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Spectroscopic characterization of B820 subunits from light-harvesting complex I of *Rhodospirillum rubrum* and *Rhodobacter sphaeroides* prepared with the detergent *n*-octyl-*rac*-2,3-dipropylsulfoxide

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B820 subunits from the light-harvesting complex 1 (LH1) were prepared by treatment of chromatophore membranes from *Rhodospirillum rubrum* with the detergent *n*-octyl-*rac*-2,3-dipropylsulfoxide (ODPS). Similar subunits were obtained by dialysis of purified LH1 complexes from *Rb. sphaeroides* mutant M2192 against a buffer containing the same detergent. In contrast to other methods, no prior extraction of carotenoids is necessary for the preparation of these subunits when using the detergent ODPS. Reassociation of the B820(ODPS) subunits resulted in an apparently native, but carotenoid depleted LH1 complex. The presence of a fourth spectral component, with an absorbance maximum at 845 nm, was demonstrated by fitting the absorbance difference spectra with a sum of gaussians. Results from absorbance, fluorescence polarization, circular dichroism and triplet-singlet spectroscopy indicate that the subunits from LH1 obtained by this method are very similar to the B820 subunit isolated from carotenoid depleted light-harvesting complex 1 by octyl glucoside titration. These results imply that the B820 subunit structure created by detergent treatment is inherent to the structure of the functional LH1 antenna both for *Rs. rubrum* and *Rb. sphaeroides*.

Introduction

The primary events of photosynthesis are light absorption, excitation energy transfer and charge separation which require antenna and reaction center complexes, respectively. In photosynthetic purple bacteria this may involve a peripheral light-harvesting complex (LH2) and a core antenna complex (LH1) located in the direct vicinity of the reaction center [1–4]. The antenna complexes consist of large aggregates (150–400 kDa) of two small hydrophobic polypeptides designated α and β, that each span the membrane once (Refs. 5,6; reviews in Refs. 7,8). The near infrared and visible light absorption of the antenna complexes is due

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Abbreviations: ODPS, *n*-octyl-*rac*-2,3-dipropylsulfoxide; bchl*a*, bacteriochlorophyll *a*; OG, *n*-octyl β-D-glucopyranoside; B873(reassoc), light-harvesting complex 1 of *Rhodospirillum rubrum* obtained by reassociating the B820 OG solubilized subunit form by dilution and chilling; B825, subunit form of light-harvesting complex 1 from *Rhodobacter sphaeroides* obtained by OG titration of light-harvesting complex 1; B820, generic name for the subunit form of light-harvesting Complex 1 obtained by detergent titration of light-harvesting complex 1; B820(OG), subunit form of light-harvesting complex 1 from *Rhodospirillum rubrum* obtained by OG titration of light-harvesting complex 1; B817(ODPS), subunit form of light-harvesting complex 1 from *Rhodospirillum rubrum* obtained by *n*-octyl-*rac*-2,3-dipropylsulfoxide titration of chromatophores; B822(ODPS), subunit form of light-harvesting complex 1 from *Rhodobacter sphaeroides* obtained by *n*-octyl-*rac*-2,3-dipropylsulfoxide titration of chromatophores; B816(OG), subunit form of light-harvesting complex 1 from *Rhodobacter capsulatus* obtained by OG titration of light-harvesting complex 1; 777(dissoc), 777 nm absorbing material formed by titrating B820 with 4–5% OG; LH1, light-harvesting complex 1, the core complex; LH2, light-harvesting complex 2, an accessory complex; NIR, near infrared; CMC, critical micellar concentration; FWHM, full width at half maximum.

to bacteriochlorophyll (BChl) and carotenoid molecules, respectively, that are bound to the polypeptides. LH2 typically has two NIR absorption bands at 800 and 850 nm, and is present in a varying ratio to the reaction center [3]. The light-harvesting complex 1 (LH1) is characterized by a single NIR absorption band around 880 nm in most purple bacteria, is present in a fixed ratio to and located in the vicinity of the reaction center [3]. Both LH2 [1] and LH1 [9,10,11] can be solubilized from the photosynthetic membrane using mild detergents and subsequently purified.

Subunit forms of LH1 have been prepared with the detergent n-octyl β-D-glucopyranoside (OG) [12–15] from carotenoid depleted *Rs. rubrum*, *Rb. sphaeroides* and *Rb. capsulatus*. These subunits exhibit spectral properties that differ from the LH1 in vivo antenna. OG concentrations near the CMC (0.73%) induce a reversible dissociation of the LH1 antenna complex, which provokes a shift of the main NIR absorption maximum from 873 nm in the carotenoid-depleted LH1 complex to approx. 820 nm in the subunit form. The exact position of the absorbance maximum is slightly different for several species of purple bacteria. The observed shift can be reversed by lowering the OG concentration below the CMC and was explained by reasociation of the LH1 complex. Higher concentrations of OG (3–5%) induce a shift of the NIR maximum from 820 to 777 nm [16,17]. These high concentrations of OG completely dissociate the BChl *a* from the α and β polypeptides. Also, this dissociation appeared to be reversible upon dilution of the sample.

