Derivative Absorption Spectroscopy of the Pigment-Protein Complexes from *Rhodopseudomonas capsulata*

Gerhard Talsky

Institut für Technische Chemie der Technischen Universität München, Lehrstuhl 1, Lichtenbergstraße 4, D-8046 Garching

Cornelis P. Rygersberg, Rien van Grondelle

Department of Biophysics, Huygens Laboratory, State University, Wassenaarseweg 78, Leiden, Netherlands

Reiner Feick, and Gerhart Drews

Institut für Biologie 2, Mikrobiologie der Universität Freiburg, Schänzlestr. 1, D-7800 Freiburg

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The near infrared absorption spectra and their fourth derivative were measured in membrane preparations or in the isolated light harvesting pigment-protein complex B 800-850 from *Rhodopseudomonas capsulata* in order to know how the underlying molecular absorption spectra contribute to the observed absorption curve. In contrast to the observations of Cogdell and Crofts [Biochim. Biophys. Acta **502,** 409 (1978)] no splitting of the 855 nm absorption bands was observed at 300 K, 100 K, and 4 K. However, a small but significant splitting of the 870 nm band in all derivates (2nd, 4th, 6th) was observed at 300 K and 4 K. The lack of splitting of the 855 nm absorption band will be discussed in the light of the molecular organization of the B 855 moiety of this light harvesting bacteriochlorophyll-protein complex.

The near infrared region of the absorption spectrum of membranes isolatd from the wild type strain *Rhodopseudomonas capsulata* 37b4 at room temperature shows two and at 77 K three prominent peaks, indicating that different spectral forms of bacteriochlorophyll (Bchl) are present. Three main pigment-protein complexes, *i.e.* the photochemical reaction center and the light harvesting complexes B 870 and B 800–850, have been isolated and partially characterized [1–4].

Chemical and spectroscopic analysis has shown that the B 800–850 complex contains one polypeptide (M_r 5100) associated with one molecule each of Bchl and carotenoid representing the 800 nm absorption band, a second polypeptide (M_r 9200) associated with two molecules of Bchl (responsible for the 850 nm absorption band) and a non-pigmented polypeptide of 11900 apparent M_r [4, 5]. The isolated complex has a molecular weight of approximately 170000 and exists as oligomers of the smallest stoichiometric unit (3 polypeptides, 3 mol Bchl, 1 mol carotenoid [3; J. Shiozawa and G. Drews, unpublished]. Due to its instability the B 870 complex has not been characterized thoroughly. Preliminary results show a molar Bchl to protein ratio of 1:1. The carotenoid:Bchl ratio is unknown.

To obtain a better understanding of the communication between antenna and reaction center complexes, it is important to know how the *in vivo*-absorption bands are constructed and which underlying molecular absorption spectra contribute to the observed absorption curve. It was shown that the fourth derivative of complex absorption spectra can enhance spectral resolution and provide information on the relative contribution of different absorbing species to one absorption band [6–8]. Moreover splitting of absorption bands due to interaction of the absorbing pigments may result in a change of the overall absorption spectrum which can be resolved in this way [9, 10].

Cogdell and Crofts [11] reported a splitting of the 850 nm absorption band of the B 800–850 complex from *R. sphaeroides* as studied with fourth derivative spectroscopy at room temperature. This is in agreement with a dimeric model for the two B 850 Bchl molecules, where the splitting of the transition moments in two different polarized components

Reprint requests to Professor G. Drews.

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causes both the observed splitting, and the CD measurements [12]. It is therefore important to study the long wavelength absorption bands of the light harvesting complex B 800–850, using the fourth derivative spectroscopy to determine the various components. It should be kept in mind that the long wavelength absorption of protein bound bacteriochlorophyll with respect to *in vitro* absorption is not necessarily due to pigment-pigment interaction but may also be due to pigment-environmental interactions [10].

Materials and Methods

The strains of *Rhodopseudomonas capsulata* and the isolation of the B 800–850 complex has been described recently [3].

Derivative spectroscopy

Absorption spectra were recorded using a single beam spectrophotometer described elsewhere [13], equipped with a cryostat which allowed us to cool the sample from 300 K down to 4 K. The 2nd and 4th derivatives of the spectra were estimated according to a method described by Butler and Hopkins [6]. The derivative spectra in Fig. 1, 2 and 4 were taken by a spectrophotometer SP 8–100 (Philips-Pye Unicam, Cambridge) and an analog differentiator TLB 6000 (Lucius and Baer, D-8142 Geretsried).

