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Characterisation of a B800/1020 antenna from the photosynthetic bacteria *Ectothiorhodospira halochloris* and *Ectothiorhodospira abdelmalekii* *

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An antenna fraction designated B800/1020 according to its near-infrared absorption maxima has been isolated from the bacteriochlorophyll *b*-containing photosynthetic bacterium, *Ectothiorhodospira halochloris*. It contains five polypeptides (approx. 4.5, 6.0, 6.5, 15.5 and 35 kDa), at least five strongly interacting BChl *b* chromophores and no carotenoids. Energy is transferred from the chromophores absorbing around 800 nm to the ones absorbing at 1020 nm. The B800/1020 fraction as well as chromatophores, sphaeroplasts or whole bacteria are reversibly transformed with acid to form B800/960 with a p*K* value of approx. 6.3. Circular dichroism and low-temperature fluorescence data of the 'low-pH form' indicate only little structural rearrangement of the chromophores and a retention of the energy transfer.

Introduction

Ectothiorhodospira halochloris and *Ectothiorhodospira abdelmalekii* are alcalophilic and extremely halophilic photosynthetic bacteria [1,2] belonging to the few organisms containing bacteriochlorophyll *b* (BChl *b*) [3–6]. In both *Ectothiorhodospira* species, it is esterified with the unusual alcohol 2,10-phytadienol [7]. The two species show the extremely red-shifted absorption maximum (1020 nm) common to all BChl *b*-containing bacteria, and in addition an unusually structured near-infrared band peaking at 800 and 830 nm, which are both due to BChl *b* [8]. There are several reports on the preparation (e.g., refs. 9–11) and even the crystallisation [12,13] of reaction centers from BChl *b*-containing bacteria, but there is less known about the light-harvesting complexes of these species [14]. Here we wish to report about the isolation and spectroscopic properties of a BChl *b*-containing antenna fraction (B800/1020) from *E. halochloris*, and a reversible pH-induced absorption change of its 1020 nm component.

Experimental

E. halochloris and *E. abdelmalekii* were grown anaerobically in 10-l bottles at 35°C in the medium of Imhoff and Trüper [1] modified by removal of sodium sulfate and the vitamine solution. *Rhodopseudomonas viridis* was grown anaerobically at 28°C in Gloe's medium [15]. The cells were disrupted by French press treatment (Aminco, U.S.A.) and chromatophores *** were isolated by the method of Okamura and Feher [16]. Spherop-

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*** The membranes isolated from *E. halochloris* do not form closed vesicles. They are termed here chromatophores for convenience only, but are rather thylakoid stacks.

Abbreviations: BChl, bacteriochlorophyll; CD, circular dichroism.

lasts were isolated according to a modified method of Michels [17]. For the fractionation of the antenna pigment proteins, chromatophores (A_{1020} = 50) were treated with 1% Triton X-100 (Serva, Heidelberg), dialysed overnight against 0.1% Triton, and fractionated on a DEAE cellulose column (DE 52, Whatman) with a NaCl gradient (0–300 mM). The B800/850 antenna complex of *Rhodopseudomonas sphaeroides* strain 2.4.1 was prepared by the method of Cogdell [18]. Electrophoresis was done on SDS-polyacrylamide slab gels modified from Laemmli [19], with a linear gradient from 11.5 to 16.5% acrylamide, and a 25% layer on the bottom [20]. Buoyant densities were determined in a sucrose density gradient in a preparative ultracentrifuge (100000 × *g*, Hitachi WKF). Absorption spectra were recorded on a model ZWS 11 (Sigma, Berlin) or a model DMR 22 (Zeiss, Oberkochen) photometer. Fluorescence-emission spectra were kindly provided by Dipl. Phys. A. Angerhofer in the group of Prof. H.C. Wolf (Stuttgart). They were obtained on a home-built fluorimeter equipped with a liquid-helium cryostat, and are uncorrected. Circular dichroism spectra were obtained on a dichrograph V (ISA, Unterhaching) equipped with a silex data-handling system (Leanord, Lille) with a modified software.

