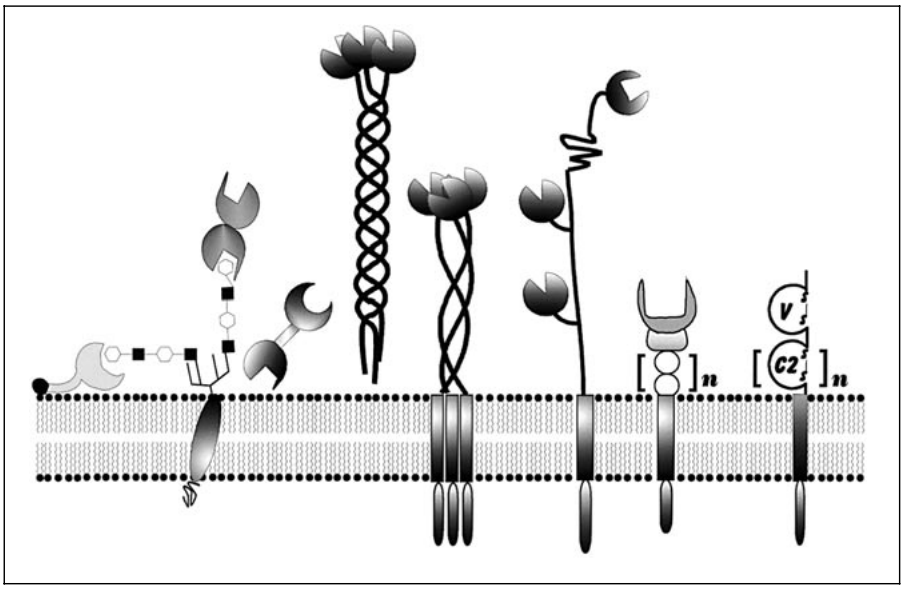

Fig. 5. Schematic illustration of the relative (strategic) positioning of CRDs in several types of animal lectins (from left to right): chimeric, prototype and tandem-repeat-type galectins with variations in CRD features for the two modules of the latter subgroup, C-type lectins in serum and surfactant with collagenous stalks forming triple helices, as transmembrane proteins with α-helical coiled-coil stalks (such as the hetero-oligomeric hepatic asialoglycoprotein receptor), with a tandem-repeat display of CRDs (and two further modules, i.e. a fibronectin type II repeat and an N-terminal cysteine-rich domain with GalNAc-4-SO_4 specificity, in the case of the C-type mannose receptor) and C-type lectins with a single CRD in a different modular arrangement [i.e. selectins with the CRD positioned for intercellular contacts at the tip of the molecule followed by the epidermal-growth-factor-like domain and by two (L-selectin), six (E-selectin) or nine (P-selectin) complement-binding consensus repeats mainly as spacers] as well as I-type lectins (such as the members of the sialoadhesin subgroup, also referred to as siglecs, which use a distinct type of V-set domain as CRD and 1–16 C2-set immunoglobulin-like domains as spacers to modulate potential for either *trans*- or *cis*-interactions on the cell surface).

tering the problems of artificial glycan design by chemoenzymatic synthesis such probes have even become accessible with complex N-glycan chains as constituents to launch routine cell biological and histochemical monitoring [André et al., 1997]. Adequate tailoring of the sugar (sensor) part of these tools toward the in situ receptor fosters the range of applications of these probes, historically first introduced as artificial antigens to raise carbohydrate-specific antibodies [Avery and Göbel, 1929; Göbel and Avery, 1929], which continues to be a field of active research [Jennings and Sood, 1994]. By addressing the challenge to unveil the intimate details of the molecular rendezvous and its driving forces, another important step to steer the concept of the sugar code down the road to connect structure to function will be taken. The success of this project depends critically on the integration of a set of sophisticated techniques, as already emphasized in the chapter on the relation between carbohydrate sequence and shape. Main strategies to crack the sugar code are compiled in figure 6 and will be outlined in the next chapter.

How Lectins and Carbohydrate Ligands Interact

The variability in linking sugar monomers to obtain linear and branched chains (fig. 1) is based upon the sugar's characteristic abundance of hydroxyl groups (fig. 2). Automatically, basic chemical knowledge comes to mind on sp^3-hybridized oxygen atom offering two lone electron pairs as acceptor and the proton as donor of hydrogen bonds, when musing about implications for the interaction. As underscored in figure 7, this bonding is highly directional and, when integrated into bidentate or cooperative bonds, acts as an inherent specificity control distinguishing epimers [Quiocho and Vyas, 1984; Quiocho, 1986; Vyas, 1991; Cambillau, 1995; Rini, 1995; Quiocho and Ledvina, 1996; Weis and Drickamer, 1996; Bundle, 1997; Gabius and Romero, 1998; Lis and Sharon, 1998; Loris et al., 1998; Rüdiger and Rougé, 1998]. Figure 7 also draws attention to another salient factor in carbohydrate interaction.

The chemical character of the saccharide naturally entails a distinct pattern of hydroxyl groups and less polar

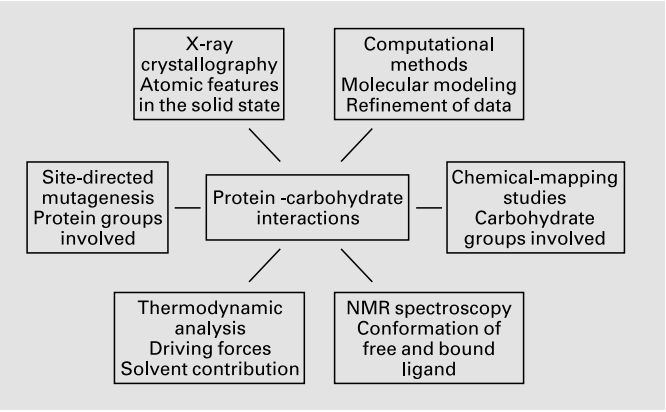

Fig. 6. Experimental approaches to examine different aspects of protein-carbohydrate interactions.

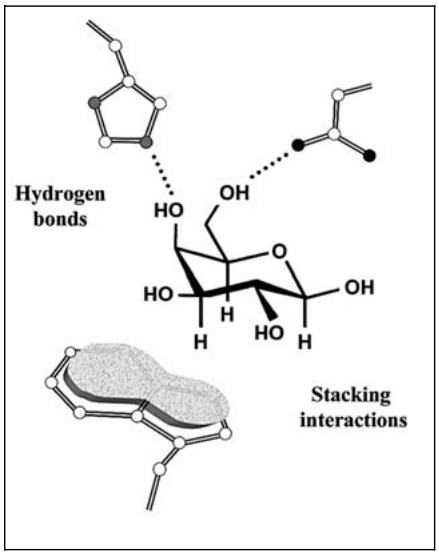

Fig. 7. Main enthalpic features to facilitate binding of a sugar to a protein: hydrogen bonds using lone electron pairs of sugar oxygen atoms as acceptors and the hydrogen atoms of hydroxyl groups as donors as well as C-H/π-electron interactions between patches of positively polarized character (in this case the B-face of *D*-Gal) and the delocalized π-electron cloud of a suitably positioned aromatic residue (here Trp).

aliphatic C-H patches. Therefore, sections of different degrees of hydrophilicity are formed [Lemieux, 1996; Sundari and Balasubramanian, 1997]. In the case of *D*-galactose, this positioning of rather apolar axial C-H groups constitutes an area stretch which should not be overlooked in the quest of dissecting driving forces for complex formation (fig. 7). Already at this stage, it thus is reasonable to refute the preconception that exclusively hydrogen bonds are responsible for ligand binding. By the way, van der Waals interactions and hydrophobic effects markedly come into play to bring about notable portions of the free enthalpy changes in various cases of drug-receptor binding [Davis and Teague, 1999; Kuntz et al., 1999]. How will this part of a saccharide contribute to affinity?

When the B face of *D*-galactose can come into contact with a delocalized π-electron cloud, the emergence of two affinity-enhancing factors can be counted upon: the mutual shielding of the nonpolar surfaces from bulk solvent is entropically favorable, and the electrostatic interaction between the positive net charge of the C-H groups and the quadrupole created by the π-system of the aromatic ring adds an enthalpic contribution. Indeed, the polarizability of the aromatic electrons and the polarizing nature of a dipol (or a cation) will lead to an attractive force which is frequently encountered in protein structures and ligand-accommodating mechanisms [Burley and Petsko, 1986; Nishio et al., 1995; Dougherty, 1996]. It should not be neglected that proper orientation furthers the generation of net enthalpic gain and thereby improves epimer selection. As a general rule, biorecognition e.g. in cell migration is not supposed to be too strong or even permanent but definitely specific, distinguishing epimers reliably. On the molecular level, the strategic placement of a tryptophan moiety in the binding site is a means to this end. Only very rarely, e.g. in glycogen phosphorylase, mannan-binding lectin, *Galanthus nivalis* agglutinin and the cation-dependent mannose-6-phosphate receptor [Martin et al., 1990; Hester et al., 1995; Weis and Drickamer, 1996; Roberts et al., 1998], can sugar receptors manage ligand binding without taking advantage of matching these surfaces. The propensity to place aromatic residues in contact sites is convincingly demonstrated by a bacterial periplasmic maltose receptor, engulfing its ligand and wedging it between aromatic side chains [Spurlino et al., 1991; Quiocho et al., 1997]. Even rather small surface patches of a sugar moiety can be exploited by van der Waals contacts such as the acetamidomethyl group and the terminal carbon of the glycerol side chain of N-acetylneuraminic acid (see fig. 2 for structure) with $Trp^{2/106}$ in the I-type lectin sialoadhesin [May et al., 1998].

To list the occurrence of the discussed interactions systematically, X-ray crystallography is commonly performed. Starting with the growth of suitable crystals (fig. 8) and the collection of diffraction data (fig. 9), the global architecture of the sugar receptor (fig. 10) and the topology of the ligand-binding site (fig. 11) can be present-

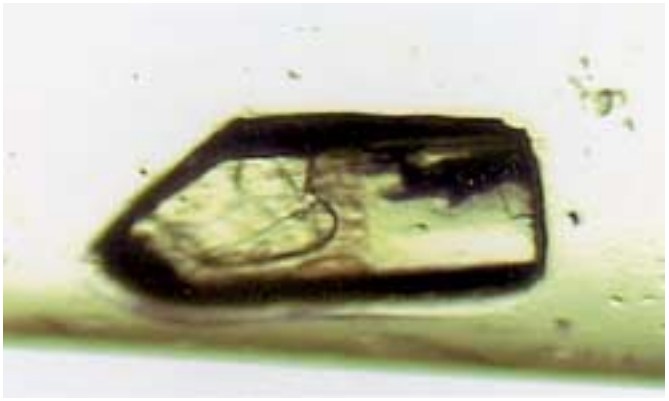

Fig. 8. Orthorhombic crystal ($C222_1$) of the developmentally regulated homodimeric galectin from chicken liver (CG-16) grown in 2 *M* ammonium sulfate, 5% (v/v) isopropanol and 1% β-mercaptoethanol, pH 5.6, at an estimated final protein concentration of 10 mg/ml. The crystal size is 0.4 × 0.6 mm^3.

ed, as documented by the given series of figures for the first analyzed avian galectin [Varela et al., 1999]. The enormous promise of this impressive technique, however, comes with two caveats: the conditions for crystal growth and crystal packing may alter the solution structure, and the actual consequences of the inferred interactions for the free energy change cannot readily be predicted. The already mentioned figure 6 offers advice on how to sort out these problems, focusing first on ligand derivatives.

