Alkaline Denaturation of the Light-Harvesting Complex II from the Purple Bacterium *Ectothiorhodospira Sp.*: Kinetic Evidence of the Existence of the 780 nm Upper Exciton Component of the B850 Bacteriochlorophylls[†]

André Buche* and Rafael Picorel

Estación Experimental de Aula Dei (CSIC), Apdo. 202, 50080 Zaragoza, Spain

Received June 30, 2000; Revised Manuscript Received November 28, 2000

ABSTRACT: 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 the bacteriochlorophyll *a*. In the present work, high concentrations of NaOH were used to study the destabilization of the complex of the *Ectothiorhodospira sp.* The majority of the bacteriochlorophylls were monomerized within 90 min of treatment. However, the kinetic patterns of the two near-infrared bands were remarkably different. After an instantaneous blue shift from 853 to 828 nm, B850 showed a first-order monomerization with a rate constant of -0.016 min^{-1}. This instantaneous blue shift was previously attributed to the deprotonation of a lysine and was independent of the monomerization process. The observed native B800 is in fact composed of two bands, one at 796 nm and the other at 780 nm. The band absorbing at 780 nm red shifted also instantaneously to 786−788 nm and then disappeared in a first-order process as B850. The other band absorbing at 796 nm has a two-step process of monomerization; after a rapid conversion a slower first-order process occurred with a rate constant of -0.025 min^{-1}. The similarity between the kinetic behaviors of B850 and the 780 nm band indicated a strong relationship between these two bands. Our interpretation of the results considers the 780 nm band as the upper exciton component of the B850 bacteriochlorophylls.

In photosynthesis, light is absorb by the pigment−protein antenna complexes and the corresponding excitation energy transferred to the reaction center complexes within the photosynthetic membranes (*1*). Three different types of antenna complexes can be isolated from purple photosynthetic bacteria. The LHI or B880 (*1*−*3*) is present in all purple bacteria and remains intimately associated with the reaction center. The LHII or B800−850 (*4*−*8*) and LHIII or B800−820 (*9*, *10*) are arranged more peripherally (*11*). 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 (NIR)[1] electronic absorption bands arising from the Q_y transition of the bacteriochlorophyll (Bchl) *a*. The LHII has two bands around 800 (B800) and 850 (B850) nm in its native state (*2*).

The crystal structures of the LHII complex from *Rhodopseudomonas* (*Rps.*) *acidophila* (*11*) and *Rhodospirillum* (*Rs.*) *molischianum* (*12*) 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 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 Bchls (depending on the species) are positioned toward the cytoplasmic side. The Bchls absorbing around 850 nm are excitonically coupled in all photosynthetic bacteria, but the Bchls absorbing around 800 nm are normally nonexcitonically coupled (*14*). However, in certain cases such as *Rps. palustris* (*15*), *Rs. molischianum* (*16*), and *Ectothiorhodospira sp.* (*17*) these Bchls seem to be excitonically coupled on the basis of circular dichroism data.

An interesting feature of the native biological photosynthetic systems is the red shifting of their pigment absorption bands compared to the pigments in solution. In the case of the LHII of *Ectothiorhodospira sp.*, B800 and B850 show maxima at 797 and 857 nm, respectively, with a ratio A_{857}/A_{797} around 1.5−1.6 when bound to the membrane. In addition, specific blue shifts of the NIR bands induced by physical, chemical, or biochemical treatments of the LHII complex are very well documented in a large variety of bacteria (*5*, *6*, *18*−*20*). Different hypotheses were proposed to explain this phenomenon. Some authors attribute the in vivo band red shifting to strong excitonic interactions between Bchls (*21*−*24*), but others relate the band red shifting to strong interactions between the apoprotein and Bchls (*25*−*27*). Both theoretical (*27*−*30*) and experimental (*12*, *20*, *31*−*34*) evidence of electrostatic effects or hydrogen bonding between protein and Bchls is extensively documented.

[†] This work was supported by EC Contract CT93-0278 and the DGICYT (Grant PB98-1632).

* Corresponding author. Phone: 34-976-576511. Fax: 34-976-575620. E-mail: abuche@eead.csic.es.

[1] Abbreviations: Bchl, bacteriochlorophyll; B850, electronic absorption band with maximum around 850 nm; B800, electronic absorption band with maximum around 800 nm; B760, electronic absorption band with maximum around 760 nm; LH, light harvesting; NIR, near-infrared; UV, ultraviolet; VIS, visible.

10.1021/bi001499c CCC: $20.00
Published on Web 02/02/2001

The present work was aimed to investigate the absorption properties of the Bchls from the *Ectothiorhodospira sp.* LHII complex induced by high concentrations of NaOH. The results show that the observed B800 is in fact composed of two bands. On the basis of kinetic analyses and in virtue of the work by Koolhaas and collaborators (*36*), one of this band absorbing at 780 nm is identified as the upper exciton component of the B850 Bchls.

MATERIALS AND METHODS

LHII Complex Preparation. Ectothiorhodospira sp. was grown photosynthetically as described by Lefebvre et al. (*36*). Preparations of the LHII antenna complex were obtained as described previously (*20*) with some modifications (*17*).

Sample Preparation. Highly concentrated solutions of the LHII complex were diluted into 1 mL 10 mM Tris-HCl and adequate NaOH solutions. Final sample concentrations corresponded to an OD of 0.5−0.75 measured at B800 maximum with 1-cm path-length cuvettes. The reversion experiments were carried out by injecting concentrated HCl directly into the LHII/Tris-HCl/NaOH solutions. The samples were then extensively dialyzed against 10 mM Tris-HCl, pH 8.

