Wavelength Shifts Correlation between Near Infrared and Ultraviolet Regions of the LHII Bacteriochlorophyll Spectrum from *Ectothiorhodospira sp.*

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Bacteriochlorophyll, *Ectothiorhodospira*, Light-Harvesting Complex

Bacteriochlorophyll *a* has a maximum at 258 nm previously related to the ring E ester system interacting with the π-system of the macrocycle. In this work, we compared the effect of lauryldimethylamine-N-oxide (LDAO) and alkaline pH on both the near infrared and the ultraviolet region of the LHII spectrum from *Ectothiorhodospira sp.* While LDAO induces only a shift of the 850 nm band arising from the *Qy* transition of the bacteriochlorophyll *a*, alkaline pH also causes a concomitant and reversible 10-nm shift from 258 to 248 nm. Both shifts have similar apparent pKs (12.3 and 12.6, respectively). Interestingly, the presence of NaCl reduces these pKs values to 11.4 and 11.7.

Introduction

The primary processes of photosynthesis involve light absorption, excitation energy transfer and primary charge separation across the photosynthetic membrane. In purple photosynthetic bacteria, light energy is gathered by an extensive system of light-harvesting (LH) complexes ensuring the efficient funneling of excitation energy toward the photochemical reaction centers where the transduction into chemical potential energy takes place (Sundström and van Grondelle, 1996). The LHI or B880 (Cogdell, 1986; Picorel and Gingras, 1988; Sundstöm and van Grondelle, 1996) is present in all purple bacteria and is intimately associated with the reaction center. The LHII or B800–850 (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). 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*.

An engaging feature in photosynthesis constists to understand how the local environnement and the spatial environnement of the pigments influence the energy of their lowest-energy electronic transitions (*Qy*). In this context, several methods inducing a reversible blue shift of *Ectothiorhodospira sp.* B850 were recently described. The detergent lauryldimethylamine N-oxide (LDAO) was reported as inducing a specific blue shift of B850 from 857 to 839–837 nm, and a hypochromism (Ortiz de Zarate and Picorel, 1994). This effect was correlated with a loss of one or two H links between BChl and polypeptides (Ortiz de Zarate, 1995). Similar results were reported in alkaline and to a less extend in acid conditions of buffer conditions of buffer (Buche and Picorel, 1998; Buche *et al.*, 2000). These pH effects were attributed to the (de)protonation of some aminoacids. Especially, the chemical modification of Lys by salicylaldehyde established a correlation between the Lys deprotonation and alkaline induced blue shift of B850 (Buche and Picorel, 1998).

The current work was aimed to compare the effect of LDAO and alkaline pH on the absorption properties of the antenna complex II of *Ectothiorhodospira sp.* The results indicate a shift correlation between B850 and a band around 260 nm when the B850 blue shift is induced by alkaline pH. On the other hand, LDAO has no effect on the UV region of the spectrum. The alkaline induced shift of the 260 nm band is interpreted as a result of the Lys deprotonation on the ring E ester system interacting with the π-system of the macrocycle.

Abbreviations: NIR, near infrared; UV, ultraviolet; BChl, bacteriochlorophyll; LH, light-harvesting; B800, 800-nm absorption band; B850, 850-nm absorption band; LDAO, lauryldimethylamine N-oxide.

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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). Several LHII preparations were then concentrated to obtain an optical density corresponding to an absorbance of 75 at 800 nm and under 1 cm path length. After overnight dialysis against 10 mM (tris-[hydroxymethyl]aminomethane)-HCl (Tris-HCl) pH 8.0, LDAO 0.5% and NaCl 400 mM 200 µl of concentrated sample were introduced onto an FPLC column (Pharmacia column HR 10/30) previously equilibrated with the same buffer. The sample was eluted using the same buffer.