The spectroscopic properties of the B820 subunits are now well established [18–20]. A number of results indicate that the B820 subunit consists of an interacting BChl *a* dimer linked to one αβ polypeptide pair. A model has been presented in which the two BChl *a* molecules are approx. 1.2 nm apart, have a small angle between their Q_y transition dipoles and are excitonically coupled with the low and high energy exciton component at 820 and 795 nm resp. [18]. Time-resolved triplet-singlet spectroscopy [19] showed that, upon excitation with a short laser flash at cryogenic temperatures, a triplet is produced that apparently is localized on one of the BChl *a* molecules. The absorbance at 820 nm is bleached and replaced by absorbance from a protein bound BChl *a* monomer at 809 nm, supporting the notion that the B820 absorption band arises from an interacting BChl *a* dimer.

Hitherto, the preparation of B820 subunits has required either the use of carotenoid-less mutants (*Rs. rubrum* G9) or the extraction of carotenoids from the membranes prior to solubilization with OG. Moreover, no other detergent completely converted the LH1 antenna into its subunit form, although it was found in [13] that several derivatives of *N*-methylglucamide stabilized the B820 subunit. In addition unstable B820 intermediates were observed after treatment of membranes from *Rs. rubrum* (G9) with the detergent LDAO [21]. Reversible effects from detergent treatment on spectroscopic properties of photosynthetic pigment-protein complexes have been studied intensively [2,12–23] and have yielded much valuable information about the structure and function of bacterial light-harvesting complexes. Here we report a simple preparation method for B820 subunits that does not require depletion of carotenoids from the chromatophores. Solubilization of chromatophore membranes from wild-type *Rs. rubrum* with the detergent ODPS caused a shift of NIR absorbance similar to that observed previously when OG was used. In addition the complex is irreversibly depleted of carotenoids. Since the obtained subunits have spectroscopic properties identical to the subunits obtained by solubilization with OG a simplified method for the preparation of B820 subunits from normal carotenoid containing photosynthetic purple bacteria has become available.

Materials and Methods

Chromatophore membranes from *Rs. rubrum* and *Rb. sphaeroides* M2192 were prepared by two passes through a French press of anaerobically grown *Rs. rubrum* cells or semi anaerobically grown *Rb. sphaeroides* M2192 [24] in the presence of DNAase I (1 μg/ml). The *Rb. sphaeroides* strain M2192 contains LH1 as the sole pigment-protein complex. The disrupted cells were layered onto a three-step sucrose gradient (45/40/15% w/w) and spun for 4 h at 80000 × *g*. Chromatophores were harvested from the 15/40% interface and stored at −70°C in the sucrose medium until used. For the preparation of B820 subunits from *Rs. rubrum* membranes were thawed and diluted in 10 mM Hepes buffer (pH 8.0) to an A_{880} of 10–15. Addition of 0.9% ODPS (Bachem, Zurich, Switzerland) or overnight dialysis against buffer containing 0.75% ODPS resulted in a complete shift of the NIR absorbance maximum from 880 to 817 nm. The chemical structure of ODPS is given in Fig. 1. Aggregates of insoluble protein matter that appear during the solubilization were removed by centrifugation (3000 × *g*, 15

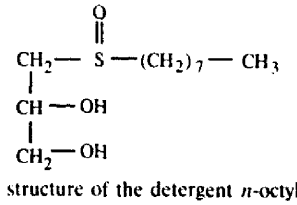

Fig. 1. Chemical structure of the detergent *n*-octyl-*rac*-2,3-dioxypropylsulfoxide (personal communication, U. Bartelstein, Bachem, Switzerland) that has been used for the preparation of subunit forms of the core antenna complex.

min). Aliquots of 0.5 ml were filtered (0.2 μm filter) and subsequently applied to a Superose®-6 FPLC column (Pharmacia, Uppsala, Sweden). The column was eluted with 10 mM Hepes buffer (pH 8.0) containing 400 mM KCl and 0.6% ODPS at 0.4 ml/min. Since the subunits are sensitive to prolonged exposure to light, chromatographic procedures were performed in the dark. The peak fraction containing B817(ODPS) was used immediately for spectroscopy without further purification. For calibration of the molecular weight, standard proteins (ferrittin, bovine serum albumin, alcohol dehydrogenase, cytochrome *c*) were applied in a separate run after equilibration of the column with the same buffer as used for the B820 subunits.

