Absorption Properties of the Carotenoids after Alkaline Denaturation of the Light-Harvesting Complex II from Ectothiorhodospira sp.

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Alkaline treatment of the Ectothiorhodospira sp. light harvesting system II induces monomerisation of the bacteriochlorophylls and a bleaching of the carotenoid absorption bands in the visible region. Concomitantly, the maximum of absorption observed around 373 nm shifts towards 354 nm. This shift does not result from the Soret band but from a change of the absorption properties of the carotenoids. Furthermore, these pigments are not modified chemically but the spectral conversion results from environmental changes. It is assumed that the dissociation of the bacteriochlorophylls in alkaline medium is accompanied by a structural reorganisation of the complex which reinforces the interactions between the polypeptides and the carotenoids.

Introduction

In photosynthesis, light is absorbed by the pigment-protein antenna complexes and the corresponding excitation energy transferred to the pigment-protein reaction center complexes within the photosynthetic membranes (Sundström and van Grondelle, 1996). Three different types of antenna complexes can be isolated from purple photosynthetic bacteria. The LHII or B880 (Cogdell, 1986; Picorel and Gingras, 1988; Sundström and van Grondelle, 1996) is present in all purple bacteria and remains intimately associated with the reaction center. This antenna is present in all photosynthetic bacteria. The LHIII or B800–850 (Clayton and Clayton, 1979; Feick and Drews, 1978; Picorel et al., 1984; Walker et al., 1988; Doi 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 characterized by one or two strong near infrared electronic absorption bands arising from the Q transition of the bacteriochlorophyll (Bchl) a. The LHII has two bands around 800 (B800) and 850 (B850) nm in its native state (Cogdell, 1986). The crystal structure of the LHII complex from Rhodopseudomonas (Rps.) acidiphila (McDermott et al., 1995) and Rhodospirillum (Rs.) molsichianum (Koepke et al., 1996) were established. The active assembly consists of two concentric cylinders of 90/90 helical protein subunits in Rps. acidiphila or 84/84 in Rs. molsichianum which hold the pigment molecules. Eighteen or sixteen Bchls (depending on the species) are sandwiched between the protein helices near the periplasmic side of the cytoplasmic membrane and another set of nine or eight Bchls (depending on the species) are positioned towards the cytoplasmic side. The Bchls absorbing around 850 nm are excitonically coupled in all photosynthetic bacteria. The Bchls absorbing around 800 nm are normally non-excitonically coupled (Fowler et al., 1992). In certain cases, however, as with Rps. palustris (van Mourik et al., 1992), Rs. molsichianum (Visschers et al., 1995) and Ectothiorhodospira sp. (Buche and Picorel, 1998) these Bchls seem to be excitonically coupled based on circular dichroism (CD) data. The Q transitions present a band around 590 nm. Towards 373 nm, the BxBy transitions generate the “Soret band”.

Abbreviations: Bchl, Bacteriochlorophyll; LH, Light-harvesting; B800, bacteriochlorophylls that absorb around 800 nm; B850, bacteriochlorophylls that absorb around 850 nm; Lys, lysine.
Another class of pigments which contributes to the structure of the light harvesting complexes is the carotenoid (McDermott et al., 1995; Koepke et al., 1996). Generally, the presence of this class of compounds in the LHII complex is easily detected by its absorbance in the visible region (see for example Fowler et al., 1997). However, the carotenoid can strongly absorb in the 340-400 nm region depending on the type, the medium, or on the chemical modification (Boucher et al., 1977; Truscott, 1990). Depending on the species, a light-harvesting complex may contain several types of carotenoids. The LHII complex of Ectothiorhodospira sp. contains spirilloxanthin as the major carotenoid (Ortiz de Zarate, 1995).

Recently, we have investigated the spectral modifications of the LHII Bchl in the presence of high concentrations of NaOH (in preparation). In the present work, it was observed that this process is accompanied by a shift of the absorbance maximum from 373 to 354 nm which would arise from a conversion of the absorption properties of the carotenoids.

**Materials and Methods**

**LHII complex preparation**

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

**Sample preparation**

Highly concentrated solutions of LHII antenna complex were diluted into 1 ml of 10 mM (tris-[hydroxymethyl]aminomethane)-HCl (Tris-HCl) buffer or adequate NaOH solutions. Final NaOH concentration was 9 mM. Final sample concentrations corresponded to 0.4–0.6 absorption units measured at the B800 band. The reversion experiments were carried out first by a 10 fold dilution with 10 mM Tris-HCl, pH 8. The sample was then neutralised either by dialysis against 10 mM Tris-HCl, pH 8 or by injecting HCl directly into the LHII complex-NaOH solutions.

**Spectroscopic measurements**

Absorption spectra were performed by a Beckman DU-640 spectrophotometer at room temperature under 1 cm pathlength. Results reproducibility was verified 3 times. For the analysis of the spectra the GRAMS software (Galactic Industries Co., Salem, NH) was used.

**Pigment extraction**

Pigments were extracted with an acetone/methanol (7/2, v/v) mixture. After sample sonication for 1 min, the extract was centrifuged in a microfuge for 2 min to pellet the undissolved material and the supernatant recovered for further analyses.

**HPLC analysis**

The extracted pigments were analyzed by HPLC basically as in Evans et al. (1988) using the System Gold (Beckman Instrument) equipped with a diode-array detector 168. Pigment separation was achieved with a reversed phase column (25 × 0.46 cm) (Beckman UltraspHERE ODS 5 mm). A linear gradient of 0–100% solvent B (ethyl acetate) and 100–0% solvent A (90% acetonitrile/water, 0.5% triethylamine) was used at a flow rate of 1 ml/min over 25 min.