Sample treatment

Adequate pH was adjusted with convenient volumes of HCl or NaOH solution to 10 mM Tris-HCl buffer. A few microliters of highly concentrated solution of LHII antenna complex were then injected into 1 ml buffer solution at chosen pH and LDAO concentration. Final sample concentration corresponded to an absorbance of 0.125 and 0.4 at 797 nm of isolated LHII complexe. The pH reversion from alkaline conditions to more neutral pH was obtained by dialysis against 10 mM Tris-HCl, pH 8.0.

Spectroscopic measurements

Absorption spectra were measured in a Beckman DU-640 spectrophotometer. 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.

Pigment extraction and HPLC analysis

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 insoluble material and the supernatant recovered for further analyses. The extracted pigments were analyzed by HPLC basically as reported (Evans *et al.*, 1988) using the System Gold (Beckman Instrument), equipped with a diode-array detector 168. Pigment separation was achieved by a reverse-phase column (25 × 0.46 cm) (Beckman Ultrasphere ODS 5 µm). 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-1 over 25 min.

Results

As mentioned in the Introduction both LDAO (Ortiz de Zarate and Picorel, 1994) and alkaline pH (Buche and Picorel, 1998) induce a reversible blue shift of LHIIB850 from *Ectothiorhodospira sp.* Both treatments affect poorly the spectral properties of the complex in a wavelength range from the Soret band to B800. However, alkaline pH affects the UV part of the spectrum (Fig. 1A). In this region the maximum of absorption shifts (reversibly) from 258 nm to 248 nm. On the other hand, LDAO (0.05%) has no effect in this spectral region (not shown).

The effect of alkaline pH in different conditions of solvent on B850 and the 258 nm band position is summarized in Figs. 1 B,C. In standard conditions of solvent the 10 nm pH induced shift of the 258 nm band occurs concomitantly to a 17 nm shift of B850 from 852 to 835 nm. The apparent pKs have very close values; 12.6 and 12.3 respectively. As previously reported (Ortiz de Zarate and Picorel, 1994), LDAO 0.05% induces a 15 nm blue shift of B850. But the presence of LDAO in the buffer has a poor effect on the pH induced shift of the 258 nm band. On the other hand, pH completes the B850 shift induced by LDAO. In the presence of NaCl, the apparent pKs are reduced from 12.3 to 11.4 and from 12.6 to 11.7 for B850 and the 258 nm band, respectively.

Figure 2 represents the second derivative of the complex spectrum from 240 to 300 nm taken under different conditions of solvent and pH. The derivative profile of the sample in standard conditions of buffer and at pH 8 shows two bands around 282–288 nm corresponding to Tyr absorbance (Fig. 2 A). A maximum of absorption at 258 nm which is related to the ring E ester of the (B)Chl interacting with the π-system of the macrocycle (Wolf and Scheer, 1973). The absorption spectrum of the HPLC isolated Bchl a (Buche *et*