For *Rb. sphaeroides* M2192 a different approach was necessary, since dialysis of membranes against different concentrations of ODPS failed to produce reasonable amounts of B820(ODPS); instead a straightforward conversion to B777 was usually observed. However, purified LH1 complexes could successfully be used to obtain subunits from *Rb. sphaeroides*. To purify LH1 complexes chromatophore membranes with A_{880} of 10–15 were solubilized in Hepes buffer containing 1% OG. Insoluble matter was removed by centrifugation ($3000 \times g$, 30 min, 4°C). One volume of saturated ammonium sulfate solution was added and the precipitate was removed by centrifugation ($3000 \times g$, 30 min, 4°C). LH1 was subsequently precipitated by addition of an equal amount of saturated ammonium sulfate solution, this time containing 1% OG. LH1 was pelleted, resuspended in buffer and excess ammonium sulfate was removed by two ultrafiltration/dilution steps (Amicon, YM10 filter). The sample was subsequently filtered (0.2 μM) and applied to a Mono-Q FPLC column (Pharmacia) equilibrated with 10 mM Hepes buffer containing 0.9% OG. The LH1 complexes were eluted by applying a linear gradient of NaCl. The LH1 complexes eluted at approx. 360 mM NaCl. The samples were pooled, concentrated by ultrafiltration (Amicon, YM10 filter) and applied to a Superose-6 FPLC column, which was eluted with buffer containing 0.9% OG. The LH1 complexes appeared as a single band with a molecular weight of approx. 350000. To obtain B820(ODPS) the purified LH1 complexes were dialyzed in small volumes (100 μl to 1 ml) against 400 mM potassium phosphate buffer (pH 7.5) containing 0.75% ODPS. Dissociation usually took place within 5 h at room temperature. The subunits from LH1 *Rb. sphaeroides* obtained this way were characterized by an absorbance maximum at 822 nm and are therefore indicated as B822(ODPS), and used without further purification.

Absorption spectra were measured on a standard Cary219 spectrophotometer with a bandwidth of 1 nm. Circular dichroism and fluorescence spectra were measured on home-built spectrophotometer described elsewhere [25]. Triplet-singlet spectroscopy was performed on the instrument described in Ref. 19. For these measurements chromatophore membranes of *Rs. rubrum*, to which 1% ODPS was added, were used. The membranes where centrifuged to remove aggregates, and suspended in a buffer containing 50% (v/v) glycerol and 1.7% ODPS and cooled in a liquid nitrogen cryostat (Oxford Ltd., UK). The exposure of the subunits to light caused some degradation of the subunits. The total decrease of absorbance during the measurements was never higher than 10%. The sensitivity for light dissapeared when the subunits were frozen to 77 K. All spectrophotometers were interfaced to a SUN3/SUN4 computer system for data analysis.

Results

Addition of 0.9% ODPS to chromatophores of wild-type *Rs. rubrum* induced a dissociation of the LH1 antenna complex. This dissociation is accompanied by a disappearance of the main NIR absorbance band at 880 nm, and by the appearance of a band at 817 nm (see Fig. 2). Spirilloxanthin is removed from the LH1 antenna complex during the solubilization and its visual absorption disappeared, indicating a modification of the carotenoid molecule. Consequently the color of the sample changed from red to gray during dissociation. Since no peaks appear in the 900–1050 nm region where oxidized carotenoids have an absorbance maximum, the possibility of oxidizing the carotenoid during the solubilization seems unlikely. The dissociation process occurred within a few hours yielding a preparation that usually retained less than 10% of the original 880 nm absorption. The sample contained a mixture of subunits absorbing at 817 nm and BChl *a* absorbing around 775 nm. The presence of high potassium chloride or potassium phosphate concentrations (400 mM) enhanced the dissociation of the LH1 antenna complex and stabilized the B817(ODPS) subunit form. In buffers containing less than 400 mM of either potassiumchloride or potassium phosphate no dissociation was observed at low ODPS concentrations, whereas an immediate appearance of B777 was observed at higher concentrations of ODPS.

