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ABSORPTION AND STRUCTURE OF AN LM UNIT ISOLATED WITH SODIUM DODECYL SULFATE FROM REACTION CENTERS OF *RHODOPSEUDOMONAS SPHAEROIDES*

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Low-temperature absorption, circular dichroism and resonance Raman spectra of the LM units isolated with sodium dodecyl sulfate from wild-type *Rhodopseudomonas sphaeroides* reaction centers (Agalidis, I. and Reiss-Husson, F. (1983) Biochim. Biophys. Acta 724, 340–351) are described in comparison with those of intact reaction centers. In LM unit, the Q_y absorption band of P-870 at 77 K shifted from 890 nm (in reaction center) to 870 nm and was broadened by about 30%. In contrast, the 800 nm bacteriochlorophyll absorption band including the 810 species remained unmodified. It was concluded that the 810 nm transition is not the higher excitonic component of P-870. The Q_x band of P-870 shifted from 602 nm (in reaction center) to 598 nm in LM, whereas the Q_x band of the other bacteriochlorophylls was the same in reaction center and LM and had two components at about 605 and 598 nm. The Q_{xII} band of bacteriopheophytin was upshifted to 538 nm and a slight blue shift of the Q_y band of bacteriopheophytin was observed. Resonance Raman spectra of spheroidene in LM showed that its native *cis*-conformation was preserved. Resonance Raman spectroscopy also demonstrated that in LM the molecular interactions assumed by the conjugated carbonyls of bacteriochlorophyll molecules were altered, but not those assumed by the bacteriopheophytins carbonyls. In particular at least one Keto group of bacteriochlorophyll free in reaction center, becomes intermolecularly bounded in LM (possibly with extraneous water). This group may belong to the primary donor molecules.

Introduction

In a preceding paper [1] we have described several characteristics of a preparation derived from wild type *Rhodopseudomonas sphaeroides* reaction centers after treatment with sodium dodecyl sulfate and lauryldimethylamine *N*-oxide; it was devoid of the heaviest polypeptide chain (H) and consisted of the two lighter chains only (L and M), associated with the pigments. The photochemical activity of this LM unit was impaired, mainly because of the loss of the primary quinone acceptor UQ_I and the denaturing action of the detergents. However, a partial restoration of photochemistry was observed (up to 50%) in the presence of an excess of various quinones, which formed in the light very unstable ubisemiquinone anions. The flash-induced $P\text{-}870^+$ radical decayed in the dark with multiexponential kinetics involving apparent half-times ranging from 60 ms to 100 s. These functional alterations were attended by several spectral modifications in the infrared and visible ranges at 25 °C: (1) the Q_y band of the donor was blue-shifted to 850 nm (870 nm in

Abbreviations: BChl, bacteriochlorophyll; Bph, bacteriopheophytin; CD., circular dichroism; L, M and H, light, medium and heavy polypeptide chain.

reaction center); (2) the Q_x band of the Bphs sharpened; (3) the maxima of the vibrational components of the visible absorption band of spheroidene were slightly blue-shifted in LM by about 1–2 nm.

In the present work we attempted to characterize in more detail modifications in electronic interactions brought in LM at the level of the pigments with respect to the intact reaction center. For this purpose we used absorption and circular dichroism. In order to get information on the structural changes which must be at the origin of the spectral modifications, we used resonance Raman spectroscopy, which has been demonstrated to constitute a sensitive tool for characterizing both the molecular structures of the pigments and their environmental ground state interactions within the bacterial reaction center [2–5]. We will show that the main structural and resulting spectral changes in LM probably originate from the primary donor BChls.

Materials and Methods

Reaction centers of wild type *Rps. sphaeroides* Y were prepared as described [6] with an additional DEAE cellulose chromatography. The LM unit was prepared by incubation of purified reaction center from wild type *Rps. sphaeroides* (strain Y) at 20 °C with 1% SDS in the presence of 0.03% lauryldimethylamine *N*-oxide in Tris-HCl buffer and centrifugation in a sucrose gradient (5–20%) as previously described [1].

Direct and first derivative absorption spectra were recorded at 293 and 77 K using a Aminco DW-2A spectrophotometer provided with the low temperature compartment. In this compartment was inserted a transparent Dewar filled with liquid nitrogen into which cuvettes with plexiglass windows (1 or 2 mm optical path) were plunged. Samples were frozen either in the aqueous buffer (50 mM Tris-HCl/0.03% lauryldimethylamine *N*-oxide/1 mM EDTA, pH 8.0) or after addition of glycerol to 60% (v/v). Light-induced difference spectra were recorded on a Cary 14 R spectrophotometer equipped for cross-illumination; saturating infrared actinic light was provided by a tungsten-iodide lamp and a Wratten 89 B filter, the photomultiplier being protected by a 10% $CuSO_4$ solution (1 cm optical path).

Sodium ascorbate and potassium ferricyanide were added to put the primary donor P-870 in a reduced and oxidized state, respectively.

Circular dichroism spectra were recorded with a computerized Jobin-Yvon Mark-III dichrograph. At 25 °C rectangular cuvettes with 5 mm optical path were used. At low temperature a Variocryostat (regulating in the range −196 °C to 40 °C) was used, and cuvettes with plexiglass windows (1 mm optical path).

Resonance Raman (RR) spectra were recorded at 30 K as already described [4,7]. Selective excitations of the various pigments were achieved using laser lines at 496.5 nm (spheroidene), 528.7 and 530–547 nm (Bphs) and 363.8 nm (BChl and Bph). Reaction center and LM samples were concentrated to approx. 300–700 μM by ultracentrifugation and were examined either in presence of excess ferricyanide or untreated.