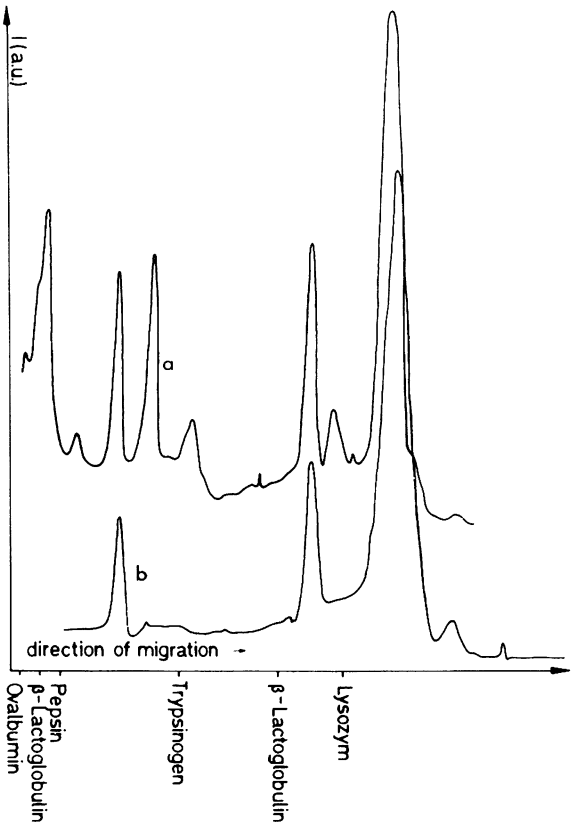

Fig. 1. Polypeptide pattern of chromatophores (from *E. halochloris*) (a, upper trace) and the antenna complex eluting with 0.15 M NaCl from DEAE cellulose (b, lower trace). Densitometric scan of SDS-polyacrylamide gels stained with Coomassie blue. The position of hydrophilic marker proteins are given on the abscissa.

Peptide composition

Two fractions with identical absorption spectra were obtained upon column chromatography of the chromatophores dissociated with Triton X-100. According to SDS gel electrophoresis, the fraction eluting with 0.3 M sodium chloride (Fig. 1) has a composition similar to whole chromatophores. It contains three sharp bands and a topmost diffuse one in the molecular weight range of 20 kDa or more, which were assigned in analogy to *Rps. viridis* [9–12] to the reaction center. In the low-molecular-weight range, there are two intense bands corresponding to sizes of 13.5 and 13 kDa, if calibrated with a standard set of globular, hydrophilic proteins. When compared with two antenna complexes of *Rps. sphaeroides* 2.4.1, the two bands move between the 6.8 kDa band of B875 and the 5.4 kDa band of B800/850, and have an estimated weight of 6.5 and 6 kDa, respectively. The electrophoresis also shows three additional, weakly stained bands in this range. One moves at approx. 12 or 4.5 kDa, respectively, depending on the calibration standards as discussed above. It should be noted, that a peptide of similar size has been found too in another BChl *b*-containing species, e.g., *Rps. viridis* [14]. The two others have weights of 15.5 and 18 kDa.

The fraction eluting at 0.15 M NaCl has essentially the same polypeptide composition in the range up to 20 kDa, with the exception of the 18 kDa band, but contains generally only one larger polypeptide with an apparent weight of 35 kDa (Fig. 1). A peptide of this weight is unusual for bacterial antennas. From a comparison with SDS-polyacrylamide electrophoresis gels of chromatophores and of the 0.3 M NaCl fraction, this band

rather appears to be the 'H' subunit of the reaction center.

The polypeptide pattern of chromatophores in the 20–40 kDa range and the light-induced near-infrared absorption changes (data not shown) are rather similar in *E. halochloris* and *Rps. viridis*, but all attempts to isolate the reaction centers from *E. halochloris* by standard procedures were unsuccessful. It then appears, that the 'H' subunit is retained with the antenna rather than the reaction center in the former.

Absorption spectra

The two fractions obtained from DEAE-cellulose chromatography of the dissociated chromatophores are identical with respect to their absorption spectra. Like the whole cells and chromatophores, they have near-infrared absorption maxima at 1018, 830 and 800 nm (Fig. 2a). According to the SDS gel analysis, the fraction eluting with 150 mM NaCl is designated as antenna fraction.

When whole cells, sphaeroplasts or chromatophores of *E. halochloris* or *E. abdelmalekii*, or isolated antenna fractions of both bacteria are titrated with acid in the pH range between 7.5 and 5.7, the 1018 nm absorption is gradually replaced by a new absorption peaking at 964 nm (Fig. 2b). The 830/800 nm band is essentially unaffected by this treatment, the minor increase being due to scattering. In chromatophores, the pH-induced absorption change is accompanied by an increase in buoyant density from 1.04 (pH 7.5) to 1.08 (pH 5.7).