Fig. 2 shows the room-temperature absorption spectrum of *Rs. rubrum* chromatophores before and 4 h after addition of 0.9% ODPS. The latter sample was centrifuged to remove aggregated material. The main NIR absorption band at 881 nm has disappeared and has been replaced by two bands, one with maximal absorption at 817 nm with a width of 30 nm (FWHM) and the other at 777 nm with a width of 63 nm. The intensity ratio of the two bands was dependent on the concentration of ODPS used to solubilize the membranes; the ratio of the B817(ODPS):B777 absorption maxima was usually in the order of 1:1, but prepara-

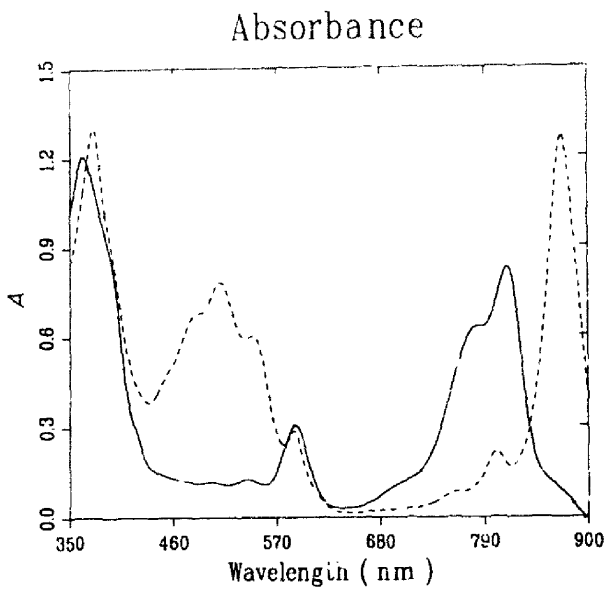

Fig. 2. Room-temperature absorbance spectra of wild-type *Rs. rubrum* chromatophores before (dashed) and 4 h after (solid) addition of 0.9% ODPS. The chromatophores were suspended in 400 mM KP buffer (pH 7.5). The spectral bandwidth was 1 nm. Since the sample containing ODPS was centrifuged, the absorbances can not be directly compared.

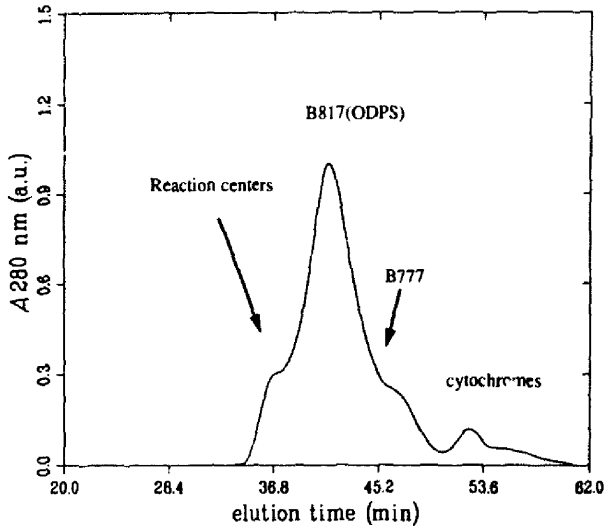

Fig. 3. Elution pattern of a Superose®-6 FPLC column. The column was equilibrated with 10 mM Hepes buffer (pH 8) containing 0.6% ODPS and 400 mM KCl at a continuous flow of 0.4 ml/min. A sample of 200 μl with A_{817} of 10 was applied. The B817(ODPS) subunits elute 42 min after sample application just after a smaller peak at 36 min that contains reaction centers. This corresponds to a molecular mass of the B817(ODPS) subunits of 71 kDa and 260 kDa for the detergent-solubilized reaction centers.

tions containing relatively more B817 were obtained when concentrations of 0.6 or 0.7% ODPS were used. However, in this case the dissociation was limited, leaving some residual absorbance around 880 nm. The Q_x band at 590 nm shifted slightly to the red, whereas the soret maximum is shifted to the blue by 10–12 nm

