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Spectroscopical studies on the light-harvesting pigment-protein complex II from dark-aerobic and light-anaerobic grown cells of *Rhodobacter sulfidophilus*

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The photosynthetic bacterium *Rhodobacter sulfidophilus* can grow and synthesize photosynthetic pigments under dark-aerobic as well as light-anaerobic growth conditions. Under both growth conditions intracytoplasmic membrane vesicles (diameter about 35 nm) are formed. The light-harvesting (LH) pigment-protein complex II, isolated from dark-aerobic and light-anaerobic grown cells, consists of two small polypeptides (< 5 kDa) and the pigments bacteriochlorophyll and carotenoids. The carotenoid composition of the LHII complex, as shown by absorption spectroscopy in the 450 to 550 nm region, changed when the cells were transferred from aerobic-dark to anaerobic-light conditions. The absorption maxima in the near infrared regions varied reversibly depending on such factors as salt concentration and detergent. These shifts of absorption bands either reflect a change of tertiary structure of the polypeptides, which then modify pigment-pigment and/or reflect a direct influence of the ions onto the conjugated system of the pigments.

Introduction

The nonsulfur purple bacterium *Rhodobacter (Rb.) sulfidophilus* (formerly *Rhodopseudomonas sulfidophila*) has been described as a new species by Hansen and Veldkamp [1]. The cells contain bacteriochlorophyll (Bchl) *a* and carotenoids of the spheroidene type as photosynthetic pigments. It is a marine bacterium tolerant to high sulfide concentrations and grows photolithotrophically on hydrogen, hydrogen sulfide and thiosulfate as well as heterotrophically on a wide range of organic compounds [1]. The bacterium grows and synthesizes Bchl under high aeration in the dark, in contrast to *Rhodobacter sphaeroides* [2]. Both dark-aerobic and light-anaerobic grown cells of *Rb. sulfidophilus* showed two strong absorption bands around 800 and 850 nm, suggesting the assembly of Bchl into membranes as pigment-protein complexes. Oxygen tension in the growth medium is the major environmental factor which regulates the rate of the biosynthesis of Bchl in most purple-sulfur bacteria. The increase in oxygen tension above a species-specific threshold value results in inhibition of Bchl synthesis [3,4]. Therefore, it is interesting to study the formation of Bchl in *Rb. sulfidophilus* under conditions of high aeration.

The photosynthetic apparatus of most purple sulfur and nonsulfur bacteria contains two light-harvesting pigment-protein complexes (LHI or B870 and LHII or B800-850) which differ in absorption maxima in near infrared regions. The structure of these complexes from photosynthetic bacteria has recently been reviewed [5]. The LHI complexes are usually arranged in membranes in a fixed stoichiometry relative to the reaction center, while the LHII is present in variable amounts. Under different growth conditions the amount and composition of intracytoplasmic membrane varied. The marine photosynthetic bacterium *Erythrobacter* sp. OCh 114 produces a unique pigment-protein complex under high aeration, the B806 complex [6], which probably corresponds to LHII. B806 contains only one polypeptide and shows a single strong absorption band at 806 nm.

Abbreviations: Bchl, bacteriochlorophyll; LDAO, lauryldimethylamine *N*-oxide; LH, light-harvesting.

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Thus, the spectral form of LHII complexes reveals a great variability in the near infrared region depending on the growth conditions and/or bacterial species. The variability of absorption in LHII complexes has been investigated and discussed in relation to their efficiency of energy transfer and organization of pigments and proteins [7–9]. In this report we describe the ultrastructure of cells and the isolation and partial characterization of the LHII complex from dark-aerobic and light-anaerobic grown cells of *Rb. sulfidophilus*. The isolated complexes showed reversible spectral changes in Bchl and carotenoid absorption, depending on salt and/or detergent.

Materials and Methods

Culture of bacteria and electron microscopical methods

Rhodobacter sulfidophilus (strain W4) was a gift from Dr. Moritoshi Aizaki (The National Institute for Environmental Studies, Tsukuba). *Rb. sulfidophilus* was grown aerobically in the dark and anaerobically in the light in the following medium (g/l): glycerol, 1.84; NaCl, 23.5; $MgSO_4 \cdot 7H_2O$, 2.0; $MgCl_2 \cdot 6H_2O$, 10; $CaCl_2 \cdot 2\ H_2O$, 1.46; KCl, 0.6; Peptone casein, 1.0; yeast extract, 1.0; Bacto peptone, 1.0, trace elements, 10 ml/l [10], pH 7.7. Photosynthetic cultures were grown in screw-cap bottles illuminated on one side with 150 W tungsten bulbs. Cells were harvested in early stationary phase. Dark-aerobic grown cells were cultured in 500-ml Erlenmeyer flasks with baffles filled to 30% capacity on a rotatory shaker.