Results and Discussion

The absorption spectrum of wild type membrane in Fig. 1 shows an asymmetric peak at 858 nm, which is due to an overlapping of two different signals, which result in the fourth derivative a second maximum at 880 nm. The fourth derivative of the absorption spectrum does not show a splitting of the 800 nm or 855 nm absorption bands, but it does seem to indicate a splitting of the 870 nm band (Fig. 1). The mutant strain A 1 a$^+$, which is free of B 800–850 complex was used to study the 870 nm band more directly. In all derivatives (2nd, 4th, 6th) of different absorption spectra from different preparations a small but significant splitting of the 870 nm band was observed at room temperature (Fig. 2) and 4 K (Fig. 3). The distance of both peaks is similar as the maximum half width of the curve. The 800 nm band, however, seemed to consist of a single absorption band. The small shoulder in Fig. 2 at

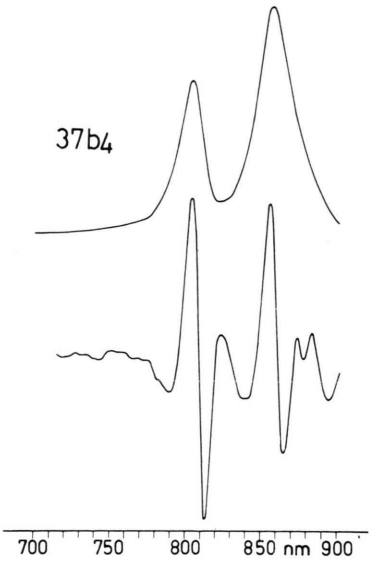

Fig. 1. Absorption spectrum (upper curve) and the fourth derivative (lower curve) at room temperature of membranes from *Rhodopseudomonas capsulate* strain 37 b 4 grown anaerobically in the light. The asymmetric peak near 850 nm indicates that more than one spectral form contributes to that peak. This is shown by the derivative absorption spectrum. The peak at 850 nm belongs to the 850 nm band of the B 800–850 light harvesting complex, while the splitted band at 870 to 880 nm belongs to the B 870 bacteriochlorophyll light harvesting complex (see introduction).

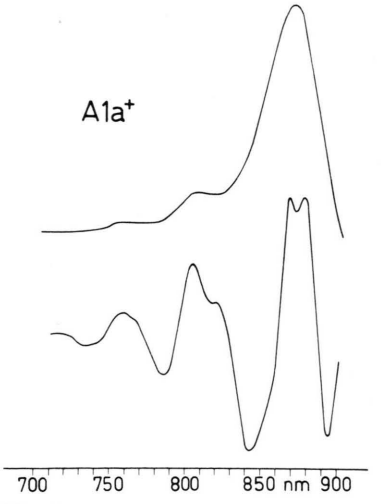

Fig. 2. Absorption spectrum (uper curve) and the fourth derivative spectrum (lower curve) at room temperature of membranes of the blue-green mutant strain A 1 a$^+$ of *R. capsulata*, lacking carotenoids and the light harvesting complex B 800–850. The dominant peak at 870 nm is mainly due to the light harvesting bacteriochlorophyll-protein complex B 870, while the peak at 800 nm is contributed by the reaction center. All derivative spectra show a splitting of the 870 nm band.

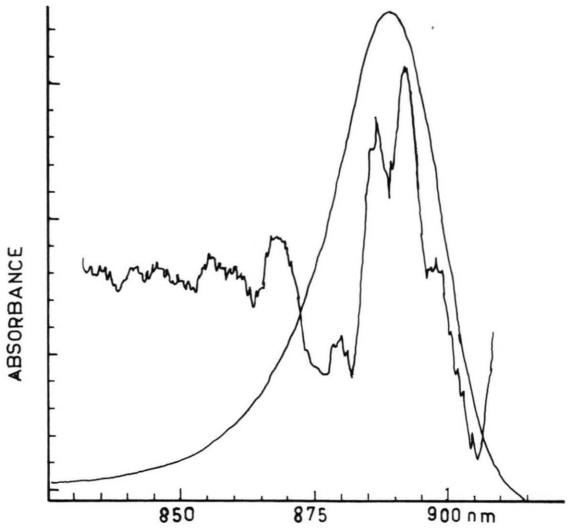

Fig. 3. Absorption spectrum (upper curve) and the fourth derivative spectrum (lower curve), taken at 4 K, of membranes from the mutant strain A 1 a$^+$ (see legend for Fig. 2). The 870 nm peak is splitted in the derivative spectrum.