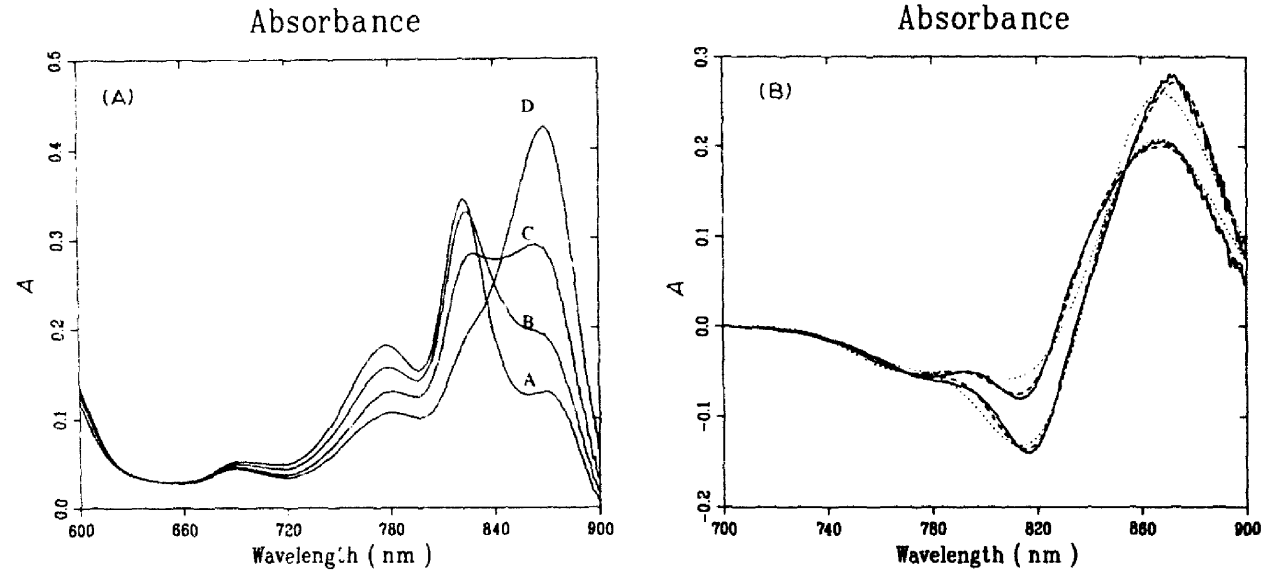

Fig. 4. Panel a: Reassociation study of *Rb. sphaeroides* B817(ODPS). A sample of B817(ODPS) was diluted with buffer free of detergent and salt. Reassociation takes place immediately upon dilution. The spectra were recorded 5 min after addition of the buffer. Trace A: diluted 2.0-fold, B: 2.25-fold, C: 3.1-fold, D: 3.6-fold. No clear isosbestic point can be observed. This indicates the presence of a fourth component (see text). Panel b: Absorbance difference spectra of two steps of a titration of B817(ODPS) from *Rs. rubrum*. The two absorption differences (solid line) were simultanously fitted with gaussian shaped bands. The use of four bands (dashed line) significantly improves the best fit obtained with three bands (dotted line). The four bands used in these fits were positioned at 873 nm (40 nm fwhm), 845 nm (56 nm fwhm), 818 nm (28 nm fwhm) and 782 nm (62 nm fwhm).

upon dissociation. Cooling to 77 K caused a red-shift of 6 nm of the 817 nm band that also sharpens to a width of 20 nm (spectrum not shown). Increasing the ODPS concentration above 1% yielded a further dissociation and complete conversion of the Q_y absorption band into B777

Fig. 3 shows the elution profile of a Superose®-6 column, onto which we applied a B817(ODPS) sample, ($A_{820} = 5$) obtained from *Rs. rubrum* wild-type membranes. B777, B817(ODPS) and reaction centers are separated on the basis of their molecular weight. Interestingly, the absorption spectrum of the reaction center fraction showed that the reaction center carontenoids were retained during the solubilization procedure. The elution time of the B817(ODPS) complexes suggested a molecular weight of about 70000 (±10000), which is slightly more than the molecular weight of the B820(ODPS) complexes reported earlier [13]. The difference may be attributed to a different amount of detergent bound to the subunits or to small differences in the size of the detergent. Large variations in the size of detergent-protein complexes depending on the actual detergent used have been reported previously [26].

In order to analyze the reassociation properties of B817(ODPS), we diluted the sample with detergent-free buffer and monitored the spectroscopic changes. As with B820(OG) the spectral changes are reversed and an absorbance band at 873 nm appears upon dilution. We interpret this as reaggregation of the B817(ODPS) into a complex that is similar to the native LH1 antenna. The reassociation of B817(ODPS) took place within the time necessary for mixing and measuring the absorbance spectrum (on a conventional spectrophotometer). In Fig. 4 we show the room-temperature spectra of the reassociation of B817(ODPS). The NIR absorbance band at 817 nm is replaced by a band with a maximum absorbance at 873 nm and a higher integrated intensity. It is clear that, as for the B820(OG), the dissociation of the LH1 antenna by the detergent ODPS can be largely (> 90%) reversed by dilution of the sample. We note that the ratio of B817(ODPS) and reassociated B873 is dependent on the degree of dilution of the sample, in contrast to B820(OG) where complete reassociation into B873 takes place as soon as the sample is diluted to OG concentration slightly below the CMC. Very similar spectra were obtained for B822(ODPS) complexes from *Rb. sphaeroides* upon dilution with detergent free buffer, with a progressive decrease in B777 accompanied by a rise in B822(ODPS), finally yielding complete conversion into the B873 spectral form.

