

85-1

#

ZEITSCHRIFT FÜR NATURFORSCHUNG

Section c

B I O S C I E N C E S

Council

E. BÜNNING, Tübingen
A. BUTENANDT, München
M. EIGEN, Göttingen
W. GENTNER, Heidelberg

Editorial Board

A. HAGER, Tübingen
W. HASSELBACH, Heidelberg
E. HELMREICH, Würzburg
F. KAUDEWITZ, München
W. SCHÄFER, Tübingen

Advisory Editorial Board

P. BÖGER, Konstanz
K. G. GÖTZ, Tübingen
G. GOTTSCHALK, Göttingen
H. HOFFMANN-BERLING, Heidelberg
R. JAENICKE, Regensburg
P. KARLSON, Marburg
G. F. MEYER, Tübingen
D. SCHULTE-FROHLINDE, Mülheim/R.

G. SCHWARZ, Basel
F. F. SEELIG, Tübingen
H. SIMON, München
W. STEGLICH, Bonn
W. TANNER, Regensburg
A. TREBST, Bochum
E. WECKER, Würzburg

EDITED IN COLLABORATION

WITH THE INSTITUTES OF THE MAX-PLANCK-GESELLSCHAFT

VOLUME 32c

NUMBER 1/2

JANUARY/FEBRUARY 1977

VERLAG DER ZEITSCHRIFT FÜR NATURFORSCHUNG
TÜBINGEN

32, c
1977

126

The Zeitschrift für Naturforschung is published monthly in three separate sections:

Section a: Physics, Physical Chemistry, Cosmic Physics.

Section b: Inorganic and Organic Chemistry.

Section c: Biochemistry, Biophysics, Biology, Immunology, Virology.

The journal includes four types of contributions:

- (1) Original papers.
- (2) Notes. These are short original papers not exceeding two pages in small print which are to be published within six weeks after submission.
- (3) Reports on current research of special interest.
- (4) Book reviews (in Section b only).

Information for Contributors to Section c:

- (1) Please send two copies of the manuscript with your contribution to the managing editor Dr. H. Hausen, P.O. Box 2645, D-7400 Tübingen.
(Section a: Mrs. T. Littmann, Lenzhalde 21, D-7082 Oberkochen, Section b: Dr. H. Voelter, P.O. Box 2645, D-7400 Tübingen).
- (2) The manuscripts should be type written using double spacing throughout.
- (3) The title should be concise but informative. The names of the author and of the institution where the work was carried out follow the title. A running title with not more than 60 characters should be indicated if the title is longer than this.
- (4) In a footnote on the first page, please give an address for reprint requests.
- (5) A title, an abstract suitable for direct use by the abstracting journals, and five keywords, all in English, must precede the main text of each contribution.
- (6) The main text should meet the highest standards as to novelty of the material, organization and conciseness. A qualified colleague and, if the text is in a foreign language, a person who thoroughly knows that language, should have been given the opportunity to check the paper before its submission for publication.
- (7) References and footnotes should be numbered (e.g. Meyer¹) and listed at the end of the paper.
- (8) Tables with the appropriate captions and a list of the figure legends should follow at the end of the paper.
- (9) Allowance should be made for the reduction in printing of the drawings (line thickness, lettering!). Original drawings larger than 21×30 cm should be replaced by copies of reduced size when the manuscript is submitted and only be sent in when the paper is accepted. On all illustrations, the figure number and the author's name must be written in pencil.
- (10) The author will receive two page proofs.
- (11) Changes in the text after acceptance of the paper and drawings not fit for direct reproduction cause delays and create extra costs which may be charged to the author.
- (12) Orders for reprints must be made when the page proof is returned. 50 reprints are free of charge.

Information for Subscribers:

The subscription prices per year are

	Section a	Section b	Section c
For normal subscribers	DM 430.—	DM 430.—	DM 270.—
For authors and their institutions if they order directly from the publishers	DM 344.—	DM 344.—	DM 216.—
Single copies and back-numbers are available.		(+ postage)	

Subscriptions will remain standing for the following year unless cancellations are made by postage October 1st.

Information for Advertisers

The price for a small advertisement (breadth 43 mm, height 57 mm) in all three sections of the journal is DM 60.—. For larger advertisements please order the price list from the publishers.

Verlag der Zeitschrift für Naturforschung, Tübingen

P.O. Box 2645, D-7400 Tübingen
(Postscheck-Konto Stuttgart 8039-700)

ZEITSCHRIFT FÜR NATURFORSCHUNG

Section c

B I O S C I E N C E S

Council

E. BÜNNING, Tübingen
A. BUTENANDT, München
M. EIGEN, Göttingen
W. GENTNER, Heidelberg

Editorial Board

A. HAGER, Tübingen
W. HASSELBACH, Heidelberg
E. HELMREICH, Würzburg
F. KAUDEWITZ, München
W. SCHÄFER, Tübingen

Advisory Editorial Board

P. BÖGER, Konstanz
K. G. GÖTZ, Tübingen
G. GOTTSCHALK, Göttingen
H. HOFFMANN-BERLING, Heidelberg
R. JAENICKE, Regensburg
P. KARLSON, Marburg
G. F. MEYER, Tübingen
D. SCHULTE-FROHLINDE, Mülheim/R.

G. SCHWARZ, Basel
F. F. SEELIG, Tübingen
H. SIMON, München
W. STEGLICH, Bonn
W. TANNER, Regensburg
A. TREBST, Bochum
E. WECKER, Würzburg

EDITED IN COLLABORATION

WITH THE INSTITUTES OF THE MAX-PLANCK-GESELLSCHAFT

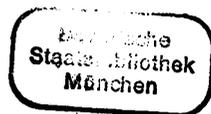
VOLUME 32 c

1977

VERLAG DER ZEITSCHRIFT FÜR NATURFORSCHUNG
TÜBINGEN

Anschrift des Verlages: 74 Tübingen, Postfach 2645
Satz und Druck: Konrad Triltsch, Würzburg

Nachdruck — auch auszugsweise — nur mit schriftlicher Genehmigung des Verlags



Section a
Physics, Physical Chemistry, Cosmic Physics

Section b
Inorganic and Organic Chemistry

Contents

Contents of Number 1/2

Original Communications

- H. Zehner, E. Westhof, W. Flossmann, and A. Müller, Formation of H-Addition Radicals in Adenine Derivatives: Part II 1
- U. F. Thomanek, F. Parak, and B. Wintergerst, The Active Center of Methemoglobin Hb(H₂O) Investigated by Mössbauer and Susceptibility Experiments 11
- U. P. Fringeli, The Structure of Lipids and Proteins Studied by Attenuated Total Reflection (ATR) Infrared Spectroscopy. II. Oriented Layers of a Homologous Series: Phosphatidylethanolamine to Phosphatidylcholine 20
- W. Steglich, A. Thilman, H. Besl, and A. Bresinsky, Pigments of Fungi, 29. 2,5-Diarylcyclopentane-1,3-diones from *Chamonixia caespitosa* (Basidiomycetes) (In German) 46
- B. V. Burger, Maritha le Roux, C. F. Garbers, H. S. C. Spies, R. C. Bigalke, K. G. R. Pachler, P. L. Wesels, V. Christ, and K. H. Maurer, Studies on Mammalian Pheromones, II. Further Compounds from the Pedal Gland of the Bontebok (*Damaliscus dorcas dorcas*) 49
- H. Röper, Analytical Investigations of the Defensive Secretion from *Peripatopsis moseleyi* (Onychophora) (In German) 57
- H. Röper and K. Heyns, Trace Analysis of *p*-Benzoquinone- and Hydroquinone Derivatives by Gas-Liquid Chromatography and Gas-Liquid Chromatography/Mass-Spectrometry. Identification of Defensive Secretion Components from European Julids (In German) 61
- H. Schiechl, Preparative Isolation of Protein III of the Human Erythrocyte Membrane (In German) 67
- O. Oster and G. Buchlow, Purification of Histone F3 by Covalent Chromatography 72
- H.-J. Lach and P. Böger, Isolation and Some Molecular Properties of Plastidic Algal Cytochrome b-559 75
- W. Trowitzsch and H. Sahn, On Amino Acid Antagonists: β -Diketooesters and Their Corresponding γ -Enol ethers (In German) 78
- G. Dietz, Chr. Woenckhaus, R. Jaenicke, and I. Schuster, Modification of Glycerinaldehyde-3-phosphate Dehydrogenase from Rabbit Skeletal Muscle by [3-(3-Bromoacetylpyridinio)-propyl]-Adenosine Pyrophosphate (In German) 85
- R. K. Sinha, P. Talapatra, A. Mitra, and S. Mazumdar, Renaturation of Alkali-Denatured T7 DNA Molecules Complexed with Ethidium Bromide 93
- H. W. Heger and H. W. Peter, Effects of Phospholipids in the Action of Acetyl-CoA Carboxylase from Rat Liver 97
- F. J. Fehrenbach and H. Eibl, Interaction of Streptolysin-O with Natural and Artificial Membranes 101
- I. Fry, G. Papageorgiou, E. Tel-Or, and L. Packer, Reconstitution of a System for H₂ Evolution with Chloroplasts, Ferredoxin, and Hydrogenase 110
- G. H. Schmid, H. List, and A. Radunz, Inhibition of Photosystem II-Reactions in Blue-Green Algae by the Antisera to Lutein and Neoxanthin 118
- K. G. Götz and S. Götz, Normal Development of the Fruitfly *Drosophila* in VLF Magnetic Fields (In German) 125