Electron microscopic preparation and examination of thin sections were performed after osmium tetroxide fixation and Epon embedding as described [11].

Isolation of the light-harvesting complex

The cells were harvested in the early exponential phase before cultures reached the absorbance of 0.5 at 660 nm. The cells were washed once in 10 mM Tris-HCl buffer (pH 7.8) and resuspended in the same buffer. After addition of phenylmethylsulfonyl fluoride (1 mM) and DNase (1 mg/ml), cells were disrupted in a French pressure cell. Unbroken cells and large debris were removed by centrifugation at $12\,000 \times g$ for 20 min. The supernatant was layered on a discontinuous gradient of 0.6, 1.0, 1.2 and 1.5 M sucrose in the same buffer. After centrifugation for 16 h at $90\,000 \times g$ in a Beckman Ti 60 rotor, the major pigmented band was collected, diluted about 4-times with buffer and spun down at $90\,000 \times g$ for 4 h. The resultant membrane fraction (intracytoplasmic membrane) was resuspended in Tris-HCl buffer at a concentration of 400 μM Bchl and mixed with an equal volume of 1.0% (w/v) lauryldimethylamine *N*-oxide (LDAO) in the same buffer. The suspension was incubated at room temperature in the dark under gentle stirring. After 30 min the mixture was layered on a sucrose step gradient (0.3, 0.6 and 1.2 M sucrose in Tris-HCl-buffer containing 0.6% LDAO). The tubes were centrifuged at $90\,000 \times g$ in the rotor Ti 60 for 16 h at 4°C.

The solubilized LHII complex banded between 0.3 and 0.6 M sucrose. For further purification the LHII-enriched fraction was directly applied to a DEAE-Toyopearl column (1.6×25 cm) equilibrated with the same buffer containing 0.05% LDAO. After washing with 200 ml of 20 mM Tris-HCl (pH 8.0) containing 100 mM NaCl and 0.05% LDAO, the fraction was eluted with 150 ml of a 100 to 300 mM NaCl linear-gradient in the same buffer containing 0.05% LDAO. The purified LHII complex was eluted at a concentration of 210–230 mM NaCl.

Analytical measurements

Absorption spectra were measured with a Kontron Uvikon spectrophotometer 860 and a Shimadzu UV-260 spectrophotometer. Bchl and carotenoids were extracted with a mixture of acetone/methanol (7 : 2, v/v) and assayed spectrophotometrically [12,13]. Membrane proteins were separated by SDS-PAGE on slab gels [14] on a 15–25% linear gradient of acrylamide. Gels were stained with Coomassie brilliant blue G-250.

LKB 1860-101 molecular weight markers were used for estimation of molecular weight of polypeptides. Protein was determined according to the method of Lowry et al. [15] using bovine serum albumin as a standard.

Results

Cytology and pigment content

The cells of *Rhodobacter sulfidophilus* are of rod-like to ovoid shape. The intracytoplasmic membrane consists of vesicle-like structures, which have diameters of about 35 nm and are clustered in peripheral and polar regions of the cell (Fig. 1). The vesicles are present in cells grown heterotrophically under strict aerobic or semiaerobic conditions in the dark as well as grown photosynthetically under anaerobic conditions. But cells kept under high oxygen tension contain less vesicles than cells grown photosynthetically (Fig. 1). The pigment content of cells and membranes varied with growth conditions but not in the same range as in cells of *Rb. sphaeroides* (Table I). *Rb. sulfidophilus* belongs to the group of photosynthetic bacteria which do not respond strongly to variations of oxygen partial pressure. The absorption spectra of intracytoplasmic membranes, isolated from *Rb. sulfidophilus*, grown either under dark-aerobic or light-anaerobic conditions are dominated by the characteristic absorption bands of the LHII complex at 802 and 853 nm (Fig. 2). The absorption ratio of 853/802 nm was about 1.62 in both types of membrane. Around 890 nm a weak shoulder was observed that can

