Photosynthetic Light-Harvesting Systems

Organization and Function

Proceedings of an International Workshop
October 12–16, 1987
Freising, Fed. Rep. of Germany

Editors
Hugo Scheer · Siegfried Schneider

Walter de Gruyter · Berlin · New York 1988

CONTENTS

EFFECT OF PROTEIN ENVIRONMENT AND EXCITONIC COUPLING ON THE EXCITED-STATE PROPERTIES OF THE BILINCHROMOPHORES IN C-PHYCOCYANIN

S. Schneider, Ch.Scharnagl
Institut für Physikalische und Theoretische Chemie der Technischen Universität München, D-8046 Garching

M. Duerring, T. Schirmer, W. Bode
Max-Planck-Institut für Biochemie, D-8033 Martinsried

Introduction

C-Phycocyanin (C-PC), one of the light-harvesting pigments of cyanobacteria, is composed of two subunits, α and β. The former contains one, the latter two open-chain tetrayrrole chromophores (phycocyanobilin), covalently bound to the apoprotein via thioether linkages (see e.g. (1)).
The geometries and individual environments of the three chromophores in the phycocyanin of Mastigocladus laminosus have recently been determined by peptide sequencing (2) and high resolution X-ray crystallography (3a,b,c). The crystal structure displays a strong similiarity of geometries, so that different spectroscopic, biochemical and functional properties must be mainly attributed to the different protein surroundings. Lately it has been possible to assign the absorption bands (maxima) to the different phycocyanin chromophores unambiguously in the following manner (4,5):

A84:	λ_{max}(abs)= 616 - 618 nm (4) ;		618 nm (5)
B84:	622 - 624 nm (4) ;	624 - 632 nm (5)	
B155:	598 - 600 nm (4) ;	594 - 598 nm (5)	
		582 - 598 nm (5,CD)	

Photosynthetic Light-Harvesting Systems

CD-spectroscopy is an important source of information to elucidate the conformation of bilin chromophores and a sensitive probe to noncovalent interactions. Therefore we studied the effect of protein-chromophore and chromophore-chromophore interactions on the chiroptic properties by means of quantum mechanical model calculations, in order to improve our knowledge about the contribution of these interactions to the spectral and functional properties of the phycobilinproteins and to get an estimate of the significance of these interactions.

Methods

Our calculations are based on the high-resolution X-ray data for chromophore and protein (3c). Approximative wavefunctions result from a π-electron model calculation (PPP) with standard parametrisation and configuration interaction (CI) of singly excited states (CI-basis: 6 occupied, 6 virtual molecular orbitals; a larger CI-basis leads to a 10 to 15 nm redshift and small variations in the other calculated properties). The rotatory strengths and f-values are calculated without further approximations (6a,6b,7).
The chromophores and their surroundings are treated as one "supermolecule". Therefore, all interactions between permanent and transition charge distributions are included. Polar and aromatic amino acid residues lying in a sphere with 10 Å radius around C10 were taken into account.
Hydrogen bonds are simulated by varying the effective ionization potential (IP') of the heteroatom since a withdrawal of the hydrogen from a pyrrole nitrogen causes the π-electrons of this center to delocalize more easily. The parameter IP' was changed accordingly from 19.6 eV (no bonding) to 16.5 eV (strong bonding, IP' midway between the values for pyrrole and pyridine nitrogen). The values of all other parameters are given elsewhere (6,7).

Results and Discussion

Single Chromophores

Figures 1 and 2 show the variations of the absorption wavelength and rotatory strength R of the red band of B84 and B155, respectively, due to chromophore-protein interactions. We restrict our discussion to this band because it is well separated from other transitions and therefore easier to analyse - theoretically as well as experimentally.