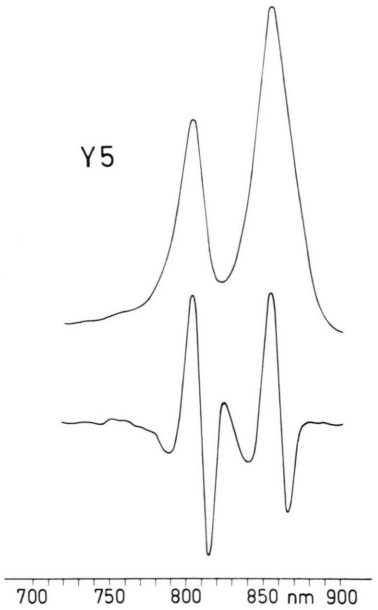

Fig. 4. Absorption spectrum (upper curve) and the fourth derivative spectrum (lower curve), taken at room temperature, of membranes from the mutant strain Y 5, lacking photochemical reaction center and the B 870 complex. The same spectrum was obtained with the isolated light harvesting complex B 800–850. The band at 850 shows no splitting.

820 nm is a virtual peak as shown by computer simulation and has its origin in an inflection point of the ground signal. Derivatives of absorption spectra of reaction center preparations did not show a splitting of the 800 or 860 nm bands (not shown here).

The B 870 complex of the carotenoid-less mutant strain A 1 a$^+$ contains approximately one molecule Bchl per polypeptide [3]. The dominating polypeptide in the complex has an apparent molecular weight of 12000 (Dodecylsulfat-polyacrylamide gel electrophoresis). It has to be studied whether the small amounts of a second polypeptide of an apparent molecular weight of 8000 contributes to the pigment-protein binding, or whether two 12000 polypeptides are associated with two Bchl molecules. This result will be discussed elsewhere with measurements of linear and curcular dichroism carried out in the laboratory of K. Sauer.

Membranes of the mutant strain Y 5, which contain only the B 800–850 complex, and the complex B 800–850, isolated from the membranes of this strain, show identical absorption bands at 800 and 855 nm (Fig. 4) at room temperature and 4 K at 802 and 857 nm (Fig. 5). Although the peaks are asymmetric, the fourth derivatives of the absorption spectra do not show a clear splitting of either of the

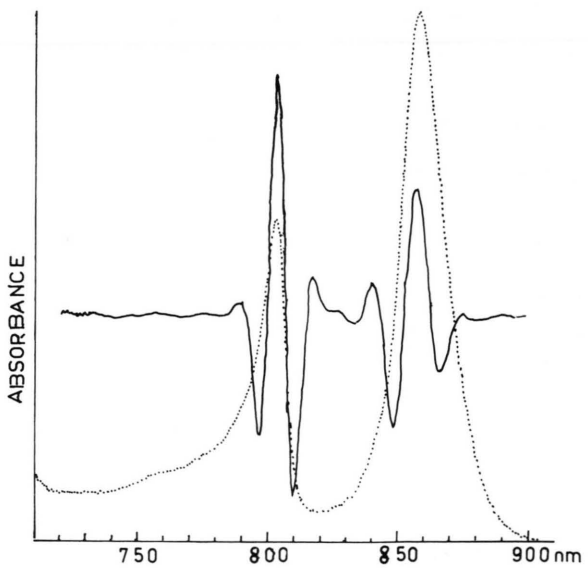

Fig. 5. Absorption spectrum (upper curve, dotted line) and the fourth derivative spectrum (lower curve), taken at 4 K, of the isolated complex B 800–850 from the mutant strain Y 5 of *R. capsulata* (see legend for Fig. 4). No splitting of both absorption band could be observed. Absorption bands at 802 and 858 nm.

bands (Fig. 4, 5). Derivatives (up to th 6th) of absorption spectra obtained at 300 K, 100 K, and 4 K, respectively, using different preparations confirmed this result. In contrast, Cogdell and Crofts [11] observed a splitting of the 850 nm band in membrane preparations from *R. sphaeroides* at room temperature.

Circular dichroism analysis of the B 800–850 nm complex from *R. sphaeroides* revealed that the absorption band at 850 nm consists of two bacteriochlorophylls with coupled absorption transition moments [9, 12].

The derivative of the B 800–850 absorption spectrum and the analytical results with this complex indicate that the transitions of the two Bchl bound to the 9200 polypeptide occur at approximately the same frequency which then does not allow a spectroscopic resolution of the two absorption bands.

Nevertheless, clear evidence is present for the existence of two transitions in the 850 nm region for the mutant strain Y 5 when examined using circular dichroism or, for oriented samples, linear dichroism (J. Bolt, K. Sauer, and G. Drews, unpublished). The CD spectra of both the membranes and the isolated B 800–850 complex show a pronounced double CD and a zero crossing near 855 nm, closely resembling the corresponding spectra reported previously for *R. sphaeroides*, strain 2.4.1 (Sauer and Austin [9], Fig. 4). A change in the linear dichroism value also occurs somewhat on the long wavelength side of the absorption maximum.

Acknowledgements

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