Notes

- D. W. Lübbers, N. Opitz, P. P. Speiser, and H. J. Bisson, Nanoencapsulated Fluorescence Indicator Molecules Measuring pH and pO₂ Down to Submicroscopical Regions on the Basis of the Optode-Principle 133
- J. G. R. Elferink, Fluorescence and Membrane-Action of Tetracaine 135
- W. Ch. Choi and W. Nagl, Ribosome Crystals in the Oocyte of *Gerris najas* (Heteroptera) 137
- B. Sprey, G. Gliem, and A. G. S. Jánossy, Changes in the Iron and Phosphorus Content of Stroma Inclusions during Etioplast-Chloroplast Development in *Nicotiana* 138
- K. Burger and R. Süßmuth, Prophage Induction by Alkylating Ethyl Methylaminosulfonate 140

Contents of Number 3/4

Original Communications

- K. Atallah, P. Rauschenbach, H. Simon, F. Berthold, and W. Kolbe, Determination of the Liquid Scintillation Counting Efficiency of ³H and/or ¹⁴C Labelled Samples Independently of the Degree of Colour and/or Chemical Quenching 143
- J. Rieser, A. Höckendorf, G. Abou-Elenien, and K. Wallenfels, Chemical and Biochemical Characterization of α -Benzyl- α -bromo-malononitrile (BBMD) as Oxidizing Agent (In German) 150
- Ch. Giese, K. D. Spindler, and H. Emmerich, The Solubility of Insect Juvenile Hormone in Aqueous Solutions and Its Adsorption by Glassware and Plastics 158
- K.-U. Sewe and R. Reich, The Effects of Molecular Polarization on the Electrochromisms of Carotenoids. II. Lutein-Chlorophyll Complexes: The Origin of the Field-Indicating Absorption-Change at 520 nm in the Membranes of Photosynthesis 161
- H.-U. Meisch, J. A. Schmitt, and W. Reinle, Heavy Metals in Higher Fungi. Cadmium, Zinc, and Copper (In German) 172
- Y. Solberg, Studies on the Chemistry of Lichens, XVI. Chemical Investigation of the Lichen Species *Alectoria ochroleuca*, *Stereocaulon vesuvianum* var. *pulvinatum* and *Icmadophila ericedorum* 182
- H. Kneifel, I. Rolle, and B. Paschold, Amines of Unicellular Green Algae, III. Identification of Homologues of Spermidine in the Green Alga *Scenedesmus acutus* 276-3a (In German) 190
- H. P. Siebertz and E. Heinz, Labelling Experiments on the Origin of Hexa- and Octa-decatrienoic Acids in Galactolipids from Leaves 193
- M. Ilyas, O. Seligmann, and H. Wagner, Biflavones from the Leaves of *Araucaria rulei* F. Muell. and a Survey on Biflavanoids of the *Araucaria* Genus 206
- A. Geller and J. Berghäuser, The Preparation of Three Dehydrogenases and Two Kinases from Pig Heart by a Single Procedure (In German) 210
- A. Schmidt, Protein-Catalyzed Isotopic Exchange Reaction between Cysteine and Sulfide in Spinach Leaves 219
- G. F. Wildner and J. Henkel, Temperature Dependent Conformation Changes of Ribulose-1,5-bisphosphate Carboxylase Studied by the Use of 1-Anilino-8-naphthalene Sulfonate 226
- G. Kahl and M. Wechselberger, Activation of Chromatin-Bound DNA-Dependent RNA Polymerase (E.C. 2.7.7.6) in Plant Storage Tissue Slices 229

H. K. Lichtenthaler and H. K. Kleudgen, Effect of the Herbicide San 6706 on Biosynthesis of Photosynthetic Pigments and Prenylquinones in <i>Raphanus</i> and in <i>Hordeum</i> Seedlings	236	H. Harms, W. Dehnen, and W. Mönch, Benzo(a)pyrene Metabolites Formed by Plant Cells	321
H. Hanauske-Abel and V. Günzler, Inhibition of Human Prolyl Hydroxylase as Common Biochemical Denominator of the Non-Sedative Effects of Thalidomide in Man	241	H. Kayser, Metabolites of β,β -Carotene in the Stick Insect, <i>Carausius morosus</i> Br.: Compounds with 2-One and 3,4-Didehydro-2-one Structure	327
J. Hoshino, U. Kühne, B. Filjak, and H. Kröger, Regulation and Characterization of L-Serine: Pyruvate Aminotransferase in Rat Liver Cytosol and Mitochondria	249	W. Kördel and F. Schneider, Identification of Essential Histidine Residues of Aminoacylase by Photooxidation and by Reaction with Diethylpyrocarbonate	337
K. Kaminski, Photosynthetic Control in Chloroplasts Suspensions Frozen in Liquid Nitrogen in the Presence of Glycerol	254	W. Kördel and F. Schneider, Renal Aminoacylase, a Zinc Enzyme	342
D. Hoffmann, R. Thauer, and A. Trebst, Photosynthetic Hydrogen Evolution by Spinach Chloroplasts Coupled to a <i>Clostridium</i> Hydrogenase	257	W. Löffelhardt, The Biosynthesis of Phenylacetic Acids in the Blue-Green Alga <i>Anacystis nidulans</i> : Evidence for the Involvement of a Thylakoid-Bound L-Amino Acid Oxidase	345
Chr. Giersch, A Kinetic Model for Translocators in the Chloroplast Envelope as an Element of Computersimulation of the Dark Reaction of Photosynthesis	263	R. Lüderitz and J.-H. Klemme, Isolation and Characterization of a Membrane-Bound Pyruvate Dehydrogenase Complex from the Phototrophic Bacterium <i>Rhodospirillum rubrum</i> (In German)	351
G. H. Schmid, A. Radunz, and W. Menke, Localization and Function of Cytochrome f in the Thylakoid Membrane	271	S. Fernandez-Bermudez, J. Loboda-Čačković, H. Čačković, and R. Hosemann, Structure of Cerebrosides. I. Phrenosine at 23 °C and 66 °C	362
F.-U. Beil, D. von Chak, W. Hasselbach, and H.-H. Weber, Competition between Oxalate and Phosphate during Active Calcium Accumulation by Sarcoplasmic Vesicles	281	H. Bleuel, G. Wiedner, and D. Schubert, Variability of Conductivity Changes in Black Phosphatidylserine Membranes Induced by Proteins from Erythrocyte Membranes	375
<i>Notes</i>		R. T. C. Huang, Transfer of Glycolipid between Membranes of Tissue Culture Cells, Using Dansylcerebroside as a Model	379
H. Stocker and H. Wanner, Long Chain Alkanes and Alcohols from the Leaf Waxes of Different <i>Coffea</i> Species (In German)	288	H. Craubner, F. Koenig, and G. H. Schmid, Molecular Weight and the Dodecyl Sulphate Binding of a Thylakoid Membrane Polypeptide Involved in a Reaction on the Oxygen-Evolving Side of Photosystem II	384
D. J. Robeson and J. B. Harborne, Pisatin as a Major Phytoalexin in <i>Lathyrus</i>	289	G. Harnischfeger and R. Schopf, A Fluorescence Method for Measuring the Retention of Coupling Factor (CF ₁) in Reconstitution Experiments of Photophosphorylation	392
R. Reinhardt and R. Hänsel, X-Ray Structure of Two Naturally Occurring Cinnamylidene-butenolides	290	R. Schopf and G. Harnischfeger, Studies on the Retention of CF ₁ with or without Induced ATPase Activity by Pyrophosphate Treated Thylakoids and Its Relation to the Regeneration of Photophosphorylation	398
Y. Solberg, Studies on the Chemistry of Lichens, XV. New Synthetic Nitrogen-Derivatives of Pulvinic Acid	292	O. Schmut, H. Katschnig, and M. Zirm, Investigations of Body Fluids with Low Protein Content by the Combination of Micro-Disc-Electrophoresis and Electroimmunodiffusion (In German)	405
G. Seibert, B. Schöbel, and R. K. Zahn, The Separation of High and Low Molecular Weight RNA by Precipitation with N-Cetyl-N,N,N-trimethyl-ammonium-bromide	294	G. Brandner and M.-S. Cho, African Green Monkey Fibroblast Actin Morphology during SV40 Infection	409
A. Brennicke and H. D. Frey, Properties of an Adenosine Cyclic Phosphates Degrading Enzyme in <i>Nicotiana tabacum</i> L. var. <i>Xanthi</i>	297	S. Erhan, L. D. Greller, and B. Rasco, Evolution of the Transfer RNA Molecule	413
B. Frenzel, O. R. Kaaden, and M. Mussgay, Purification of a Glycoprotein from Bovine Leukemia Virus (BLV)	301	J.-P. Liautard, D. Tromm, and K. Köhler, Isolation of Messenger Ribonucleoproteins from HeLa Cells by Affinity Chromatography on Poly (U) Sepharose	419
R. Riehl, Paracrystalline Bodies in Young Oocytes of the Loach, <i>Neomacheilus barbatus</i> (L.) (Teleostei, Cobitidae) (In German)	305	A. R. Trim, J. R. O. Dawson, P. E. Dickerson, F. Sakai, J. W. Watts, W. Hirst, and R. A. Cox, A Method for the Purification of Large Quantities of Biologically Active Ribonucleic Acid Components from Cowpea Chlorotic Mottle Virus, a Multicomponent Plant Virus	424
E. Schulte and R. Riehl, Electron Microscopical Studies of Olfactory Organ of <i>Ophicephalus (Channa) obscurus</i> (GUENTHER, 1861 (Teleostei, Ophicephalidae) (In German)	307	A. Taketo, Sensitivity of <i>Escherichia coli</i> to Viral Nucleic Acid, XII. Ca ²⁺ - or Ba ²⁺ -Facilitated Transfection of Cell Envelope Mutants	429
L.-J. Adam, H. Dahmen, and P. Fastrich, Oscillatory Nervous Response and Transient Vibration (Ear of <i>Locusta migratoria</i> Acrididae, Insecta)	309	P. Mascheck, Kl. Scheller, and P. Karlson, Changes in Gene Expression during Larval Development of <i>Calliphora vicina</i> Induced by Ecdysterone	434
J. Haupt, Preliminary Report on the Mating Behaviour of the Primitive Spider <i>Heptathela kimurai</i> (Kishida) (Araneae, Liphistiomorphae)	312	K. Kirschfeld and B. Lutz, The Spectral Sensitivity of the Ocelli of <i>Calliphora</i> (Diptera)	439
Contents of Number 5/6			
<i>Original Communications</i>			
W. Wray and K. G. Wagner, ¹³ C NMR Investigations on the Stacking of 5'-AMP with Tryptamine	315		

