THE ANTENNA SYSTEM OF *RHODOSPIRILLUM RUBRUM*: DERIVATIVE ANALYSIS OF THE MAJOR NEAR INFRARED ABSORPTION BAND OF CHROMATOPHORES

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1. Introduction

The photosynthetic pigments are associated with specific apoproteins to form several types of complexes. Only one of such complexes has been found in the light-harvesting antenna system of the purple bacterium *Rhodospirillum rubrum* [1], containing spirilloxantin, bacteriochlorophyll *a* and a hydrophobic polypeptide of low M_r. The solubilized complex exhibits a single near infrared absorption band which is very similar to that of bulk bacteriochlorophyll in the intact membrane. Circular dichroism spectra of the band show exciton coupling [2], suggesting a dimeric state of bacteriochlorophyll in the complex. Fourth-derivative analysis of the band at room temperature [3] indicates also that it is composed of 2 spectral forms of bacteriochlorophyll.

We have shown [4] that mild oxidation of *Rds. rubrum* chromatophores elicits the sequential and reversible bleaching of two distinct components of the near infrared band of antenna bacteriochlorophyll. Although the existence of several spectral forms of bacteriochlorophyll might be interpreted in terms of pigment–pigment interaction within a single antenna complex, the redox behaviour of the resolved constituents suggests the presence of different antenna complexes in the *Rds. rubrum* membrane. To obtain information about this point, we have carried out fourth-derivative analysis of the near infrared absorption band at room and at liquid nitrogen temperatures. The results of such analysis, performed on intact and on detergent-treated chromatophores, again suggest that the band is composed of several constituents which belong to at least 2 different antenna complexes.

2. Methods

The wild-type strains S1 and T of the purple photosynthetic bacterium *Rds. rubrum* have been described before [5,6]. The growth medium and other culture conditions have also been described [7]. Photosynthetic membrane vesicles were prepared according to [5] and stored at −25°C in 50% (v/v) glycerol, 50 mM NaOH–Tricine (pH 7.7). The absorbance of the suspension at 880 nm (1 cm lightpath) was ~10.

Dodecyldimethylamine *N*-oxide treated chromatophores were obtained as a byproduct of photoreaction center purification [8]. The preparations retained <5% of the original photoreaction center level as estimated from the intensity of the 800 nm absorption band.

Near infrared absorption spectra were obtained with a Hitachi model 356 spectrophotometer using a half bandwidth of 3 nm. The deuterium line at 656.1 nm was used to correct the wavelength scale of the instrument. Prior to measurements, the chromatophore suspensions were diluted with 50 mM potassium phosphate (pH 7.0) and glycerol to obtain a final glycerol concentration of 60% (v/v) and an absorbance of 1.0 at 880 nm. Standard 1 cm optical path cells were used at room temperature. At low temperature, 1 mm lucite cells mounted on an aluminum frame were used. The samples were quickly frozen by immersion in boiling liquid nitrogen. The metal frame of the cuvette assembly was kept submerged in liquid nitrogen till scanning was completed.

The output of the spectrophotometer was connected to a microprocessor controlled recorder (Bascom Turner, model 8120-ER) which performed digitizing and storing of the spectra. Digital records (one absorbance reading every 0.2 nm) of a large number of scans were added together to improve the sig-

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nal-to-noise ratio. In order to obtain the fourth derivatives of the spectra, a linear method [9] was used and several sets of differentiating intervals were tried for each particular spectrum. In some cases, a simplified least-squares procedure [10] was also used. Numerical computations were carried out by the Bascom Turner recorder. For this purpose, appropriate programs were developed.

3. Results

The fourth-derivative of the near infrared absorption band of bulk bacteriochlorophyll in *Rds. rubrum* chromatophores at room temperature is shown in fig.1. For comparison, the derivative spectra of chromatophores isolated from two different wild-type strains (S1 and T) are given. Both show a prominent peak at 882 nm and two others at 875 and 890 nm, as well as some unresolved shoulders. There are also several minor peaks at wavelengths longer than 896 nm but, since their positions change from preparation to preparation, it is likely that they result from periodic reinforcement of photometric noise.