It is a feat of synthetic chemistry to enable to tinker with the basic structure of saccharides. Any hydroxyl group can be chosen as target for site-specific modification, and the binding properties of the derivatives can be assessed in relation to the natural ligand [Lemieux, 1989; Solís and Díaz-Mauriño, 1997]. The types of chemical replacements and their expectable impacts on the regular donor-acceptor profile of hydroxyl groups are compiled in figure 12. The strict directionality in this figure attests the

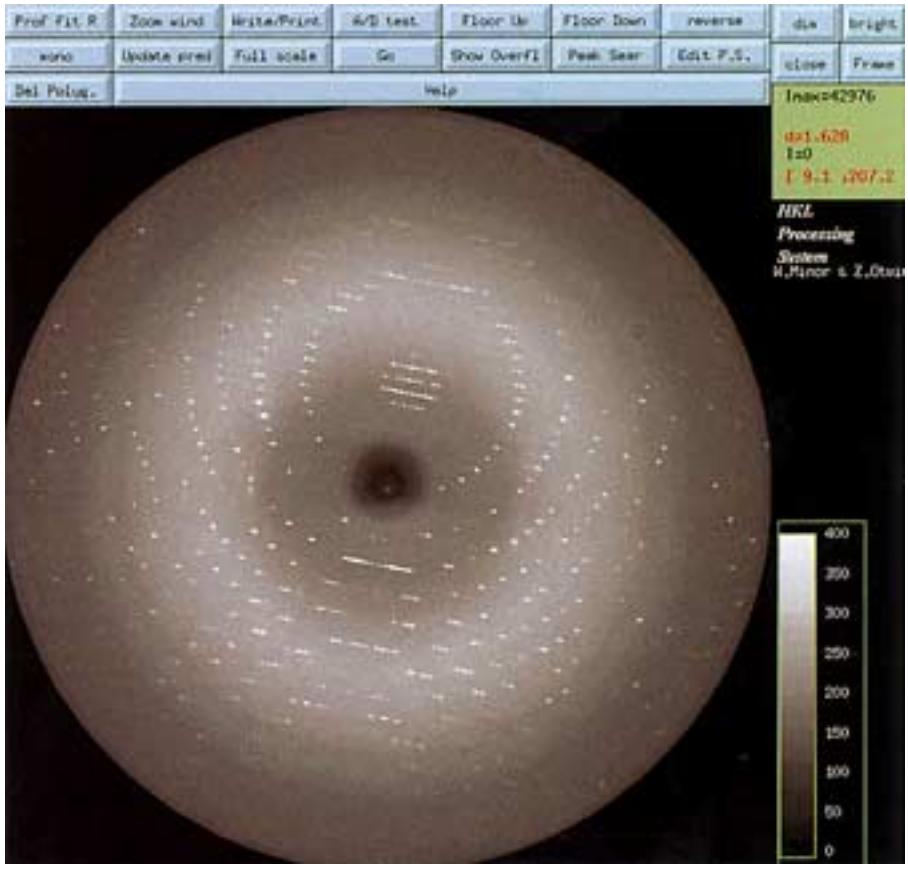

Fig. 9. A 1° oscillation photograph of CG-16 collected on a Rigaku RU200 rotating anode generator operating at 5.4 kW. The diffraction pattern in this film extends to 2.1 Å resolution.

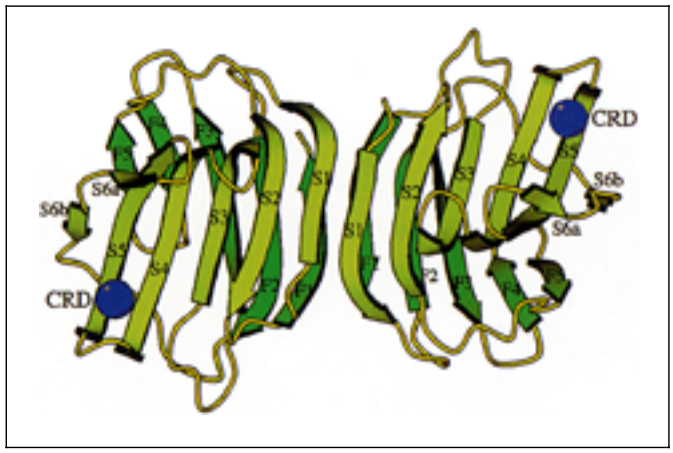

Fig. 10. Ribbon diagram of CG-16, prepared with MOLSCRIPT [Kraulis, 1991]. The β-strands in the five-stranded (F1–F5) and six-stranded (S1–S6a/S6b) β-sheets are denoted by the letter-number code. The two carbohydrate-binding sites located at the opposite ends of the homodimer are indicated by spheres.

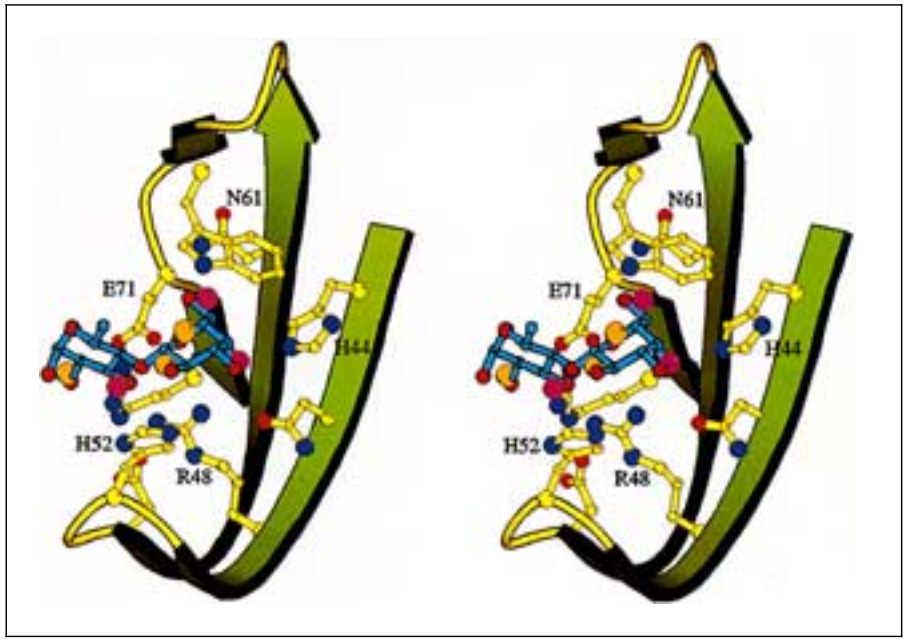

Fig. 11. Stereoview of the carbohydrate-binding site in ligand-free CG-16 showing the positions of the bound water molecules [Varela et al., 1999]. The three water molecules making direct contact with the protein are drawn as spheres. The relative position of a sugar ligand, i.e. N-acetyllactosamine, in the complex, as derived from molecular modelling, is shown for reference, documenting how ligand accommodation will lead to release of the water molecules and to stacking with Trp68 above the B-face of the galactose moiety (see fig. 7 for a scheme of the C-H cluster/π-electron cloud interaction).

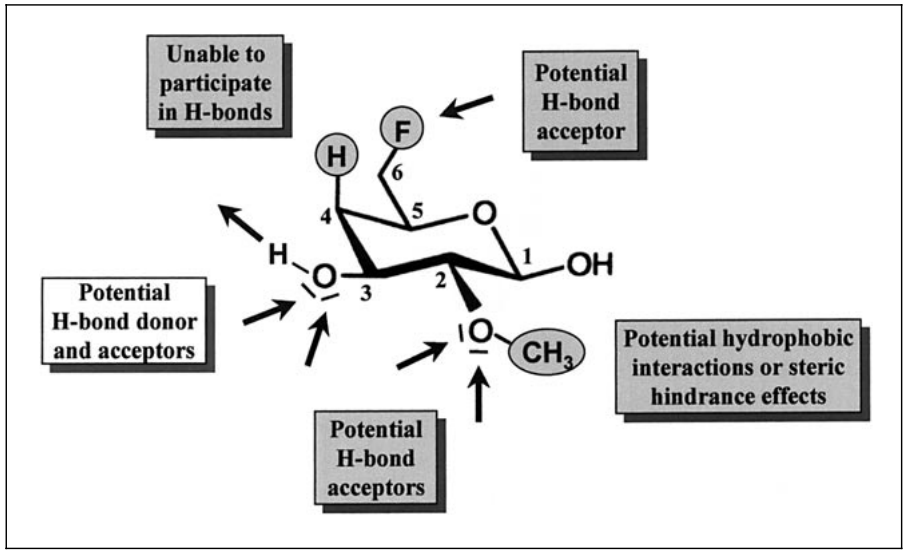

Fig. 12. Binding potential of different functional groups in synthetically engineered carbohydrate derivatives. The arrows indicate the capacity to be engaged in hydrogen bonding and point from donor to acceptor. Numbering according to the ring skeleton atoms of *D*-Gal starting at the anomeric C1 atom (see also fig. 7) is shown.

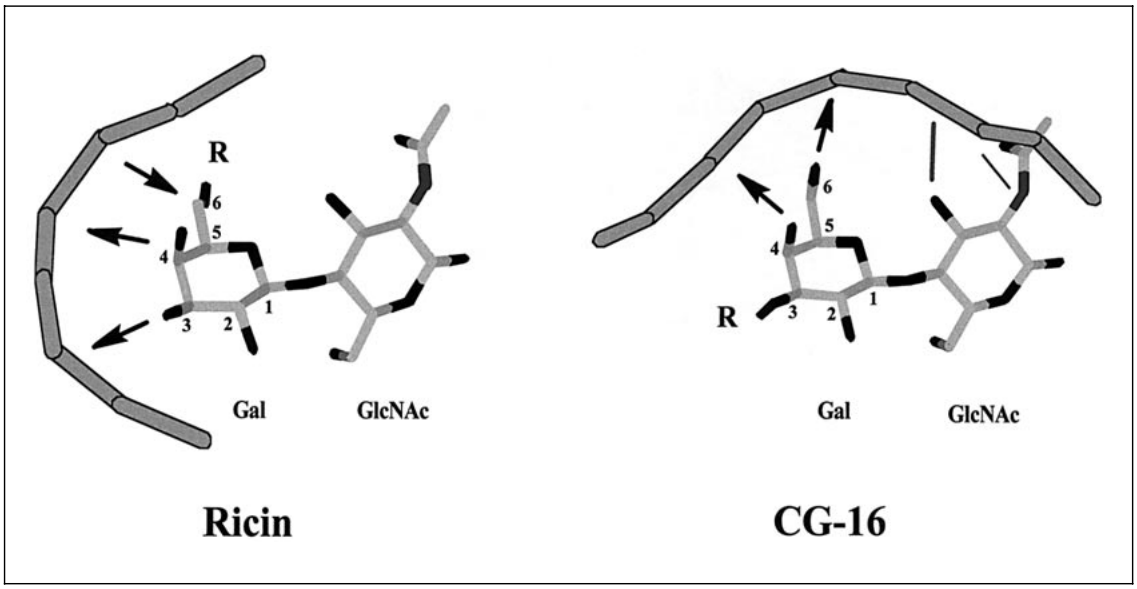

Fig. 13. Depiction of the differential patterns of key polar contacts between lectin and ligand, explaining the different oligosaccharide specificities of the plant AB toxin ricin and the chicken galectin CG-16 (see fig. 10, 11 for its crystal structure). As shown in figure 12, the arrow points from the donor to the acceptor in a hydrogen bond, when these relationships are known. The differential involvement of the protons of the 3′- and 6′-hydroxyl groups as donors in hydrogen bonds with the binding sites translates into preferred selection of 6-sialyllacNAc (Neu5Acα2,6Galβ1,4GlcNAc; R = Neu5Ac, see also fig. 2 for complete structure of sialyllactose) by the plant lectin and the 3′-isomer by the animal lectin (CG-16). Notably, further contacts are established in this case by the GlcNAc moiety.

exquisite specificity control imposed by the binding pocket. Having prepared a panel of these derivatives, key hydroxyl groups are identified, and the number of contributing hydrogen bonds is readily enumerated.