Notes

P. Fährnich and L. Chenuaux, Studies on Carotenoids in Gametangia and Spores of <i>Allomyces arbuscula</i> (In German)	442
Mohamed A. El-Ansari, Moheb S. Ishak, Ahmed A. Amed, and Nabel A. M. Saleh, Flavonol Glycosides of <i>Carya pecan</i> and <i>Casuarina equisetifolia</i>	444
J. L. Ingham and P. M. Dewick, Isoflavonoid Phytoalexins from Leaves of <i>Trifolium arvense</i>	446
J. L. Ingham, Medicarpin as a Phytoalexin of the Genus <i>Melilotus</i>	449
K. Dettner and G. Schwinger, High 3-Indoleacetic Acid- and Phenylacetic Acid Concentrations in the Pygidial Glands of Water Beetles (Ditiscidae) (In German)	453
P.-J. Enzmann and H. Rehberg, The Structural Components of Hog Cholera Virus	456
H. Schwarz, H.-J. Thiel, and W. Schäfer, Spontaneous Leukemia of AKR Mice. Successful Passive Immunization with Goat Antibodies against Isolated Glycoprotein gp71 of Friend Leukemia Virus (In German)	459
F. Schlecht, Electron-Microscope Study of the Peritrophic Membrane in Phyllopora (Crustacea) (In German)	462
G. Wiedenmann, Weak and Strong Phase Shifting in the Activity Rhythm of <i>Leucophaea maderae</i> (Blaberidae) after Light Pulses of High Intensity	464
Kl. Vogt, Ray Path and Reflection Mechanism in Crayfish Eyes	466
R. Wehner and I. Flatt, Visual Fixation in Freely Flying Bees	469

Contents of Number 7/8

Original Communications

M. Wenzel, R. Herken, and W. Klose, Biochemistry of Metalloenes, II. Organ-Distribution and Thymus Affinity of Cinnamoyl- ¹⁰³ Ru]Ruthenocene (In German)	473
J. Kopoldová and Št. Hrnčíř, Gamma-Radiolysis of Aqueous Solution of Histidine	482
B. Czochralska, H. Fritsche, and D. Shugar, Photochemically Reversible Dimer Electroreduction Product of 2-Oxopurine	488
E. Wünsch, G. Wendlberger, A. Hallet, E. Jaeger, S. Knof, L. Moroder, R. Scharf, I. Schmidt, P. Thamm, and L. Wilschowitz, Total Synthesis of Human Big Gastrin I and the 32-Leucine Analogue (Preliminary Communication) (In German)	495
F. Parak, U. F. Thomanek, D. Bade, and B. Wintergerst, The Orientation of the Electric Field Gradient Tensor in CO-Liganded Myoglobin	507
H. Scheer and W. Kufer, Studies on Plant Bile Pigments, IV: Conformational Studies on C-Phycocyanin from <i>Spirulina platensis</i>	513
W. Steglich and L. Zechlin, Pigments of Fungi, XXXII. 3-Methylriboflavine from <i>Panellus serotinus</i> (Agaricales) (In German)	520
P. Renz, R. Wurm, and J. Hörig, Nonenzymatic Transformation of Riboflavin into 5,6-Dimethylbenzimidazole	523
H. Lüdemann and E. Westhof, Conformations of the Nucleoside Analogs Formycin, 2-Azaadenosine, and Nebularine in Solution	528
M. F. Macchiato, G. F. Grossi, G. C. Gialanella, and A. Cascino, Sucrose Gradient Analysis: Computer Simulation and Measurement of the Parameters Involved in the Sedimentation of DNA Molecules	539
A. Fink and G. Hotz, Immunological Reaction of UV-Induced Radiation Damage in Coliphage DNA	544

R. Jeck, The Properties of [ω -(3-Acetylpyridinio)- <i>n</i> -alkyl]Adenosine Pyrophosphates, Structural Analogs of the Coenzyme NAD (In German)	550
K. Sauber, R. Müller, E. Keller, J. Eberspächer, and F. Lingens, Degradation of Antipyrin by Pyrazon-Degrading Bacteria (In German)	557
W. Kuhnz and H. Rembold, Application of Lathanide Induced Shifts in Proton Magnetic Resonance Spectroscopy of Juvenile Hormones	563
H. B. Leising and D. O. Schachtschabel, Stimulation of Tyrosinase Activity and Melanin Formation of Cultured Melanoma Cells by Serum Deprivation Alone or in Combination with Dibutyryl Cyclic AMP and Theophylline	567
D. L. Dorset and A. J. Hancock, Glycerol Conformation and the Crystal Structure of Lipids I. An Electron Diffraction Study of Tripalmitin and Conformationally Fixed Analogs	573
C. Gebhardt, H. Gruler, and E. Sackmann, On Domain Structure and Local Curvature in Lipid Bilayers and Biological Membranes	581
A. Radunz, Binding of Antibodies onto the Thylakoid Membrane. II. Distribution of Lipids and Proteins at the Outer Surface of the Thylakoid Membrane	597
R. Schopf, The Degree of CF ₁ Release and the Reconstituting Capacity of the Depleted Membranes	600
G. Vierke and P. Struckmeier, Binding of Copper(II) to Proteins of the Photosynthetic Membrane and its Correlation with Inhibition of Electron Transport in Class II Chloroplasts of Spinach	605
K.-P. Heise and G. Krapf, Comparison of Lipid Biosynthesis of Normal and Dark Kept Spinach Leaves in Photosynthetically Active Light	611
S. Saphon and A. R. Crofts, Protolytic Reactions in Photosystem II: a New Model for the Release of Protons Accompanying the Photooxidation of Water	617
M. Saleemuddin, U. Zimmermann, and F. Schneeweiß, Preparation of Human Erythrocyte Ghosts in Isotonic Solution: Haemoglobin Content and Polypeptide Composition	627
U. Yamaguchi-Koll, K. J. Wieggers, and R. Drzeniek, Dissociation and Reassociation of Poliovirus, II. Protein Components Obtained by Urea Treatment of the Virus Particle	632
G. S. Dogra, G. M. Ulrich, and H. Rembold, A Comparative Study of the Endocrine System of the Honey Bee Larvae under Normal and Experimental Conditions	637

