A Study on the Heterogeneity of the Light-Harvesting Complex II from *Ectothiorhodospira sp.* after Acid/Chaotropic Treatment

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The light-harvesting complex II of the purple bacteria has two strong near infrared electronic absorption bands, around 800 (B800) and 850 (B850) nm, arising from the Q_y transitions of bacteriochlorophyll *a*. It was previously reported that under some specific acid/chaotropic conditions the B850 bacteriochlorophylls of the light-harvesting complex II *of Ectothiorhodospira sp.* are strongly reorganised. Part of these pigments absorbs at 843 nm while another set absorbs around 858 nm. The current work should investigate whether a mix of two different complexes could generate the 843- and 858-nm bands. Acid/chaotropic conditions inducing the reorganisation of B850 were reproduced on a sample bound to an ionic-exchange column. The chromatographic pattern was found strongly homogeneous. The findings indicate that the heterogeneity of the reorganised B850 results from two forms of differently structured bacteriochlorophylls bound to the same polypeptide backbone.

Introduction

Three different types of antenna complexes can be isolated from photosynthetic purple bacteria. The LHI or B880 (Cogdell, 1986**;** Picorel and Gingras, 1988; Sundström and van Grondelle, 1996) is present in all purple bacteria and is intimately associated with the reaction center. The LHII or B800-850 (Feick and Drews, 1978; Clayton and Clayton, 1981; Doi *et al.*, 1991; Walker *et al.*, 1991) and LHIII or B800-820 (Hayashi and Morita, 1980; Cogdell *et al.*, 1983) are arranged more peripherally (Monger and Parson, 1977). Their concentration in the cells can vary with growth conditions. All of these antenna complexes are spectrally characterised by one or two strong near infrared (NIR) electronic absorption bands arising from the Q_y transition of the bacteriochlorophyll (BChl) *a*.

The crystal structure of the LHII complex from *Rhodopseudomonas* (*Rps.*) *acidophila* (McDermott *et al.*, 1995) and *Rhodospirillum* (*Rs.*) *molischianum* (Koepke *et al.*, 1996) were established. The active assembly consists of two concentric cylinders of 9α/9β helical protein subunits in *Rps. acidophila* or 8α/8β in *Rs. molischianum* that hold the pigment molecules. Eighteen or sixteen B850 BChls (depending on the species) are sandwiched between the protein helices near the periplasmic side of the cytoplasmic membrane and other set of nine or eight B800 BChls (depending on the species) are positioned towards the cytoplasmic side. Two B850 BChls are located at a typical center-to-center distance of about 1 nm, two B800 BChls at about 2.1 nm, and the two rings are about 1.8 nm apart (McDermott *et al.*, 1995).

Recently, we studied the effect of acid pH, both in the absence or presence of urea on the LHII complex from *Ectothiorhodospira sp.* (Buche *et al.*, 2000; Buche, 2000). Around pH 5.5-6.0 B850 was specifically and reversibly affected by a blue shift suggesting that a histidine residue most probably participated in the *in vivo* shifting of the absorption in the red. This transition was effective with or without urea present. Under strong chaotropic conditions, a second transition occurred around pH 2.0, affecting the B800 band irreversibly and B850 reversibly. Under these conditions a blue shift from 856 to 842 nm occurred and a new and strong circular dichroism signal from the new 842-nm band was observed. Reversion to the original

Abbreviations: BChl, bacteriochlorophyll; BPheo, bacteriopheophytin; LH, light-harvesting; B800, 800-nm absorption band; B850, 850-nm absorption band; LDAO, lauryldimethylamine N-oxide.

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experimental conditions induced a red shift up to 856 nm of the B850 band but the circular dichroism signal remained mostly unaffected. Furthermore, gaussian deconvolution and second derivative of the reverted spectra at pH 8.0 after strong acid treatment indicated that the new B850 band was actually composed of two bands centred at 843 and 858 nm. We ascribed the 858-nm band to BChls that underwent a reversible spectral shift, and that the 843-nm band is due to oligomeric bacteriopheophytin (Bpheo) formed from a part of the B850 BChls. This new oligomer should be responsible for the observed strong and mostly conservative circular dichroism signal.

The current work was aimed to investigate the possibility that the reverted form could be a mix of both native and reorganised complexes. The results indicate that the B850 BChls of the reverted complex are fixed to the same polypeptide backbone.

Materials and Methods

Preparation of the LHII complex

The photosynthetic bacterium *Ectothiorhodospira sp.* was grown photosynthetically as described by Lefebvre *et al.* (1984). Preparations of the LHII antenna were obtained as described previously (Ortiz de Zarate and Picorel, 1994) with some modifications (Buche and Picorel, 1998).

Sample treatment

One ml of LHII complex at a concentration corresponding to 4 absorption units measured at 795 nm under a 1 cm pathlength was loaded onto a Fractogel TSK-DEAE 650s column of 5 × 1cm equilibrated with 50 mM phosphate buffer, pH 8.0 and including 0.05% (wt/vol) lauryldimethylamine N-oxide (LDAO). The sample was then washed with 45 ml phosphate buffer pH 2 and 8.9 M urea. The column was successively washed by phosphate buffer pH 8 and, phosphate buffer, pH 8/400 mM NaCl. The sample was then eluted with phosphate buffer 400 mM NaCl and 0.2% LDAO.