In detail, replacement of a hydroxyl group by deoxygenation with a hydrogen will abrogate the donor-acceptor potential at this site. Thus, a reduction in binding affinity upon deoxygenation at a certain position is indicative of involvement of the missing group in the hydrogen-bonding network. To address the question on the relative degrees of participation of the donor and/or acceptor qualities, another type of derivative is helpful. The electronegative fluorine atom maintains a capacity to serve as acceptor. Its incorporation at a distinct site and assays with fluoro and deoxy derivatives can dissect the hydroxyl group's activity as donor and/or acceptor. Finally, synthesis of derivatives with bulky substituents within former hydroxyl groups extends the probing into this aspect and also serves as sensor for nonpolar patches and spatial restrictions to mobility. As an example, this strategy has been crucial to pinpoint the differential involvement of hydroxyl groups in the course of complex formation between nonhomologous lectins and lactose [Rivera-Sagredo et al., 1991, 1992; Solís et al., 1993, 1994, 1996; Fernández et al., 1994]. Besides the salient role of the axial 4′-OH group distinguishing galactose from glucose and mannose the plant agglutinin ricin and galectins use either the 3′-OH or 6′-OH group as topological reference point [Rivera-Sagredo et al., 1991; Solís et al., 1994, 1996]. Fittingly, the natural α2,3/6-sialylated galactosides will exhibit conspicuous differences in docking capacity (fig. 13). The tolerance of α2,3-sialylation by galectins explains their binding to poly-N-acetyllactosamine chains (Galβ1,4GlcNAcβ1,3 units) and possibly also to internal sites, for example presented by the ganglioside GM_1, a major galectin ligand on human neuroblastoma cells [Gabius, 1997a; Kopitz et al., 1998; André et al., 1999b]. With respect to the mentioned influence of the often non-physiological conditions for crystallization it is notable that a thorough comparison of ricin's mode of ligand binding by crystallographic analysis and chemical mapping intimates a difference attributed to the pH in the crystallization protocol [Solís et al., 1993; Solís and Díaz-Mauriño, 1997]. These examples allow to insinuate that exploitation of engineered ligands is a great asset for mapping the hydrogen-bonding pattern with implications for drug design [Solís and Díaz-Mauriño, 1997; Rüdiger et al., 2000]. As similarly added to the section on crystal-

lography, voicing sources of potential complications is nonetheless essential to avoid to be led astray in special instances. Implicit assumptions on steric conservation despite the substitution under scrutiny may not always be fully valid, as seen for the 6-fluoro-6-deoxy derivative of galactose and the *Escherichia coli* periplasmic monopyranoside transporter [Vermersch et al., 1992], and involvement of differential ligand and protein solvation has been suggested to lead to nonlinear relationships of $\Delta\Delta H$ and $\Delta\Delta G$ values in the case of two related plant lectins [Dam et al., 1998].

This type of preeminent concern must be raised for the interpretation of results using protein mutants with intentional single-site substitution. Although it is often tacitly considered as true that this protein engineering will not distort any topological parameter, caution is not to be abandoned prematurely and supportive evidence needs to be collected. For example a recent modeling study with a plant agglutinin reveals that even long-range effects for surface accessibility of side chains have to be reckoned with upon mutagenesis [Siebert et al., 1997b]. Growing insights into the structure of the C-type carbohydrate recognition and the C-type lectin-like domains and their 'completely unanticipated variations' have served 'to emphasize both the limits of homology modeling and the need for continued structure determination, even within a family of homologous protein modules' [Drickamer, 1999]. Since modeling is evidently still prone to errors, spectroscopic analysis lent credibility to the results of the computations in the case of the two mentioned lectins [Siebert et al., 1997b]. Fluorescence and NMR spectroscopy with lectins in solution have also been conducive to underscore the already emphasized role of aromatic residues in the binding site of lectins for stacking, e.g. for galectins, a legume lectin and hevein-domain-harboring plant lectins [Levi and Teichberg, 1981; Siebert et al., 1997b, c]. By taking advantage of the principles of the already introduced NOE measurements the range of NMR spectroscopy – mutatis mutandis – can be extended to deliver parameters on ligand topology in the complex.

To glean information on conformational aspects of the ligand, interresidual proximity of protons of a ligand rapidly moving between the free and bound state is assessed. Monitoring of magnetization transfer for a ligand in the presence of less than stoichiometric amounts of receptor can yield transferred NOE (trNOE) signals [Lian et al., 1994; Ni and Scheraga, 1994]. The double-resonance experiments in the presence of a lectin furnish access to a molecular ruler of interproton distances in the complex [Peters and Pinto, 1996; Siebert et al., 1997a; Gabius, 1998; Poveda and Jiménez-Barbero, 1998; von der Lieth et al., 1998; Jiménez-Barbero et al., 1999; Rüdiger et al., 2000]. In fact, the more of these contacts are seen, the smaller the respective area section in the plots (see fig. 3) will be [von der Lieth et al., 1998]. To this end, measurements in aprotic solvents which do not impair the lectin's functionality have recently been shown to be a new strategy [Siebert et al., 2000].

Under all circumstances, adequate precautions must be taken to follow the pathway of energy transfer. Due to the intimate interaction with the protein, an indirect interresidual contact can be mediated via a proton of the binding site, establishing a three-spin process (fig. 14). Graphically denoted as spin diffusion, this mechanism can account for cross-peaks in the spectrum which do not arise from a direct contact (H1′–H1/H3 vs. H1′–H2 in fig. 14). When the free-ligand features have been assessed, a comparison between them and those of the bound state is feasible. In combination with results from molecular mechanics and dynamics simulations, as already discussed for the free ligand, the trNOE signals furnish the experimental input to assign parameters of free and bound ligand to distinct sections in the φ, ψ, E plot, given in figure 3. With this technique it is possible to answer the question as to the biological relevance of a 'bunch of keys'.

Remarkably, conformers from distinct low-energy sections of this plot (keys) can indeed fit into individual locks (lectins) sharing the same monosaccharide specificity. These results for plant/animal lectins and the C-type lectin subfamily of selectins (see fig. 5) establish the concept of differential conformer selection by lectins [Siebert et al., 1996; Poppe et al., 1997; Gilleron et al., 1998; von der Lieth et al., 1998; Harris et al., 1999]. Not only different sections of functional groups of a carbohydrate ligand are relevant for binding, as documented for example for plant, animal and bacterial galactoside- or plant blood group H (type 2)-trisaccharide-binding lectins by chemical mapping [Rivera-Sagredo et al., 1991; Du et al., 1994; Haataja et al., 1994; Solís et al., 1994; Solís and Díaz-Mauriño, 1997]. Importantly, each of the conformations in solution can be selected by different receptors as bioactive. The two conformers of the disaccharide Galβ1,2Gal which prevail in solution and are subject to differential selection by a plant and an animal lectin are shown in figure 15.

The same principle of selection of the right key from a conformer equilibrium also holds true for synthetic carbohydrate analogues, i.e. the methylene-bridged C lactose. Its three conformers can be categorized according to the

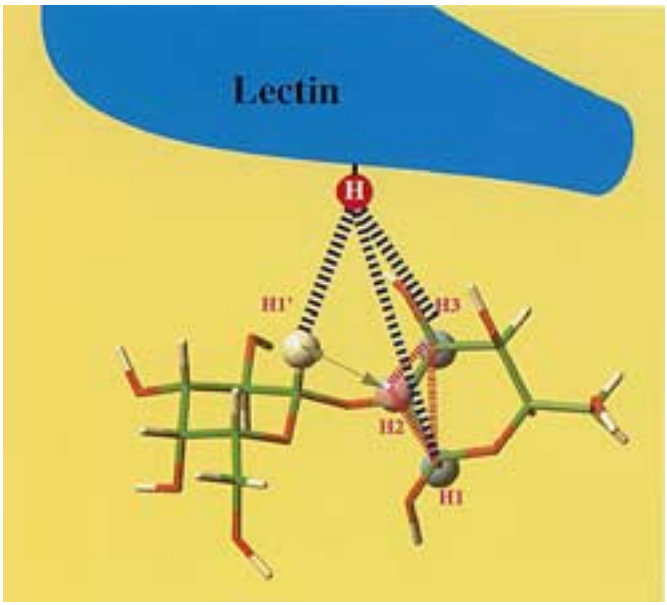

Fig. 14. Illustration of spin-diffusion pathways hampering immediate interpretation of trNOE-spectra. In addition to direct interresidual magnetization transfer from proton H1′ to proton H2 three-spin processes involving a suitably positioned proton of an amino acid in the lectin's binding pocket or a resonance between intraresidual protons (H2–H1, H2–H3) are theoretically possible.

expectable interresidual contacts and the methylene-proton-dependent contacts with pyranose ring protons (fig. 16). This compilation allows to infer that each conformer will be characterized by a distinct set of signals. Signal generation by binding to a galectin, a plant agglutinin or a bacterial β-galactosidase will thus readily be ascribed to any of these conformers, unless a major deviation from relative low-energy positions occurs. Indeed, the experimental data with receptors tested in the given order could be reconciled with the syn (left), anti (middle) and gauche-gauche (right) conformation, respectively [Espinosa et al., 1996, 1998; Ravishankar et al., 1998; Asensio et al., 1999]. The delineation of the selection process of the nonhydrolyzable pseudosubstrate for the glycosidase warrants attention for the design of inhibitors with adapted conformational properties. Whether conformationally constrained derivatives with frozen degrees of freedom copied from the bound-state topology can live up to the promise of enhanced binding is currently a topic of initial studies [Bundle et al., 1998; Navarre et al., 1999; Wacowich-Sgarbi and Bundle, 1999].