Notes

L. Sportelli, H. Neubacher, and W. Lohmann, On the Influence of Aromatic Residues on the Interaction of Copper(II) with Small Peptides Containing Aromatic Amino Acids: ESR and Optical Studies	643
J. Marañón and O. M. Sorarrain, The Adenine and Thymine Molecules. Some Excited Singlet and Triplet Levels of the Normal and Tautomeric Forms	647
H. Esterbauer and E. Schwarzl, Aerobic Oxidation of <i>p</i> -Hydroquinone by Horse Radish Peroxidase in the Presence of a Thiol and MnCl ₂	650
K. Bürcky, Gibberellin Activity of Different Antheridiogens in the Dwarf Pea Bioassay (In German)	652
G. Reimer and D. Drahovsky, Chromosomal Structures of <i>Pseudomonas testosteroni</i> . IV. Effect of Testosterone on RNA-Synthesis (In German)	654
R. T. C. Huang, Artificial Phospholipid and Glycolipid Particles, Visualization of Their Structure by Fluorescence Markers and Some Biological Properties Expressed by These Particles	656

- T. M. Maida, Microvillar Orientation in the Retina of a Pierid Butterfly 660
 W. C. Gordon, Microvillar Orientation in the Retina of the Nymphalid Butterfly 662

Contents of Number 9/10

Original Communications

- M. A. Haleem and K. D. Parker, Studies on the Helical Structure of β -D-1,3 Xylan 665
 M. A. Haleem and K. D. Parker, Scattering of X-Rays by Parallel and Antiparallel Layers in Disordered Stacking and Statistical Layer Shifts for α -Chitin 669
 D. A. Adamiak, W. Saenger, R. Kinas, and W. J. Stec, X-Ray-Diffraction Study and Determination of Absolute Configuration of the Anticancer Drug S(-)-Cyclophosphamide (Endoxan, Cytosan, NSC-26271) 672
 H. Lotter, A. Jones, and M. Sturm, X-Ray Structure Analysis of Mezerein from *Daphne mezereum* L. (In German) 678
 H. E. Marcolin, R. Reschke, and A. Trautwein, Investigation of the Recombination Kinetic of Photo-dissociated Myoglobin-CO at Low Temperatures by Mössbauer Spectroscopy (In German) 683
 H. Röper and K. Heyns, On the Problem of Dimethylnitrosamine Formation from Tetracycline-Derivatives by Nitrosation Reaction in Acidic Medium (In German) 696
 G. Grimmer and H. Böhnke, Investigation on Drilling Cores of Sediments of Lake Constance. I. Profiles of the Polycyclic Aromatic Hydrocarbons (In German) 703
 J. A. Schmitt, H.-U. Meisch, and W. Reinle, Heavy Metals in Higher Fungi, II. Manganese and Iron (In German) 712
 H. Rimpler and I. Christiansen, Tectograndinol, a New Diterpene from *Tectona grandis* L. fil. (In German) 724
 T. Hirata and T. Suga, Biologically Active Constituents of Leaves and Roots of *Aloe arborescens* var. *natalensis* 731
 J. Jacob and U. Green, Composition of the Ventral Gland-Pad Sebum from the Mongolian Gerbil, *Meriones unguiculatus* 735
 A. Keim, Electrophoretic Analyses of the Crop Contents of *Helobdella stagnalis* (L.) (Hirudinea) 739
 R. Jonak, Ch. M. Lapière, A. Meinel, H. Nemetschek-Gansler, Th. Nemetschek, and H. Riedl, Structure and Mechanical Propertiel of Dermatosparactic Collagen (In German) 743
 Ch. Gähwiller, C. von Planta, D. Schmidt, and H. Steffen, Size, Structure, and Dynamics of Bile Salt/Lecithin Mixed Micelles (In German) 748
 L. Bornmann and B. Hess, Interaction of Cibacron Dyes with Dehydrogenases and Kinases 756
 A. M. Relimpio, Structure and Anticholinesterase Activity of Series of Ethyl Substituted Phenyl Methylphosphonates 760
 M. H. Saylor and R. L. Mansell, Hydroxycinnamoyl: Coenzyme A Transferase Involved in the Biosynthesis of Kaempferol-3-(*p*-coumaroyl Triglucoiside) in *Pisum sativum* 765
 J. Frey, W. Kördel, and F. Schneider, The Reaction of Aminoacylase with Chloromethylketone Analogs of Amino Acids 769
 H.-H. Hamm and W. Seubert †, On the Mechanism of Inactivation and ATP-Dependent Reactivation of Rat Liver Tyrosine Aminotransferase 777
 K. Okabe, Properties of Ribulose Diphosphate Carboxylase/Oxygenase in the Tobacco Aurea Mutant Su/su var. Aurea 781
 J. Köhrle, J. Lüstorf, and E. Schlimme, P¹,P⁵-Bis-(5'-adenosyl)pentaphosphate: Is this Adenylate Kinase Inhibitor Substrate for Mitochondrial Processes? 786
 J.-D. Schwenn and B. Depka, Assimilatory Sulfate Reduction by Chloroplasts: The Regulatory Influence of Adenosine-mono- and Adenosine-diphosphate 792
 C. Buschmann and H. K. Lichtenthaler, Hill-Activity and P700 Concentration of Chloroplasts Isolated from Radish Seedlings Treated with Indoleacetic Acid, Kinetin or Gibberellic Acid 798
 B. Huchzermeyer and H. Strotmann, Acid/Base-Induced Exchange of Adenic Nucleotides on Chloroplast Coupling Factor (CF₁) 803
 S. Saphon and A. R. Crofts, The H⁺/e Ratio in Chloroplasts is 2. Possible Errors in Its Determination 810
 F. Koenig, W. Menke, A. Radunz, and G. H. Schmid, Localization and Functional Characterization of Three Thylakoid Membrane Polypeptides of the Molecular Weight 66 000 817
 B. Rauch, D. v. Chak, and W. Hasselbach, Phosphorylation by Inorganic Phosphate of Sarcoplasmic Membranes 828
 H. K. Samanta and S. B. Bhattacharjee, Thymineless Death in *Escherichia coli* 835
 H. A. B. Linke, Growth Inhibition of Glucose-Grown Cariogenic and Other Streptococci by Saccharin *in vitro* 839
 G. F. Grossi, G. Cesareni, and F. Liello, Development of Phage Populations in a Bacterial Culture: a Mathematical Model 844
 R. Figueroa, A. Sepúlveda, M. A. Sato, and J. Tohá, Genetic Information Analysis of Bacteriophage Φ x 174 850
 H. Stieve, M. Bruns, and H. Gaube, Ability to Light-Induced Conductance Change of Arthropod Visual Cell Membrane, Indirectly Depending on Membrane Potential, during Depolarization by External Potassium or Ouabain 855
- Notes*
- J. Marañón and O. M. Sorarrain, The Tautomeric Conformers for the Molecules of Guanine and Cytosine. Some Remarks about Their Stability 870
 D. A. Alizade and K. Gaede, Chirality of the Hydrogen Transfer to NAD Catalyzed by (3R)Hydroxybutyrate Dehydrogenase from *Pseudomonas lemoignei* 874
 H. J. Lach and P. Böger, Some Properties of Plastidic Cytochrome b-563 877
 H. E. A. Schenk and J. Hanf, Thioacylamides and Thioacylureas as Inhibitors of Photosystem II (In German) 880
 H.-J. Thiel, C. Bergholz, H. Beug, F. Deinhardt, H. Schwarz, and W. Schäfer, Isolation of the Major Glycoprotein (gp70) of Simian Sarcoma Virus (SSV-1/SSAV-1) in Preparative Quantities 884
 R. C. Hardie, Electrophysiological Properties of R7 and R8 in Dipteran Retina 887

Contents of Number 11/12

Original Communications

- V. N. Babin, E. B. Zavelovich, and Yu. A. Belousov, The Effect of Water on Proton Transfer in Pyrazole 891
 R. Stolarski, M. Remin, and D. Shugar, Studies on Prototropic Tautomerism in Neutral and Monoanionic Forms of Pyrimidines by Nuclear Magnetic Resonance Spectroscopy 894
 H. H. Mantsch and O. Bârzu, Anomalous Base-Stacking of the N₁-Oxide of AMP 901