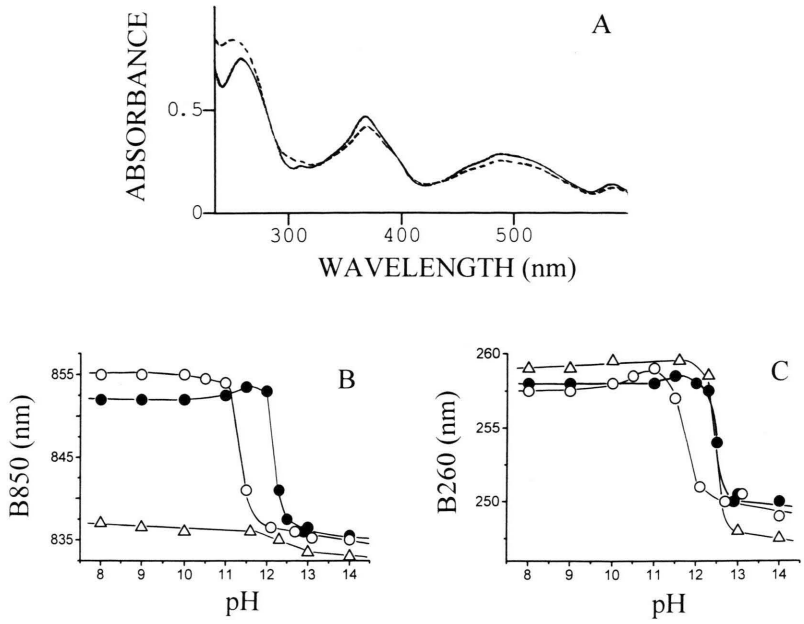

Fig. 1. Electronic absorption spectra LHII from 230 to 600 nm at pH 8 (connected curve) and pH 14 (dashed curve) (A). Blue shift of the 850 nm band (B850) (B) and of the 258 nm band (B260) (C) without any additive (●) in presence of 400 mM NaCl (○) and in presence of 0.05% LDAO (△).

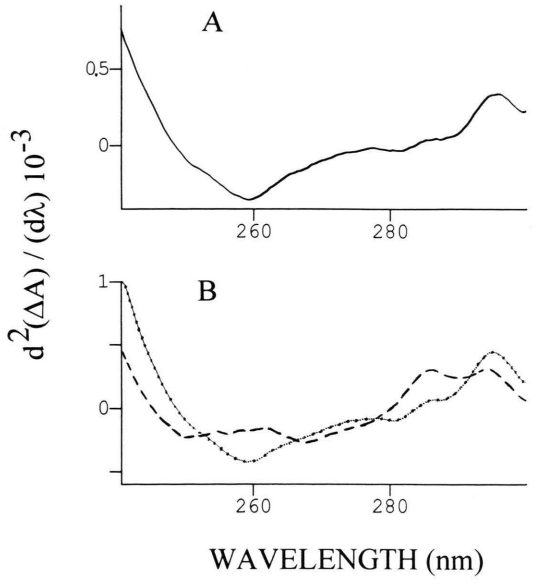

Fig. 2. Second derivative of the UV spectral region of untreated LHII (A) and treated by LDAO 0.05% (dotted curve) or by NaOH at pH 14 (dashed curve) (B).

al., 2000) shows effectively a maximum of absorption around 260 nm (Fig. 3). On the other hand, the sucessive steps of the complex purification, in-

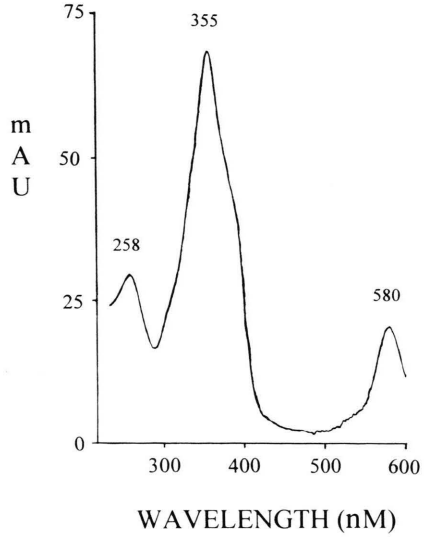

Fig. 3. Spectrum from 230 to 600 nm of Bchl *a* extracted from the complex and analysed by HPLC such a described in Materials and Methods.

cluding a supplementary FPLC procedure, render highly improbable the presence of contaminants or degradation products in the sample (see Materials and Methods). Figure 2 B compares the second derivative of the UV spectra of samples treated by LDAO 0.05% and by extreme condition of alkaline pH. (pH 14). The detergent has no effect on the UV spectral properties of the complex and the second derivative is thoroughly similar to that corresponding to the untreated sample. In alkaline condition the Tyr's bands at 282–288 nm shift to 292 which is characteristic of the deprotoned Tyr (Buche and Picorel, 1998). Previously, we demonstrated that this shift does not occur concomitantly to B850 blue shift (pK = 12.3) but at pH between 13 and 14 (Buche and Picorel, 1998). So Bchl and Tyr shifts could not be correlated. But at pH 14 it can be observed that the spectral region from 268 to 278 nm, corresponding to Trp, remains unchanged. On the other hand, the 258 nm band shifts to 248 nm. So we reported here for the first time a NIR-UV correlation suggesting that the B850 blue shift, induced by alkaline pH and by detergent, could be a result of different effects on the porphyrin ring.