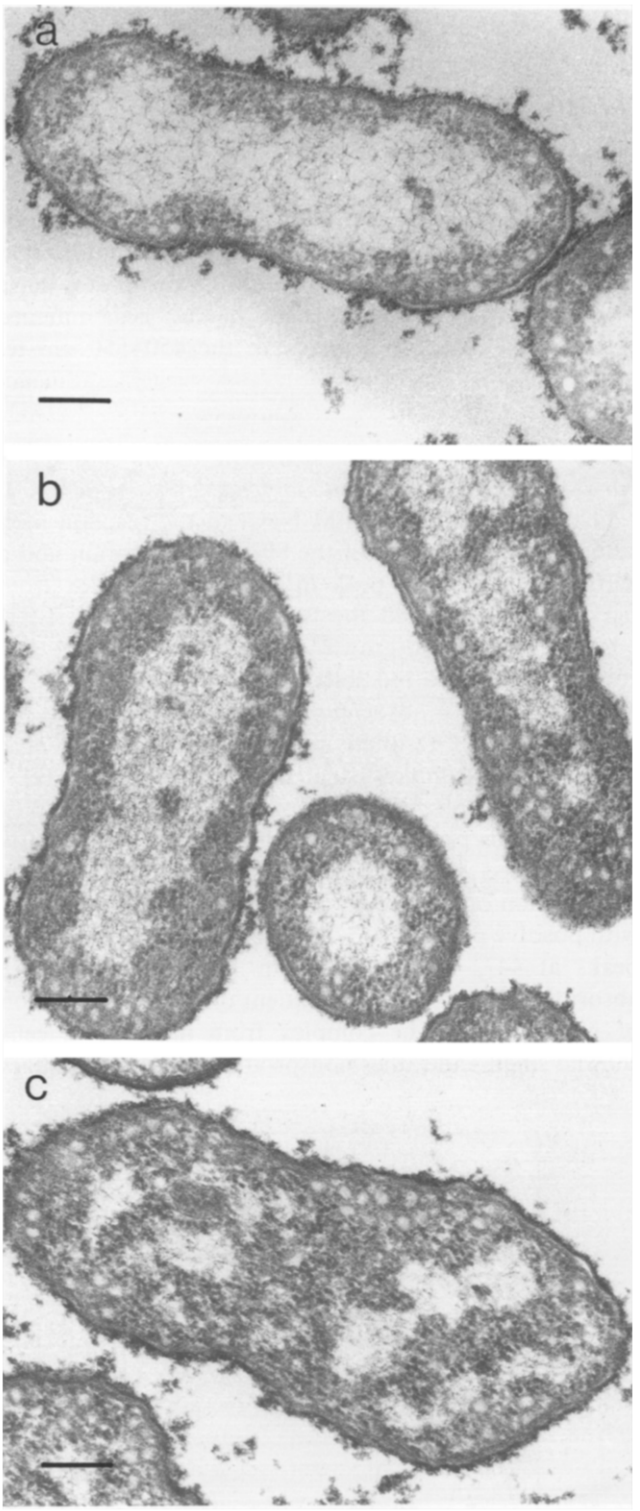

Fig. 1. *Rb. sulfidophilus*. Electron micrographs of sections show intracytoplasmic membrane vesicles bearing the photosynthetic apparatus. The vesicles are grouped and present in aerobically (a) semi-aerobically in the dark- (b) and anaerobically in the light- (c) grown cells. The bar represents 200 nm.

be attributed to the LHI complex. In the visible region both the Q_x transition of Bchl and the absorption bands of carotenoids have been seen. The difference between

TABLE I

Pigment content

Pigment content of cells and membranes in cultures of *Rb. sulfidophilus* grown strictly aerobically in the dark (ae) or anaerobically in the light (pho) at 30°C or 10°C, respectively. The aerobically grown cells were grown in Erlenmeyer flasks with buffels filled 15% of total volume and grown to an $A_{660\,nm,\,1.0\,cm}$ of 0.4.

Culture conditions	Pigment content (nmol/mg protein)		
	bacteriochlorophyll	carotenoids	
ae	0.76	0.8	cells
	5.56	3.73	membranes
pho	2.03	2.21	cells
	10.15	5.93	membranes
pho 10°C	84.04	41.54	membranes

the two spectra in the carotenoid-band region is due to the presence of different carotenoids in membranes isolated from dark-aerobic or light-anaerobic grown cells (Fig. 2).