Spectroscopic measurements

Absorption spectra were measured in a Beckman DU-640 spectrophotometer. Circular dichroism spectra were scanned in a Jasco spectropolarograph (model 720) using a 1-mm optical path cell. Spectra of dialysed samples were normalized for volume modification. The reproducibility of the results was verified at least 5 times on different preparations of the LHII complex. Spectral gaussian deconvolution was carried out using the GRAMS software (Galactic Industries Co., Salem NH).

Pigment extraction

Pigments were extracted with an acetone/methanol (7/2, vol/vol) mixture. After sonication for 1 min, the extract was centrifuged in a microfuge for 2 min to pellet the unsolved material and the supernatant recovered for further analyses. When necessary the BChl was converted to BPheo by adding a few µl of concentrated HCl to the pigment extract.

Results

As mentioned in the Introduction the 858-nm band of the reorganized LHII complex was ascribed to BChl and the 843 nm band to oligomeric BPheo (Buche *et al.*, 2000). The absorption spectra of pigments extracted in organic solvent (see Materials and Methods) both from reverted and native LHII complexes are compared (Fig. 1 A and B). Acidification of these pigments induces a total pheophytinisation of BChl. The band at 525 nm is characteristic of this process concomitantly with an increase of the Soret band intensity and a blue-shift/hypochromism of the *Qy* band. The difference between the spectra before and after acidification presented in Fig. 1C is less important in case of the reverted sample, precisely because of the presence of BPheo in this complex. From this figure and considering the modification of intensity of the Soret or the *Qy* band it could be deduced that BPheo in the reverted complex represents around 30–35% of the initial BChls.

The effect of the presence of BPheo on the heterogeneity of the reorganized complex was tested subjecting a native sample to acid/chaotropic conditions after previous fixation to the same ionic liquid chromatography column used to purify the LHII complex (see Materials and Methods). Reversion from acid/chaotropic to standard buffer at pH 8 was also achieved on the column. The elution profile of the reverted sample is pre-

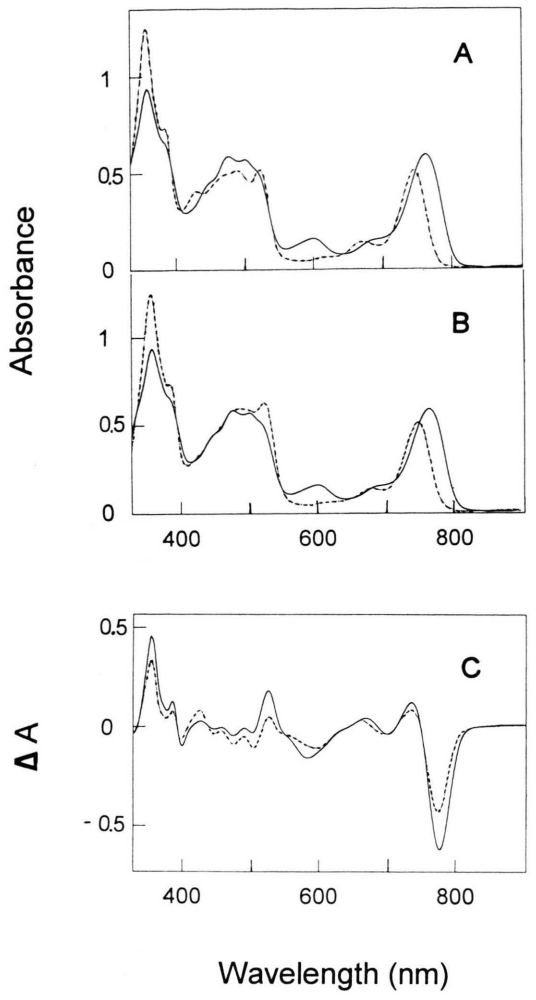

Fig. 1. Organic extraction of pigments from reorganised (A) and native (B) LHII complexes before (solid line) and after acidification (dotted line) (see Materials and Methods).
Difference between the absorption spectra of pigments before and after acidification (C). Both pigments from reorganised (dotted line) and native (solid line) complexes are presented.

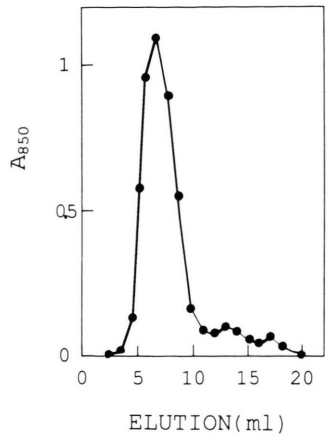

Fig. 2. Elution profile of LHII complex previously loaded onto a Fractogel TSK-DEAE 650s column, treated by acid/chaotropic conditions washed and eluted (see Materials and Methods).