H.-J. Breter, The Quantitative Determination of Metabolites of 6-Mercaptopurine in Biological Materials. IV. An Improved Separation Method for Twentytwo Compounds Related to Purine and 6-Thiopurine Metabolism Using High-Pressure Liquid Cation-Exchange Chromatography	905	J. Kiefer and B. Laske, Protein Synthesis and Amino Acid Pools in Irradiated Yeast Cells	973
H. J. Schmidt, U. Schaum, and J. P. Pichotka, The Influence of Mode and Intensity of Homogenization on the Absolute Value and Stability of Oxygen Consumption of Guinea Pig Liver Homogenates (In German)	908	E. Priesner, H.-J. Bestmann, O. Vostrowsky, and P. Rösler, Sensory Efficacy of Alkyl-Branched Pheromone Analogues in Noctuid and Tortricid Lepidoptera	979
G. Müller, Pollution Research on Dated Sediment Cores from Lake Constance. II. Historical Evolution of Heavy Metals — Relationship to the Evolution of Polycyclic Aromatic Hydrocarbons (In German)	913	W. Hasselbach and A. Migala, Calcium Gradient Dependent Pyrophosphate Formation by Sarcoplasmic Vesicles	992
G. Müller, Pollution Research on Dated Sediment Cores from Lake Constance. III. Historical Evolution of N- and P-Compounds — Relationship to the Development of Heavy Metals and Polycyclic Aromatic Hydrocarbons (In German)	920	G. Wiedner, G. Wilhelm, G. Jureit, and Z. Bojadžijev, Calcification of a Native Collagen Membrane	997
R. Kanne, Isolation and Characterization of a Potassium Specific Ionophore from <i>Streptococcus faecalis</i>	926	S. Erhan, Origins of the First Cell. A New Model for the Spontaneous Formation of the First Living Cell Based on a Novel Approach	1003
G. Metz, R. Marx, and K.-H. Röhm, The Quaternary Structure of Yeast Aminopeptidase I. 1. Molecular Forms and Subunit Size	929	<i>Notes</i>	
R. Marx, G. Metz, and K.-H. Röhm, The Quaternary Structure of Yeast Aminopeptidase I. 2. Geometric Arrangement of Subunits	938	H. W. Ludwig, 99.26 per cent Water Content in the Fresh-Water Medusa <i>Craspedacusta sowerbii</i>	1011
S. Postius and F. Schneider, Multiple Effects of Amobarbital on Ehrlich Ascites Tumor Cells. Inhibition of Pyruvate Dehydrogenase	944	E. Wollenweber, Chalcones and Dihydrochalcones as Constituents of Fern Farina (Genera <i>Cheilanthes</i> and <i>Notholaena</i>) (In German)	1013
W. Tinschert and L. Träger, Evidence for an 4-Ene-3-oxosteroid-5 α -reductase and Δ^4 - Δ^5 -Ketosteroid Isomerase Activity in Extracts of <i>Streptomyces hydrogenans</i>	949	G. J. Niemann, Flavonoids and Related Compounds in Leaves of Pinaceae. II. <i>Cedrus atlantica</i> c. v. <i>Glauca</i>	1015
K. Alef and J.-H. Klemme, Characterization of a Soluble NADH-Independent Nitrate Reductase from the Photosynthetic Bacterium <i>Rhodospseudomonas capsulata</i>	954	J. L. Ingham, Phytoalexins of Hyacinth Bean (<i>Lablab niger</i>)	1018
G. Muckle and W. Rüdiger, Chromophore Content of C-Phycocerythrin from Various Cyanobacteria	957	M. Dizdaroglu, D. Schulte-Frohlinde, and C. v. Sonntag, γ -Radiolyses of DNA in Oxygenated Aqueous Solution. Structure of an Alkali-Labile Site	1021
G. Renger and G. H. Schmid, On the Correlation between the Amplitude of the Electrochromic Absorption Changes and the Number of Bulk Pigments	963	H. C. Heinrich, J. Brüggemann, E. E. Gabbe, M. Gläser, F. Icgagic, and E. Pape, Correlation between Diagnostic $^{59}\text{Fe}^{2+}$ -Absorption and Serum Ferritin Concentration in Man	1023
G. F. W. Searle, A Chloroplast Photosystem 2 Reaction Resistant to Salicylaldehyde	968	M. Popescu, J. Löhler, and F. Lehmann-Grube, Infectious Lymphocytes in Mice Persistently Infected with Lymphocytic Choriomeningitis Virus	1026
		G. Erler and U. Thurm, A Simplified Method for Recording Receptorpotentials and Nervous Impulses of Insect Epidermal Mechanoreceptors (In German)	1029
		Erratum	1031
		To B. Rauch, D. v. Chack, and W. Hasselbach (32 c, 828 [1977])	
		Subject Index	1033
		Authors Index	1053

Conformational Studies on C-Phycocyanin from *Spirulina platensis*

Hugo Scheer and Werner Kufer

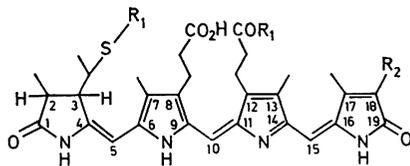
Institut für Botanik, Universität München

(Z. Naturforsch. **32 c**, 513–519 [1977]; received April 25, 1977)

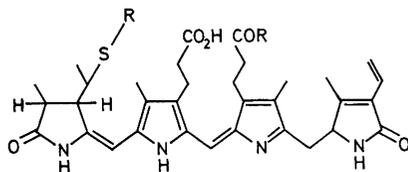
Bile Pigments, Protein Interaction, Conformation, Denaturation

The chromophore-protein interactions of C-phycocyanin (C-PC) from *Spirulina platensis* have been studied by following the partial and complete denaturation with UV-Vis spectroscopy. From comparison with published MO calculations, an elongated conformation of the chromophore is suggested for native C-PC, a cyclic one for denatured C-PC. By means of partial denaturation, a step-wise unfolding of the protein has been demonstrated. The presence of at least two sets of spectroscopically different chromophores is suggested from the partial denaturation and low temperature experiments.

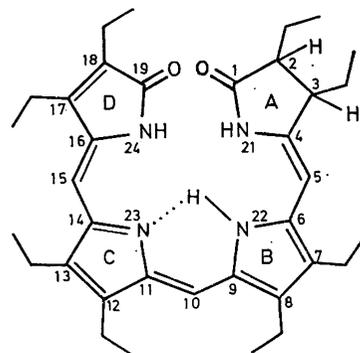
Phycocyanins and phycoerythrins are photosynthetic light harvesting pigments of blue-green, red and cryptophyten algae, which contain bile-pigment chromophores covalently bound to proteins². The absorption of the various types of these pigments cover the spectral range between about 500 and 670 nm practically completely. In spite of this spectral variety, however, the phycobiliproteins contain with only few exceptions just two chemically distinct chromophores: the blue phycocyanobilin (**1a**) and the red phycoerythrobilin (**2**)^{*, 2, 3}. Uninfluenced by the protein, the free bases of the two chromo-



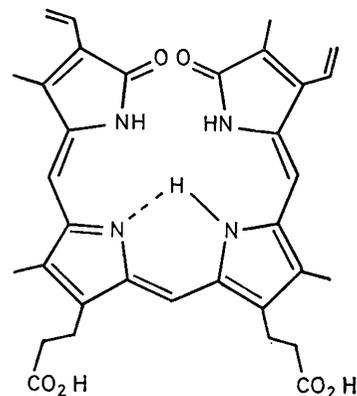
1a: R₁ = Protein; R₂ = C₂H₅
1b: R₁ = Protein; R₂ = C₂H₃



2: R = Protein



3



4

* The terms phycocyanobilin and phycoerythrobilin are used for compounds with the molecular structure of **1a** and **2**, which are characterized by a substituted ethyl-group at C-3. For a critical discussion, see ref. 2.

Reprint requests should be sent to Dr. Hugo Scheer, Institut für Botanik, Menzinger Straße 67, D-800 München 19.

phores absorb around 600 and 530 nm, respectively, in the visible spectral region, and around 350 and 320 nm in the near UV. The spectra of the free chromophores are quite dissimilar to those of the native pigments, too, in terms of intensity and shape

of the two main absorption bands (vide infra), and of their fluorescence. The specific chromophore-protein interactions which underly these profound optical changes are hitherto only little understood.

These interactions render the chromophore an intrinsic and sensitive indicator for the state of the biliproteins. Qualitatively, this built-in probe has been widely used to test any alterations of biliproteins during their isolation and purification. Brown *et al.*⁴ have demonstrated, that the renaturation of allophycocyanin subunits, depending on the buffer system used, yields pigments absorbing at either 650 nm (like allophycocyanin) or 620 nm (like C-PC). The influence of protein aggregation and isotope substitution on the chromophore absorption has been studied by several groups^{2, 3, 5-9}. The rearrangement of the protein induced by the initial photochemical reaction of the chromophore is also believed to be responsible for the sequence of intermediates identified during the phytochrome (**Ib** = **P_r**) interconversions^{10, 11}.