No isobestic point is observed in the absorbance spectra of the reassociation experiment shown in Fig. 4a. This implies that during the ODPS dilution small amounts of other spectral forms are generated which contribute to the absorbance around 850 nm. To assess the presence of other spectral components we have fitted the observed absorption difference spectra of B822 (ODPS) as a sum of three or four gaussian-shaped bands (Fig. 4b). Only when a fourth component was assumed with a maximal absorbance at 845 nm and a bandwidth of 51 nm (FWHM) could a good agreement

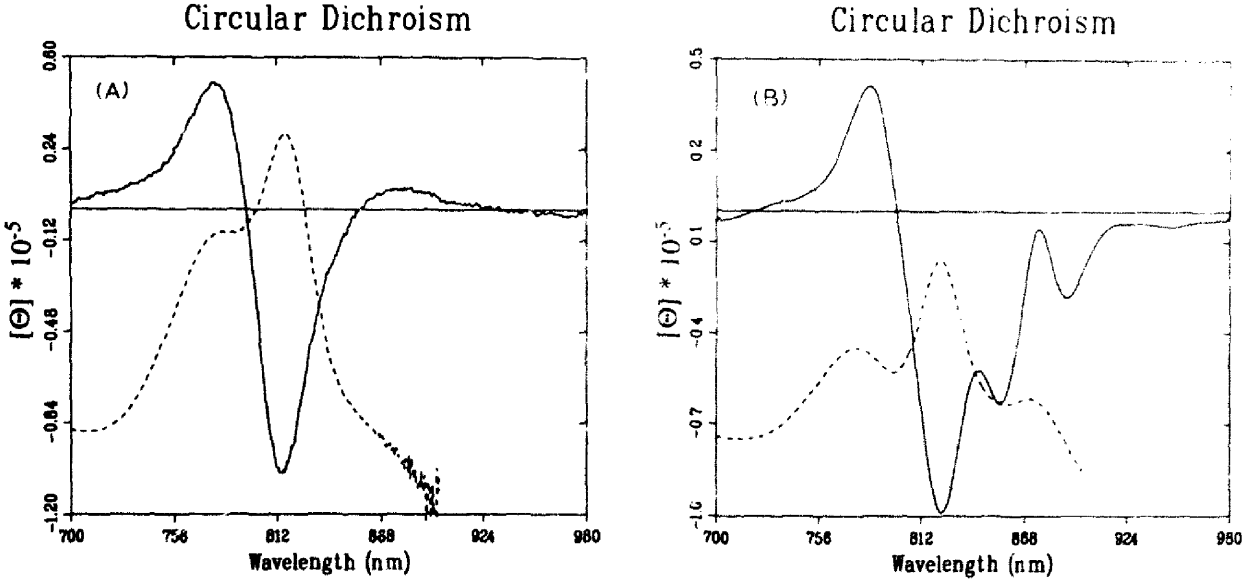

Fig. 5. Room-temperature CD spectra of subunits from LH1 antenna complexes. The spectral bandwidth was 3 nm. Panel a: B817(ODPS) subunit from *Rs. rubrum wt*. The dashed line shows the absorbance of the actual sample. Panel b: B822(ODPS) from *Rb. sphaeroides* M2192. The dashed line represents the absorbance of the sample. The additional lobes on the red side of the spectrum (850–900 nm) are due to some remaining intact LH1.

between the fits and the measured absorbance difference spectra be obtained. The presence of this component can especially be noted at ODPS concentrations around 0.4 to 0.5%. This component completely disappears upon full reassociation into B873.

The LH1 complexes of *Rb. sphaeroides* M2192 show a similar shift of the NIR absorbance upon treatment with ODPS. Addition of ODPS to membranes from this bacterium resulted in conversion to B777, rather than the conversion into B822(ODPS) subunit forms. However, when purified complexes were dialyzed against 1% ODPS B822 predominates. In these samples all three forms B777, B822(ODPS) and B880 can be observed simultaneously. A room-temperature spectrum of such a sample is shown in Fig. 5b (dashed line). No pure B822(ODPS) samples could be obtained by varying concentrations of salt or ODPS.

The room-temperature CD spectra of the Q_y region of both B817(ODPS) and B822(ODPS) have features identical to those reported for B820(OG) [14,18,19]. For B817(ODPS) a positive lobe is observed at 776 nm and a larger negative lobe is maximal at 816 nm (Fig. 5a). In Fig. 5b the room-temperature CD spectrum of B822(ODPS) is shown, which essentially is identical to the spectrum shown in Fig. 5a but with the positive lobe situated at 779 nm and the larger negative lobe at 825 nm. The additional features of this spectrum in the red flank are due to some remaining intact LH1 which can be seen in the absorbance spectrum. The general shape of the CD spectra as well as the non-conservativeness and the intensity of the spectra are in good agreement with a BChl *a* dimer model describing the spectroscopic features of the B820(OG) subunit [18].