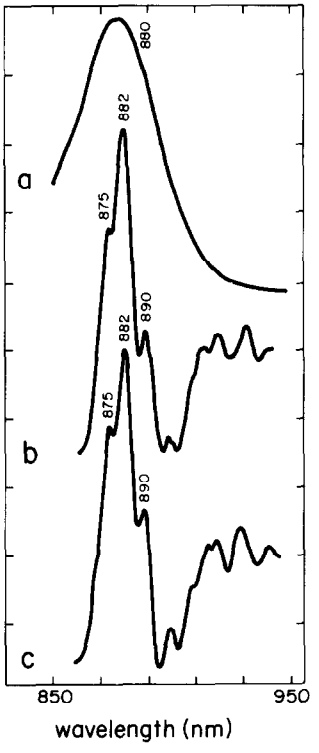

Fig.1. Absorption spectra and fourth derivatives of chromatophores at 293 K: (a) absorption spectra of strains T and S1; (b,c) fourth derivatives of strains T and S1, respectively. Derivatives were obtained by the linear method with intervals of 5.2, 4.8, 4.6 and 4.4 nm.

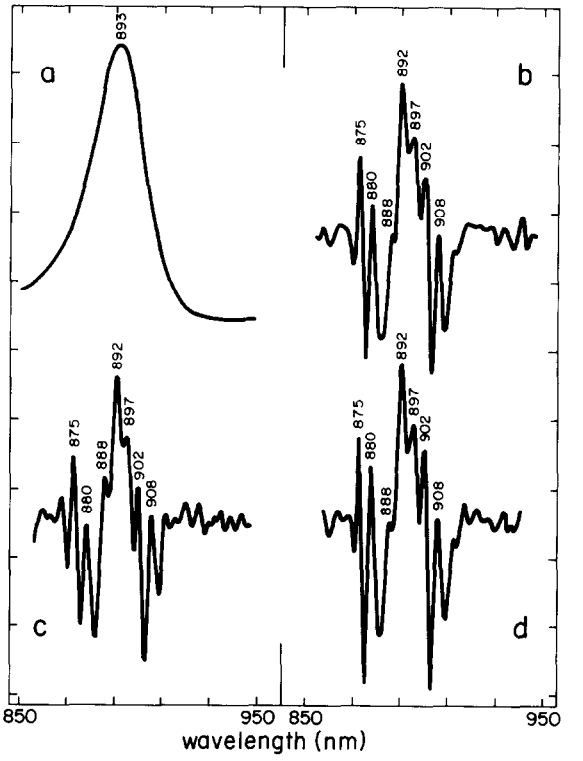

Fig.2. Absorption spectra and fourth derivatives of chromatophores at 77 K: (a) absorption spectra of strains T and S1; (b,c) fourth derivatives of strains T and S1, respectively, obtained by the linear method with intervals of 3.0, 2.6, 2.4 and 2.2 nm; (d) fourth derivative of strain T, obtained by the simplified least squares procedure by using a quadratic convolute with 5 points spaced 0.6 and 0.7 nm for odd and even derivatives, respectively.

In agreement with [11], we found that cooling of the membranes to 77 K enhanced and sharpened the undifferentiated band and shifted the location of its maximum toward longer wavelengths (fig.2). The fourth derivative of the low temperature spectrum exhibits highly increased resolution, probably because of reduction of thermal broadening in the constituent transitions. Thus, at least 7 distinct peaks are observed at 875, 880, 888, 892, 897, 902 and 908 nm in the derivative spectra of both strains S1 and T (fig.2). As in the room temperature measurements (fig.1), other minor spectral features are probably artifacts. Essentially the same results are obtained when the least

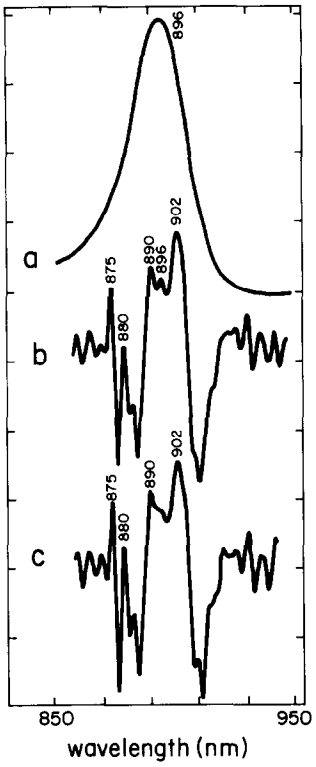

Fig.3. Absorption spectra and fourth derivatives of detergent-treated chromatophores at 77 K: (a) absorption spectrum of strain T chromatophores after treatment with 0.35% (v/v) dodecyldimethylamine *N*-oxide; (b,c) fourth derivatives of (a) obtained by the linear and the least squares procedures, respectively. Conditions as in fig.2.

squares method [10] is used to obtain the derivatives (fig.2).