The influence of the protein moiety in biliproteins can be abolished completely by denaturation with 8 M urea, 6 M guanidinium chloride, or heat. Spectral comparison of the thus exposed chromophores with free bile pigments of known structure has been widely used as a sensitive and very mild method to obtain structural information on biliproteins which are less accessible or unstable^{12, 13}.

To obtain more detailed information on the chromophore-protein interaction, we have studied by UV-Vis spectroscopy the controlled (partial) denaturation and renaturation of C-phycoyanin (C-PC) from *Spirulina platensis*. To exclude aggregation effects, these studies were performed under conditions where C-PC is monomeric⁵⁻⁹. Spectra have been measured in the range between 320 and 700 nm. Thus, spectral changes have not only followed for the long wavelength band of the chromophore, but also for the less studied short wavelength band. The results indicate a stepwise denaturation of C-PC, the presence of at least two chromophore populations, and a predominant influence of the protein *via* conformational modifications of the flexible bile pigment chromophore.

Materials and Methods

Frozen cells of *Spirulina platensis* (110 g) were thawed, and broken in a beaker-type cell mill with

glass beads (350 g, 0.25 mm ϕ). The pigments were extracted twice with sodium phosphate buffer (0.1 M, pH 7.0) containing NaN_3 (10^{-3} M) and EDTA potassium salt (10^{-3} M). The crude extract was freed from chlorophyll by centrifugation for 1 h at $78\,000 \times g$, and the pigments precipitated by 50% saturation with $(\text{NH}_4)_2\text{SO}_4$. Purification on Ultrogel AcA22 (LKB, Sweden) and subsequently DEAE cellulose Servacel CM23 (Serva, W. Germany) yielded C-PC with a E_{620}/E_{280} value of 4.1.

Spectra were recorded on a DMR 22 spectrophotometer (Zeiss, Germany) equipped with a pair of cryostats model 600 (Thor, England) and a temperature controller (Kisch, Germany). Gelchromatographic determinations of molecular weights were carried out on thermostated Sephadex G-200 columns (1.5×60 cm at 4°C , and 2.5×50 cm at 39°C) calibrated with Dextran blue, catalase, bovine serum albumin, and cytochrome c. Sedimentation velocity (S_{20}) determinations were run in a Beckmann E analytical ultracentrifuge (UV analyzer at 350 nm), and corrected for temperature changes, solvent viscosity, solvent density and the partial molar volume of the protein. For the denaturation experiments a stock solution of C-PC in potassium phosphate buffer (0.05 M, pH 7.5) containing NaN_3 , EDTA and sodium ascorbate (5×10^{-4} M each) was prepared. In the case of heat denaturation, a fresh sample ($\sim 50 \mu\text{l}$) of this solution was added to the thermostated cell containing 2.0 ml of buffer to yield a final concentration of 0.25×10^{-6} M, and the spectrum followed in time. In the case of urea denaturation, aliquots were added to buffer containing increasing amounts of urea. The low temperature experiments were carried out in a 1:1 buffer:glycerol mixture, the sedimentation experiments in a buffer without ascorbate, and the Sephadex MW determinations in a Tris buffer (0.01 M, pH 7.8) containing KCl (0.1 M), NaN_3 (10^{-3} M) and EDTA potassium salt (10^{-3} M). All spectroscopic measurements were performed under N_2 .

Results

Complete denaturation

The UV-Vis spectral changes of C-PC upon complete denaturation by either heat (Fig. 1) or urea at pH 7.5* are similar. The intensity (ϵ_{max}) of the long-wavelength band is decreased by a factor of 4.5, that of the near UV-band is increased by a factor of 2. The red band is shifted by about 20 nm

* For denaturation at different pH values leading to either cations or anions, or denaturation in the presence of Zn^{2+} , c. f. ref. 12.

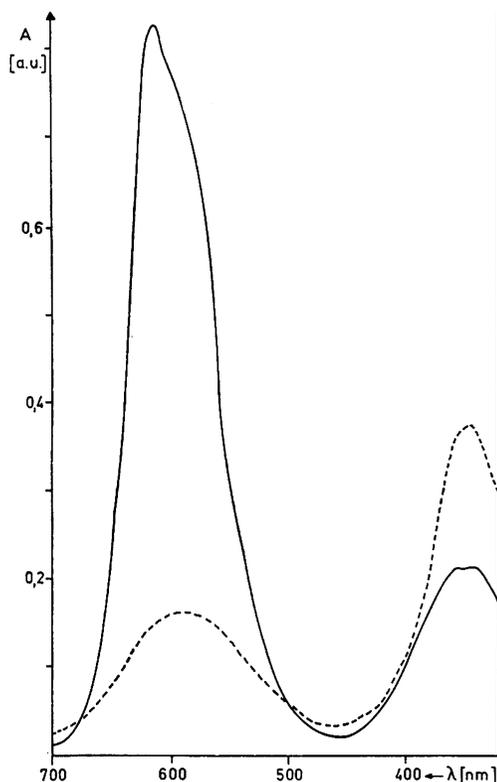


Fig. 1. UV-Vis absorption spectra of C-phycoerythrin: — native, at 4 °C and pH 7.5, - - heat denatured at 71 °C.

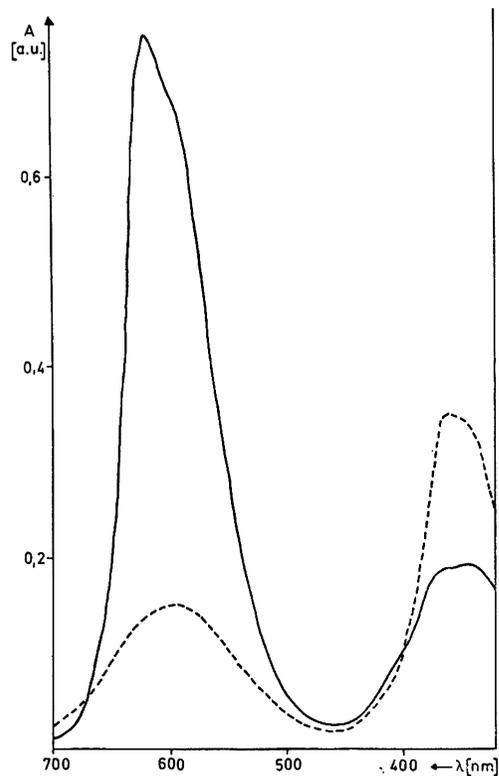


Fig. 2. UV-Vis absorption spectra of C-phycoerythrin: — native, solution in sodium phosphate buffer at 4 °C and pH 7.5, - - denatured with 8 M urea.

to 600 nm, the near UV-band remains at roughly the same position. The fine structure of both bands is diminished. The long-wavelength band of native C-PC has a pronounced peak at 620 nm, with a broader shoulder to shorter wavelengths (see below), the near UV-band is just resolved into two bands of about equal intensity at 360 and 347 nm. The respective bands in denatured C-PC are considerably broadened. Except for a slight redshift due to the different substituents, the spectrum is similar to that of the free base of the synthetic A-Dihydrobilin (3)^{1a}. Like in other bilins, the spectrum probably constitutes but an envelope of various conformers of the flexible bilin chromophore. On this basis, the comparably narrow bands in the native pigments indicate an increased rigidity, and the presence of only few conformers of the tetrapyrrole skeleton. Increased rigidity is evidenced, too, by the strong fluorescence of C-PC^{**}.

However, the spectral changes upon denaturation do not only indicate an increased flexibility of the free bilin chromophore, but also a profound change

in the average chromophore conformation. MO calculations of several groups¹⁴⁻¹⁶ agree that the relative intensity of the two main bands is an indicator for the chromophore conformation. The results predict a weak long-wavelength and a more intense near UV-band for cyclic "porphyrin-type" conformations. This situation is gradually reversed upon stretching the chromophore (*c.f.* Fig. 3). The trends predicted from these calculations¹⁵ gain strong support from the recent x-ray analysis of the dimethylester of biliverdin (4)¹⁷. A cyclic "porphyrin-type" structure has been found for 4, and its UV-Vis spectrum is indeed similar to that in Fig. 3 a ($E_{655}/E_{370} \approx 1:4$). The presence of cyclic conformations in bile pigment solutions, possibly among other conformations, had already been suggested in 1964 by Moscovitz *et al.*¹⁸ from chiroptical data^{***}. In the cyclic conformation, the two terminal O-atoms overlap thus rendering the

^{**} This rigidity is a prerequisite, too, for the efficient photo-reactivity of P_r . In both cases, the radiationless, energy degrading decay by internal conversion is slowed down sufficiently by conformational fixation.