While protein-ligand proton contacts can be bothering in the interpretation of trNOE experiments (see fig. 13 for a three-spin process), these distance constraints are essen-

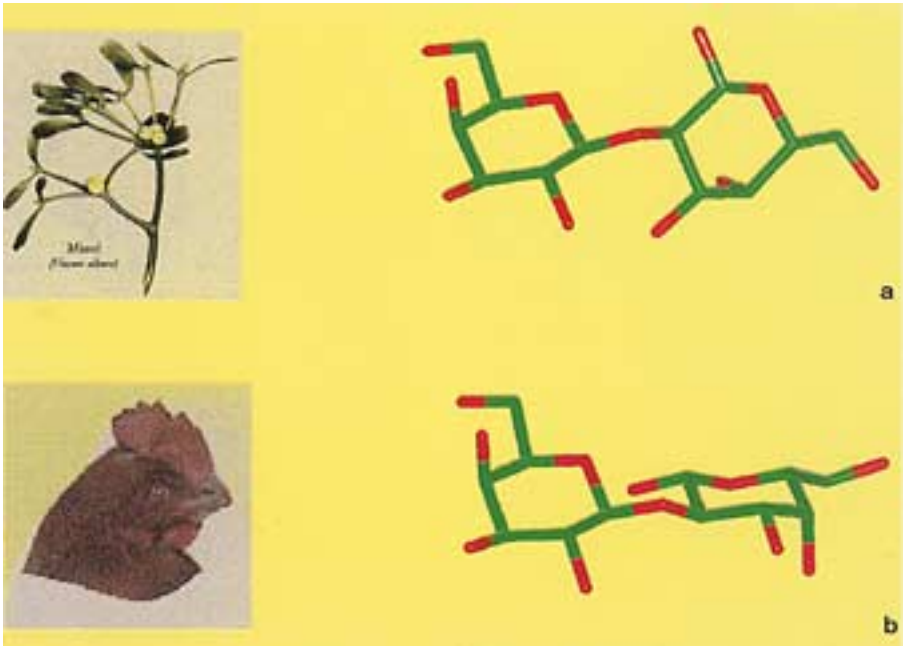

Fig. 15. Illustration of the geometry of the two low-energy conformers of Galβ1,2Gal serving as ligand either for the mistletoe agglutinin (**a**) or for the avian galectin (**b**). The position of the nonreducing Gal unit is deliberately kept constant to depict the remarkable topological difference as also done for highlighting the impact of rotation of one torsion angle in figure 2.

tial to gain insight into the way the ligand makes contacts with functional groups in the binding pocket. By completing the assignment of all measurable connectivities (an example of a part from a spectrum is shown in fig. 17) topological details of lectin structure and the interaction with the ligand are amenable. In view of the fact that accurate assignment of up to 375 distance constraints had to be completed [Asensio et al., 1998], it is evident that small lectins with 30–43 amino acids in total have so far been preferential targets for this demanding task [Asensio et al., 1995, 1998, 2000; Martins et al., 1996; Lü et al., 1999]. Access to isotope-enriched lectins and ligands with ensuing dispersion of signal overlap in multidimensional analysis is expected to expand the range of molecular weight stepwisely. Albeit without sugar-binding property, work on the type II antifreeze protein from sea raven presumably homologous to the CRD of C-type lectins presents an example of how ^{15}N-heteronuclear NMR spectroscopy using 785 NOE distance constraints and 47 angular constraints has been instrumental for solution structure assessment of a 14-kDa protein [Gronwald et al., 1998; Sönnichsen et al., 1999]. By the way, research on the C-type lectin domain appears to be rewarding due to the remarkable versatility for ligand binding in this module [Drickamer, 1999]. Distinct changes in certain sections of the common fold can be correlated to differential ways of binding ice crystals in the type II antifreeze proteins documented by site-directed mutations instead of sugar hydroxyl groups [Ewart et al., 1998, 1999; Loewen et al., 1998], to the promiscuity towards several carbohydrates or to carbohydrate and peptide motifs seen for the modular proteoglycans brevican, versican and aggrecan [Ng et al., 1996; Aspberg et al., 1997, 1999; Miura et al., 1999; Mann et al., 2000] or to the transition from carbohydrate to peptide accommodation in the case of the natural killer

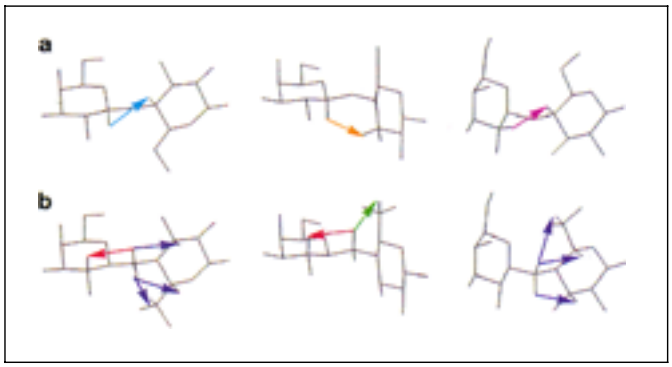

Fig. 16. Illustration of the three conformers of C-lactose, the methylene-bridged lactose derivative, present in equilibrium in D_2O and depiction of the expectable interresidual NOE contacts (**a**) and the contacts between protons on the pyranose ring and the methylene bridge of the nonhydrolyzable lactose derivative (**b**).

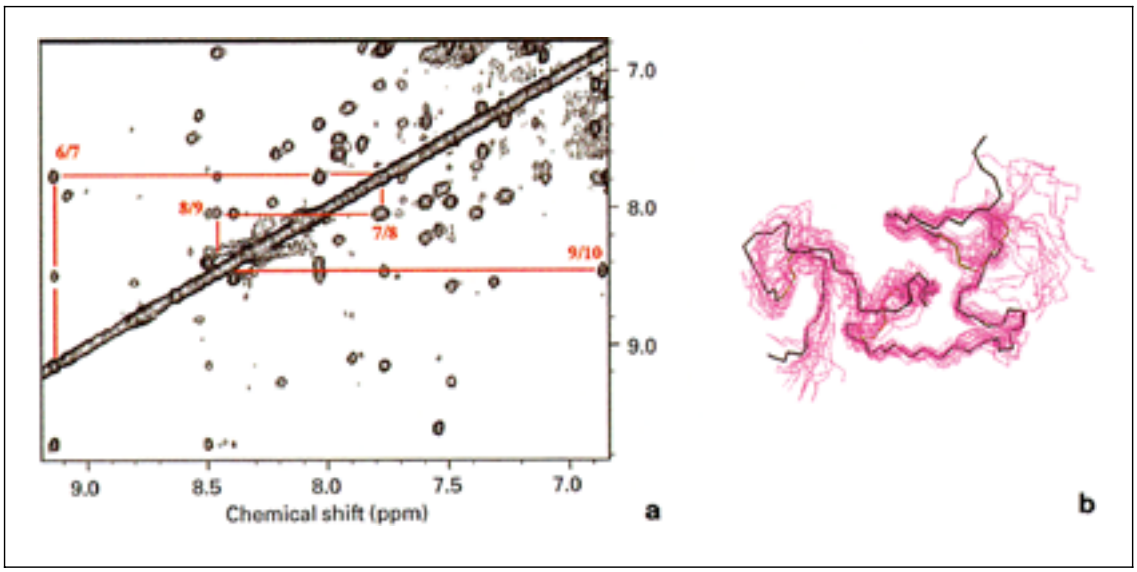

Fig. 17. Section of a NOESY spectrum (mixing time of 150 ms) of pseudohevein for assignment of -NH-Hα and NH-NH connectivities (**a**) and the ensemble of 20 simulated annealed structures based on 342 measured NOEs and their conversion to distance constraints (**b**) [Asensio et al., 2000].

cell receptor Ly49A [Tormo et al., 1999]. Again, a caveat also on this technique is appropriate.

The isotopic substitution to D_2O instead of H_2O yields deuterium bonds which are both stronger and more localized than hydrogen bonds. In terms of free energy, an enthalpic gain by nearly 10% is balanced by an equivalent unfavorable entropic contribution, leaving the overall free energy unchanged. Nonetheless, the possibility for an impact on structure by isotopic substitution should be kept in mind. For binding of a carbohydrate ligand, the decrease in enthalpy of binding of mannosides to two legume lectins in D_2O has been attributed to solvent reorganization [Chervenak and Toone, 1994]. This example guides to the enormous challenge [recently referred to as 'horrendous task', Doyle, 1997] to unambiguously relate thermodynamic parameters to driving forces and events on the level of molecules.

Globally, lectin-sugar interaction is not different from any other recognition mode and exhibits enthalpy/entropy compensation [Toone, 1994; Gabius, 1998]. Also, the parameters accessible by isothermal titration calorimetry are in principle attributable to individual contributions of the ligand, the receptor and the solvent in the process of complex formation [Gabius, 1998]. It is, for example, necessary to compare ligand-free and ligand-containing receptor to infer any change in flexibility and/or solvent population. Such a process is operative via a disorder $\rightarrow$ order transition for the loop containing residues 38–43 in the cation-dependent mannose-6-phosphate receptor [Roberts et al., 1998] or by replacement of the water molecules by the ligand in the avian galectin CG-16 (fig. 11) [Varela et al., 1999].

Actually, an ordering process in the course of mutual accommodation is a recurring theme in protein-RNA interaction. It supposedly maintains affinity at a level compatible with transient interactions [Draper, 1999]. Augmentation of stability by multivalent binding will then modulate this factor, as seen with increased ligand display in lectin research [Lee, 1982, 1992; Gabius et al., 1990, 1994, 1996; Lee and Lee, 1994a; Varki, 1994; Bovin and Gabius, 1995; Kiessling and Pohl, 1996; Roy, 1997; Mammen et al., 1998; Horan et al., 1999]. Clustering will thus be an efficient means of transiently switching on the capacity for tight binding. Already the reversible introduction of an acetyl or sulfate group may also be a key factor for triggering significant effects. To give an idea on the influence of structural modifications for thermodynamics, the avian galectins and the bacterial maltose receptor are proper models. Which factors actually account for the reduction of entropic penalty from 17.2 $\pm$ 0.5 to 7.0 $\pm$ 0.5 kJ/mol when testing lactose and N-acetyllactosamine, respectively, for this lectin [Bharadwaj et al., 1999] or the shift from an entirely entropy-driven process with maltodextrin to binding of maltose with favorable enthalpy and entropy by the bacterial protein [Thomson et al., 1998] will only become apparent with complete systematic studies, acting in concert as shown in figure 6.

Conclusions

Although peptide motifs are participants in virtually every biological process, their versatility in information transfer should not delude into the notion that they cover these multifaceted processes completely. New ideas and concepts on glycans as a new dimension in this area have been sparked off by diligent glycoconjugate analysis and the discovery of the diversity of animal lectins. Inevitably, this development to view oligosaccharides as manifestation of a code system, the sugar code, has taken on at a slow pace. But research over the last decade has delivered ample justification of why this concept is now at a high premium. There is good reason to expect that the outlined integrated approach, highlighted in figure 6, will be the basis to enhance our understanding of complex biological reaction cascades starting with fertilization. Unquestionably, it is becoming a foregone conclusion that the scope and versatility of glycocoding render the exploitation of the sugar code likely in more instances than currently described. Since the impact of protein-carbohydrate interaction is thus beginning to be fully appreciated by intense efforts in cutting-edge research, further interest will be engendered by the ensuing perspectives. Opportunities are likely to be opened at the interface of chemistry and biology to turn these insights into the how and why of protein-carbohydrate interaction eventually into rational medical applications.