Case I: isolated chromophore, neglecting all side chains
Case II: I + propionic acid side chains attached to C8 + C12
Case III: II + aspartate residue near pyrrole rings B and C (B87 and B39, respectively)
Case IV: III + residues of polar amino acids near the propionic side chains
Case V: IV + all polar and aromatic residues in a sphere of 10 Å around C10
Case VI: V + amide groups of the protein backbone within the same sphere (in brackets)

The calculated transition energies for the isolated chromophores (I) are around or below 550 nm. The origin for this hypsochromic shift, when compared to prior calculations (6a,6b,7), is the significant deviation from a planar geometry. Coulombic interaction (static coupling in terms of first order perturbation theory) effects the transition energies (bathochromic shift) mainly because of the different stabilization of the various molecular orbitals. The rotatory strength is altered because of changed coefficients in the CI-wavefunctions and the concomitant modified mixing of electric and magnetic transition moments.
Inclusion of the carboxylate groups of the propionic acid side chains (II) exhibits only small effects (the proton tautomerism is fixed by the interaction with adjacent amino acids (3c)).
Both chromophores (as well as the third, not displayed, at A84) show a common principle of interaction with the protein.

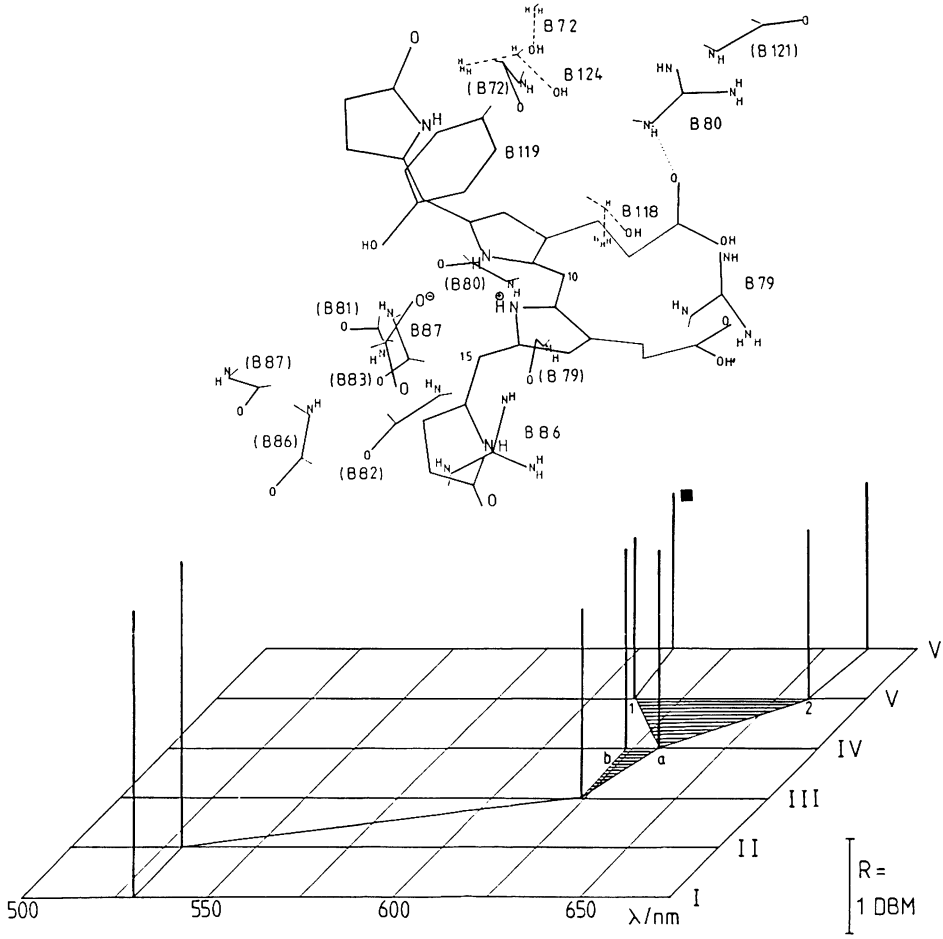

Figure 1: Calculated rotatory strength R of the longwave-length absorption band of the B84 chromophore (1 DBM=0.93 10^{-39} cgs; case I to VI: see text). The shaded regions show the spectral variations with the tautomerism of the arginine groups:
a) B80 as shown b) tautomeric form
1) B79 and B86 as shown 2) tautomeric form