Light-harvesting complex II

LHII complexes were isolated from the intracytoplasmic membranes of dark-aerobically and light-anaerobically grown cells by LDAO treatment, sucrose gradient centrifugation and DEAE chromatography as described in Materials and Methods. The polypeptide composition of isolated LHII complexes was analyzed by SDS-PAGE (Fig. 3). Both complexes consisted of two polypeptides with an apparent molecular weight of about 5000 and 3000. These polypeptides were also

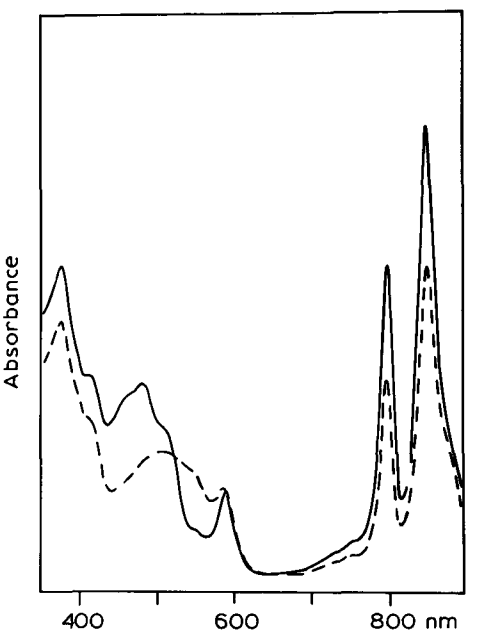

Fig. 2. Absorption spectra of membranes isolated from cells grown aerobically in the dark (broken line) or anaerobically in the light (full line).

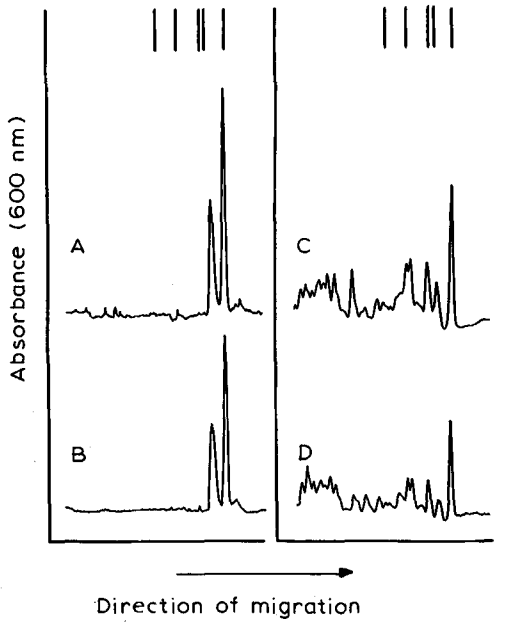

Fig. 3. Comparison of polypeptides from the purified membranes and the isolated complexes on a SDS-PAGE (15–25% polyacrylamide gradient): (A) LHII complex from dark-grown cells; (B) LHII complex from light-grown cells; (C) purified membranes from dark-grown cells; (D) purified membranes from light-grown cells. Markers are indicated as bars on the top of figures.

detected in membrane fractions under non-denaturing conditions, the LHII complex migrated as a pigmented band with an apparent molecular weight of greater than 100 000 and retained the original absorption spectrum. The carotenoid composition of the complexes was analyzed spectrophotometrically after separation of components by a cellulose gel thin-layer plate. The major components were spheroidenone (over 95%) and neurosporene (4%) in dark-complex, and OH-spheroidene (46%), spheroidene (25%), neurosporene (24%) and spheroidenone (4%) in the light-complex. Some minor component (less than 1%) was observed on the TLC plates in both complexes.

Fig. 4 shows the absorption spectra of isolated LHII complexes measured in 20 mM Tris-HCl buffer (pH 8.0) containing 0.05% LDAO. Both complexes have absorption bands due to Bchl in the near infrared regions and carotenoid bands in the 450–550 nm regions.

The influence of salt and detergent on the absorption bands of the LHII complex

The addition of 200 mM NaCl to the reaction mixture caused a lowering of the 803 nm absorption and a shift of the 827 nm peak in the LHII complex from dark grown cells and the 834 nm band in the LHII complex from light-grown cells to 850 and 852 nm, respectively, and a red shift of Q_x and Soret bands. In addition to these absorption changes due to Bchl, the addition of salt resulted in a red shift of carotenoid bands in the complex isolated from light-grown cells. The red shift of the carotenoid band in the complex isolated from photosynthetically grown cells is clearly demonstrated by the difference spectrum induced by the addition of NaCl (Fig. 5) giving absorption changes with positive peaks at 463, 493 and 526 nm and negative peaks at 447, 480 and 510 nm. The extent of these absorption changes was dependent on the concentration of NaCl added. The complex from dark-grown cells showed small and rather nonspecific absorption changes