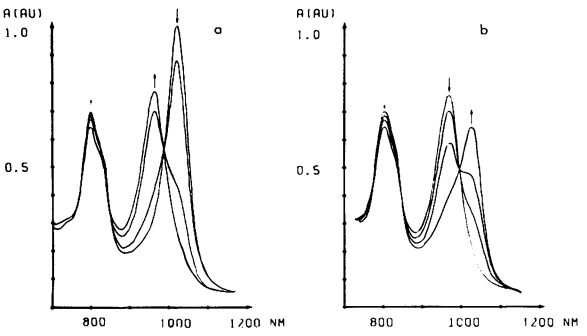

Fig. 2. Titration of the isolated antenna fraction of *E. halochloris* ($A_{1020} = 0.6$) with hydrochloric acid in the pH range from 8.0 to 5.7 (a). Back titration in Fig. 2a with NaOH to pH 8.0. The time elapsed from the beginning of experiment (a) to the end of experiment (b) was approx. 30 min.

Whereas the 'high-pH form' (λmax $\approx$ 1020 nm) is stable over extended times, the 'low-pH form' (λmax $\approx$ 960 nm) is unstable, in particular upon addition of more acid. The decrease of the 960 nm band is accompanied by the evolution of a new band around 680 nm (data not shown), which is typical for the oxidation products of BChl *b* [21]. If judged from the spectra in the range of the Q_x band (500–600 nm) no bacteriopheophytin is produced during the degradation, nor has it been present in the original preparations. This is in agreement with earlier observations on free BChl *b*, where the demetalation is generally slower than the reactions involving ring *b*.

The 800/830 nm band is again unaffected under these conditions. At pH 5.7, the species absorbing at 960 nm has a half-life of approx. 1 h. If excessive decay of the 'low-pH form' is avoided by an immediate back titration with base, the spectrum of the 1020 nm 'high-pH form' is at least partially restored (up to 70%) (Fig. 2b). The observed pigment losses are then probably due to the instability of the 960 nm form during the titration. The instability interferes also with a precise determination of the p*K* of the transformation, which is estimated in the range 6.3–6.5 from rapid titration experiments.

Fluorescence spectra

The natural 'high-pH form' (pH 8.0) has an emission band peaking at 1066 nm ($T = 5$ K). The acid induced 'low-pH form' (pH 5.7) has its emission maximum blue shifted to 1007 nm. Stokes' shift is similar in both forms (approx. 46 nm). Both bands have the same excitation spectrum below 920 nm. It is characteristic for BChl *b* (Soret band, Q_x band at 600 nm) and includes in particular the 800/830 nm band system, which is well resolved at low temperatures. Since the fluorescence spectra are uncorrected, a quantitative evaluation is not possible. We estimate, however, from the identical relative intensities in both forms a similar efficiency of the energy transfer from the BChl *b* chromophores absorbing around 830 nm to the ones absorbing at more than 900 nm.