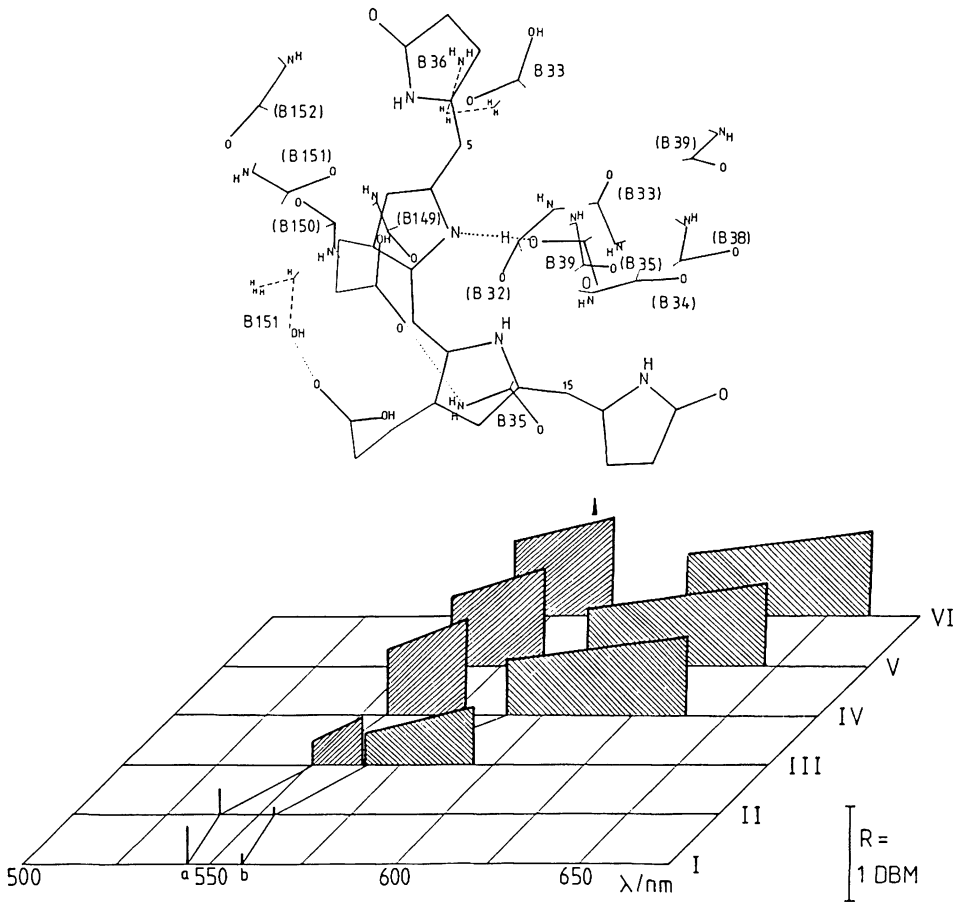

Figure 2: Calculated rotatory strength R of the longwavelength absorption band of the B155 chromophore. The shaded area reflects the bathochromic shift calculated for an increasing of the hydrogen bond strength to the B39 aspartate residue.
a) N23-H-tautomer b) N22-H-tautomer

They arch around an aspartate residue (B87 and B39, resp.), the nitrogens of pyrrole rings B and C (N22 and N23) being within hydrogen bonding distance of one of the carboxylate oxygens. This interaction dominates the wavelength adjustment (III).

For B84 (and A84) the assumption of a proton transfer to the chromophore, leaving a negative aspartate counterion, is necessary to shift the calculated electronic excitation energy into the experimentally established region.

B155 deviates from this behaviour; a proton transfer would result in a transition near 700 nm. Therefore we postulate that B155 and aspartate B39 are coupled via a hydrogen bond. Only a positive partial charge near N22 and N23 (OD2 treated as -O-H) yields a redshift. Depending on the methen tautomerism (N22-H or N23-H) and the hydrogen bond strength, the visual absorption band varies in a wavelength region of about 50 nm width (shaded areas in figure 2).

Inclusion of the charged and aromatic residues around B84 (case IV: Arg B80; case V: Arg B79, Arg B86, Ser B72, Tyr B119, Thr B124, Thr B118) leads to further shifts. There is a strong indication, that the arginine residues play a vital role in tuning the transition energy. Small variations in geometry (e.g. a rotation of the $-C(NH_2)(NH)$-groups by 180°, equivalent to an exchange of positive and negative partial charges) result in a drastic variation of the transition wavelength. Therefore, these residues may be responsible for the heterogeneity observed in the fluorescence kinetics of smaller aggregates (8).