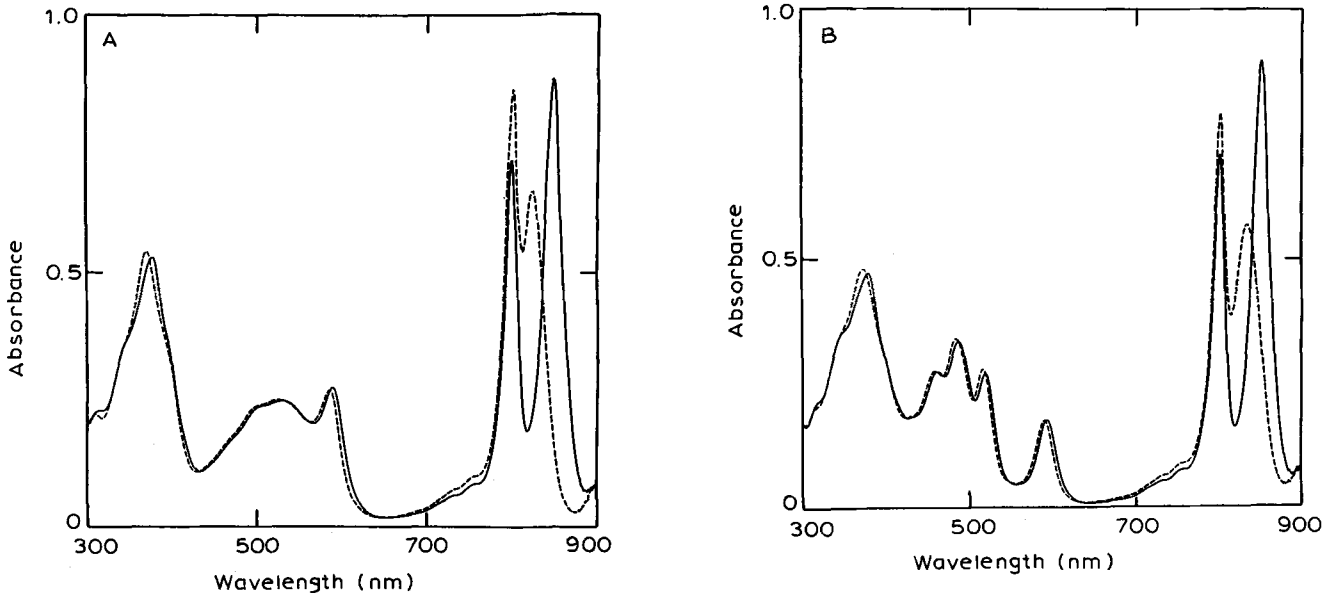

Fig. 4. Absorption spectra of light-harvesting pigment-protein complexes isolated from light- (A) and dark-grown (B) cells of *Rb. sulfidophilus*. The complexes (about 50 μg protein/ml) were suspended in 10 mM Tris-HCl containing 0.05% LDAO either in the presence (———) or absence of 200 mM NaCl (— — —).

in carotenoid band regions. Besides NaCl, KCl, $MgCl_2$, ammonium sulfate and sodium phosphate were also effective. The influence of salts, their ionic radii and concentration will be described in a further paper. The absorption spectra of 'high-salt forms' of both complexes were similar to those of intracytoplasmic membranes, suggesting that the complexes are assembled into membranes as 'high-salt form'. The conversion between 'high-salt form' and 'low-salt form' in isolated complexes occurred instantly and was completely reversible. It remains to be clarified how these complexes are fixed as 'high-salt form' in vivo. No spectral change depending on NaCl was observed in vivo unless the complexes were solubilized from membranes. Analogous complexes which showed similar spectral changes were prepared with the aid of Triton X-100 or Nonidet P-40 as detergents for solubilization instead of LDAO.

The dependency on NaCl concentration of NaCl-induced spectral shifts in the near infrared region was investigated (Fig. 6). Increasing of NaCl concentration in the reaction mixture caused a large red-shift of absorption peak of the long wavelength band in both complexes with different dependency on NaCl concentration. The 834 nm band in the complex from