Both 'low-' and 'high-' pH forms contain an

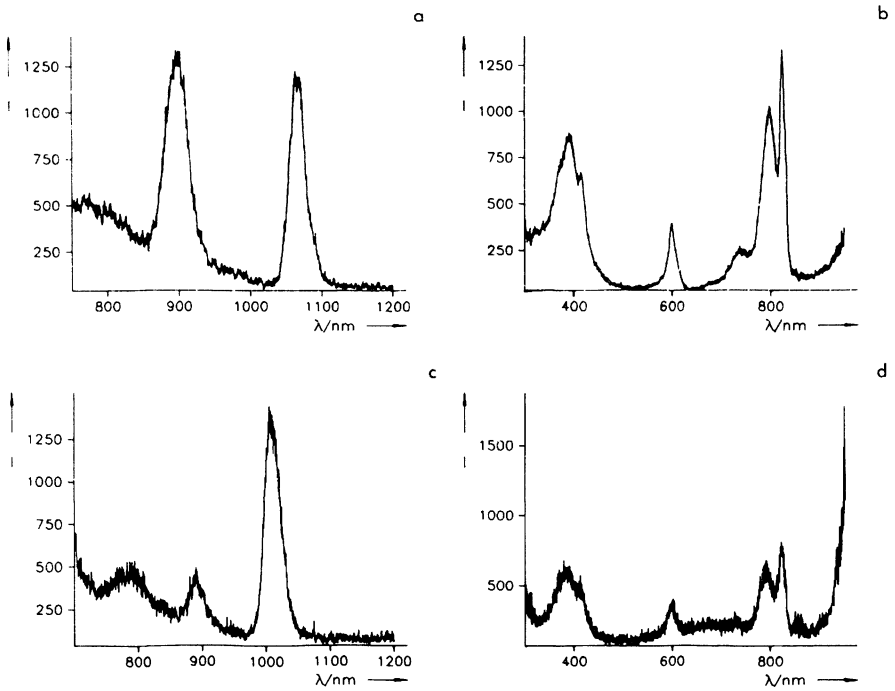

Fig. 3. Fluorescence excitation (b, d) and emission spectra (a, c) of the isolated antenna fraction of *E. halochloris* in the 'high-pH form' (a, b) and the 'low-pH form' (c, d), determined at 5 K. The spectra are not corrected for the intrument response.

additional emission band of varying intensity at 900 nm, the low pH form has also one at 800 nm. The latter has a BChl *b* excitation spectrum, and is probably due to some dissociated pigment. The emission band at 900 nm has a very unusual excitation spectrum in which the Q_x band in the 500–600 nm range is greatly reduced in intensity. A component of this type has been found in whole cells of this and another organism (*Rps. sphaeroides*) and assigned to a bacteriopheophytin aggregate (Angerhofer, A. and Wolf, H.C., personal communication). Detergent complexes with a reduced Q_x band have also been observed with bacteriochlorophyll *a* derivatives [22,23], and treated theoretically [24].

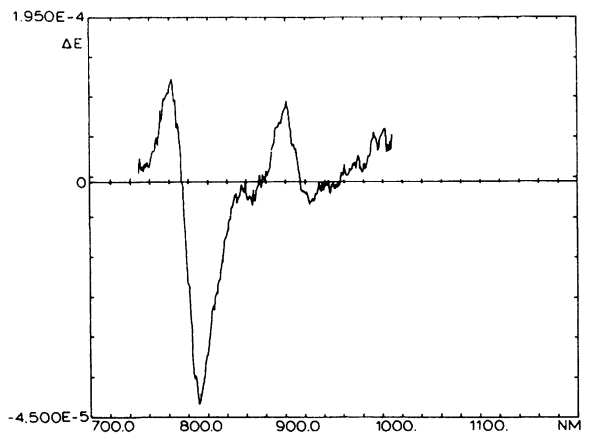

Fig. 4. Circular dichroism spectra of the 'high-pH form' of the isolated antenna fraction of *E. halochloris* at ambient temperature ($A_{830} = 0.3$).

Circular dichroism

The CD spectrum (Fig. 4) of the 'high-pH form' shows an 'M'-shaped pattern * in the 780–850 nm range, and a wing of a positive band

* The long-wavelength wing of the 'M' is superimposed by an inflection of varying size in different preparations. It can grow to an 'S'-shaped pattern dominating the near-infrared part of the spectrum. In such preparations, a minor band around 900 nm becomes noticeable in the absorption, too. It is thus likely, that this inflection is due to a minor, but strongly Cd-active contaminant.

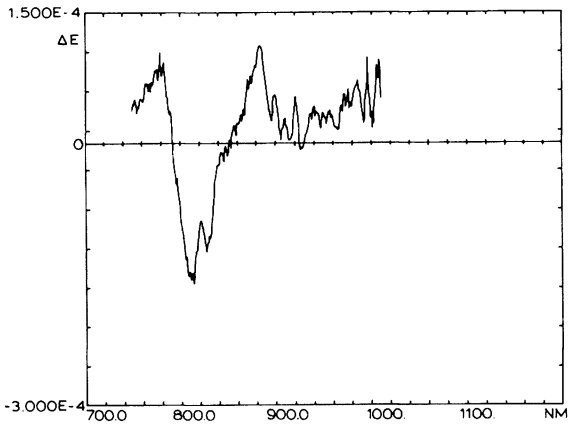

Fig. 5. Circular dichroism spectra of *E. abdelmalekii* chromatophores.

