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SEVEN-FOLD EXCITON SPLITTING OF THE 810-nm BAND IN BACTERIOCHLOROPHYLL *A*-PROTEINS FROM GREEN PHOTOSYNTHETIC BACTERIA

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Summary

We report comparative absorbance and fourth derivative absorbance spectra of two different bacteriochlorophyll *a*-proteins at 5 K in each of two different cryogenic solvent mixtures. In previous studies at 5 K each protein was observed in only one of these mixtures (not the same one). For the protein from *Prosthecochloris aestuarii* strain 2K, whose structure is known, the solvent effect is relatively small; for the protein from *Chlorobium limicola* f. sp. *thiosulfatophilum* strain 6230 (Tassajara), the effect is much more pronounced. From these results together with earlier results at 300 K, we conclude there may be slight conformational differences of the *Prosthecochloris* protein between the crystalline form used for X-ray diffraction studies and that in a cryogenic solvent. By comparing spectral features of the two proteins in the same solvent, we are able for the first time to assign all seven of the expected exciton levels in each protein. These occur at 793, 801, 806, 810, 814, 819, and 825 nm in the *Prosthecochloris* protein, and at 793, 802, 806, 810, 816, 820, and 823 nm in the *Chlorobium* protein.

The bacteriochlorophyll (BChl) *a*-protein from the green photosynthetic bacterium *Chlorobium limicola* f. sp. *thiosulfatophilum* strain 6230 (Tassajara) is quite similar to the analogous protein from *Prosthecochloris*

Abbreviation: BChl, bacteriochlorophyll.

aestuarii strain 2K [1]. Both proteins have the same subunit size and similar absorption spectra at room temperature; they differ only slightly in amino acid composition and circular dichroism characteristics (810-nm band). The trimeric structure of the *Prosthecochloris* protein is known to a resolution of 2.8 Å [2—5], and we assume that the structure of the *Chlorobium* protein is similar. On the basis that each subunit of the trimeric protein contains 7 molecules of BChl *a*, exciton theory predicts the splitting of each absorbance band in the protein, corresponding to one BChl electronic transition, into 7 exciton levels. Because of the relatively close spacing of these levels, they cannot all be resolved in absorbance spectra, even at low temperature and with the aid of derivative techniques. We report here the first, tentative, assignment of all 7 exciton levels for the lowest-energy singlet transition (810-nm band) based on a comparative analysis of spectral features of the two proteins in the same solvent.

Spectroscopic studies of these two BChl *a*-proteins in two different solvent mixtures were carried out at 5 K. One mixture (A) consisted of 75% potassium glycerophosphate (4:7, v/v), glycerol (1:7, v/v), and BChl *a*-protein in buffer (2:7, v/v) [6]. The other mixture (B) consisted of glycerol (1:2, v/v) and BChl *a*-protein in buffer (1:2, v/v) [7]. Absorption and CD spectra at 77 K [6] and absorption spectra at 5 K [8] have been reported for the *Prosthecochloris* protein in mixture A. Absorption and CD spectra have been reported for both proteins in mixture B at 77 K, and absorption spectra for the *Chlorobium* protein in mixture B have been reported at 5 K [9]. The 5 K absorption spectra of the two proteins were not measured in the same solvent. Because we have observed a solvent effect on the absorption spectra of these proteins at 5 K, we present here the 5 K absorption of the *Prosthecochloris* protein in mixture B, and that of the *Chlorobium* protein in mixture A.

The experimental methods and procedures for purifying materials used here have all been given previously. Bacteria were grown and proteins isolated as described by Olson [1]. The spectroscopic techniques were the same as those used previously [8, 9], including methods of numerical differentiation [8].

Fig. 1 shows the absorption spectra of each of the proteins in both solvent mixtures at 5 K. For the *Prosthecochloris* protein the solvent effect is relatively small; but spectral features are somewhat sharper in the mixture B. The solvent effect on the *Chlorobium* protein spectra is much more pronounced; some peaks are shifted by as much as 0.8 nm, and relative peak heights are altered so that the 816-nm peak is dominant in mixture A while the 806-nm peak is dominant in mixture B.