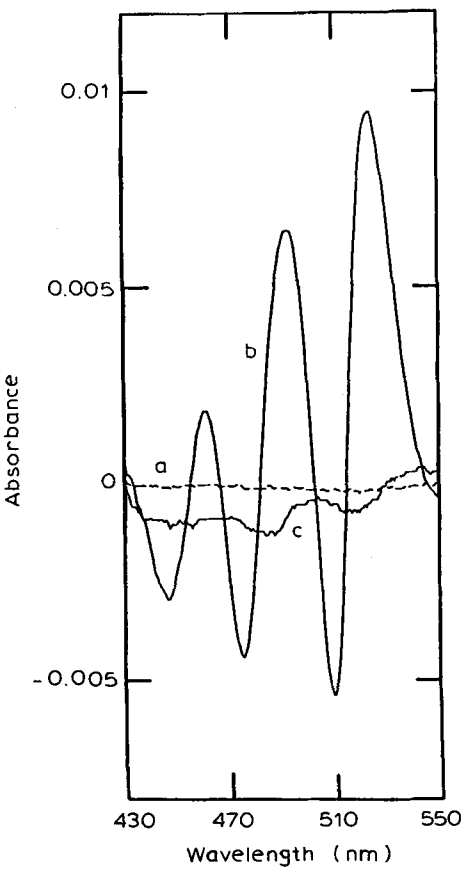

Fig. 5. Difference absorption spectra of carotenoid(s) induced by the addition of NaCl in the isolated complexes. (a) Base line; (b) LHII complex from light-grown cells; (c) LHII complex from dark-grown cells, Other conditions were the same as in Fig. 4.

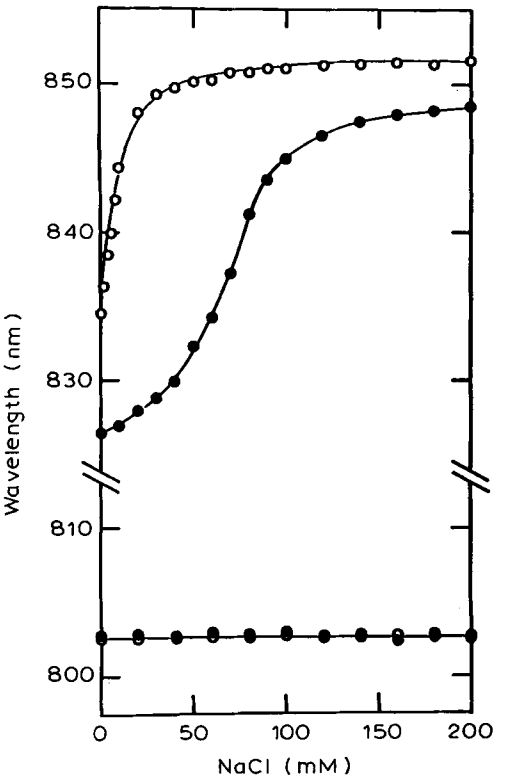

Fig. 6. Dependence of band shift in near infrared regions on NaCl concentrations. Absorption spectra were measured at 25°C with a Shimadzu UV 260 spectrophotometer. The complexes were suspended (20 μg protein/ml) in 10 mM Tris-HCl (pH 8) containing 0.05% LDAO. ●, Complex from dark-grown cells; ○, complex from light-grown cells.

light-grown cells shifted up to 852 nm and the 827 nm band in the complex from dark-grown cells shifted up to 850 nm upon increasing NaCl concentration. A further shift of these bands to longer wavelength was not observed. The position of the 803 nm band was essentially unaffected by the addition of NaCl up to 1 M in both complexes, the minor shift of less than 1 nm being probably due to the changes of long-wavelength bands. The lowering of absorption at 803 nm by the addition of NaCl might be due to reduction of spectral overlap with the long wavelength band (Fig. 4). The ratio of absorption of the shorter to the longer infrared wavelength band increased from 0.72 to 1.26 and from 0.76 to 1.21 depending on increasing NaCl concentration, respectively, in complexes from light- and dark-grown cells. All intermediate spectral forms of both complexes were fairly stable over 24 h at 0°C in the dark.

Similar spectral changes of Bchl were observed in both complexes, depending on the concentration of LDAO (Fig. 7). Lowering of LDAO concentration in the reaction mixture resulted in a red-shift of the long wavelength band. At a concentration of less than $2 \cdot 10^{-4}$% LDAO, the 834 nm band in the LH complex from light-grown cells shifted to 851 nm and the 827 nm band in the complex from dark-grown cells to 839