Discussion

Previously, several treatments inducing a reversible and specific blue shift of the *Ectothiorhodospira sp.* B850 were reported (Ortiz de Zarate and Picorel, 1994; Buche and Picorel, 1998; Buche *et al.*, 2000; Buche, 2000). The detergent LDAO generates a 19 nm blue shift of this band which was attributed to the loss of one or two H links (Ortiz de Zarate and Picorel, 1995). The amino acid(s) involved in these links are not known. However, from the sequence of both the α and β (β1 and β2) polypeptides of this complex (unpublished results) we know that both Trp and a Tyr are close to the B850 binding site. On the other hand, a strong correlation exists between alkaline pH and chemical modification of Lys by salicylaldehyde (Buche and Picorel, 1998). Both treatments induce a reversible blue shift of B850, totally comparable to that induced by LDAO, indicating the deprotonation of Lys as the cause of the alkaline induced blue shift. Because Lys is poor at H bonding, this observation indicates that the positive charge of the proton is responsable for blue shift control. Consequently, charge or H bonding modifications could exert the same control on the red shifting mechanism of B850. Nevertheless, in this work we observed that both LDAO and alkaline treatments differ in their effect in the UV region of the complex spectra. While LDAO induces only a reversible shift of B850, alkaline pH also causes a concomitant and reversible 10 nm shift from 258 to 248 nm. Both shifts have similar apparent pKs (12.3–12.6). Interestingly, the presence of NaCl reduces this pK values to 11.4–11.7 confirming that a charge process related to the 258 nm band exerts a control on the B850 redshifting mechanism. This 258 nm band is obviously originated by Bchl *a* (Fig. 3). While main of the Bchl absorption bands were ascribed to π-π* transitions of the aromatic system isoconjugated to the [18]-annulene (Wolf and Scheer, 1973; Hanson, 1988) some of them, located in the UV region between 220–370 nm, have not been completely correlated with defined transitions. However, a comparison of the ORD spectra of the enantiomeric 10(S) and 10 (R) methoxy pheophorphyrins shows an inversion of the sign of these bands including the 260 nm with is the more important band between 220 and 300 nm (Wolf and Scheer, 1973). This indicates that the ring E ester system interacts with the π system and that the 260 nm band is in fact a manifestation of this interaction.

The fact that NaOH but not LDAO induces this 260 nm band shift suggests that the energetical level of B850 is under punctual charge(s) control via the ring E ester. It is worth noting that the electrochromic effect upon (B)chl chromophores of charged amino acid residues were modeled by calculations with point charges (Eccles and Honig, 1983; Pancoska *et al.*, 1983; Hanson *et al.*, 1987 a,b,c; Eccles *et al.*, 1988; Hanson, 1991). For example a 70 nm redshift of the Chl a *Qy* band could be induced by a single point charge placed 3.5 Ä above the macrocycle (Eccles and Honig, 1983). In this context, it was previously reported in *Rb. sphaeroides* that a Lys situated at −17 amino acids from the putative B850 binding site of the β polypeptide exerts a 17 nm redshift on B850 (Fowler *et al.*, 1992).