References

André, S., P.J. Cejas Ortega, M. Alamino Perez, R. Roy, H.-J. Gabius (1999a) Lactose-containing starburst dendrimers: Influence of dendrimer generation and binding-site orientation of receptors (plant/animal lectins and immunoglobulins) on binding properties. Glycobiology *9:* 1253–1261.

André, S., S. Kojima, N. Yamazaki, C. Fink, H. Kaltner, K. Kayser, H.-J. Gabius (1999b) Galectins-1 and -3 and their ligands in tumor biology. Non-uniform properties in cell surface presentation and modulation of adhesion to matrix glycoproteins for various tumor cell lines, in biodistribution of free and liposome-bound galectins and in their expression by breast and colorectal carcinomas with/without metastatic propensity. J Cancer Res Clin Oncol *125:* 461–474.

André, S., C. Unverzagt, S. Kojima, X. Dong, C. Fink, K. Kayser, H.-J. Gabius (1997) Neoglycoproteins with the synthetic complex biantennary nonasaccharide or its α2,3-/α2,6-sialylated derivatives: Their preparation, the assessment of their ligand properties for purified lectins, for tumor cells in vitro and in tissue sections and their biodistribution in tumor-bearing mice. Bioconjug Chem *8:* 845–855.

Aplin, J.D., J.C. Wriston (1981) Preparation, properties and applications of carbohydrate conjugates of proteins and lipids. CRC Crit Rev Biochem *10:* 259–306.

Asensio, J.L., F.J. Cañada, M. Bruix, A. Rodriguez-Romero, J. Jiménez-Barbero (1995) The interaction of hevein with N-acetylglucosamine-containing oligosaccharides. Solution structure of hevein complexed with chitobiose. Eur J Biochem *230:* 621–633.

Asensio, J.L., F.J. Cañada, M. Bruix, C. Gonzalez, N. Khiar, A. Rodriguez-Romero, J. Jiménez-Barbero (1998) NMR investigations of protein-carbohydrate interactions: Refined three-dimensional structure of the complex between hevein and methyl β-chitobioside. Glycobiology *8:* 569–577.

Asensio, J.L., J.F. Espinosa, H. Dietrich, F.J. Cañada, R.R. Schmidt, M. Martín-Lomas, S. André, H.-J. Gabius, J. Jiménez-Barbero (1999) Bovine heart galectin-1 selects a distinct (syn) conformation of C-lactose, a flexible lactose analogue. J Am Chem Soc *121:* 8995–9000.

Asensio, J.L., H.-C. Siebert, C.-W. von der Lieth, J. Laynez, M. Bruix, J.J. Beintema, F.J. Cañada, H.-J. Gabius, J. Jiménez-Barbero (2000) NMR investigations of protein-carbohydrate interactions. Structure determination of the complex between pseudohevein and N,N′,N″-tri-O-acetylchitotriose and delineation of two different binding modes. Proteins, in press.

Ashwell, G., J. Harford (1982) Carbohydrate-specific receptors of the liver. Annu Rev Biochem *51:* 531–554.

Aspberg, A., S. Adam, G. Kostka, R. Timpl, D. Heinegård (1999) Fibulin-1 is a ligand for the C-type lectin domains of aggrecan and versican. J Biol Chem *274:* 20444–20449.

Aspberg, A., R. Miura, S. Bourdoulous, M. Shimonaka, D. Heinegård, M. Schachner, E. Ruoslahti, Y. Yamaguchi (1997) The C-type lectin domains of lecticans, a family of aggregating chondroitin sulfate proteoglycans, bind tenascin-R by protein-protein interactions independent of carbohydrate moiety. Proc Natl Acad Sci USA *94:* 10116–10121.

Avery, O.T., W.F. Göbel (1929) Chemo-immunological studies on conjugated carbohydrate proteins. II. Immunological specificity of synthetic sugar protein antigens. J Exp Med *50:* 533–550.

Barondes, S.H. (1981) Lectins: Their multiple endogenous cellular functions. Annu Rev Biochem *50:* 207–231.

Barondes, S.H. (1988) Bifunctional properties of lectins: Lectins redefined. Trends Biochem Sci *13:* 480–482.

Berger, E.G., E. Buddecke, J.P. Kamerling, A. Kobata, J.C. Paulson, J.F.G. Vliegenthart (1982) Structure, biosynthesis and function of glycoprotein glycans. Experientia *38:* 1129–1162.

Bharadwaj, S., H. Kaltner, E.Y. Korchagina, N.V. Bovin, H.-J. Gabius, A. Surolia (1999) Microcalorimetric indications for ligand binding as a function of the protein for galactoside-specific plant and avian lectins. Biochim Biophys Acta *1472:* 191–196.

Biermann, L., H.-J. Gabius, H.-W. Denker (1997) Neoglycoprotein-binding sites (endogenous lectins) in the fallopian tube, uterus and blastocyst of the rabbit during the preimplantation phase and implantation. Acta Anat *160:* 159–171.

Bovin, N.V., H.-J. Gabius (1995) Polymer-immobilized carbohydrate ligands: Versatile chemical tools for biochemistry and medical sciences. Chem Soc Rev *24:* 413–421.

Brockhausen, I., H. Schachter (1997) Glycosyltransferases involved in N- and O-glycan biosynthesis; in Gabius, H.-J., S. Gabius (eds): Glycosciences: Status and Perspectives. London, Chapman & Hall, pp 79–113.

Brockhausen, I., J. Schutzbach, W. Kuhns (1998) Glycoproteins and their relationship to human disease. Acta Anat *161:* 36–78.

Bundle, D.R. (1997) Antibody-oligosaccharide interactions determined by crystallography; in Gabius, H.-J., S. Gabius (eds): Glycosciences: Status and Perspectives. London, Chapman & Hall, pp 311–331.

Bundle, D.R., R. Alibés, S. Nilar, A. Otter, M. Warwas, P. Zhang (1998) Thermodynamic and conformational implications of glycosidic rotamers preorganized for binding. J Am Chem Soc *120:* 5317–5318.

Burley, S.K., G.A. Petsko (1986) Amino-aromatic interactions in proteins. FEBS Lett *203:* 139–143.

Bush, C.A., M. Martin-Pastor, A. Imberty (1999) Structure and conformation of complex carbohydrates of glycoproteins, glycolipids, and bacterial polysaccharides. Annu Rev Biophys Biomol Struct *28:* 269–293.

Cambillau, C. (1995) 3D structure. 1. The structural features of protein-carbohydrate interactions revealed by X-ray crystallography; in Montreuil, J., J.F.G. Vliegenthart, H. Schachter (eds): Glycoproteins. Amsterdam, Elsevier, pp 29–65.

Carver, J.P. (1991) Experimental structure determination of oligosaccharides. Curr Opin Struct Biol *1:* 716–720.

Chervenak, M.C., E.J. Toone (1994) A direct measure of the contribution of solvent reorganization to the enthalpy of ligand binding. J Am Chem Soc *116:* 10533–10539.

Cook, G.M.W. (1994) Recognising the attraction of sugars at the cell surface. Bioessays *16:* 287–295.

Cumming, D.A., J.P. Carver (1987) Virtual and solution conformations of oligosaccharides. Biochemistry *26:* 6664–6676.

Dam, T.K., B.S. Cavada, T.B. Grangeiro, C.F. Santos, F.A.M. de Sousa, S. Oscarson, C.F. Brewer (1998) Diocleinae lectins are a group of proteins with conserved binding sites for the core trimannoside of asparagine-linked oligosaccharides and differential specificities for complex carbohydrates. J Biol Chem *273:* 12082–12088.

Danguy, A., C. Decaestecker, F. Genten, I. Salmon, R. Kiss (1998) Application of lectins and neoglycoconjugates in histology and pathology. Acta Anat *161:* 206–218.

Danguy, A., F. Genten, H.-J. Gabius (1991) Histochemical evaluations of application of biotinylated neoglycoproteins for the detection of endogenous sugar receptors in fish skin. Eur J Basic Appl Histochem *35:* 341–357.

Danguy, A., K. Kayser, N.V. Bovin, H.-J. Gabius (1995) The relevance of neoglycoconjugates for histology and pathology. Trends Glycosci Glycotechnol *7:* 261–275.

Davis, A.M., S.J. Teague (1999) Die Bedeutung der Balance von Wasserstoffbrückenbindungen und hydrophoben Wechselwirkungen im Wirkstoff-Rezeptor-Komplex. Angew Chem *111:* 778–792.

Denker, H.-W. (1970a) Topochemie hochmolekularer Kohlenhydratsubstanzen in Frühentwicklung und Implantation des Kaninchens. I. Allgemeine Lokalisierung und Charakterisierung hochmolekularer Kohlenhydratsubstanzen in frühen Embryonalstadien. Zool Jahrb Abt Allg Zool Physiol *75:* 141–245.

Denker, H.-W. (1970b) Topochemie hochmolekularer Kohlenhydratsubstanzen in Frühentwicklung und Implantation des Kaninchens. II. Beiträge zu entwicklungsphysiologischen Fragestellungen. Zool Jahrb Abt Allg Zool Physiol *75:* 246–308.

Denker, H.-W. (1983) Basic aspects of ovoimplantation; in Wynn, R.M. (ed): Obstetrics and Gynecology Annual. Norwalk, Appleton Century Crofts, vol 12, pp 15–42.

Denker, H.-W. (1993) Implantation: A cell biological paradox. J Exp Zool *266:* 541–558.

Denker, H.-W., J.D. Aplin (1990) Trophoblast Invasion and Endometrial Receptivity. Novel Aspects of the Cell Biology of Embryo Implantation. Trophoblast Research. New York, Plenum Press, vol 4.

Dougherty, D.A. (1996) Cation-π interactions in chemistry and biology: A new view of benzene, Phe, Tyr, and Trp. Science *271:* 163–168.

Doyle, M.L. (1997) Characterization of binding interactions by isothermal titration calorimetry. Curr Opin Biotechnol *8:* 31–35.

Draper, D.E. (1999) Themes in RNA-protein recognition. J Mol Biol *293:* 255–270.

Drickamer, K. (1999) C-type lectin-like domains. Curr Opin Struct Biol *9:* 585–590.

Drickamer, K., M.E. Taylor (1993) Biology of animal lectins. Annu Rev Cell Biol *9:* 237–264.

Du, M.-H., U. Spohr, R.U. Lemieux (1994) The recognition of three different epitopes for the H-type 2 human blood group determinant by lectins of *Ulex europaeus, Galactia tenuiflora* and *Psophocarpus tetragonolobus* (winged bean). Glycoconj J *11:* 443–461.