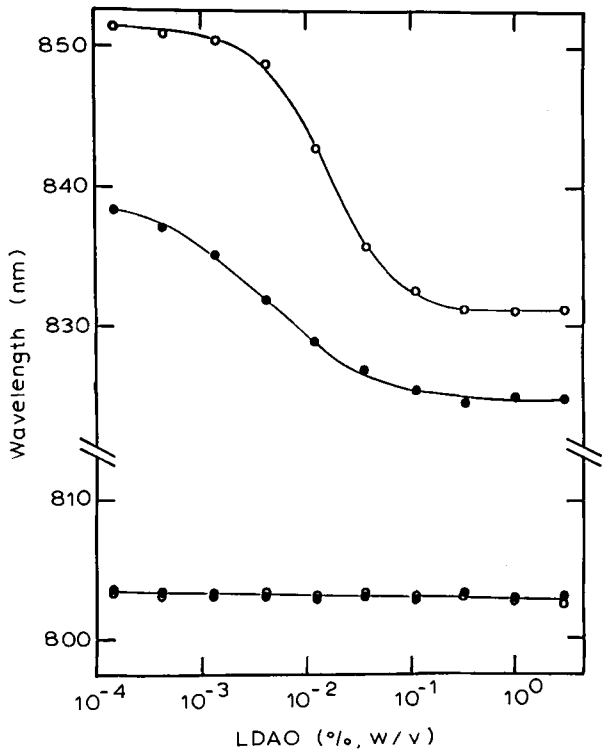

Fig. 7. Dependence of band shift in near infrared regions on LDAO concentrations. The complexes (20 μg protein/ml) were suspended in 10 mM Tris-HCl (pH 8). ●, LHII complex from dark-grown cells; ○, LHII complex from light-grown cells.

nm. The peak position of short-wavelength band at 803 nm did not change in either complex in the concentration ranges of LDAO used. The absorption ratio at 851 to 803 nm was 1.29 in the complex from light-grown cells, and 0.86 at 839 to 803 nm in the complex from dark-grown cells. The spectral shift induced by decreasing of LDAO concentration in the LHII complex from dark-grown cells was incomplete compared with the LHII from light-grown cells. Prolonged dialysis with detergent-free buffer caused aggregation of complexes.

Discussion

The light-harvesting complex II was isolated from *Rhodobacter sulfidophilus* grown either chemotrophically or phototrophically. The carotenoid composition changed under the two growth conditions as known from other purple nonsulfur bacteria. The LHII complex isolated from cells grown at one of the described conditions consisted of two polypeptides having apparent molecular weights of about 5000 and 3000. These values, especially the low one, were smaller than those reported for LHII complexes isolated from other purple photosynthetic bacteria [7]. The amino acid sequence analysis which is in progress will result in the precise determination of molecular weight.

The isolated LHII complexes showed characteristic spectral shifts of the long-wavelength band in the near-infrared region, which represents dimeric Bchl. The shift of this band is probably due to change of excitonic interactions between two Bchl molecules. In fact, the monomeric Bchl band at 803 nm was not affected by salt and detergent. Reversibility of the shift reflects specific changes in complexes. According to structural models [5,16,17], Bchl molecules are bound to polypeptides by histidine-residues and other conservative amino acids. Reconstitution experiments have shown that the spectral feature of a pigment-protein complex depends from the interaction of Bchl and polypeptides [18]. One possible explanation for changing of interactions between Bchls is a change of tertiary structure of polypeptides which bind Bchls.

Conformational changes of polypeptides could modify the excitonic interactions between Bchls by changing the distance and/or relative orientation between them. Salt and detergent may change ionic and hydrophobic interactions between polypeptides in isolated complexes, respectively. Specific effects of salt and detergent on the interactions between Bchl–Bchl or Bchl–polypeptide are not excluded at present. The different dependence of band-shifts on salt and detergent concentration suggests different organization of Bchl and polypeptides between dark and light complexes.

In addition to changes of absorption due to Bchl, a carotenoid band shift was observed in the complex from light-grown cells. The different absorption changes induced by NaCl are similar to the electrochromic band shift of carotenoids found in membranes and whole cells of photosyntetic bacteria [19]. This shift reflects the change of absorption of special carotenoid molecules responding to a local-field change. Salt-induced spectral-changes of carotenoids in the complex from light-grown cells is particularly interesting for such carotenoids which bind to the LHII complex in vivo [20,21]. Although carotenoids are not essential for the assembly of the LHII [22], carotenoids seem to be bound to the complex in a specific way which makes possible an effective energy transfer between the pigment molecules [8,23]. The carotenoid band shift accompanied by the absorption change of Bchl supports close interaction of these pigments in the LHII complex.

There are some variations in the spectral form of the LHII complex isolated from purple photosynthetic bacteria [23–29]. The absorption spectra of B800-850 complexes, which have been well characterized in *Rb. sphaeroides*, *Rb. capsulatus* and *Rhodocyclus gelatinosus*, are similar to that of the 'high-salt' form of LHII isolated from *Rb. sulfidophilus*. The ratio of $A_{850/800}$ is typically in the range from 1.2 to 1.5. On the other hand, another type of LHII presented by the B800-820 complex in *Chromatium vinosum* and *Rhodopseudomonas acidophila*, which has a high absorption at 800 nm and low at 820 nm resembled the 'low-salt' form.