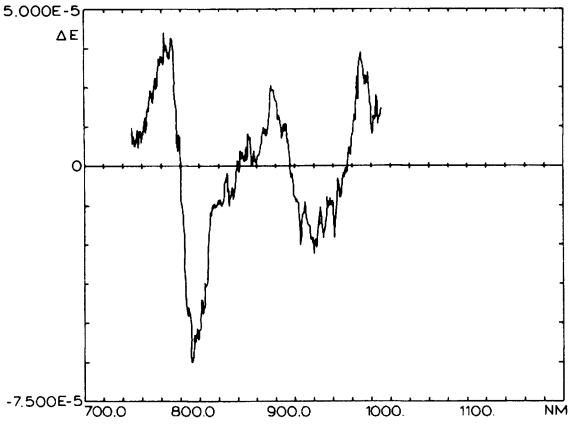

Fig. 6. Circular dichroism spectra of the 'low-pH form' of the antenna of *E. halochloris*; $E_{960} = 0.25$, corresponding to $E_{1020} = 0.42$ in the original preparation.

extending beyond 1010 nm (which is the limiting wavelength of our dichrograph). It is more complex than that of *Rps. viridis* membranes, and resembles the CD spectra of BChl *a*-containing bacteria with a complex antenna structure, e.g., *Chromatium* [25]. The spectrum of the closely related *E. abdelmalekii* containing also BChl *b* is very similar (Fig. 5).

The complexity reflects the complex absorption spectrum of *E. halochloris,* in addition to the spread-out absorption range typical for BChl *b*-containing bacteria. The 'M'-shaped pattern around 800 nm can arise either from strong exciton coupling among several (three or more) chromophores, or from a variety of isolated, non-interacting chromophores being situated in different environments. The former is more likely from the magnitude of the signals. Monomeric bacteriochlorophylls give only very weak signals (anisotropy, approx. $6 \cdot 10^{-6}$), which upon aggregation become strongly anisotropic and complex due to exciton coupling [22]. The anisotropy of the *E. halochloris* antenna (positive extremum at 783 minus negative extremum at 812 nm) is $5.7 \cdot 10^{-4}$. This is similar to *Chromatium* [25], but smaller than the most strongly CD-active complexes [22,23] of light-harvesting proteins containing BChl *a* (see, e.g., Refs. 26 and 27).

The shape of 800 nm band system of *E. halochloris* is similar, but its anisotropy is somewhat reduced ($4.6 \cdot 10^{-4}$) in the 'low-pH form' (Fig. 6). The region above 900 nm is dominated by an 'S'-shaped band centered around 974 nm (anisotropy $1.9 \cdot 10^{-4}$ taken from the extrema at 987 and 942 nm). The spectrum can be rationalized by (at least) a pair of strongly interacting bacteriochlorophylls absorbing around 960 nm, and a minimum of three interacting pigments in the 800/830 nm region. The CD data indicate only moderate changes among the chromophores absorbing around 800 nm. No conclusions can be drawn with regard to the redmost absorbing chromophores, since the wavelength range of ≥ 1010 nm or more was inaccessible to us, thus precluding a CD study of the 1020 nm band of the 'high-pH form'.

Discussion

The BChl *b* containing antenna complex B800/1020 isolated from *E. halochloris* has an absorption spectrum very similar to that of whole cells and chromatophores of this species. It retained in particular the absorption in the 800 nm range, which is typical for *E. halochloris* and the closely related *E. abdelmalekii.* Complex near-infrared absorption bands are common for BChl *a*-containing bacteria, but have not been observed in the (few) other species with BChl *b*.

In the species containing BChl *a*, the complex absorption reflect a complex composition with generally two types of antenna present: one is

formed in a fixed ratio to the reaction centers (see, e.g., Ref. 29) and has a single absorption band at approx. 870 nm; the second is formed in variable amounts and has already a structured near-infrared maximum at approx. 800 and 850 nm. It seemed then possible, that the antenna systems of the two *Ectothiorhodospira* species are more closely related to BChl *a*-containing than to other BChl *b*-containing species, only with the near-infrared absorptions spaced further apart to gap the strong water absorption around 960 nm.

We did not observe any variations in the ratio of the absorptions at 1020 and 600/800 nm in *E. halochloris* during fractionation, and it has not been possible to fractionate the antenna by chromatography on ion exchange cellulose into forms with different absorption spectra or polypeptide patterns. There are also distinct differences to the variable B800/850 type BChl *a*-containing light-harvesting complexes, which have already by themselves two near infrared absorptions. These are in particular the larger number of polypeptides and interacting chromophores, the absence of carotenoids *, and the acid lability of B800/1020.