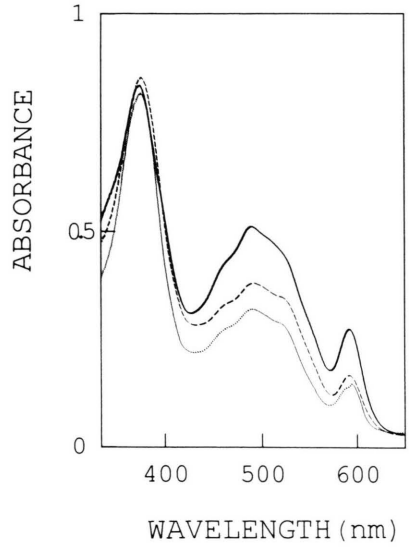

Fig. 3. UV-VIS spectra of the native complex (solid line), the sample corresponding to the maximum of elution of Fig. 2 (dotted line) and a sample submitted 45 min to acid/chaotropic treatment (dashed line). Spectra were normalised on B850.

sented by Fig. 2. Elution of the complex occurs uniformly. The sample corresponding to the maximum of elution presents similar near infrared spectroscopic properties as that of the reverted sample directly submitted to acid/chaotropic conditions (not shown). Figure 3 compares *Qx*, carotenoid and the Soret bands of reverted samples (directly treated by acid/chaotropic conditions or previously fixed to the column) with the native sample before treatment. Both reverted samples are similarly affected by a loss of the carotenoid and the *Qx* bands. The Soret band is poorly affected but a definitive conclusion about this band is difficult because of the presence of different contributions in this wavelength region: BChl, BPheo but also the ($^1C \rightarrow {}^1A$) transition of the carotenoid (Buche and Picorel, 2000). The absorp-

tion spectra of the samples constituing the main peak of elution are similar to that of the maximum of elution (not shown). Some differences could be found in the last samples of this peak. For example, Fig. 4 compares the sample corresponding to the maximum of elution to the samples after 9 and 10 ml of elution. This heterogeneity does not relate to the position of B850 which stays centered around 853 nm but is rather due to the B850/B800 ratio, the presence of monomeric BChl and the presence of a band around 360 nm which could correspond to the intensification of the ($^1C \rightarrow {}^1A$) transition of the carotenoid (Buche and Picorel, 2000). The chromatographic analysis reveals that the great majority of the reverted complex is homogeneous.

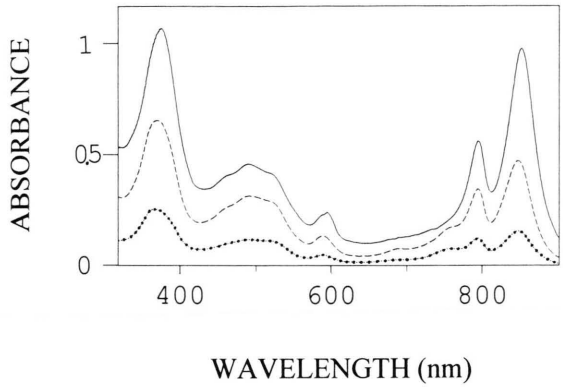

Fig. 4. Comparison of absorption spectra corresponding to different steps of the elution profile: the maximum of elution (solid line), after 9 (dashed line) and 10 (dotted line) ml of elution.

Discussion

The current work was aimed to investigate the possibility that the heterogeneity of B850 induced by the acid/chaotropic treatment occurs concomitantly to the distribution of the complex into two distinct populations comprising BChl absorbing at 858 nm and BPheo absorbing at 843 nm respectively. It is demonstrated here that after acid/chaotropic treatment and reversion to standard conditions of buffer about 30–35% of the BChls are transformed into BPheo. Knowing the B800 BChl/B850 BChl ratio (1:2) (McDermott *et al.*, 1995; Koepke *et al.*, 1996) and by virtue of previous spectral analysis of the reorganized LHII complex (Buche *et al.*, 2000) it could be conclude that the transformation of 1/3 of the BChls into BPheo induces the formation of the 843-nm band while an other 1/3 fraction constitutes the 858-nm band. The remaining BChls are from B800, in dimeric or monomerised form (Buche *et al.*, 2000). We proposed that the BPheos were responsible of the strong circular dichroism signal while B858 was assumed to arise from B850 BChls exciton interactions (Buche *et al.*, 2000). If the heterogeneity of the reorganized B850 is a result of two distinct complexes the electronic interactions between the pigments must be radically different (Somsen *et al.*, 1995; Koolhaas *et al.*, 1997). In this case, it could be expected to separate these two complexes using an ionic liquid chromatography column.

However, the elution profile of a sample previously fixed, treated by acid/chaotropic conditions and reverted to standard conditions of buffer and pH reveals a great uniformity of B850. This result suggests that the B850 heterogeneity of the reorganized complex is not the effect of a distribution of the pigments into two different complexes. Alternatively, we propose that 843- and 858-nm bands are related to BChls fixed to two slightly different binding sites of the polypeptide.

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