In the case of *Ectothiorhodospira sp.* LHII, β1Lys and β2Lys are situated at −17 and −14 amino acids respectively from the His co-ordinated to B850Bchl magnesium (unpublished re-

sults). These Lys have probably no effect on the red shifting of B800 because of their central position with regard to the porphyrin (Eccles and Honig, 1983; Pancoska *et al.*, 1983; Eccles *et al.*, 1988). On the other hand, in view of the structural data of Mc Dermott and collaborators (1995) *on Rps. acidophila*, the distance center to center between both B800 and B850 Bchls is 17 Ä. Basically, the architecture of LH2 from *Rsp. molischianum* was found analogous (Koepke *et al.,* 1996) and it should be assumed that the distance between both Bchls rings is similar for LH2 from purple bacteria (for review see Leupolds *et al.,* 2000). Consequently, on *Ectothiorhodospira sp.* LHII, β1 Lys and β2 Lys could be situated at less that 13–15 Ä from the keto ester of ring E. In view of the electrochromic theories this situation could perfectly control a 20 nm red shift of B850.

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Buche A. and Picorel R. (1998), Spectral changes induced by alkaline pH and specific chemical modification of amino acid residues in the light-harvesting II antenna complex from *Ectothiorhodospira sp.* Photochem. Photobiol. **69**, 275–281.

Buche A. and Picorel R. (2000), Absorption properties of the carotenoids after alkaline denaturation of the light-harvesting complex II from *Ectothiorhodospira sp.*, Z. Naturforsch. **55c**, 576–581.

Buche A. (2000), On the reorganisation of the LHII 850 nm band of *Ectothiorhodospira sp.* after acidic/chaotropic treatment. J Biochem. Mol. Biol. Biophys (in press).

Buche A., Ramirez J. M. and Picorel R. (2000), Effects of acid pH and urea on the spectral properties of the LHII antenna complex from the photosynthetic bacterium *Ectothiorhodospira sp.* Eur. J. Biochem. **267**, 3235–3243.

Clayton R. K. and Clayton B. J. (1981), B850 pigment-protein complex of *Rhodopseudomonas sphaeroides* extinction coefficients, circular dichroism, and the reversible binding of bacteriochlorophyll. Proc. Natl. Acad. Sci. USA **78**, 5583–5587.

Cogdell R. J. (1986), Light-harvesting complexes in the purple bacteria. In: Encyclopaedia of Plant Physiology, Photosynthesis, III vol. **19** (Staehelin, L. A. & Arntzen, C. J., eds). Springer-Publ., Berlin, pp. 252–259.

McDermott G., Prince S. M., Freer A. A., Hawthornthwaite-Lawless A. M., Papiz M. Z., Cogdell R. J. and Isaacs N. W. (1995), Crystal structure of an integral membrane light harvesting complex from photosynthetic bacteria. Nature **374**, 517–521.

Doi M., Shioi Y. Gad'on N., Golecki J. R. and Drews G. (1991), Spectroscopical studies on the light-harvesting pigment-protein complex II from dark-aerobic grown cells of *Rhodobacter sulfidophilus.* Biochim. Biophys. Acta **1058,** 235–241.

Eccles J. and Honig B. (1983), Charged amino acids as spectroscopic determinants for chlorophyll in vivo. Proc. Natl. Acad. Sci. USA **80**, 4959–4962.

Eccles J., Honig B. and Schulten K. (1988), Spectroscopic determinants in the reaction center of *Rhodopseudomonas viridis.* Biophys. J. **53**, 137–144.

Evans M. B., Cogdell R. J. and Britton G. (1988), Determination of the bacteriochlorophyll: carotenoid ratios of the B890 antenna complex of *Rhodospirillum rubrum* and the B800–850 complex of *Rhodobacter sphaeroides.* Biochim. Biophys. Acta **935**, 292–298.

Fowler G. J. S., Crielaard W., Visschers R. W., van Grondelle R., and Hunter C. N. (1992), Site-directed mutagenesis of the LH2 light-harvesting complex of *Rhodobacter sphaeroides* changing bLys22 to Gln results in a shift in the 850 nm absorption peak Photochem. Photobiol. **57**, 2–5.

Hanson L. K., Fajer J., Thompson M. A. and Zerner M. (1987), Electrochromic effects of charge separation in bacterial photosynthesis: theoreticals models. J. Am. Chem. Soc. **109**, 4728–4731.