Spectroscopic Measurements. Absorption spectra were carried out in a Beckman DU-640 spectrophotometer at room temperature with a 1-cm path-length cuvette. The reproducibility of the results was verified three times.

Spectral Deconvolutions and Derivatives. The spectral treatments were carried out using the GRAMS software (Galactic Industries Co., Salem, NH). The second derivatives were calculated using the Gap procedure of this software. Spectral deconvolutions were done using the interactive method with a linear baseline.

Extinction Coefficient and Bchl Concentration Determinations. In a preliminary step, the LHII pigments were extracted with acetone−metanol (7:2, by volume) as previously described (*37*). Using an extinction coefficient of 70 $mM^{-1}\cdot cm^{-1}$ at 771 nm (*3*), a correlation could be established between the absorption of the different bands and their corresponding Bchl concentrations. The monomerization process of the LHII Bchls induced by the organic mixture could be related to the alkaline treatment product by

$$A_{\max}^{\ B771} = A_{\max}^{\ B760} K$$

where K is a coefficient of proportionality.

Consequently, the total Bchl concentration in LHII is

$$C_{\mathrm{Bchl}}^{\ B760} = A_{\max}(K/70)$$

Knowing that the ratio $Bchl_{800}$:$Bchl_{850}$ is 1:2 (*12, 13*), this relation is sufficient to calculate both the extinction coefficients and Bchl concentrations of the different absorption bands.

RESULTS

The absorption spectra of the LHII complex both in the absence and in the presence of 4 M NaOH are shown in Figure 1A. Sodium hydroxide at a concentration of 4 M induces a 25 nm blue shift and hypochromism of B850 with some minor modifications of the Q_x and Soret bands. These

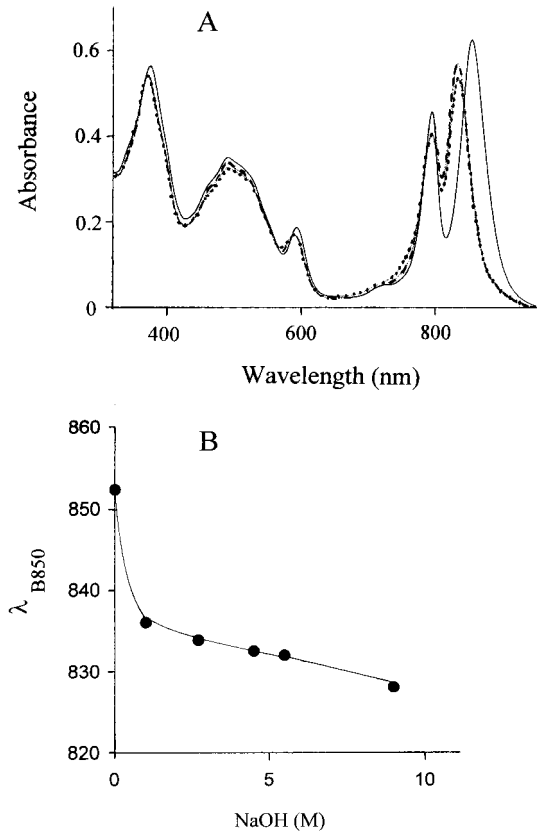

FIGURE 1: Electronic absorption spectra of the LHII antenna complex in standard buffer conditions (continuous curve), immediately (dashed-dotted curve) or 30 min (dotted curve) after 4 M NaOH treatment (A). Instantaneous effect of different NaOH concentration treatment on the wavelength position of B850 (B).

modifications consist in a 2−3 nm blue shift with a small hypochromism. The intensity of the carotenoid bands also decreases slightly. After 30 min of alkaline treatment only a small additional hypochromism of the Q_y and carotenoid bands was observed.

Figure 1B presents the instantaneous blue shift of the LHII B850 as a function of the NaOH concentration. Below 4 M, this effect is the main modification observed in the 300−950 nm spectral region (not shown). At higher NaOH concentrations a number of more pronounced spectral changes appear. The time dependence of these changes in 9 M NaOH is shown in Figure 2. After an instantaneous blue shift of the B850 from 853 to 828 nm both B800 and the new band at 828 nm are converted to a spectral form absorbing at 763 nm (B760) (Figure 2A). The presence of an isosbestic point around 778 nm suggests that this conversion takes place without intermediate states. In fact, with the exception of the first step that corresponds to the instantaneous blue shift of B850, the decrease of the area under the 800 and 828 nm bands remains proportional to the increase of the area under B760 during all the conversion processes (not shown). To further characterize the different spectral forms present, the second derivatives of the spectra were obtained (Figure 2B). B800 shows an asymmetrical shape and conserves it during all of the conversion process. Figure 2C presents the spectral evolution of the LHII complex in the 300−620 nm region. The Q_x band shifts from 592 to 571 nm with concomitant hypochromism. The carotenoid absorbance in the 430−550 nm spectral region