While these data tend to disprove a close relation of *E. halochloris* with these species, a definite conclusion is presently difficult. We may well be dealing with antennas which are similar in their chromopeptides, but have differently strong associations within the membrane. Such an unusual fragmentation of the *E. halochloris* photosynthetic membrane is indicated by the preferential retention of the 35 kDa peptide in the antenna fraction, which is tentatively assigned to the 'H' subunit of the reaction center. It is known from cross-linking studies in several bacteria, that the 'H' subunit is associated with both antenna and the reaction center polypeptides [27–30], but is generally isolated as a subunit of the latter. The increased affinity to the antenna could at the same time explain the lability of the reaction centers in *E. halochloris,* because the 'LM' complex of the reaction center devoid of the 'H' subunit is less stable than the integral reaction center [16].

* The two *Ectothiorhodospira* species have a low carotenoid content (Refs. 1 and 2, and references cited therein). In the antenna complexes, the spectra region from 400 to 550 nm is devoid of carotenoid absorptions (data not shown).

The native form of the B800/1020 antenna is stable at a pH of 7 or more, but is reversibly transformed at lower pH to a form absorbing at 960 and 800/830 nm (B800/960). This 'low-pH form' has an increased buoyant density and changed interactions among the chromophores, which points to structural rearrangements within the membrane. The apparent pK value of the transformation is approx. 6.3, which is close to that of histidine (6.8), or possibly carboxylic acid side chains (p$K \approx 4.7$). Attempts to modify histidine residue(s) with the hydrophilic reagent α-bromopropionate and also with its more lipophilic methyl ester [32,33] did neither affect the 'high-pH form' (1020 nm) nor the 'low-pH form' (960 nm), nor the interconversion of the two. This tends to argue against the participation of histidine, but further experiments with other modification reagents are required to resolve this question.

The pH-dependent spectral changes are not only observed in the isolated antenna fractions, but also in whole cells, sphaeroplasts (namely, 'right-side-out vesicles') and chromatophores. Whichever group is affected appears therefore to be accessible from either side of the membrane. Biological membranes are, however, fairly permeable to protons. It has therefore been attempted to induce similar changes by other means than pH. Preliminary results show that proteolysis with, e.g., trypsin has the same effect, but works with chromatophores only, and not with sphaeroplasts. If the low pH and proteolysis are acting at the same site, this would indicate that it is located on the cytoplasmic side of the membrane in a hydrophilic environment.

There is evidence from several bacteria, that only approx. 20 amino acids of each antenna polypeptide are actually within the hydrophobic interior of the membrane, and that both the N- and the C-terminal parts are in a hydrophilic environment (see Ref. 31 for a recent review). It has furthermore been shown in *Rhodospirillum rubrum,* that the N-terminus is on the cytoplasmatic side of the photosynthetic membrane and subject to proteolysis [34]. Those parts of the membrane peptides extending into the aqueous phase may then play a rather crucial role in the association of the antenna complexes to their native structures.

The (low-temperature) fluorescence data indicate for both the high and low pH forms an efficient energy transfer from the chromophores absorbing in the 830 nm region to the ones at longer wavelengths. While this makes large structural changes among the two forms unlikely, short range rearrangements from a tightly to a weakly coupled state are indicated by the CD spectra. One possible explanation would be the assignment of the 1020 and the 800/830 nm band to two (sets of) BChl *b*-polypeptides, which are in close association. Only the long wavelength absorbing chromopeptide is sensitive to acid. This is again reminiscent of B800/850 from BChl *a*-containing bacteria. In the complex from *Rps. spheroides*, the two absorptions are assigned to one and two chromophores, respectively, bound to two polypeptides. The spectrum and energy transfer in this complex are sensitive to various treatments [26,27,35,36]. These do not include acid, however, since the absorptions and CD maxima (although not the relative absorptions and CD signal magnitudes) of the two bands are acid insensitive down to pH 1.7 (Steiner, R., unpublished data). The increased sensitivity of *E. halochloris* to low pH may reflect a specific adaptation to the high pH value (approx. 11) prevailing in its natural environment.