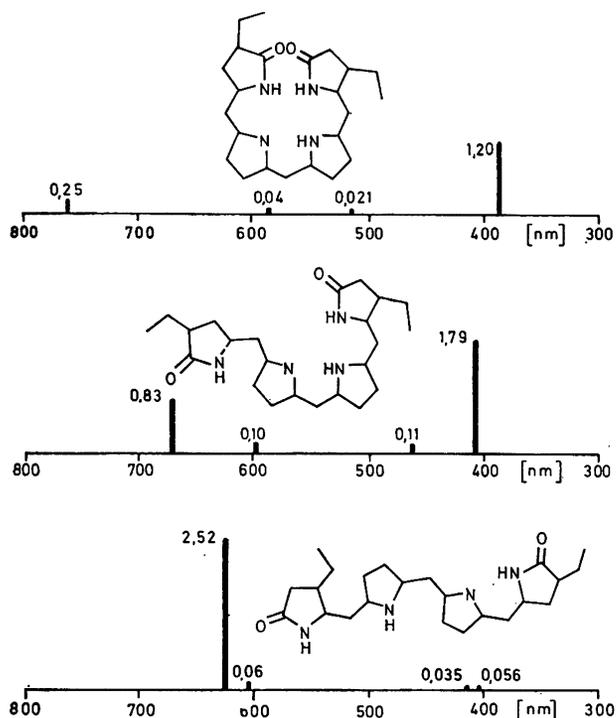


Fig. 3. Calculated oscillator strengths for the biliverdin chromophore in three different conformations: a: cyclic, "porphyrine type", b: semicyclic, c: elongated, "polyene type" (modified from Chae and Song, 1975).

chromophore inherently dissymmetric. The predominance of only one helicality in bilins containing asymmetric α -pyrrole C-atoms then leads to unusually large Cotton effects ****.

Thus, the intensity ratio calculated by Chae and Song¹⁵ for the cyclic conformation(s) is well supported by experimental evidence. Based on the same calculations, the intensity ratio observed in the spectra of native biliproteins would then suggest the chromophores to be in a linear, polyene-type conformation (Fig. 3 c). The conformation shown in Fig. 3 c is chosen from the set used by Chae and Song¹⁵ with regard to minimal steric hindrance at

*** "Porphyrin type" structures have been found for tripyrins, too, both in the crystal^{19a} and in solution^{19b}.

**** A more elongated structure for 4 has been proposed recently from comparison of MO results with low temperature absorption and fluorescence data¹⁵. However, biliverdin and other bilins are protonated at low temperatures in ethanol (-196°C)²⁰. The increase of the long-wavelength band in bilin cations, as compared to the free bases, would then simulate a more elongated structure.

the β -pyrrolic substituents. Conformations like the ones used in formulas 1 and 2 are sterically much more hindered, but the relief of steric strain by twisting the methine bridges is not expected to change the intensity ratio significantly¹⁴. Although other influences cannot be ruled out, no presently published calculations indicate similar pronounced influences, *e.g.* of electric fields^{16a}. However, these factors may be responsible for the large shifts observed among native biliproteins with the same chromophore 1a.

Partial denaturation

At 4°C the red absorption band of C-phycoyanin from *S. platensis* ($0.25 \times 10^{-6}\text{ M}$) is distinctly asymmetric with a narrow peak at 620 nm, which is superimposed to a broader band centered around 615 nm. At 40°C the red band exhibits no longer this asymmetry, and the maximum absorption is reduced by about 20% (Fig. 4 a). If the decrease of the long wavelength absorption maximum is followed as a function of temperature (Fig. 4 b), there is a distinct transition between 20 and 40°C , and after a plateau a second decrease above 50°C . The second decrease corresponds to the complete unfolding of the protein chain^{5b}. The first decrease must then be related to a conformational change from the "low temperature" form, which is stable below 20°C , to a "high temperature" form stable between 35 and 55°C . A two-step unfolding is observed, too, by adding C-PC to increasing concentrations of urea, a denaturing agent (Fig. 5). There is again a distinct transition between 1.5 and 4 M, followed by a plateau and a second decrease above 5 M which again corresponds to the onset of complete unfolding of the peptide chain. As long as the temperature does not exceed 50°C , and the urea concentration is kept below 5 M, respectively, these spectral changes are completely reversible by cooling to 4°C , and by dialyzing the urea out of the solution.

That these spectral changes are due to conformational changes in the monomeric protein, rather than to aggregation, is suggested from molecular weight measurements. On calibrated Sephadex columns, PC has an apparent molecular weight of 44,000 and 47,000 daltons at 4°C and 40°C , respectively. Similarly, ultracentrifugation experiments yield sedimentation coefficients of 2.99 S at 5°C and 3.15 S at 35°C , comparable to the 3 S found for monomeric C-PC⁵⁻⁹.

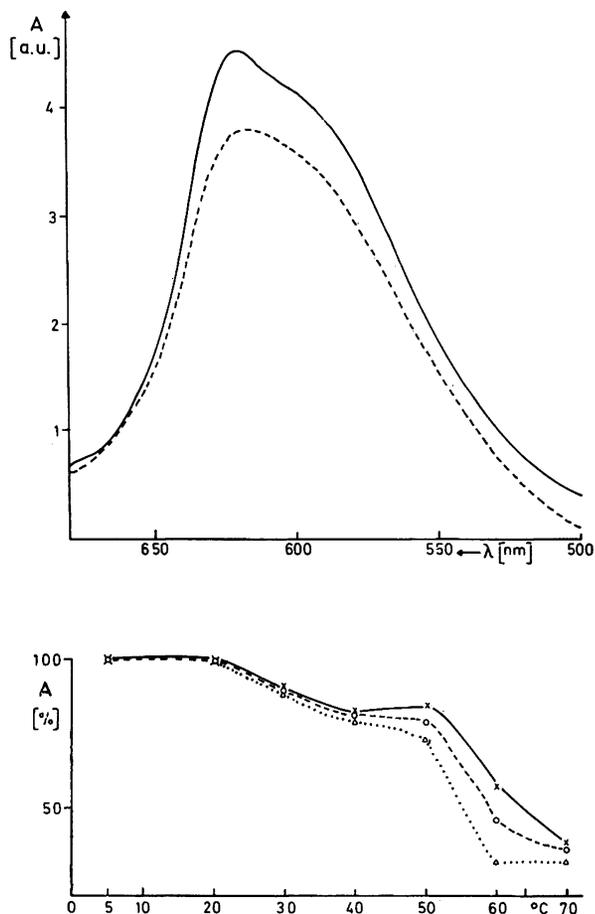


Fig. 4. Partial denaturation of C-phycoerythrin by heat
 a) long wavelength band of the "low temperature" conformation at 5 °C (—), and of the "high temperature" conformation at 40 °C (---).
 b) Decrease of the long wavelength absorption maximum relative to $E_{620}^{100} = 100\%$. The three points at each temperature correspond to a time of 1, 3 and 15 min, respectively. Prolonged treatment, especially at higher temperatures, leads to irreversible side reactions.

Spectroscopic differences between the two intermediate forms of C-PC, *viz.* the "high temperature" form obtained by heating to 40 °C, and the form obtained by adding 4 M urea, respectively, indicate that the two forms are somewhat different from each other. With urea, the intermediate still has an asymmetric red band, although the maximum is shifted to 617 nm. In the spectrum of the "high temperature" intermediate, such fine structure is no longer discernible at all, but the peak originally present at 620 nm is decreased in intensity and/or shifted to shorter wavelengths. These differences would indicate the presence of (at least) two sets of chromo-