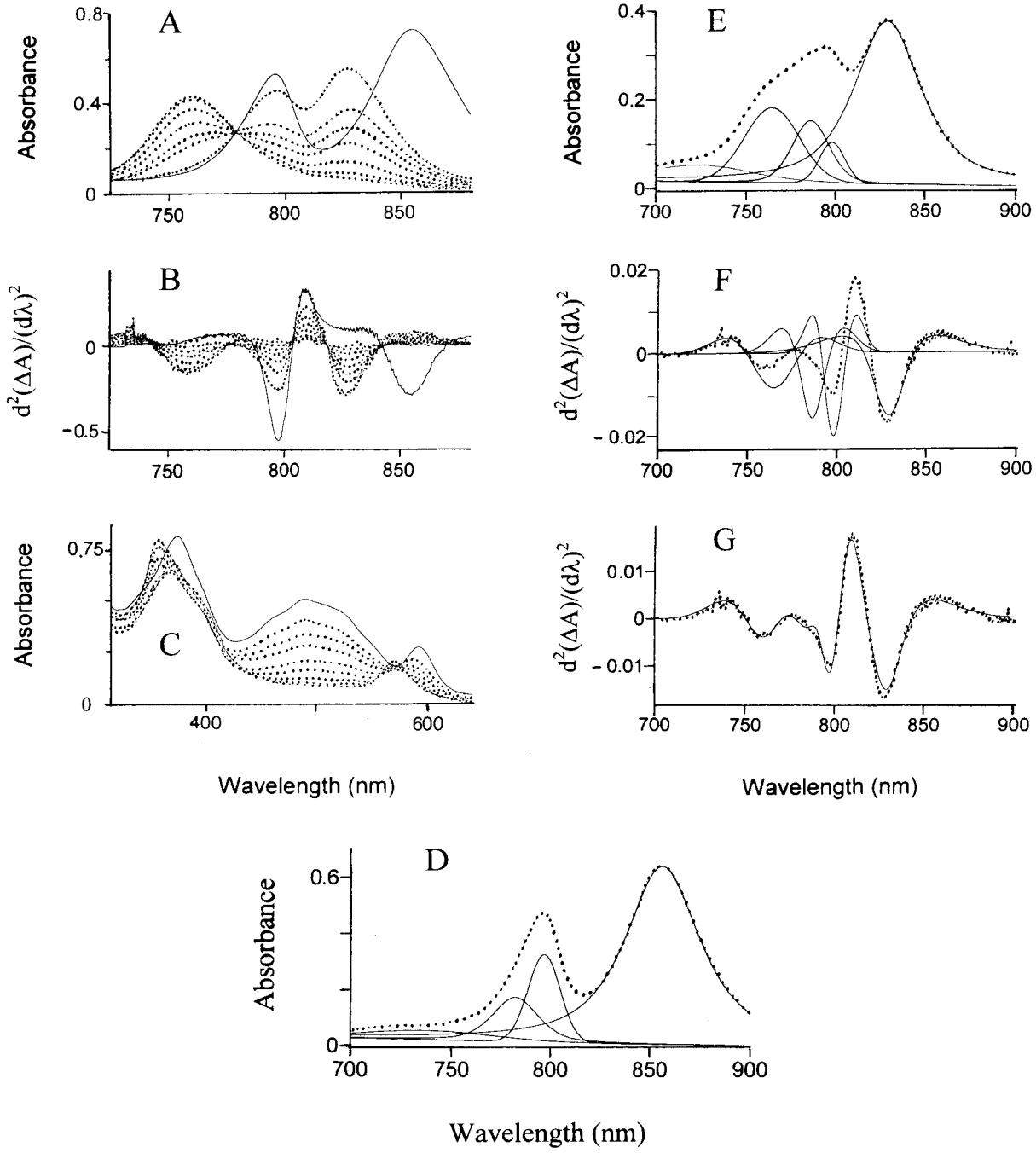

FIGURE 2: Effect of 9 M NaOH on the NIR spectral properties (A), the corresponding second derivative (B), and the 320−620 nm spectral region (C). The continuous line represents the LHII complex spectrum at $t = 0$. The dotted lines represent the complex after several times of 9 M NaOH treatment from 0.5 to 90 min. Electronic absorption spectrum (dotted curve) and deconvolution bands at 780, 796, and 856 nm, respectively (continuous curves), of the untreated LHII complex (D). Deconvolution bands at 762, 786, 797, and 829 nm (continuous curves) of the LHII NIR absorption spectrum (dotted curve) after 20 min of 9 M NaOH treatment (E). Comparison between second derivatives of the complex (dotted curve) and the deconvolution bands (continuous curves) (F). Comparison between the second derivative of the complex (dotted curve) and the sum of the derivatives of deconvolution bands (continuous curve) (G).

disappears almost completely within 90 min of treatment. In the meantime, an absorption band appears around 354 nm. Recently, we demonstrated that the emergence of a band around 354 nm during the alkaline treatment did not result from a shift of the Bchl Soret band but rather from a change of the spectral properties of the carotenoids (primarily spirilloxanthin) resulting from modifications in the environment of these pigments (*38*).

Further analysis of the kinetic patterns of the processes presented in Figure 2A involves spectral deconvolution methods. An example of this method is presented in Figure 2D. As mentioned in the introduction, the LHII complex in standard buffer conditions at pH 8.0 shows two NIR bands corresponding to B800 and B850. A small band is observed near 725 nm, probably due to some Bchl degradation occurring during the LHII isolation and purification process (*39*). However, the interactive process presented in Materials and Methods does not converge if we assume only the presence of these three bands (not shown). In fact, as mentioned above the second derivative of the LHII NIR spectrum (Figure 2B) indicates an asymmetrical shape of B800. In this context and assuming two transitions for B800,

the interactive process converges, showing two bands around 780 and 796 nm (Figure 2D).