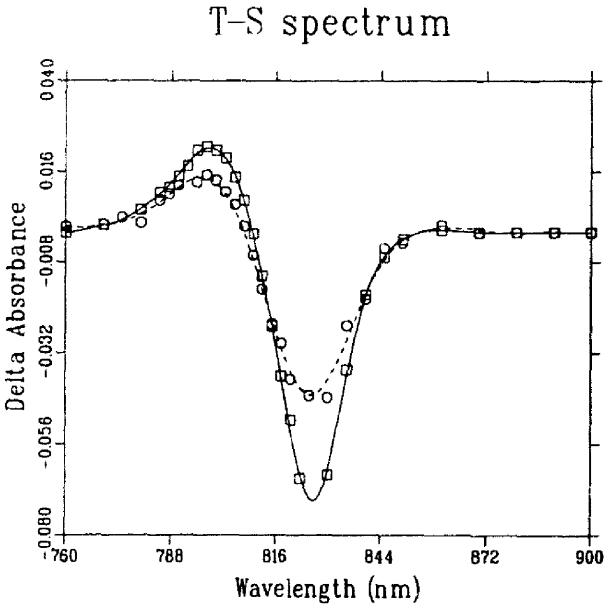

Fig. 7. 77 K triplet-singlet spectra of B822(ODPS) of *Rs. rubrum*. Excitation at 822 nm (solid, squares), excitation at 835 nm (dashed, open circles). The spectrum shows a bleaching of the original dimer band around 822 nm and a generation of the monomer band on the blue side of the dimer band. The individual points in the absorption difference spectrum were obtained by fitting the flash induced, transient absorbance signal between 20 and 300 μs after the flash with a single exponential function and extrapolating the amplitude to time zero. This signal decayed with a time constant of 106 ± 5 μs and is due to the formation of triplets in the B817(ODPS) subunit. A much longer-lived signal due to the closing of reaction centers is corrected for by allowing a time-independent offset.

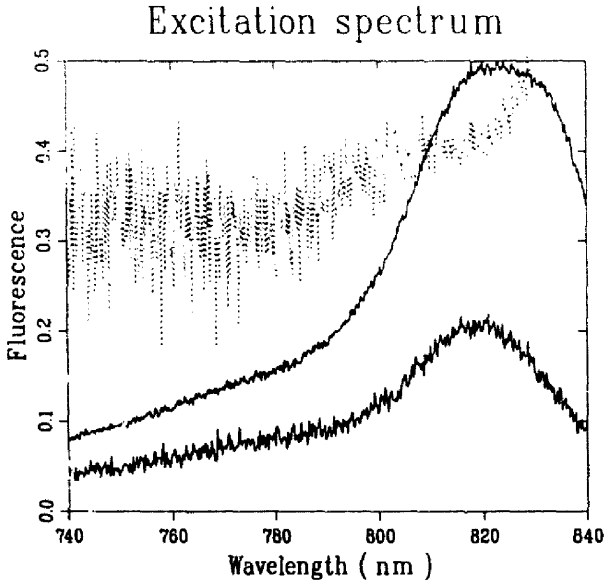

Fig. 6. The room-temperature fluorescence excitation spectrum of B817(ODPS) from *Rs. rubrum*. The solid lines represents the polarized excitation spectra. The dotted line shows the polarization calculated from the polarized spectra. The bandwidth was 12 nm and the detection wavelength was 845 nm.

Fig. 6 shows the fluorescence excitation spectrum at room temperature of the B817(ODPS). The polarization of the fluorescence upon excitation over the B817 absorbance band is high (0.4), and shows a clear decrease on the blue side of the B817 band. Due to some scattering at the red side of the excitation spectrum the 817 nm absorbance band looks broader than expected, and the polarization increases to values above 0.5 in the red wing of the B817(ODPS) excitation spectrum. In our model proposed for the B820(OG) subunit form, the two exciton components of an interacting bacteriochlorophyll *a* dimer were placed at 820 and 795 nm [18]. Since these two transitions are perpendicular, the high energy exciton component of the dimer absorbing at 795 nm causes a decrease of the polarization around this wavelength. The polarization of the B817(ODPS) absorption band shows a similar feature at room temperature, although it is expectedly less pronounced than in the case of B820(OG) at 77 K.

The dimeric nature of the B817(ODPS) absorption band was also probed by singlet-triplet spectroscopy

[19]. By exciting one of the two participating BChl *a* molecules to a triplet-state by means of a short (5 ns), intense laser pulse, the exciton interaction is disrupted and the original dimer spectrum is replaced by a monomer band and a BChl *a* triplet, the latter has no significant absorption in the monomer and dimer NIR wavelength region. Fig. 7 shows the transient absorbance difference spectrum generated by laser flashes at two different wavelengths. The decay time of this signal was 106 ± 5 μs. The spectrum is obtained after correction for the absorbance changes in this region due to the closing of the reaction center, and is independent of the excitation wavelength. It can be described by a bleaching of the original band at 817 nm and generation of a broad monomer band at 805 nm with approx. half the intensity of the original signal.