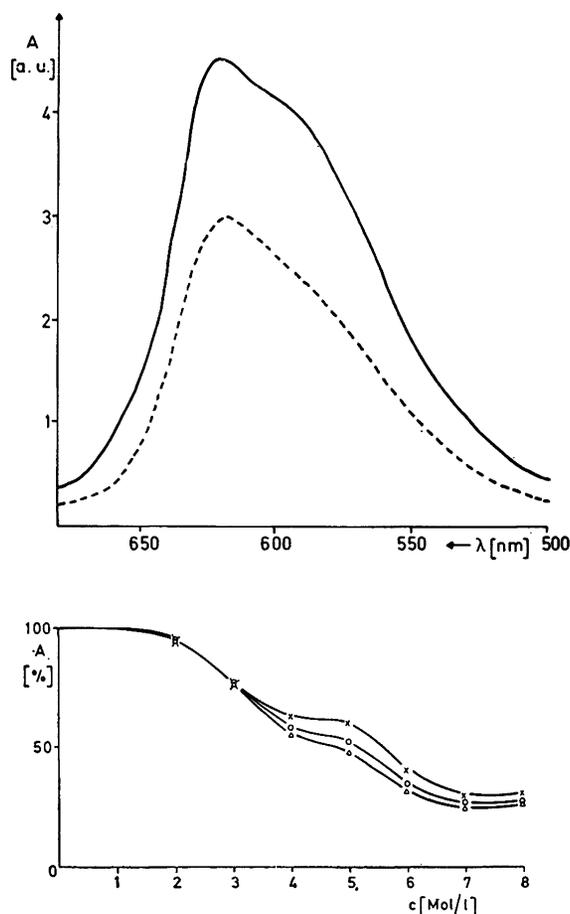


Fig. 5. Partial denaturation of C-phycoerythrin by urea.
 a) Long wavelength band of the conformation stable in the absence of urea at 4 °C (—), and in the presence of 4 M urea (---);
 b) decrease of the long wavelength absorption band ($E_{620}^{100} = 100\%$). The three points correspond to denaturation times of 3, 15 and 30 min.

phores in C-PC. The two sets are affected to a different degree by heat, but they are less discriminated by partial denaturation with urea. Monomeric C-PC generally contains three phycoerythrin chromophores, one in the light (or α), and two in the heavy (or β) subunit^{2, 3}. The spectral changes upon partial heat denaturation may then reflect the preferential unfolding of the peptide chain in the environment of one chromophore. Two different chromophore populations for C-PC had been suggested, too, from fluorescence data^{21, 22}. Both sets are efficiently coupled to allow excitation energy transfer from the chromophore(s) absorbing at shorter wavelengths (termed sensitizers, "s"), to the one(s) absorbing at longer wavelengths (termed fluorescers, "f"). Experiments

identical to the ones described above, but utilizing fluorescence rather than absorption spectroscopy as a probe^{5b}, are under way.

The presence of two different chromophore populations is supported, too, by low temperature absorption spectroscopy of C-PC. The UV-Vis spectrum of a solution of C-PC ($0.3 \times 10^{-4} M$) in a 1 : 1 mixture of buffer and glycerol at 4 °C is similar to that in buffer. In particular, the long-wavelength band is again asymmetric (*c. f.* Fig. 1). If this solution is cooled, both components of the band gradually sharpen, and eventually split into two bands centered

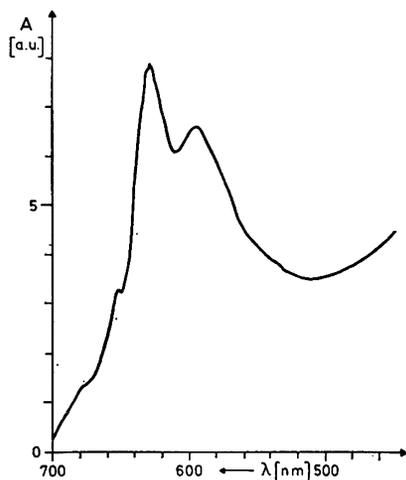


Fig. 6. Low temperature UV-Vis absorption spectrum ($-196^{\circ}C$) of C-phycocyanin in a 1 : 1 mixture of sodium phosphate buffer, pH 7.5, and glycerol.

at 630 and 595 nm below $-100^{\circ}C$ (Fig. 6)²³. The end spectrum ($-196^{\circ}C$) is the same, irrespective of the time required for cooling down (15–180 min), and upon warming up to $4^{\circ}C$ the original spectrum is retained. Presently, it cannot be excluded that both bands observed at low temperatures are due to a single chromophore in a defined conformation. This is unlikely, however, in view of the low temperature spectra of free bilins. If care is taken to avoid protonation during cool-down (see above), the spectra still remain broad, and only little fine structure appears down to $-196^{\circ}C$. There is only one type of bile pigment known to have a two-peaked long wavelength absorption maximum. Purpurins are characterized by spectra with two comparably narrow bands centered around 540 and 500 nm. This spectrum has been interpreted, too, as to arise from two distinct forms present in solution^{1b}.

The results indicate, that the bilin chromophore is held in an elongated conformation in the interior of biliproteins. They also demonstrate, that partial denaturation is a sensitive method to study the interaction between the bile-pigment chromophores and the protein in biliproteins, and to differentiate between distinct chromophore populations.

We acknowledge a grant from the Deutsche Forschungsgemeinschaft, Bonn-Bad Godesberg. We thank Prof. Rüdiger for continuing support and helpful discussions. We are indebted to Prof. C. J. Soeder for generous supply of *Spirulina platensis*, and Dr. R. Loewe for the ultracentrifugation measurements.

- 1 a: No. 1; H. Scheer, Z. Naturforsch. **31c**, 413 [1976]; b: No. 2; H. Scheer, U. Linsenmeier, and C. Krauss, Hoppe-Seyler's Z. Physiol. Chem. **358**, 185 [1977]; c: No. 3; H. Scheer and C. Krauss, Photochem. Photobiol. **25**, 311 [1977].
- 2 W. Rüdiger, Fortschr. Chem. Org. Naturst. **29**, 60 [1971]; W. Rüdiger, Ber. Dt. Bot. Ges. **88**, 125 [1975].
- 3 L. Bogorad, Ann. Rev. Plant Physiol. **26**, 369 [1975].
- 4 A. S. Brown, J. A. Foster, P. V. Voynow, C. Franzblau, and R. F. Troxler, Biochemistry **14**, 3581 [1975].
- 5 a: D. S. Berns, Subunits in Biological Systems, part A (S. N. Timasheff and G. D. Fasman, ed.), p. 105, Marcel Dekker, New York 1971; b: D. S. Berns, H. L. Crespi, and J. J. Katz, J. Amer. Chem. Soc. **85**, 8 [1963].
- 6 A. Hattori, H. L. Crespi, and J. J. Katz, Biochemistry **4**, 1225 [1965].
- 7 G. J. Neufeld and A. F. Riggs, Biochim. Biophys. Acta **181**, 234 [1969].
- 8 C. Vernotte, Photochem. Photobiol. **14**, 163 [1971].
- 9 B. H. Gray, J. Cosner, and E. Gantt, Photochem. Photobiol. **24**, 299 [1976].
- 10 C. J. P. Spruit, R. E. Kendrick, and R. J. Cooke, Planta **127**, 121 [1975].
- 11 E. M. Tobin, W. R. Briggs, and P. K. Brown, Photochem. Photobiol. **18**, 497 [1973].
- 12 H. P. Köst, W. Rüdiger, and D. J. Chapman, Justus Liebig's Ann. Chem. **1975**, 1582 [1975]; S. Grombein, W. Rüdiger, and H. Zimmermann, Hoppe-Seyler's Z. Physiol. Chem. **356**, 1706 [1975].
- 13 A. N. Glazer and C. S. Hixson, J. Biol. Chem. **250**, 5487 [1975].
- 14 G. Blauer and G. Wagniere, J. Amer. Chem. Soc. **97**, 1949 [1975].
- 15 Qu. Chae and P. S. Song, J. Amer. Chem. Soc. **97**, 4176 [1975].
- 16 a: T. Sugimoto, K. Ishikawa, and H. Suzuki, J. Phys. Soc. Japan **40**, 268 [1976]; b: M. J. Burke, D. C. Pratt, and A. Moscowitz, Biochemistry **11**, 4025 [1975]; J.-H. Fuhrhop, P. K. W. Wasser, J. Subramaniam, and U. Schrader, Justus Liebig's Ann. Chem. **1974**, 1450 [1974].
- 17 W. S. Sheldrick, J. Chem. Soc. Perkin Trans II, p. 1457 [1976].
- 18 A. Moscowitz, W. C. Krueger, I. T. Kay, G. Skewes, and S. Bruckenstein, Proc. Nat. Acad. Sci. U.S. **52**, 1190 [1964].
- 19 a: D. Cullen and K. M. Smith, private communication,

- 1976; b: H. Falk, S. Gergely, K. Grubmayr, and O. Hofer, *Z. Naturforsch.* **32 b**, 299 [1977].
- ²⁰ H. Scheer and W. Kufer, to be published.
- ²¹ R. E. Dale and F. W. J. Teale, *Photochem. Photobiol.* **12**, 99 [1970].
- ²² C. Vernotte and I. Moya, *Photochem. Photobiol.* **17**, 245 [1973].
- ²³ This split has been observed for C-PC from other organisms by: D. Frackowiak and J. Grabowski, *Photosynthetica* **5**, 146 [1971].