As shown in Figure 2A, a concentration of 9 M NaOH induces a progressive conversion of B800 and B850 into B760, but the deconvolution procedure has to take into account the presence of this new transition at 780 nm. Figure 2E presents the results of the spectral deconvolution after 20 min of alkaline treatment, i.e., the B850 blue shifted up to 828 nm, the B800 decomposed in two contributions at 796 and 786 nm, and a band at 763 (B760) were apparent (Figure 2E). Figure 2F compares the second derivative of the original spectrum with the second derivative of every single deconvoluted band. It is noticeable to observe that the sum of the second derivative of every deconvoluted band is very similar to the second derivative of the original spectrum (Figure 2G).

The same deconvolution method was applied to all of the spectra of Figure 2A. The absorption spectra of these three bands, B850, B800 (i.e., the sum of 796 and 780 nm band contributions), and B760, are presented in Figure 3A. Both B850 and B760 show strong variations in the maximum band wavelengths. In fact, these changes were already partially discernible in the original spectra of Figure 2A. B850 blue shifts instantaneously from 853 to 828 nm. B760 appears initially around 770 nm but shifts progressively to 763 nm within the 30 min NaOH incubation period (Figure 3A). Figure 3B represents the kinetics of the absorption maximum shift of B800, B850, and B760 as a function of time. The absorbance evolution of both B850 and B760 is perturbed between the 0 and 30 min period. These perturbations probably are related to the absorption maximum shifts observed for these two bands during this time period. On the other hand, B800 presents a good exponential decay. Figure 3C represents the time dependence of both the 780 and the 796 nm bands which constitute the observed B800. The 796 nm band is initially the main component of the observed B800 but in 0.5 min of 9 M NaOH treatment the 780 nm band appears as the main B800 component due to a strong decrease of the 796 nm band. The 780 nm band shows a 8 nm red shift with hyperchromism occurring concomitantly to the instantaneous blue shift with hypochromism of B850 (Figure 3D). Figure 3E summarizes the time course of the maximum position of every band. After 30 s of treatment the wavelength position of B780 stays at 786 nm. On the contrary, B796 shows poor modification of its wavelength position which is stabilized at 797−797.5 nm after 30 s. The different behavior between the 780 and the 796 nm bands is a first demonstration that the asymmetry of the observed B800 results in fact from the presence of different absorption bands.

To avoid the effect of the blue/red shifting phenomena on the kinetic patterns, it may be interesting to convert the absorbance data of Figure 3B to pigment concentration values. To calculate these values, we need essentially two things: (i) One is the absorbance value of B760 after total conversion (A_{max} of B760). This value can be obtained by extrapolating the four last points of Figure 3B such as expressed in Figure 3F ($A^{763\ nm} = 0.49$). Using the procedure described in Materials and Methods, we deduce K and the corresponding concentration (C) of Bchls (7.8 μM). (ii) The other is a correction of the time-dependent curve of B760. This was carried out using a logarithmic extrapolation of the four last points of B760 absorbance of Figure 3B (not shown). From this procedure, the evolution of B760, the observed B800 sum, and B850, expressed in Bchls concentrations, were plotted as a function of time (Figure 3G).

The results of Figure 3G were plotted using a first- or second-order process, and good straight lines were obtained (not shown). However, in the case of B800 and B760 the value at $t = 0$ remains away from the straight line. This confirms that the conversion process is a two-step process: (i) a rapid step occurring within 30 s and (ii) a second step that needs more than 90 min to obtain a total conversion. As observed in Figure 3G, this two-step process does not practically concern B850, which is essentially involved in an instantaneous blue shift (Figure 3A) during the first 30 s. In Figure 4, $t = 0.5$ min is considered as the first time of the second step of the kinetic process. B850 and B760 are well expressed by a first-order plot (Figure 4A) while B800 is better expressed by a second-order plot (Figure 4C). In this procedure, we have treated the observed B800 as one population. But the observation that the conversion of B800 into B760 is a second-order process points at once to the fact that the observed B800 may be composed of two different bands. Thus, we reproduced the calculation expressed in Figures 3G and 4A, substituting the absorption time dependence of B800 by the corresponding plot of the 796 nm band. The final results, expressed in Figure 4B, show that the time-dependent plots of B760, B850, and the 795 nm band conversion are all first-order processes. These results not only confirm the presence of two bands in the observed B800 but also demonstrate that both the 780 nm band and B850 are absorption bands of the same Bchls. Indeed, from the current study the monomerization process could be summarized by the following first-order relation:

$$\delta[\mathrm{B760}]/\delta t = k_1[\mathrm{B760}] = -k_2[\mathrm{B796}] - k_3[\mathrm{B850}] \qquad (1)$$

where [B760], [B796], and [B850] are the concentrations of the different Bchls and k_1, k_2, and k_3 the corresponding rate constants. Furthermore, from the Bchl ratio normally found in LHII systems (*12*, *13*) we know that this equation should respect the relation

$$k_1 = -(k_2 + 2k_3)/3 \qquad (2)$$

The rate constants deduced from Figure 4, i.e., $k_1 = 0.018$ (± 0.002) min^{-1}, $k_2 = -0.025$ (± 0.003) min^{-1}, and $k_3 = -0.015$ (± 0.002) min^{-1}, confirm eq 2. Consequently, eq 1 describes effectively the monomerization process, and the 780 nm band could be related to B850 Bchls. The interest of these results will be estimated in the Discussion.