The simplest interpretation of these solvent effects is that the exciton interactions [3, 6, 10] among the BChl molecules in each protein are slightly different in the two solvents. In general, changes in exciton interactions produce comparable changes in both absorption intensities and peak separations [11], such as are observed here. Changes in exciton interaction energies of this magnitude, i.e. less than approx. 15 cm^{-1}, could be caused by small changes in protein conformation with solvent. A change in separation of a pair of BChl molecules in a protein of no more than 0.6 A would be sufficient

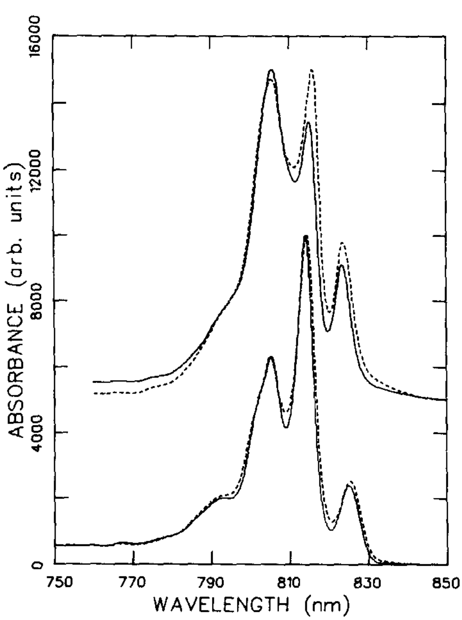

Fig. 1. Absorption spectra at 5 K of *Chlorobium* BChl *a*-protein (upper set of curves) and *Prosthecochloris* protein (lower set of curves). In each case the dashed curve represents the spectrum of the protein in mixture A (glycerophosphate), the solid curve in mixture B (glycerol).

to produce such an effect. (Because of the inverse cube dependence of exciton interaction energy on the separation of the two interacting dipoles [12], a fractional change in energy of 0.15 can result from a fractional change in separation of only 0.05. Thus, a 15% change in one of the BChl-BChl exciton interaction energies, typically approx. 100 cm^{-1} in the *Prosthecochloris* protein [10], can result from a 5% change in the BChl-BChl nearest neighbor distance of approx. 12 Å. If several of the nearest neighbor distances change, or if there are changes in relative BChl orientations as well, the observed spectral changes could be produced with mean distance changes substantially less than 0.6 Å.) If there is such a conformational change, one would expect a bigger change in the CD spectrum than in absorption. In fact, for the *Prosthecochloris* protein there are noticeable differences between the 77 K CD spectrum observed in mixture A [6] and that in mixture B [7]. (The CD spectrum of the *Chlorobium* protein in mixture A has not been recorded at 77 K.)

A solvent effect on the 300 K absorption spectra of the *Prosthecochloris* protein has been reported previously [8]. In that case, one of the two solvents was mixture A, and the other was just the standard buffer solution used in purifying the protein. The observed shift in the largest peak between these two solvents was comparable, approx. 1 nm, to that observed at 5 K for the *Chlorobium* protein between the two cryogenic solvents. (Because of the reduced resolution at 300 K it was not obvious whether the changes in absorption intensities were comparable to those in peak separations.) Hence, it is possible that there are slight conformational differences between the *Prosthecochloris* protein in the crystal and in a cryogenic solvent. While these differences would not be enough to account for the gross difficulties in fitting exciton-theoretical absorption and CD spectra [10], they would preclude an extremely close fit to the low temperature spectra based on the crystal structure.