The position of the long-wavelength band is rather variable among the complexes isolated from different bacteria. Recently, Brunisholz and Zuber [9] suggested the different spectral forms of Bchl to be the result of complexes with specific apoproteins and the important role of aromatic amino acid residues located in the C-terminal portions of the complexes. As described here, the conversion among different spectral forms of pigments occurred also by rearrangements due to polypeptide conformational changes. The isolated LHII complex of *Rb. sulfidophilus* is, therefore, interesting and useful as model to elucidate the relationship between spectral forms and pigment organization in complexes in this connection.

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References

1 Hansen T.A. and Veldkamp H. (1973) Arch. Mikrobiol. 92, 45–58.
2 Shioi, Y. and Doi, M. (1990) in Current Research in Photosynthesis (Baltscheffsky, M., ed.), Vol. III, pp. 853–856, Kluwer, Dordrecht.
3 Drews, G. (1988) in Oxygen Sensing in Tissues (Acker, H., ed.), pp. 3–11, Springer, Berlin.
4 Drews, G. and Oelze, J. (1981) Adv. Microbiol. Physiol. 22, 1–92.
5 Zuber, H. (1986) Trends Biochem. Sci. 11, 414–419.
6 Shimada, K., Hayashi, H. and Tasumi, M. (1985) Arch. Microbiol. 143, 244–247.
7 Drews, G. (1985) Microbiol. Rev. 49: 59–70.
8 Angerhofer, A., Cogdell, R.J. and Hipkins, M.F. (1986) Biochim. Biophys. Acta 848, 333–341.
9 Brunisholz, R.A. and Zuber, H. (1988) in Light-Energy Transduction in Photosynthesis (Stevens, S.E. and Bryant, D.A., eds.), pp. 47–61, The American Society of Plant Physiologists, Rockville.
10 Drews, G. (1983) Mikrobiologisches Praktikum, p. 11, Springer, Berlin.
11 Golecki, J.R., Schumacher, A. and Drews, G. (1980) Eur. J. Cell Biol. 23, 1–5.
12 Clayton, R.K. (1966) Photochem. Photobiol. 5, 669–677.
13 Shneour, E.A. (1962) Biochim. Biophys. Acta 62, 534–540.
14 Laemmli, U.K. (1970) Nature 227, 680–685.
15 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J., (1951) J. Biol. Chem. 193, 265–275.
16 Zuber, H. (1990) in Molecular Biology of Membrane-Bound Complexes in Phototrophic Bacteria (Drews, G., and Dawson, E., eds.), pp. 161–180, Plenum, New York.
17 Tadros, M.H. and Drews, G. (1990) in Molecular Biology of Membrane-Bound Complexes in Phototrophic Bacteria (Drews, G., and Dawson, E., eds.), pp. 181–192, Plenum, New York.
18 Loach, P.A., Parkes-Loach, P.S., Chang, M.C., Heller, B., Bustamante, P.L. and Michalski, T. (1990) in Molecular Biology of Membrane-Bound Complexes in Phototrophic Bacteria (Drews, G., and Dawson, E., eds.), pp. 235–244, Plenum, New York.
19 Wraight, C.A., Cogdell, R.J. and Chance, B. (1978) in The Photosynthetic Bacteria (Clayton, R.K. and Sistrom, W.R., eds.), pp. 471–511, Plenum Press, New York.
20 Holmes, N.G., Hunter, C.N., Niederman, R.A. and Crofts, A.R. (1980) FEBS Lett. 115, 43–48.
21 Symons, M. and Swysen, C. (1983) Biochim. Biophys. Acta 723, 454–457.
22 Dörge, B., Klug, G. and Drews, G. (1987) Biochim. Biophys. Acta 892, 68–74.
23 Van Grondelle, R., Kramer, H.J.M. and Rijgersberg, C.P. (1982) Biochim. Biophys. Acta 682, 208–215.
24 Thornber, J.P. (1970) Biochemistry 9, 2688–2698.
25 Feick, R. and Drews, G. (1978) Biochim. Biophys. Acta 501, 499–513.
26 Sauer, K. and Austin, L.A. (1978) Biochemistry 17, 2011–2019.
27 Webster, G.D., Cogdell, R.J. and Lindsay, G.J. (1980) FEBS Lett. 111, 391–394.
28 Thornber, J.P., Cogdell, R.J., Pierson, B.K. and Seftor, R.E.B. (1983) J. Cell. Biochem. 23, 159–169.
29 Wacker, T., Gad'on, N., Steck, K., Welte, W. and Drews, G. (1988) Biochim. Biophys. Acta 933, 299–305.