DISCUSSION

The aim of this work was to study the spectral modifications of the LHII complex of *Ectothiorhodospira sp*. under denaturation induced with strong NaOH concentrations. These experimental conditions were chosen on the basis of previous observations that suggested a strong stability of this LHII complex in various chaotropic media. For instance, 8.9 M urea had no significant effect on the NIR spectral properties of the complex at normal pH (*40*). Furthermore, high concentrations of urea were necessary to induce denaturation of this antenna complex at pH as low as 2.1−

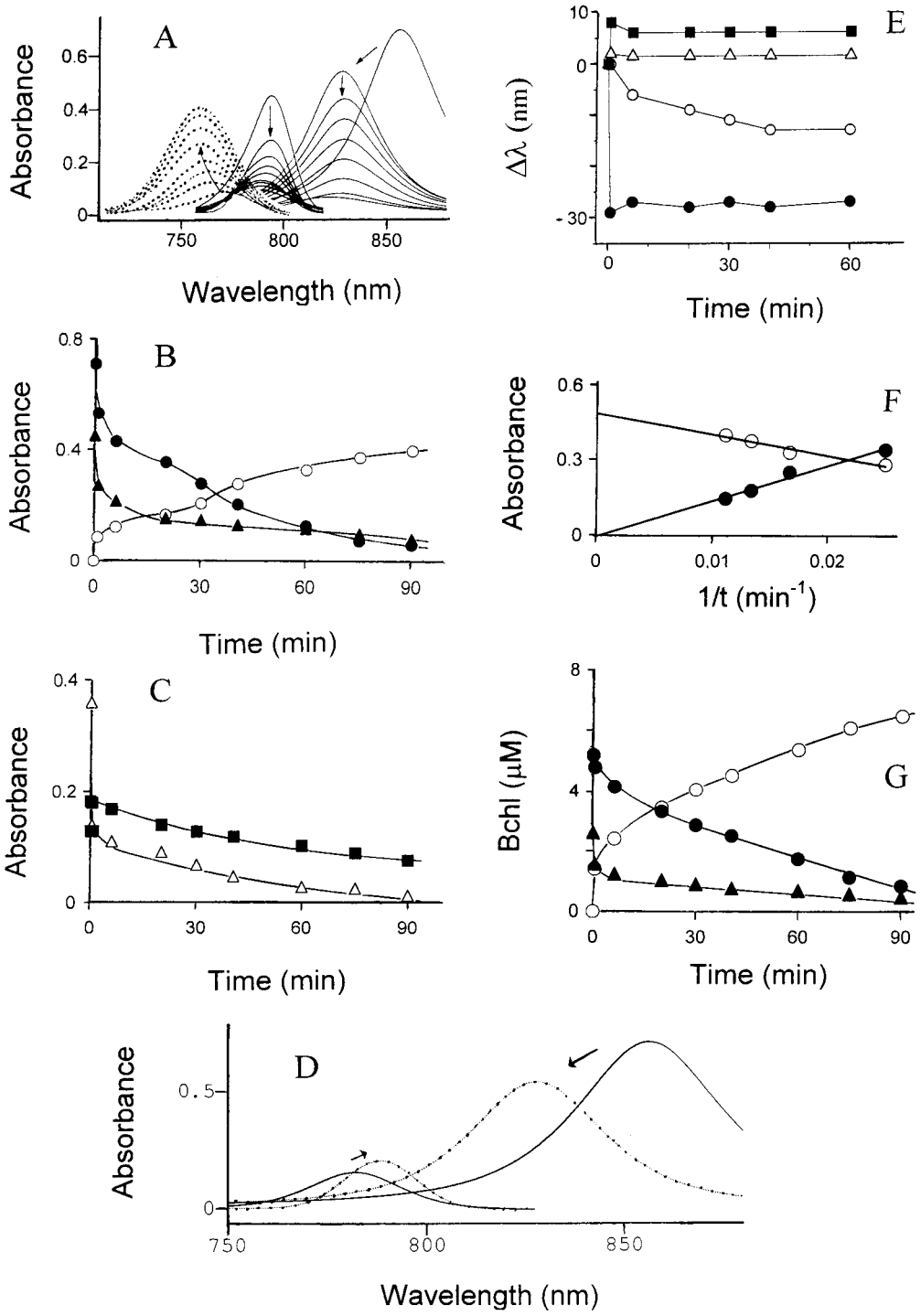

FIGURE 3: Deconvolution bands of B850, B800 (continuous curves), and B760 (dotted curves) at different times of 9 M NaOH treatment: 0, 0.5, 6, 20, 30, 40, 60, 75, and 90 min (A). Time dependence of the absorption band intensity of B850 (●), B800 (▲), and B760 (○) (B). Time dependence of the absorption band intensity of B780 (■) and the 796 nm band (△) (C). Profiles of deconvoluted bands of B780 and B850 before (continuous curves) and after 30 s of alkaline treatment (dotted curve) (D). Time course of the shift ($\Delta\lambda$ expressed in nm) of B760 (○), B780 (■), B850 (●), and the 796 nm band (△) (red shift and blue shift correspond to positive and negative values of $\Delta\lambda$, respectively) (E). Extrapolation of the absorption band maximum of B760 (○) and B800 + B850 (●) to higher time values (F). Time dependence of B850 (●), B800 (▲), and B760 (○) expressed in μM Bchl (G).