Discussion

The use of the detergent ODPS for the preparation of subunit forms of LH1 of purple bacteria circumvents the need to extract carotenoids from the membranes. Consequently, B820 subunits that may be used for spectroscopical experiments can be obtained by a simplified procedure requiring only minimal biochemical effort. The loss of the carotenoid molecules upon solubilization with ODPS again shows that the absence of the carotenoid molecule is essential for dissociation of the LH1 complex as suggested earlier [13]. Since the loss of carotenoid is irreversible, it is likely that the carotenoid molecule is completely removed from the LH1 complex and that the mechanism of dissociation of LH1 into subunit forms is similar for OG and ODPS.

The preparation method for ODPS solubilized subunit form of LH1, presented in this manuscript, yields samples that can be used for spectroscopical studies. In some cases mixtures of several species are obtained that will require further purification when homogeneous samples are necessary. The intermediate form absorbing at 845 nm, shown to be present in the reassociation studies, may be an ODPS solubilized BChl *a* dimer similar to the LDAO solubilized BChl *a* dimer [27]. The bandwidth of this band is found to be comparable to the bandwidth of the B777 form and much broader than either the B820 band or the B875 band. The absorbance difference spectra can be described satisfactorily by four gaussian shaped bands at 873, 845, 822 and 777 nm.

An important issue is whether the subunit forms from LH1 obtained by solubilization with ODPS are 'identical' to those obtained with OG. Clearly, both preparations have a number of features in common: in both cases the observed spectral changes are fully reversible upon dilution; the main absorbance bands of the ODPS-solubilized subunits are hypochromic compared to the B880 band; hypochromism is comparable to the B820(OG) although no unambiguous estimate can be made due to the presence of other spectral forms; higher concentrations of both detergents stabilize the B777 form; the intensity and shape of the CD spectra for both *Rs. rubrum* B817(ODPS) and *Rb. sphaeroides* B822(ODPS) are identical to the CD spectra reported for the subunits obtained from the LH1 from these bacteria with the detergent OG. All these similarities indicate that the BChl *a* dimer, which gives rise to the NIR absorbance of the B817(ODPS) complex, must have a configuration that is very similar to the structure proposed for the B820(OG).

The fluorescence polarization measurements also demonstrate the great similarity of B817(ODPS) to the B820(OG). Both are characterized by high fluorescence polarization values (Fig. 6 and Ref. 15). Since the depolarization caused by the high energy exciton component is very small, the oscillator strength of this component can not be very large. This points to a small angle between the Q_y transition dipoles of the two BChl a molecules, since otherwise the contribution from the high energy exciton component to the NIR absorption would be much larger. The transient triplet-singlet spectrum also indicates that the spectroscopic properties from the B817(ODPS) subunit arise from an interacting dimer of BChl *a*.

It must be noted that also a number of differences between the two systems were observed. The absorption maximum of the subunit forms in ODPS is shifted 3 nm to the blue with respect to the OG solubilized subunits. Contrary to the B820(OG), dilution of the preparation stabilizes different ratios of subunit and reassociated forms; furthermore, a fourth spectral form is observed upon dilution of the B817(ODPS). Different chemical properties of the detergents might account for most of these differences. The BChl *a* molecules within the B820(OG) subunit are known to be exposed to the environment [13]. Thus, the presence of ODPS might give a slightly different orientation of the B817(ODPS) or change the local environment of the BChl *a* molecules in such a way that the absorbance is shifted some 3 nm to the blue. Moreover, the micelle size of ODPS is probably much larger compared to OG, which forms relatively small micelles of approx. 8 kDa [28]. Thus, ODPS might therefore stabilize the different ratios of subunit and reassociated forms more efficiently. The presence of the BChl *a* dimers in the reassociation spectra might also be due to a better stabilization of these forms by ODPS than by OG.

The 77 K fluorescence polarization of B820(OG) consistently showed a small but significant decrease of the polarization around 798 nm [18] This decrease of the polarization, which has been ascribed to the high energy exciton component of the dimer system was not

present in the room-temperature fluorescence spectrum shown in Fig. 6, although an overall decrease is observed on the blue side of the spectrum. The absence of a clear dip is due to the much broader absorbance bands at room temperature. Consequently the high energy component is masked by the low energy exciton component which has much more intensity. At room temperature rotational mobility of the pigment may cause some depolarization of the fluorescence signal. The polarization of the B820 fluorescence at room temperature is equal to the polarization of the subunit at 77 K, which is indicative for a low degree of rotational mobility of the BChl *a* dimer in the B820 complex.

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