Although the solvent effects interfere with the ability of theory to fit observed spectra closely, they should not affect the interpretation of prominent spectral features. Fig. 2 shows the fourth derivative absorption spectra of the two proteins at 5 K in mixture B. The positions of seven maxima from each of these derivative spectra are given in Table I. The positions of the fitted Gaussian components of the 77 K absorption and CD spectra in the same solvent are also tabulated. With the possible exception of the relatively

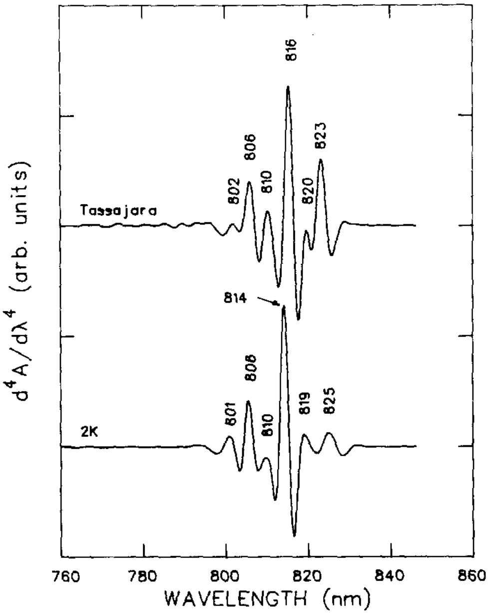

Fig. 2. Fourth derivative absorption spectra at 5 K of *Chlorobium* BChl *a*-protein (upper curve) and *Prosthecochloris* (lower curve) in mixture B (glycerol).

TABLE I

WAVELENGTHS OF PRINCIPAL SPECTRAL FEATURES OF BChl *a*-PROTEINS IN MIXTURE B

Wavelengths are in nm. The features shown are: 5 K 4th derivative maxima, present investigation (Fig. 2); components from Gaussian resolution of 77 K absorption, with 6 components assumed for *Prosthecochloris* and 5 components for *Chlorobium* (Ref. 7); and Gaussian components of 77 K CD spectrum with sign (+ or —) in parentheses (Ref. 7).

	Spectrum	1	2	3	4	5	6	7	Source
Prosthecochloris	Abs (5 K)	793	801	806	810	814	819	825	Fig. 2
	Abs (77 K)	789	800	805	810	814	—	825	Ref. 7
	CD (77 K)	791 (—)	798 (+)	—	811 (+)	814 (—)	822 (—)	—	Ref. 7
Chlorobium	Abs (5 K)	793	802	806	810	816	820	823	Fig. 2
	Abs (77 K)	789	801	807	—	816	—	824	Ref. 7
	CD (77 K)	790 (—)	799 (+)	807 (—)	812 (+)	—	821 (—)	—	Ref. 7

broad shortest-wavelength peak, which is obscured in the fourth derivative because of its breadth [8], the correspondence in wavelength of 5 K derivative and 77 K Gaussian absorption features is obvious. When the absorption and CD features are aligned in this way, the wavelengths of corresponding peaks in the three spectra for both proteins are in better agreement than in previous alignments [7]. Because of the small change (less than approx. 1 nm) in the positions of the narrow absorbance components between 5 K and 77 K, it is unlikely that the present alignment of absorption and CD spectral features would require modification were CD data at 5 K available. The original 5- or 6-component Gaussian resolutions gave wavelength differences of as much as 4 nm for corresponding features in the absorption and CD spectra. In making this new correspondence, the fourth derivative peak near 820 nm, previously considered as a likely artifact of differentiation [8], is here interpreted as a resolved exciton transition. This reinterpretation leads to an especially close correspondence of absorption and CD features for the *Chlorobium* protein. The new correspondence of CD and absorption features also provides a more consistent ordering of the signs of the CD peaks. The close chemical similarity of the two proteins [1] supports this analogous assignment of exciton levels for the two. Thus, the comparative analysis of spectral features of both proteins allows us to assign tentatively all seven of the expected [3, 10] exciton transitions within the 810-nm band.

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