2.2 (*40*, *41*). It is noticeable that in that case the denaturation was a reorganization of the complex rather than a process of randomization. Finally, the complex showed a strong stability in alkaline buffer conditions; i.e., only a reversible 17−20 nm blue shift of B850 was induced upon pH increasing to 11 (*17*).

In the presence of 4 M NaOH, the main change consisted in a 25 nm blue shift from 853 to 828 nm of B850. In these experimental conditions both the Bchl Soret and the carotenoid bands showed only minor modifications (Figure 1A). NaOH concentrations higher than 4 M were necessary to induce significant time-dependent spectral modifications of the LHII antenna complex. Data corresponding to incubation with 9 M NaOH are those presented in this work. Both B800 and B850 were converted progressively into a spectroscopic form absorbing at 763 nm (B760). The main part of the

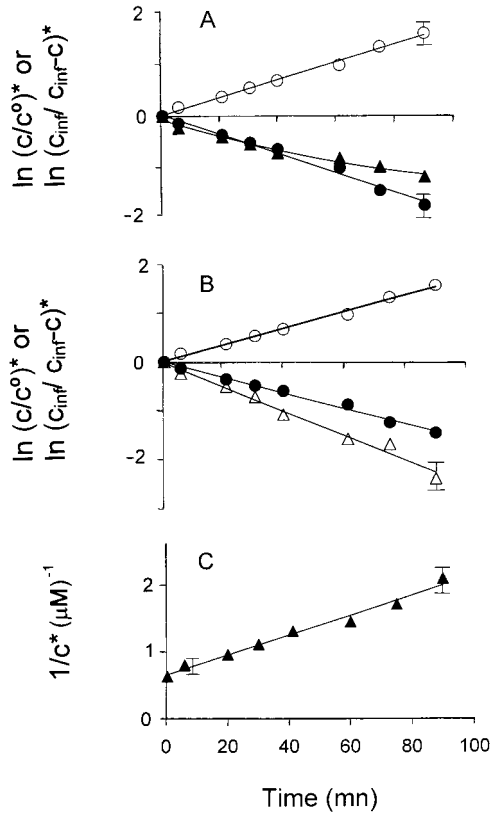

FIGURE 4: First- (A, B) and second- (C) order plots of the kinetics induced by the alkaline treatment. B800 (▲), B850 (●), and the 795 nm band (△) are expressed by $\ln(c/c^0)$, where c^0 is the initial concentration of B800 or B850 Bchl while c is the Bchl concentration as a function of time. B760 (○) is expressed by $\ln[c_{inf}/(c_{inf} - c)]$, where c_{inf} is the total Bchl concentration in the complex. The asterisk indicates that the data were corrected considering $t = 0.5$ min as the first point of the kinetics.

process occurred within the 90 min incubation period, but a total conversion took more than 24 h (Figure 3F). The new B760 band of this form was located at 763 nm instead of 771 nm, which corresponds to the band maximum of the monomeric Bchl in acetone−methanol (7:2, by volume) mixture. Most probably the 763 nm form would correspond to a slightly chemically modified form of monomeric Bchl induced by the extended treatment in strong alkaline medium. This could explain why B760 first appeared around 770 nm but shifted progressively to 763 nm within the first 30 min of the conversion process. A reaction similar to an allomerization process can occur with an enolizable β-ketoester system at ring V and a proton at C-13 (*2*, *42*−*46*). Other reactions such as hydrolysis of the propionic phytyl ester might also occur (*47*).

The kinetic patterns of the two NIR bands are strongly different. After an instantaneous blue shift from 853 to 828 nm, B850 shows a first-order process of monomerization. This instantaneous blue shift was previously attributed to the deprotonation of a lysine and was independent of the monomerization process (*17*). The observed B800 is in fact composed of two bands. One band absorbing at 780 nm shows an instantaneous red shift from 780 to 786−788 nm and then monomerizes in a first-order process. Another band absorbing at 796 nm has a two-step process of monomerization. After a rapid transformation a slower first-order process occurs. The similarity between the kinetic behavior of B850 and the 780 nm band indicates a relationship between these two bands. The analysis of the kinetics confirms these relationships. However, it could be argued that a correlation is not necessarily a demonstration. At a first sight, we could imagine that the different kinetic behaviors we observed in this work are not conclusive results because of the very strong treatment we submitted the LHII complex. On the other hand, B800 could be intrinsically asymmetric and not necessarily the results of two bands (B780 and B796). In this case, the same asymmetric pattern should be conserved during all of the denaturation process. However, this interpretation seems to be false since the asymmetrical profile of the observed B800 changes during the time course because both contributing populations (B780 and B796) have different and specific kinetic behaviors. On the other hand, the strong conditions used in this work (i.e., 9 M NaOH) should induce drastic and totally unspecific changes, destroying instantaneously many weak links in the overall complex. This means that the specific changes we observed during the denaturation process could not be correlated to the unspecific changes induced by NaOH. On the contrary, these specific changes should correspond to several microenvironments related to B796 and B850 and in the case of B780/B850 to different spectral expressions of the same chromophores.