Hanson L. K., Thompson M. A. and Fajer J. (1987), Environmental effects on the properties of chlorophylls *in vivo.* Theoretical models. In: Progress in Photosynthesis Research, Vol. **1** (Biggins, J. Ed.). Martinus Nijhoff Publ., Dordrecht, pp 331–317.

Hanson L. K., Thompson M. A., Zerner M. C. and Fajer J. (1987), Theoreticals models of electrochromic and enviromental effects on bacteriochlorophylls and -pheophytins in reaction centers. In: The Photosynthetic Bacterial Reaction Center. Structure and Dynamics (Breton, J. and Vermeglio, A., Eds.). Plenum Press, New York, pp 355–368.

Hanson L. K. (1988), Theorical calculations of photosynthetic pigments. Photochem. Photobiol. **47**, 903–921.

Hanson L. K. (1991), Molecular orbital theory of monomer pigments. In: Chlorophylls (Scheer, H., Ed.). CRC Press, Boca Raton FL. pp. 993–1046.

Hayashi H. and Morita S. (1980), Near-Infrared absorption spectra of light-harvesting bacteriochlorophyll protein-complexes from *Chromatium vinosum.* J. Biochem. **88**, 1251–1258.

Koepke J., Hu X., Muenke C., Schulten K. and Michel H. (1996), The crystal structure of the light-harvesting complex II (B800–850) from *Rhodospirillum molischianum.* Structure **4**, 581–597.

Lefebvre S., Picorel R.,Cloutier Y. and Gingras G. (1984), Photoreaction center of *Ectothiorhodospira sp.* Pigment, heme, quinone, and polypeptide composition. Biochemistry **23**, 5279–5287.

Leupold D., Voigt B., Beenken W. and Stiel H. (2000), Pigment-protein architecture in the light-harvesting antenna complexes of purple bacteria: does the crystal structure reflect the native pigment-protein arrangement. FEBS Lett. **480**, 73–78.

Monger T. G. and Parson W. W. (1977), Singlet-triplet fusion in *Rhodopseudomonas sphaeroides* chromatophores. A probe of the organization of the photosynthetic apparatus, Biochim. Biophys. Acta **460**, 393–407.

Ortiz de Zarate I. (1995), Estudio estructural de las antenas de bacterias purpuras mediante técnicas espectroscópicas, Doctoral Thesis, University of Zaragoza.

Ortiz de Zarate I. and R. Picorel (1994), Spectral changes of the B800–850 antenna complex from *Ectothiorhodospira sp.* induced by detergent and salt treatment. Photosynth. Res. **41**, 339–347.

Pancoska P., Kapoun M. and Skala L. (1983), The effect of external charges on the absorption spectra of porphyrins. Quantum mechanical discussion. Photobiochem. Photobiophys. **6**, 39–46.

Picorel R. and Gingras G. (1988), Preparative isolation and characterization of the B875 complex from *Rhodobacter sphaeroides* 2.4.1. Biochem. Cell Biol. **66**, 442–448.

Sturgis J. N. and Niederman R. (1990), Acid denaturation of the B875 light-harvesting complex in membranes of *Rhodobacter sphaeroides* M21.Photosynth. Res. **23**, 241–248.

Sundström, V. and van Grondelle, R. (1996), Kinetics of excitation transfer and trapping in purple bacteria. In: Anoxygenic Photosynthetic Bacteria, (Blankenship, R., Madigan, M. T. & Bauer, C. E., eds.). Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 349–372.

Walker T., Gad'on N., Steck K., Welte W. and Drews, G. (1988), Isolation of reaction center and antenna complexes from the halophilic purple bacterium *Rhodospirillum salexigens.* Biochim. Biophys. Acta **933**, 299–305.

Wolf H. and Scheer H. (1973), Stereochemistry and chiroptic properties of pheophorbides and related compounds. Ann. N. Y. Acad. Sci. **202**, 549–567.