Dwek, R.A., C.J. Edge, D.J. Harvey, M.R. Wormald (1993) Analysis of glycoprotein-associated oligosaccharides. Annu Rev Biochem *62:* 65–100.

Espinosa, J.-F., F.J. Cañada, J.L. Asensio, M. Martin-Pastor, H. Dietrich, M. Martin-Lomas, R.R. Schmidt, J. Jiménez-Barbero (1996) Experimental evidence of conformational differences between C-glycosides and O-glycosides in solution and in the protein-bound state: The C-lactose/O-lactose case. J Am Chem Soc *118:* 10862–10871.

Espinosa, J.-F., E. Montero, A. Vian, J.L. García, H. Dietrich, R.R. Schmidt, M. Martín-Lomas, A. Imberty, F.J. Cañada, J. Jiménez-Barbero (1998) *Escherichia coli* β-galactosidase recognizes a high-energy conformation of C-lactose, a nonhydrolyzable substrate analogue. NMR and modeling studies of the molecular complex. J Am Chem Soc *120:* 1309–1318.

Ewart, K.V., Z. Li, D.S.C. Yang, G.L. Fletcher, C.L. Hew (1998) The ice-binding site of Atlantic herring antifreeze protein corresponds to the carbohydrate-binding site of C-type lectins. Biochemistry *37:* 4080–4085.

Ewart, K.V., Q. Lin, C.L. Hew (1999) Structure, function and evolution of antifreeze proteins. Cell Mol Life Sci *55:* 271–283.

Fernández, P., J. Jiménez-Barbero, M. Martín-Lomas, D. Solís, T. Díaz-Mauriño (1994) Involvement of the glucose moiety in the molecular recognition of methyl b-lactoside by ricin: Synthesis, conformational analysis, and binding studies of different derivatives at the C-3 region. Carbohydr Res *256:* 223–244.

Fischer, E. (1894) Einfluss der Configuration auf die Wirkung der Enzyme. Ber Dtsch Chem Ges *27:* 2985–2993.

Gabius, H.-J. (1987a) Endogenous lectins in tumors and the immune system. Cancer Invest *5:* 39–46.

Gabius, H.-J. (1987b) Vertebrate lectins and their possible roles in fertilization, development and tumor biology. In Vivo *1:* 75–84.

Gabius, H.-J. (1988) Tumor lectinology: At the intersection of carbohydrate chemistry, biochemistry, cell biology and oncology. Angew Chem Int Ed *27:* 1267–1276.

Gabius, H.-J. (1991) Detection and functions of mammalian lectins – with emphasis on membrane lectins. Biochim Biophys Acta *1071:* 1–18.

Gabius, H.-J. (1994) Non-carbohydrate binding partners/domains of animal lectins. Int J Biochem *26:* 469–477.

Gabius, H.-J. (1997a) Animal lectins. Eur J Biochem *243:* 543–576.

Gabius, H.-J. (1997b) Concepts of tumor lectinology. Cancer Invest *15:* 454–464.

Gabius, H.-J. (1998) The how and why of protein-carbohydrate interaction: A primer to the theoretical concept and a guide to application in drug design. Pharm Res *15:* 23–30.

Gabius, H.-J. (2000) Biological information transfer beyond the genetic code: The sugar code. Naturwissenschaften *87:* 108–121.

Gabius, H.-J., S. André, A. Danguy, K. Kayser, S. Gabius (1994) Detection and quantification of carbohydrate-binding sites on cell surfaces and in tissue sections by neoglycoproteins. Methods Enzymol *242:* 37–46.

Gabius, H.-J., S. Gabius (1992) Chemical and biochemical strategies for the preparation of glycohistochemical probes and their application in lectinology. Adv Lectin Res *5:* 123–157.

Gabius, H.-J., S. Gabius (1993) Lectins and Glycobiology. Berlin, Springer. Gabius, H.-J., S. Gabius (1997) Glycosciences: Status and Perspectives. London, Chapman & Hall.

Gabius, H.-J., S. Gabius, T.V. Zemlyanukhina, N.V. Bovin, U. Brinck, A. Danguy, S.S. Joshi, K. Kayser, J. Schottelius, F. Sinowatz, L.F. Tietze, F. Vidal-Vanaclocha, J.-P. Zanetta (1993) Reverse lectin histochemistry: Design and application of glycoligands for detection of cell and tissue lectins. Histol Histopathol *8:* 369–383.

Gabius, S., K. Kayser, N.V. Bovin, N. Yamazaki, S. Kojima, H. Kaltner, H.-J. Gabius (1996) Endogenous lectins and neoglycoconjugates: A sweet approach to tumor diagnosis and targeted drug delivery. Eur J Pharm Biopharm *42:* 250–261.

Gabius, H.-J., A. Romero (1998) Structure and functions of animal lectins. Carbohydr Eur *23:* 16–21.

Gabius, S., V. Schirrmacher, H. Franz, S.S. Joshi, H.-J. Gabius (1990) Analysis of cell surface sugar receptor expression by neoglycoenzyme binding and adhesion to plastic-immobilized neoglycoproteins for related weakly and strongly metastatic cell lines of murine tumor model systems. Int J Cancer *46:* 500–507.

Gabius, H.-J., C. Unverzagt, K. Kayser (1998) Beyond plant lectin histochemistry: Preparation and application of markers to visualize the cellular capacity for protein-carbohydrate recognition. Biotech Histochem *73:* 263–277.

Geyer, H., R. Geyer (1998) Strategies for glycoconjugate analysis. Acta Anat *161:* 18–35.

Gilleron, M., H.-C. Siebert, H. Kaltner, C.-W. von der Lieth, T. Kozár, K.M. Halkes, E.Y. Korchagina, N.V. Bovin, H.-J. Gabius, J.F.G. Vliegenthart (1998) Conformer selection and differential restriction of ligand mobility by a plant lectin. Conformational behavior of Galβ1–3GlcNAcβ1-R, Galβ1–3GalNAcβ1-R and Galβ1–2Galβ1-R' in the free state and complexed with mistletoe lectin as revealed by random walk and conformational clustering molecular mechanics calculations, molecular dynamics simulations and nuclear Overhauser experiments. Eur J Biochem *252:* 416–427.

Göbel, W.F., O.T. Avery (1929) Chemo-immunological studies on conjugated carbohydrate proteins. I. The synthesis of *p*-aminophenyl β-glucoside, *p*-aminophenyl β-galactoside, and their coupling with serum globulin. J Exp Med *50:* 521–531.

Gronwald, W., M.C. Loewen, B. Lix, A.J. Daugulis, F.D. Sönnichsen, P.L. Davies, B.D. Sykes (1998) The solution structure of type II antifreeze protein reveals a new member of the lectin family. Biochemistry *37:* 4712–4721.

Haataja, S., K. Tikkanen, U. Nilsson, G. Magnusson, K.-A. Karlsson, J. Finne (1994) Oligosaccharide-receptor interaction of the Galα1–4Gal binding adhesin of *Streptococcus suis*. Combining site architecture and characterization of two variant adhesin specificities. J Biol Chem *269:* 27466–27472.

Hardy, B.J. (1997) The glycosidic linkage flexibility and time-scale similarity hypotheses. J Mol Struct *395–396:* 187–200.

Harris, R., G.R. Kiddle, R.A. Field, M.J. Milton, B. Ernst, J.L. Magnani, S.W. Homans (1999) Stable-isotope-assisted NMR studies on 13C-enriched sialyl Lewisx in solution and bound to E-selectin. J Am Chem Soc *121:* 2546–2551.

Hermentin, P., R. Witzel (1999) The hypothetical N-glycan charge. A number to characterize protein N-glycosylation. Pharm Pharmacol Commun *5:* 33–43.

Hester, G., H. Kaku, I.J. Goldstein, C.S. Wright (1995) Structure of mannose-specific snowdrop *(Galanthus nivalis)* lectin is representative of a new plant lectin family. Nature Struct Biol *2:* 472–479.

Hooper, L.V., S.M. Manzella, J.U. Baenziger (1997) The biology of sulfated oligosaccharides; in Gabius, H.-J., S. Gabius (eds): Glycosciences: Status and Perspectives. London, Chapman & Hall, pp 261–276.

Horan, N., L. Yan, H. Isobe, G.M. Whitesides, D. Kahne (1999) Nonstatistical binding of a protein to clustered carbohydrates. Proc Natl Acad Sci USA *96:* 11782–11786.

Hounsell, E.F. (1997) Methods of glycoconjugate analysis; in Gabius, H.-J., S. Gabius (eds): Glycosciences: Status and Perspectives. London, Chapman & Hall, pp 15–29.

Imberty, A. (1997) Oligosaccharide structures: Theory versus experiment. Curr Opin Struct Biol *7:* 617–623.

Jardetzky, O. (1980) On the nature of molecular conformations inferred from high-resolution NMR. Biochim Biophys Acta *621:* 227–232.

Jennings, H.J., R.K. Sood (1994) Synthetic glycoconjugates as human vaccines; in Lee, Y.C. R.T. Lee (eds): Neoglycoconjugates. Preparation and Applications. San Diego, Academic Press, pp 325–371.

Jiménez-Barbero, J., J.L. Asensio, F.J. Cañada, A. Poveda (1999) Free and protein-bound carbohydrate structures. Curr Opin Struct Biol *9:* 549–555.

Kaltner, H., B. Stierstorfer (1998) Animal lectins as cell adhesion molecules. Acta Anat *161:* 162–179.

Kapitonov, D., R.K. Yu (1999) Conserved domains of glycosyltransferases. Glycobiology *9:* 961–978.

Kayser, K., N.V. Bovin, E.Y. Korchagina, C. Zeilinger, F.-Y. Zeng, H.-J. Gabius (1994) Correlation of expression of binding sites for synthetic blood group A-, B-, and H-trisaccharides and for sarcolectin with survival of patients with bronchial carcinoma. Eur J Cancer *30A:* 653–657.

Kayser, K., H.-J. Gabius (1999) The application of thermodynamic principles to histochemical and morphometric tissue research: Principles and practical outline with focus on glycosciences. Cell Tissue Res *296:* 443–455.

Kiessling, L.L., N.L. Pohl (1996) Strength in numbers: Non-natural polyvalent carbohydrate derivatives. Chem Biol *3:* 71–77.

Kilpatrick, D.C., C. Green (1992) Lectins as blood typing reagents. Adv Lectin Res *5:* 51–94.

Kishore, U., P. Eggleton, K.B.M. Reid (1997) Modular organization of carbohydrate recognition domains in animal lectins. Matrix Biol *15:* 583–592.

Kopitz, J. (1997) Glycolipids: Structure and function; in Gabius, H.-J., S. Gabius (eds): Glycosciences: Status and Perspectives. London, Chapman & Hall, pp 163–189.

Kopitz, J., C. von Reitzenstein, M. Burchert, M. Cantz, H.-J. Gabius (1998) Galectin-1 is a major receptor for ganglioside GM1, a product of the growth-controlling activity of a cell surface ganglioside sialidase, on human neuroblastoma cells in culture. J Biol Chem *273:* 11205–11211.