Recently, a band at 780 nm ascribed to the high exciton component of the B850 Bchls was identified on a B800-free LHII complex (*48*) and a B800-free mutant (*35*) of *Rb. sphaeroides.* This band arises from the energy splitting due to the dimeric character of the unit in the ring of B850 Bchls. Assuming that the structure of this complex is similar to the structure of the B850 part of the *Rps. acidophila* LHII, Koolhaas and collaborators (*35*) developed a theoretical model that allows analysis of this finding. In this model, the B850 part of the LHII complex is considered a ring of coupled dimers or two interacting rings of monomers: one ring of chromophores bound to the α-polypeptide and the second ring bound to the β-polypeptide. One result of this approach is the prediction of the electronic absorption spectra of B850 as a function of ΔE, the site energy difference between α and β chromophores. A 7 nm blue shift of B850 with a concomitant 2−3 nm red shift of the 780 nm band could be simulated by a decrease of ΔE from 600 to 150 cm^{-1}. This means that an increase of the energy level of B850 should be compensated by a decrease of the energy level of the 780 nm band. In the present work, this prediction is corroborated; indeed, the 25 nm B850 blue shift is compensated by a 6−8 nm red shift of the 780 nm band (Figure 3D,E). Interestingly, the model also predicts that an hypochromism of B850 occurs with a hyperchromism of the upper exciton band. This prediction is corroborated by the fact that the instantaneous blue shift/hypochromism of B850 is related to a red shift/hyperchrosmism of B780 (Figure 3D). Furthermore, from Figure 3B,C,G it is possible to calculate the extinction coefficient of the different bands before and after 90 min alkaline treatment. A decrease from 140 to 100 $mM^{-1}\cdot cm^{-1}$ for B850 and a concomitant increase from 30 to 85 $mM^{-1}\cdot cm^{-1}$ for the 780 nm band support the model. Another engaging observation after the Koolhaas and collaborators model is the fact that the integrated surface B850/780 nm band ratio is a linear dependence of ΔE. On this basis, the average interaction strength between monomers in the B850 ring of *Rb. sphaeroides* was estimated to be

300 cm^{-1} (*35*). In the present work, this value corresponds to 650 cm^{-1}, which indicates slightly different ring structure between both LHII complexes.

At this stage of our investigation it should be beneficial to focus our interest on the CD signal of B780. But in contrast to the LHII from *Rb. sphaeroides* or *Rps. capsulatus*, B800 of the LHII from *Ectothiorhodospira sp.* has a strong CD contribution (*17*, *40*) that would complicate the B780 CD analysis and induce to wrong conclusions, and therefore, we did not try this approach.

In summary, this work not only corroborates the presence of the 780 nm band as the upper exciton component of B850 Bchls but also describes a technique that allows to distinguish between several processes in the blue/red shifting mechanism in the *Ectothiorhodospira sp*. LHII complex. First, there is an energetic tune of B850 between 853 and 828 nm which has often been related to pigment−protein interactions as mentioned in the introduction (*5*, *6*, *17*−*20*). As demonstrated by directed mutagenesis, these interactions are controlled by certain amino acid residues (*14*, *32*−*34*) and, thus, appear to be the result of punctual modifications on the protein matrix. In contrast, the conversion of the 828 and 796 nm band into B760 requires very strong chaotropic conditions and, thus, appears to be related to the structural integrity of the complex. This technique could also have interesting applications when the effect of different mutations in the LHII polypeptides is compared with respect to the different processes described above. At present, the experiments in our laboratory are focused on the preparation of these mutants by directed mutagenesis.

ACKNOWLEDGMENT

We thank Maria V. Ramiro for skillful technical assistance.