Inclusion of the charges in the surrounding of B155 (case IV: Thr B151, Asn B35; case V: Lys B36, Glu B33) induces also additional redshifts of the first electronic transition. Only for the N23-H-tautomer is the theoretical result, however, in agreement with observation.

The interaction with the amide groups of the protein backbone (case VI) produces only small corrections to the excited state properties and can therefore be ignored in first approximation studies.

β-subunit

The results described so far predict that the CD-spectrum of two, non-interacting chromophores in the β-subunit should exhibit a large rotatory strength around 620 nm. This is, however, in contradiction to the experimental finding (4) (inactive longwavelength absorption band, attributed to B84). Therefore, we extended our model calculations to take into account both chromophores and their environments together. The selected status of chromophore-protein interaction according to approximation VI is marked in figures 1 and 2 for both chromophores. It yields transition energies in the correct wavelength region and also the required energy spacing for B84 and B155. Figure 3 shows the thereby obtained theoretical CD-spectrum of the β-subunit.

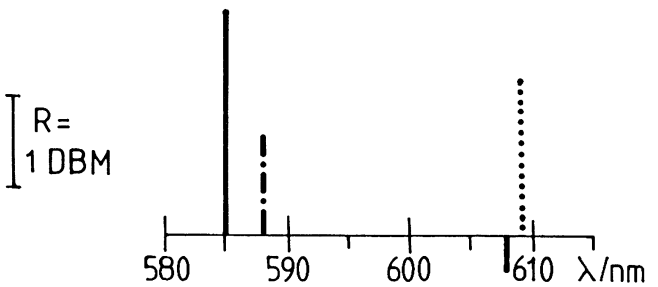

Figure 3: Calculated visible CD-spectrum of individual chromophores (B84 ·····, B155 -·-·-·-·-) and the β-subunit with excitonic coupling included (————).

Due to excitonic coupling, the shorter wavelength transition (ψ^{+}=0.9 ψ^{*}(B155) + 0.1 ψ^{*}(B84)) gains rotatory strength, which is lost in the corresponding longwavelength transition. This final result fits the experimentally found CD-bandshape very well and is a clear indication, that the interaction with charged residues controls also the chromophore-chromo-

phore coupling within the phycobiliproteins. It is furthermore a warning that the properties of isolated chromophores may not be extracted from measurements on biological systems with interacting chromophores.

Acknowledgement

We want to thank Prof. H. Scheer for many fruitful discussions. Free computer time and the access to NOS/VE by the Leibniz Rechenzentrum der Bayerischen Akademie der Wissenschaften is greatly appreciated.

References

1. Scheer, H. 1983. In: Light Reaction Path of Photosynthesis (F. K. Fong, ed.). Springer Verlag, Berlin. pp. 7-45.

2. Frank, G., W. Sidler, H. Widmer and H. Zuber. 1978. Hoppe-Seyler's Z. Physiol. Chem. 359, 1491-1507.

3a. Schirmer, T., W. Bode, R. Huber, W. Sidler and H. Zuber. 1985. J. Mol. Biol. 184, 257-277

b. Schirmer, T., R. Huber, M. Schneider, W. Bode, M. Miller and M. L. Hackert. 1986. J. Mol. Biol. 188, 651-676

c. Schirmer, T., W. Bode and R. Huber. 1987. J. Mol. Biol. 196, 677-695

4. Siebzehnrübl, S., R. Fischer and H. Scheer. 1987. Z. Naturforsch. 42c, 258-262.

5. Mimuro, M., R. Rümbeli, P. Füglistaller and H. Zuber. 1987. Biochimica et Biophysica Acta 851, 447-456.

6a. Blauer, G. and G. Wagnière. 1975. J. Am. Chem. Soc. 97, 1949-1954.

b. Blauer, G. and G. Wagnière. 1976. J. Am. Chem. Soc. 98, 7806-7810.

7. Scharnagl, Ch., E. Köst-Reyes, S. Schneider, H.-P. Köst and H. Scheer. 1983. Z. Naturforsch. 38c, 951-959.

8. Schneider, S., P. Geiselhart, F. Baumann, S. Siebzehnrübl, R. Fischer and H. Scheer. Contribution in this volume.