Kozár, T., C.-W. von der Lieth (1997) Efficient modeling protocols for oligosaccharides: From vacuum to solvent. Glycoconjug J *14:* 925–933.

Kraulis, P.J. (1991) MOLSCRIPT: A program to produce both detailed and schematic plots of protein structures. J Appl Crystallogr *24:* 946–950.

Krüpe, M. (1956) Blutgruppenspezifische pflanzliche Eiweisskörper, Phytagglutinine. Stuttgart, Enke.

Kuntz, I.D., K. Chen, K.A. Sharp, P.A. Kollman (1999) The maximal affinity of ligands. Proc Natl Acad Sci USA *96:* 9997–10002.

Laine, R.A. (1997) The information-storing potential of the sugar code; in Gabius H.-J., S. Gabius (eds): Glycosciences: Status and Perspectives. London, Chapman & Hall, pp 1–14.

Lee, R.T. (1982) Binding site of the rabbit liver lectin specific for galactose/N-acetylgalactosamine. Biochemistry *21:* 1045–1050.

Lee, Y.C. (1992) Biochemistry of carbohydrate-protein interaction. FASEB J *6:* 3193–3200.

Lee, Y.C., R.T. Lee (1991) Neoglycoconjugates: Fundamentals and recent progress; in Gabius, H.-J., S. Gabius (eds): Lectins and Cancer. Heidelberg, Springer, pp 53–70.

Lee, R.T., Y.C. Lee (1994a) Enhanced biochemical affinities of multivalent neoglycoconjugates; in Lee, Y.C., R.T. Lee (eds): Neoglycoconjugates. Preparation and Applications. San Diego, Academic Press, pp 23–50.

Lee, Y.C., R.T. Lee (1994b) Neoglycoconjugates. Preparation and Applications. San Diego, Academic Press.

Lemieux, R.U. (1989) The origin of the specificity in the recognition of oligosaccharides by proteins. Chem Soc Rev *18:* 347–374.

Lemieux, R.U. (1996) How water provides the impetus for molecular recognition in aqueous solution. Acc Chem Res *29:* 373–380.

Levi, G., V.I. Teichberg (1981) Isolation and physicochemical characterization of electrolectin, a β-*D*-galactoside-binding lectin from the electric organ of *Electrophorus electricus*. J Biol Chem *256:* 5735–5740.

Lian, L.Y., I.L. Barsukov, M.J. Sutcliffe, K.H. Sze, G.C.K. Roberts (1994) Protein-ligand interactions: Exchange processes and determination of ligand conformation and protein-ligand contacts. Methods Enzymol *239:* 657–700.

Lis, H., N. Sharon (1986) Lectins as molecules and as tools. Annu Rev Biochem *55:* 35–67.

Lis, H., N. Sharon (1998) Lectins: Carbohydrate-specific proteins that mediate cellular recognition. Chem Rev *98:* 637–674.

Loewen, M.C., W. Gronwald, F.D. Sönnichsen, B.D. Sykes, P.L. Davies (1998) The ice-binding site of sea raven antifreeze protein is distinct from the carbohydrate-binding site of the homologous C-type lectin. Biochemistry *37:* 17745–17753.

Loris, R., T. Hamelryck, J. Bouckaert, L. Wyns (1998) Legume lectin structure. Biochim Biophys Acta *1383:* 9–36.

Lü, S., S. Liang, X. Gu (1999) Three-dimensional structure of *Selenocosmia huwena* lectin-I (SHL-I) from the venom of the spider *Selenocosmia huwena* by 2D-NMR. J Protein Chem *18:* 609–617.

Mammen, M., S.-K. Choi, G.M. Whitesides (1998) Polyvalente Wechselwirkungn in biologischen Systemen: Auswirkungen auf das Design und die Verwendung multivalenter Liganden und Inhibitoren. Angew Chem *110:* 2908–2953.

Mann, K., I.M. Weiss, S. André, H.-J. Gabius, M. Fritz (2000) The amino acid sequence of the abalone *(Haliotis laevigata*) nacre protein perlucin. Detection of a functional C-type lectin domain with galactose/mannose specificity. Eur J Biochem *267:* 5257–5264.

Mann, P.L., R.E. Waterman (1998) Glycocoding as an information management system in embryonic development. Acta Anat *161:* 153–161.

Marshall, R.D. (1972) Glycoproteins. Annu Rev Biochem *41:* 673–702.

Martin, J.L., S.G. Withers, L.N. Johnson (1990) Comparison of the binding of glucose and glucose-1-phosphate derivatives to T state glycogen phosphorylase b. Biochemistry *29:* 10745–10757.

Martins, J.C., D. Maes, R. Loris, H.A.M. Pepermans, L. Wyns, R. Willem, P. Verheyden (1996) 1H NMR study of the solution structure of Ac-AMP2, a sugar binding antimicrobial protein isolated from *Amaranthus caudatus*. J Mol Biol *258:* 322–333.

May, A.P., R.C. Robinson, M. Vinson, P.R. Crocker, E.Y. Jones (1998) Crystal structure of the N-terminal domain of sialoadhesin in complex with 3′-sialyllactose at 1.85 Å resolution. Mol Cell *1:* 719–728.

Mitchell, S.W. (1860) Researches upon the venom of the rattlesnake. Smithsonian Contrib Knowledge *XII:* 89–90.

Miura, R., A. Aspberg, I.M. Ethell, K. Hagihara, R.L. Schnaar, E. Ruoslahti, Y. Yamaguchi (1999) The proteoglycan lectin domain binds sulfated cell surface glycolipids and promotes cell adhesion. J Biol Chem *274:* 11431–11438.

Munday, J., H. Floyd, P.R. Crocker (1999) Sialic acid-binding receptors (siglecs) expressed by macrophages. J Leukoc Biol *66:* 705–711.

Navarre, N., N. Amiot, A. van Oijen, A. Imberty, A. Poveda, J. Jiménez-Barbero, A. Cooper, M.A. Nutley, G.-J. Boons (1999) Synthesis and conformational analysis of a conformationally constrained trisaccharide, and complexation properties with Concanavalin A. Chem Eur J *5:* 2281–2294.

Ng, K.K.S., K. Drickamer, W.I. Weis (1996) Structural analysis of monosaccharide recognition by rat liver mannose-binding protein. J Biol Chem *271:* 663–674.

Ni, F., H.A. Scheraga (1994) Use of transferred nuclear Overhauser effect to determine the conformations of ligands bound to proteins. Acc Chem Res *27:* 257–264.

Nishio, M., Y. Umezawa, M. Hirota, Y. Takeuchi (1995) The CH/π interaction: Significance in molecular recognition. Tetrahedron *51:* 8665–8701.

Olden, K., J.B. Parent, S.L. White (1982) Carbohydrate moieties of glycoproteins. A re-evaluation of their function. Biochim Biophys Acta *650:* 209–232.

Pearlman, D.A. (1994) How is an NMR structure best defined? An analysis of molecular dynamics distance-based approaches. J Biomol NMR *4:* 1–16.

Pérez, S., A. Imberty, J.P. Carver (1994) Molecular modeling: An essential component in the structure determination of oligosaccharides and polysaccharides. Adv Comput Biol *1:* 147–202.

Pérez, S., A. Imberty, S.B. Engelsen, J. Gruza, K. Mazeau, J. Jiménez-Barbero, A. Poveda, J.-F. Espinosa, B.P. van Eyck, G. Johnson, A.D. French, M.L.C.E. Kouwijzer, P.D.J. Grootenuis, A. Bernardi, L. Raimondi, H. Senderowitz, V. Durier, G. Vergoten, K. Rasmussen (1999) A comparison and chemometric analysis of several molecular mechanics force fields and parameter sets applied to carbohydrates. Carbohydr Res *314:* 141–155.

Peters, T., B.M. Pinto (1996) Structure and dynamics of oligosaccharides: NMR and modeling studies. Curr Opin Struct Biol *6:* 710–720.

Poppe, L., G.S. Brown, J.S. Philo, P.V. Nikrad, B.H. Shah (1997) Conformation of sLex tetrasaccharide, free in solution and bound to E-, P-, and L-selectin. J Am Chem Soc *119:* 1727–1736.

Poveda, A., J. Jiménez-Barbero (1998) NMR studies of carbohydrate-protein interaction in solution. Chem Soc Rev *27:* 133–143.

Powell, L.D., A. Varki (1995) I-type lectins. J Biol Chem *270:* 14243–14246.

Probstmeier, R., P. Pesheva (1999) I-Type lectins in the nervous system. Prog Neurobiol *58:* 163–184.

Quiocho, F.A. (1986) Carbohydrate-binding proteins: Tertiary structures and protein-sugar interactions. Annu Rev Biochem *55:* 287–315.

Quiocho, F.A., P.S. Ledvina (1996) Atomic structure and specificity of bacterial periplasmic receptors for active transport and chemotaxis: Variation of common themes. Mol Microbiol *20:* 17–25.

Quiocho, F.A., J.C. Spurlino, L.E. Rodseth (1997) Extensive features of tight oligosaccharide binding revealed in high-resolution structures of the maltodextrin transport/chemosensory receptor. Structure *5:* 997–1015.

Quiocho, F.A., N.K. Vyas (1984) Novel stereospecificity of the *L*-arabinose-binding protein. Nature *310:* 381–386.

Ravishankar, R., A. Surolia, M. Vijayan, S. Lim, Y. Kishi (1998) Preferred conformation of C-lactose at the free and peanut lectin bound states. J Am Chem Soc *120:* 11297–11303.

Reuter, G., H.-J. Gabius (1999) Eukaryotic glycosylation – Whim of nature or multipurpose tool? Cell Mol Life Sci *55:* 368–422.

Rini, J.M. (1995) Lectin structure. Annu Rev Biophys Biomol Struct *24:* 551–577.

Rivera-Sagredo, A., D. Solís, T. Díaz-Mauriño, M. Jiménez-Barbero, M. Martín-Lomas (1991) Studies on the molecular recognition of synthetic methyl β-lactoside analogs by ricin, a cytotoxic plant lectin. Eur J Biochem *197:* 217–228.

Rivera-Sagredo, A., J. Jiménez-Barbero, M. Martín-Lomas, D. Solís, T. Díaz-Mauriño (1992) Studies on the molecular recognition of synthetic methyl β-lactoside analogs by *Ricinus communis* agglutinin. Carbohydr Res *232:* 207–226.

Roberts, D.L., D.J. Weix, N.M. Dahms, J.-J.P. Kim (1998) Molecular basis of lysosomal enzyme recognition: Three-dimensional structure of the cation-dependent mannose-6-phosphate receptor. Cell *93:* 639–648.

Roseman, S. (1970) The synthesis of complex carbohydrates by multiglycosyltransferase systems and their potential function in intracellular adhesion. Chem Phys Lipids *5:* 270–297.

Roy, R. (1996a) Blue-prints, synthesis and applications of glycopolymers. Trends Glycosci Glycotechnol *8:* 79–99.

Roy, R. (1996b) Synthesis and some applications of chemically defined multivalent glycoconjugates. Curr Opin Struct Biol *6:* 692–702.

Roy, R. (1997) Recent developments in the rational design of multivalent glycoconjugates. Top Curr Chem *187:* 241–274.