Chromatophores which have been treated with appopriate concentrations of dodecyldimethylamine *N*-oxide, a zwitterionic detergent, lose the photoreaction center complex but retain most of the light harvesting antenna [12,13]. The detergent modifies the spectrum of chromatophore bacteriochlorophyll, as illustrated in fig.3. Thus, the undifferentiated band at 77 K is shifted ~3 nm towards the infrared as compared to that of untreated chromatophores, and the fourth derivative shows that whereas the constituent transitions at 875, 880 and 902 nm appear to retain their locations, the other peaks are shifted or replaced by new ones. Also, the relative intensities of the peaks seem to have been altered by the detergent. It should be added here that no significant levels of antenna constituents are present in the detergent solubilized fraction. The near infrared absorption spectrum of this fraction exhibits only the characteristic features of the photoreaction center complex which, at 77 K, consist of 3 bands at 751, 801 and 892 nm (not shown). A similar spectrum was reported for *Rhodopseudomonas sphaeroides* photoreaction centers [14].

4. Discussion

The fourth derivative of the long-wavelength band of antenna bacteriochlorophyll in *Rds. rubrum* membranes shows several peaks, specially at liquid nitrogen temperature. The observation that the peaks retain their distinctive locations in different preparations seems to exclude the possibility that the complex structure may result from intensification of photometric noise, particularly since the methods of digital differentiation used here perform an intrinsic signal averaging process which reduces random noise in the higher derivatives [9]. It is possible, though, that some of the peaks are virtual, having arisen from addition of subsidiary maxima of contiguous spectral constituents [15]. However, it is dubious that such might be the origin of more than one or two of the resolved peaks.

Due to its low relative level in the membrane, photoreaction center bacteriochlorophyll accounts only for a small fraction of the total chromatophore absorption in the 850–950 nm spectral range. We have checked that, because of that, none of the resolved derivative peaks may be attributed to the photoreaction center itself unless it is assumed that the spectral properties of this constituent are largely different in the solubilized and in the membrane-bound states. Such an assumption does not seem to be supported by actual experimental evidence, though.

Comparison of the fourth-derivative spectra of native (fig.2) and detergent treated chromatophores (fig.3) suggests that the infrared shift elicited by the treatment results from the alteration of only some of the constituent transitions. If there existed just a single type of antenna bacteriochlorophyll–protein complex in the membrane, that is, if all the absorption peaks corresponded to states of a single group of interacting pigment molecules it would not be likely that a change in the relative position of those molecules would affect only some, but not all, the spectral transitions. Thus, it seems that at least two kinds of bacteriochlorophyll–protein complexes (or two kinds of independent chromophores within a single complex)

should be admitted to account for the alterations elicited by the detergent in the infrared band. This conclusion is in accordance with the appearance of two distinct bleachings, at about 882 and 888 nm, when bulk bacteriochlorophyll is reversibly oxidized in the membrane at room temperature [4].

The postulated existence of two (or more) types of antenna complexes in *Rds. rubrum* does not exclude the possibility that part of the structure which is resolved by fourth-derivative spectroscopy may arise from resonant interactions among bacteriochlorophyll molecules within each type of pigment–protein complex. In fact, independently obtained data such as circular dichroism spectra of membranes and of solubilized antenna preparations have been taken as indicative of exciton splitting of dimeric bacteriochlorophyll [2]. The appearance of an absorption band at 1230 nm upon reversible oxidation of antenna bacteriochlorophyll in the chromatophore [4] is also consistent with an oligomeric state of the pigment, since such a band does not seem to be exhibited by the oxidized monomer [16]. Therefore, the available experimental data favour both mixed complexes and exciton splitting as the origin of the diversity of peaks observed in the derivative spectra.

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