Acknowledgements

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References

1 Imhoff, J.F. and Trüper, H.G. (1977) Arch. Microbiol. 114, 115–121
2 Imhoff, J.F. and Trüper, H.G. (1981) Zbl. Bakt. Hyg., Abt. orig. C2, 288–234
3 Eimhjellen, K.E., Aasmundrud, O. and Jensen, A. (1963) Biochem. Biophys. Res. Comm. 10, 232–236
4 Eimhjellen, K.E., Steensland, H. and Traetteberg, J. (1967) Arch. Microbiol. 59, 82–92
5 Eimhjellen, K.E. (1970) Arch. Microbiol. 73, 193–194
6 Drews, G. and Giesbrecht, P. (1966) Arch. Microbiol. 53, 255–262
7 Steiner, R., Schäfer, W., Blos, I., Wieschhoff, H. and Scheer, H. (1981) Z. Naturforsch. 36c, 417–420
8 Steiner, R., Wieschhoff, H. and Scheer, H. (1982) J. Chrom. 242, 127–134
9 Thornber, J.P., Dutton, P.L., Fajer, J., Forman, A., Holten, D., Olson, J.M., Parson, W.W., Prince, R.C., Tiede, D.M. and Windsor, M.W. (1977) in Proceedings of the 4th International Congress on Photosynthesis (Hall, D.O., Coombs, J., Goodwin, T.W., eds.), pp. 55–70 Biochemical Society, London
10 Seftor, R.E.B. and Thornber, J.P. (1983) Biochim. Biophys. Acta 764, 148–159
11 Gingras, G. (1978) in The Photosynthetic Bacteria (Clayton, R.K. and Sistrom, R., eds.), pp. 119–131, Plenum Press, New York
12 Michel, H. (1982) J. Mol. Biol. 158, 567–572
13 Deisenhofer, J., Epp, O., Miki, K., Huber, R. and Michel, H. (1984) J. Mol. Biol. 180, 385–398
14 Jay, F., Lambilotte, M. and Muehlethaler, K. (1983) Eur. J. Cell Biol. 30, 1–8
15 Gloe, A. (1977) Dissertation, University of Göttingen
16 Feher, G. and Okamura, M.Y. (1978) in The Photosynthetic Bacteria (Clayton, R.K. and Sistrom, R., eds.), pp. 349–386, Plenum Press, New York
17 Michels, P.A.M. and Konings, W.N. (1978) Biochim. Biophys. Acta 507, 353–368
18 Cogdell, R.J. (1983) Annu. Rev. Plant Physiol. 34, 21–45
19 Laemmli, U.K. (1970) Nature 227, 680–685
20 Cogdell, R.J. and Thornber, J.P. (1980) FEBS Lett. 122, 1–8
21 Steiner, R., Cmiel, E. and Scheer, H. (1983) Z. Naturforsch 38c, 748–752
22 Gottstein, J. and Scheer, H. (1983) Proc. Natl. Acad. Sci. USA 80, 2231–2234
23 Scherz, A. and Parson, W.W. (1984) Biochim. Biophys. Acta 766, 653–665
24 Scherz, A. and Parson, W.W. (1984) Biochim. Biophys. Acta 766, 666–678
25 Hayashi, H., Nozawa, T., Hatano, M. and Morita, S. (1981) J. Biochem. 89, 1853–1861
26 Sauer, K. and Austin, L.A. (1978) Biochem. 17, 2011–2019
27 Vasmel, H., Swarthoff, T., Kramer, H.J.M. and Amesz, J. (1983) Biochim. Biophys. Acta 725, 361–367
28 Peters, J. and Drews, G. (1983) Eur. J. Cell Biol. 115–120
29 Bachmann, R.C. and Takemoto, I.Y. (1983) Biochem. Int. 7, 137–145
30 Schumacher, A. and Drews, G. (1978) Biochim. Biophys. Acta 501, 183–194
31 Wiemken, V., Theiler, R. and Bachofen, R. (1981) J. Bioenerg. 13, 181–194
32 Crestfield, A.M., Moore, S. and Stein, W.H. (1963) J. Biol. Chem. 238, 622–627
33 Heinrikson, R.L., Stein, W.H., Crestfield, A.M. and Moore, S. (1965) J. Biol. Chem. 240, 2921–2934
34 Brunisholz, R.A., Wiemken, V., Suter, S., Bachofen, R. and Zuber, H. (1984) Hoppe-Seylers Z. Chem. Physiol. 365, 689–701
35 Cogdell, R.J. and Valentine, J. (1983) Photochem. Photobiol. 38, 769–772
36 Clayton, R.K. and Clayton, B.J. (1981) Proc. Natl. Acad. Sci. USA 78, 5583–5587