Rudd, P.M., R.A. Dwek (1997) Glycosylation: Heterogeneity and the 3D structure of proteins. Crit Rev Biochem Mol Biol *32:* 1–100.

Rüdiger, H., P. Rougé (1998) Structure and functions of plant lectins. Carbohydr Eur *23:* 18–22.

Rüdiger, H., H.-C. Siebert, D. Solís, J. Jiménez-Barbero, A. Romero, C.-W. von der Lieth, T. Díaz-Mauriño, H.-J. Gabius (2000) Medicinal chemistry based on the sugar code: Fundamentals of lectinology and experimental strategies with lectins as targets. Curr Med Chem *7:* 389–416.

Schachter, H. (ed) (1999) Molecular basis of glycoconjugate disease. Biochim Biophys Acta *1455:* 61–418.

Sears, P., C.-H. Wong (1998) Enzyme action in glycoprotein synthesis. Cell Mol Life Sci *54:* 223–252.

Sharon, N. (1984) Glycoproteins. Trends Biochem Sci *9:* 198–202.

Sharon, N. (1998) Glycoproteins now and then: A personal account. Acta Anat *161:* 7–17.

Sharon, N., H. Lis (1972) Lectins: Cell-agglutinating and sugar-specific proteins. Science *177:* 949–958.

Sharon, N., H. Lis (1997) Glycoproteins: Structure and function; in Gabius, H.-J., S. Gabius (eds): Glycosciences: Status and Perspectives. London, Chapman & Hall, pp 133–162.

Siebert, H.-C., R. Adar, R. Arango, M. Burchert, H. Kaltner, G. Kayser, E. Tajkhorshid, C.-W. von der Lieth, R. Kaptein, N. Sharon, J.F.G. Vliegenthart, H.-J. Gabius (1997b) Involvement of laser photo CIDNP (chemically induced dynamic nuclear polarization)-reactive amino acid side chains in ligand binding by galactoside-specific lectins in solution. Similarities in the role of tryptophan/tyrosine residues for ligand binding between a plant agglutinin and mammalian/avian galectins and the detection of an influence of single-site mutagenesis on surface presentation of spatially separated residues. Eur J Biochem *249:* 27–38.

Siebert, H.-C., S. André, J.L. Asensio, F.J. Cañada, X. Dong, J.F. Espinosa, M. Frank, M. Gilleron, H. Kaltner, T. Kozár, N.V. Bovin, C.-W. von der Lieth, J.F.G. Vliegenthart, J. Jiménez-Barbero, H.-J. Gabius (2000) Experimental demonstration of potential for maintenance of carbohydrate-protein (lectin and immunoglobulin G) interactions in an aprotic solvent leading to a new combined computational and NMR-spectroscopical strategy for the identification of additional conformational constraints of the bound ligand. Submitted.

Siebert, H.-C., M. Gilleron, H. Kaltner, C.-W. von der Lieth, T. Kozár, N.V. Bovin, E.Y. Korchagina, J.F.G. Vliegenthart, H.-J. Gabius (1996) NMR-based, molecular dynamics- and random walk molecular mechanics-supported study of conformational aspects of a carbohydrate ligand (Galβ1–2Galβ1-R) for an animal galectin in the free and in the bound state. Biochem Biophys Res Commun *219:* 205–212.

Siebert, H.-C., C.-W. von der Lieth, M. Gilleron, G. Reuter, J. Wittmann, J.F.G. Vliegenthart, H.-J. Gabius (1997a) Carbohydrate-protein interaction; in Gabius H.-J., S. Gabius (eds): Glycosciences: Status and Perspectives. London, Chapman & Hall, pp 291–310.

Siebert, H.-C., C.-W. von der Lieth, R. Kaptein, J.J. Beintema, K. Dijkstra, N. van Nuland, U.M.S. Soedjanaatmadja, A. Rice, J.F.G. Vliegenthart, C.S. Wright, H.-J. Gabius (1997c) Role of aromatic amino acids in carbohydrate binding of plant lectins. Laser photo CIDNP (chemically induced dynamic nuclear polarization) study of hevein domain-containing lectins. Proteins *28:* 268–284.

Sinowatz, F., J. Plendl, S. Kölle (1998) Protein-carbohydrate interactions during fertilization. Acta Anat *161:* 196–205.

Solís, D., T. Díaz-Mauriño (1997) Analysis of protein-carbohydrate interaction by engineered ligands; in Gabius, H.-J., S. Gabius (eds): Glycosciences: Status and Perspectives. London, Chapman & Hall, pp 345–354.

Solís, D., P. Fernández, T. Díaz-Mauriño, J. Jiménez-Barbero, M. Martín-Lomas (1993) Hydrogen-bonding pattern of methyl β-lactoside binding to the *Ricinus communis* lectins. Eur J Biochem *214:* 677–683.

Solís, D., J. Jiménez-Barbero, M. Martín-Lomas, T. Díaz-Mauriño (1994) Probing hydrogen-bonding interactions of bovine heart galectin-1 and methyl β-lactoside by use of engineered ligands. Eur J Biochem *223:* 107–114.

Solís, D., A. Romero, H. Kaltner, H.-J. Gabius, T. Díaz-Mauriño (1996) Different architecture of the combining sites of two chicken galectins revealed by chemical-mapping studies with synthetic ligand derivatives. J Biol Chem *271:* 12744–12748.

Sönnichsen, F.D., P.L. Davies, B.D. Sykes (1999) NMR structural studies on antifreeze proteins. Biochem Cell Biol *76:* 284–293.

Spurlino, J.C., G.-Y. Lu, F.A. Quiocho (1991) The 2.3 Å resolution structure of the maltose- or maltodextrin-binding protein, a primary receptor of bacterial active transport and chemotaxis. J Biol Chem *266:* 5202–5219.

Stowell, C.P., Y.C. Lee (1980) Neoglycoproteins: The preparation and application of synthetic glycoproteins. Adv Carbohydr Chem Biochem *37:* 225–281.

Sundari, C.S., D. Balasubramanian (1997) Hydrophobic surfaces in saccharide chains. Prog Biophys Mol Biol *67:* 183–216.

Thomson, J., Y. Liu, J.M. Sturtevant, F.A. Quiocho (1998) A thermodynamic study of the binding of linear and cyclic oligosaccharides to the maltodextrin-binding protein of *Escherichia coli*. Biophys Chem *70:* 101–108.

Timoshenko, A.V., I.V. Gorudko, S.N. Cherenkevich, H.-J. Gabius (1999a) Differential potency of two crosslinking plant lectins to induce formation of haptenic sugar-resistant aggregates of rat thymocytes by post-binding signaling. FEBS Lett *449:* 75–78.

Timoshenko, A.V., I.V. Gorudko, H. Kaltner, H.-J. Gabius (1999b) Dissection of the impact of various intracellular signaling pathways on rat thymocytes after initial lectin-dependent cell association using a plant lectin as model and target-selective inhibitors. Mol Cell Biochem *197:* 137–145.

Toone, E.J. (1994) Structure and energetics of protein-carbohydrate complexes. Curr Opin Struct Biol *4:* 719–728.

Tormo, J., K. Natarajan, D.H. Margulies, R.A. Mariuzza (1999) Crystal structure of a lectin-like natural killer cell receptor bound to its MHC class I ligand. Nature *402:* 623–631.

Varela, P.F., D. Solís, T. Díaz-Mauriño, H. Kaltner, H.-J. Gabius, A. Romero (1999) The 2.15 Å crystal structure of CG-16, the developmentally regulated homodimeric chicken galectin. J Mol Biol *294:* 537–549.

Varki, A. (1993) Biological roles of oligosaccharides: All of the theories are correct. Glycobiology *3:* 97–130.

Varki, A. (1994) Selectin ligands. Proc Natl Acad Sci USA *91:* 7390–7397.

Varki, A. (1996) 'Unusual' modifications and variations of vertebrate oligosaccharides: Are we missing the flowers for the trees? Glycobiology *6:* 707–710.

Vasta, G.R., M. Quesenberry, H. Ahmed, N. O'Leary (1999) C-type lectins and galectins mediate innate and adaptive immune functions: Their roles in the complement activation pathway. Dev Comp Immunol *23:* 401–420.

Vermersch, P.S., J.J.G. Tesmer, F.A. Quiocho (1992) Protein-ligand energetics assessed using deoxy and fluorodeoxy sugars in equilibrium binding and high resolution crystallographic studies. J Mol Biol *226:* 923–929.

Villalobo, A., H.-J. Gabius (1998) Signaling pathways for transduction of the initial message of the glycocode into cellular responses. Acta Anat *161:* 110–129.

Vliegenthart, J.F.G., F. Casset (1998) Novel forms of protein glycosylation. Curr Opin Struct Biol *8:* 565–571.

von der Lieth, C.-W., T. Kozár, W.E. Hull (1997) A (critical) survey of modeling protocols used to explore the conformational space of oligosaccharides. J Mol Struct *395–396:* 225–244.

von der Lieth, C.-W., H.-C. Siebert, T. Kozár, M. Burchert, M. Frank, M. Gilleron, H. Kaltner, G. Kayser, E. Tajkhorshid, N.V. Bovin, J.F.G. Vliegenthart, H.-J. Gabius (1998) Lectin ligands: New insights into their conformations and their dynamic behavior and the discovery of conformer selection by lectins. Acta Anat *161:* 91–109.

Vyas, N.K. (1991) Atomic features of protein-carbohydrate interactions. Curr Opin Struct Biol *1:* 732–740.

Wacowich-Sgarbi, S.A., D.R. Bundle (1999) Constrained H-type 2 blood group trisaccharide synthesized in a bioactive conformation via intramolecular glycosylation. J Org Chem *64:* 9080–9089.

Watkins, W.M. (1955) Blood group substances. Science *152:* 172–181.

Watkins, W.M. (1999) A half century of blood-group antigen research: Some personal recollections. Trends Glycosci Glycotechnol *11:* 391–411.

Watkins, W.M., W.T.J. Morgan (1952) Neutralisation of the anti-H agglutinin in eel serum by simple sugars. Nature *169:* 825–826.

Weigel, P.H. (1992) Mechanisms and control of glycoconjugate turnover; in Allen, H.J., E.C. Kisailus (eds): Glycoconjugates: Composition, Structure and Function. New York, Dekker, pp 421–497.

Weis, W.I., K. Drickamer (1996) Structural basis of lectin-carbohydrate recognition. Annu Rev Biochem *65:* 441–473.

Winterburn, P.J., C.F. Phelps (1972) The significance of glycosylated proteins. Nature *236:* 147–151.

Woods, R.J. (1998) Computational carbohydrate chemistry: What theoretical methods can tell us. Glycoconj J *15:* 209–216.

Woods, R.J., R.A. Dwek, C.J. Edge, B. Fraser-Reid (1995) Molecular mechanical and molecular dynamical simulations of glycoproteins and oligosaccharides. J Phys Chem *99:* 3832–3846.

Zanetta, J.-P. (1998) Structure and functions of lectins in the central and peripheral nervous system. Acta Anat *161:* 180–195.