REFERENCES

1. Sundström, V., and van Grondelle (1996) in *Anoxygenic Photosynthetic Bacteria* (Blankenship, R., Madigan, M. T., and Bauer, C. E., Eds.) pp 349−372, Kluwer Academic Publishers, Dordrecht, The Netherlands.
2. Cogdell, R. J. (1986) *in Encyclopaedia of Plant Physiology, Photosynthesis III* (Staehelin, L. A., and Arntzen, C., Eds.) Vol. 19, pp 252−259, Springer-Verlag, Berlin.
3. Picorel, R., and Gingras, G. (1988) *Biochem. Cell Biol. 66*, 442−448.
4. Feick, R., and Drews, G. (1978) *Biochim. Biophys. Acta 501*, 499−513.
5. Clayton, R. K., and Clayton, B. J. (1981) *Proc. Natl. Acad. Sci. U.S.A. 78*, 5583−5587.
6. Picorel, R., Lefebvre, S., and Gingras, G. (1984) *Eur. J. Biochem. 142*, 305−311.
7. Doi, M., Shioi, Y., Gad'on, N., Golecki, J. R., and Drews, G. (1991) *Biochim. Biophys. Acta 1058*, 235−241.
8. Walker, T., Gad'on, N., Steck, K., Welte, W., and Drews, G. (1991) *Biochim. Biophys. Acta 933*, 299−305.
9. Hayashi, H., and Morita, S. (1980) *J. Biochem. 88*, 1251−1258.
10. Cogdell, R. J., Durant, Y., Valentine, Y., Lindsay, J. G., and Schmidt, K. (1983) *Biochim. Biophys. Acta 722*, 427−435.
11. Monger, T. G., and Parson, W. W. (1977) *Biochim. Biophys. Acta 460*, 393−407.
12. McDermott, G., Prince, S. M., Freer, A. A., Hawthornthwaite-Lawless, A. M., Papiz, M. Z., Cogdell, R. J., and Isaacs, N. W. (1995) *Nature 374*, 517−521.
13. Koepke, J., Hu, X., Muenke, C., Schulten, K., and Michel, H. (1996) *Structure 4*, 581−597.
14. Fowler, G. J. S., Visschers, R. W., Grief, C. G., van Grondelle, R., and Hunter, C. N. (1992) *Nature 355*, 848−850.
15. van Mourik, F., Hawthornthwaite, A. M., Vonk, C., Evans, M., Cogdell, R. J., Sundström, V., and van Grondelle, R. (1992) *Biochim. Biophys. Acta 1140*, 85−93.
16. Visschers, R., Germeroth, W., Michel, H., Monshouwer, R., and van Grondelle, R. (1995) *Biochim. Biophys. Acta 1230*, 147−154.
17. Buche, A., and Picorel, R. (1998) *Photochem. Photobiol. 69*, 275−281.
18. Sauer, K., and Austin, L. A. (1978) *Biochemistry 17*, 2011−2019.
19. Webster, G. D., Cogdell, R. J., and Lindsay, G. (1980) *FEBS Lett. 111*, 391−394.
20. Ortiz de Zarate, I., and Picorel, R. (1994) *Photosynth. Res. 41*, 339−347.
21. Gottstein, J., and Scheer, H. (1983) *Proc. Natl. Acad. Sci. U.S.A. 80*, 2231−2234.
22. Scherz, A., and Parson, W. W. (1984) *Biochim. Biophys. Acta 766*, 653−665.
23. Scherz, A., and Rosenbach-Belkin, V. (1989) *Proc. Natl. Acad. Sci. U.S.A. 86*, 505−1509.
24. Braun, P., and Scherz, A. (1991) *Biochemistry 30*, 5177−1184.
25. Rafferty, E. N., Bolt, J., Sauer, K., and Clayton, R. K. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 4429−4432.
26. Picorel, R., L'Ecuyer, A., Potier, M., and Gingras, G. (1986) *J. Biol. Chem. 261*, 3020−3024.
27. Eccles, J., and Honig, B. (1983) *Proc. Natl. Acad. Sci. U.S.A. 80*, 4959−4962.
28. Hanson, L. K., Fajer, J., Thompson, M. A, and Zerner, M. (1987) *J. Am. Chem. Soc. 109*, 4728−4731.
29. Hanson, L. K. (1988) *Photochem. Photobiol. 47*, 903−921.
30. Hanson, L. K. (1991) *in Chlorophylls* (Scheer, H., Ed.) pp 993−1058, CRC Press, Inc., Boca Raton, FL.
31. Mäntele, W., Sawatzki, J., Doi, M., Gad'on, N., and Drews, G. (1991) *Biochim. Biophys. Acta 1057*, 367−372.
32. Hunter, C. N., Fowler, G. J. S., Grief, G. G., Olsen, J. D., and Jones, M. R. (1992) *Biochem. Soc. Trans. 21*, 41−43.
33. Fowler, G. J. S., Crielaard, W., Visschers, R. W., van Grondelle, R., and Hunter, C. N. (1993) *Photochem. Photobiol. 57*, 2−5.
34. Fowler, G. J. S., Sockalingum, G. D., Robert, B., and Hunter, C. N. (1994) *Biochem. J. 299*, 695−449.
35. Koolhaas, M. H. C., Frese, R. N., Fowler, G. J. S., Bibby, T. S., Georgakopoulou, S., van der Zwan, G., Hunter, C. N., and van Grondelle, R. (1998) *Biochemistry 37*, 4693−4698.
36. Lefebvre, S., Picorel, R., Cloutier, Y., and Gingras, G. (1984) *Biochemistry 23*, 5279−5294.
37. van der Rest, M., and Gingras, G. (1974) *J. Biol. Chem. 249*, 6446−6453.
38. Buche, A., and Picorel, R. (2000) *Z. Naturforsch. C 55*, 576−581.
39. Ortiz de Zarate, I. (1995) Ph.D. Thesis, University of Saragosse, Spain.
40. Buche, A., Ramirez, J. M., and Picorel, R. (2000) *Eur. J. Biochem. 267*, 3235−3243.
41. Buche, A. (2000) *J. Biochem. Mol. Biol. Biophys*. (in press).
42. Schaber, P. M., Hunt, J. E., Fries, R., and Katz, J. J. (1984) *J. Chromatogr. 316*, 25−41.
43. Rahmani, A., Eckhardt, C. B., Brereton, R. G., and Maxwell, J. R. (1993) *Photochem. Photobiol. 57*, 1048−1052.
44. Brereton, R. G., Rahmani, A., Liang, Y.-Z., and Kvalheim, O. M. (1994) *Photochem. Photobiol. 59*, 99−110.
45. Hynninen, P. H. (1991) in *Chlorophylls* (Scheer, H., Ed.) pp 145−209, CRC Press, Inc., Boca Raton, FL.
46. Hynninen, P. S., Wooley, B. J., Keely R., and Hester, R. E (1997) *J. Chem. Soc., Perkin Trans. 2*, 1731−1734.
47. Fisher, M. J. (1997) Ph.D. Thesis, Ludwig-Maximilians Universität München.
48. Bandilla, M., Ücker, Ram, M., Simonin, I., Gelhaye, E., McDermott, G., Cogdell, R. J., and Scheer, H. (1998) *Biochim. Biophys. Acta 1364*, 390−402.

BI001499C