**Results**

The denaturation effect of 9 mM NaOH on the LHII complex is presented in Figure 1A. After 90 min treatment the carotenoid absorption bands in the 430-550 nm spectral region disappeared completely and the Q_x band of the Bchls blue-shifted from 590 to 569 nm. This corresponded to the monomerisation of the B800 and B850 Bchls (not shown). After 6 min of alkaline treatment a reduction of the Soret band intensity was observed and between 6 and 90 min the absorbance around 354 nm reappeared. At a first glance one could assume that the Soret band has blueshifted from 373 to 354 nm. However, the second derivatives of the absorption spectra (Fig. 1B) suggest that the Soret band, although decreased in intensity, is maintained around 373 nm whereas the 354 band could be generated by the carotenoids.

As shown in Fig. 2, the reversion at pH 8.0 was analysed: After a 90-min alkaline treatment of the LHII complex, the sample was diluted tenfold and
then neutralised to pH 8.0. The Qε band disappeared completely, just as the Qγ bands (not show). The second derivative confirms the removal of the Soret band as well as the bands located between 389 and 402 nm which possibly resulted from Bchl degradation (Ortiz de Zarate, 1995). On the other hand, the 354 nm band remains, its maximum being 3-nm redshifted. In order to check which pigments are responsible for the absorption changes, the pigments of the reverted sample were extracted with an acetone/methanol (7/2) mixture and compared with the pigments of an untreated (control) LHII complex (Fig. 3A,B). Pigments of both treated and untreated samples show the same carotenoid bands in the VIS region. This indicates that the 354 nm band of Fig. 1 does not result from a shift of the Bchl Soret band but from a conversion of the carotenoid bands. However, the HPLC runs of these
two samples were practically identical (Fig. 4) proving that the carotenoids were not modified chemically by the alkaline treatment.

**Discussion**

Recently, we performed a study focused on the effect of various alkaline treatments on the LHII complex of *Ectothiorhodospira sp.* Using high NaOH concentration, we observed that it was very difficult to denature this complex; i.e., up to 1.5 m NaOH the only effect on the absorption spectrum was a reversible blueshift of the B850 which we ascribed to a Lys deprotonation (Buche and Picorel, 1998). The monomerisation of the Bchls was induced using a NaOH concentration higher than 4 m. Concomitantly to the Bchl monomerisation some other spectral modifications appear as a bleaching of the carotenoid bands in the 400–550 nm spectral region. The question arises whether the carotenoids are destroyed or there is only a band shift phenomenon. And also is the
Fig. 3. Pigments extract in acetone/methanol (7/2) of the LHII complex without (dash-dot) or after 90 min NaOH exposure and neutralisation to pH 8 by HCl addition (solid).
A. Spectra from 340 to 830 nm.
B. Second derivatives from 400 to 600 nm.

Absorption maximum shifting from 373 nm to 354 nm the result of a shift of the Bchl Soret band? The spectra presented in the Fig. 1 indicate that the Bchl Soret band is maintained around 373 nm and there is a correlation between the disappearance of the carotenoid bands in the 400–550 nm spectral region and the appearance of the 354 nm band. In addition, after neutralisation of NaOH, the band at 354 nm is practically the only one to remain while the Bchls signals disappear. It should be recalled that in aqueous medium the (B)chl normally cannot stay in its monomeric state. For example, under certain experimental conditions Bchl a aggregates giving both circular dichroism and absorption spectra (Scherz et al., 1991; Uehara, et al., 1995) most similar to those of type II chlorosomes (Somsen and van Grondelle, 1996). In the present work, the maintenance of the monomeric form in aqueous solution seems to be directly related to the presence of a high NaOH concentration. Neutralising the solution seems to induce a total and irreversible loss of solubility. The mechanism of Bchl a self-assembly in aqueous media can be considered as a manifestation of the Hofmeister effect (Koynova et al., 1997; Vladkova, 2000). It relates to the (co)solvent ability to distribute water in the vicinity of the macromolecule.

Fig. 4. HPLC pigment extract chromatograms detected at 485 nm from untreated LHII complex (A) and treated/reverted complex (B).
Analysis of treated sample pigments reveals only the presence of carotenoids. This clearly states that the 354 nm band is due to these pigments. Moreover, the HPLC study indicates that both treated and native samples have the same carotenoid composition. Thus, the carotenoid band shifts from 400-550 nm region towards 354 nm cannot be explained by a chemical modification of these pigments. This phenomenon thus results from an effect of the environment similar to that previously reported for spirilloxanthin in the photoreaction center of Rs. rubrum (Boucher et al., 1977). Differential spectra studies of both wild-type and carotenoid-less mutants show that when spirilloxanthin is attached to the complex, it absorbs relatively more in the 340-400 nm region (\(1^1\text{C} \rightarrow 1^1\text{A} \) transitions) than in the visible region (\(1^3\text{B} \rightarrow 1^3\text{A} \) transitions). On the other hand, if the spirilloxanthin is dispersed in organic or aqueous solution this ratio is markedly reversed. In the present work, apparently the Chl dissociation by NaOH induces a strengthening of the interactions between the protein matrix and the carotenoids.

When a sample is neutralized by HCl or dialysis (see Materials and Methods) identical results are obtained: the 354 nm band is always accompanied by a band around 280 nm in a constant proportion (not shown). This may indicate that the carotenoids remain in contact with the protein matrix throughout the alkaline treatment.

In conclusion, the emergence of a band around 354 nm during the alkaline treatment does not result from a shift of the Chl Soret band but rather from a change of the spectral properties of the carotenoids (primarily spirilloxanthin) resulting from modifications of the environment of these pigments. The new properties of the carotenoids suggest a reinforcement of the binding of these pigments to the protein matrix.

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