Results

Electronic absorption and circular dichroism spectra at 77 K

In intact reaction centers, the lowering of the temperature from 293 K to 77 K resulted in a red shift of the Q_y absorption band of P-870 from 870 to 890 nm, as previously reported [8]. Similarly in LM, the Q_y band was shifted from 850 nm at 293 K to 870 nm at 77 K in aqueous buffer (Fig. 1); we observed that in the presence of 60% glycerol it was shifted further to 888 nm, while glycerol did not affect the absorption spectrum at 77 K of intact reaction center. Similarly, we have shown in a preceding paper [1] that addition of UQ_6 or UQ_{10} to LM at normal temperature shifted the Q_y peak from 850 to 860 nm. Both the Q_y bands of P-870 and of the Bphs at 77 K were broader in LM than in intact reaction center, by about 33% and 25%, respectively. This was established using four independent reaction centers and LM preparations, by measuring in the reduced state the ratio of the half bandwidths (σ) between the P-870 and Bph Q_y absorptions, relative to that of the 800 nm band. The following values were obtained for reaction center and LM respectively: $\sigma_{760nm}/\sigma_{800nm} = 1.16 \pm 0.1$ and 1.46 ± 0.1; $\sigma_{870nm}/\sigma_{800nm} = 1.9 \pm 0.17$ and 2.53 ± 0.22. The broadening of the Q_y

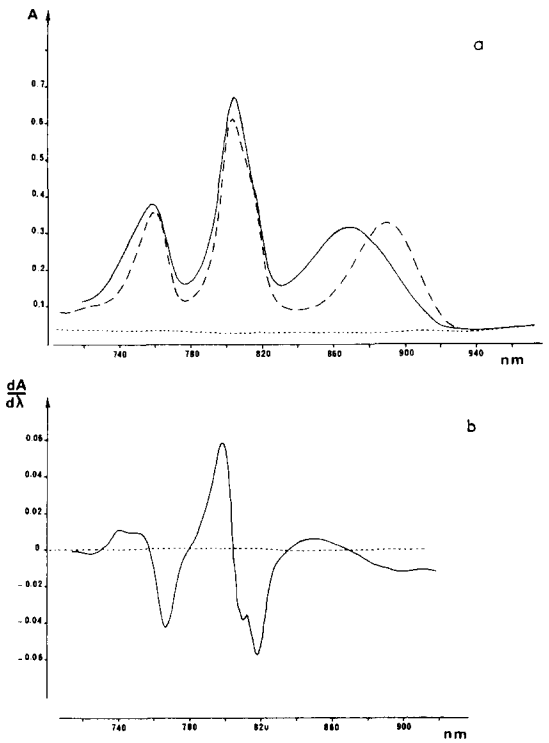

Fig. 1. (a) Electronic absorption spectra of LM (———) and reaction center (– – –) recorded at 77 K with an Aminco DW2A spectrophotometer. (· · · · · ·), base line. 6.3 μM LM and 6 μM reaction center were suspended in 50 mM Tris-HCl/1 mM EDTA/0.03% LDAO (pH 8.0), in the presence of 10 mM sodium ascorbate. (b) First derivative absorption spectrum of LM.

absorption of Bphs was otherwise evidenced by the first derivative absorption spectrum of LM (oxidized or reduced) at 77 K (Fig. 1; Fig. 2, right trace). Indeed the positive absorption was broadened and sometimes resolved in two weak shoulders (when P-870 was reduced) with maxima at 742 and 754 nm (Fig. 1b), whereas for reaction center the band was narrower and pointed at 754 nm (Fig. 2, left).

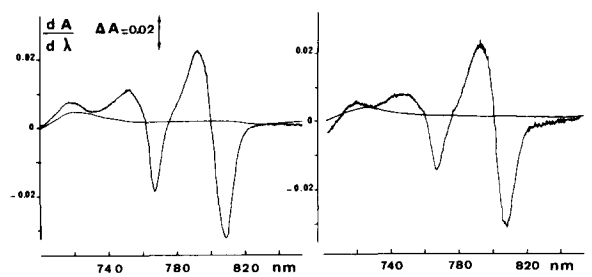

Fig. 2. First derivative absorption spectra of 6 μM LM (right trace) and 6 μM reaction center (left trace), oxidized by 1 mM potassium ferricyanide; 77 K. Other conditions as in Fig. 1.

In contrast to the modifications observed in Bphs and P-870 Q_y bands, the complex BChl Q_y absorption band at 800 nm (which also includes the 810 nm transition) was similar in LM and in intact reaction center as regards the position of its main maximum and its half bandwidth (seen Fig. 1a). In the first derivative spectrum of reduced preparations, the presence of the 810 nm component was as conspicuous in LM (Fig. 1b) as in intact reaction center (not shown); this component was apparently bleached in both samples in the oxidized state (Fig. 2) as well established for reaction center [9].

The infrared circular dichroism spectra of wild type *Rps. sphaeroides* reaction center in oxidized and reduced states at 293 and 77 K were similar to those published by Reed and Ke [10] for *Rps. sphaeroides* R26 reaction center. A comparison of these CD spectra with those of LM showed some small differences, which affected mainly the P-870 absorption band (see Fig. 3). Namely in LM the positive P-870 dichroic band was blue-shifted by 12–14 nm and broadened; the negative dichroic band of Bphs at 750 nm was also broadened. On

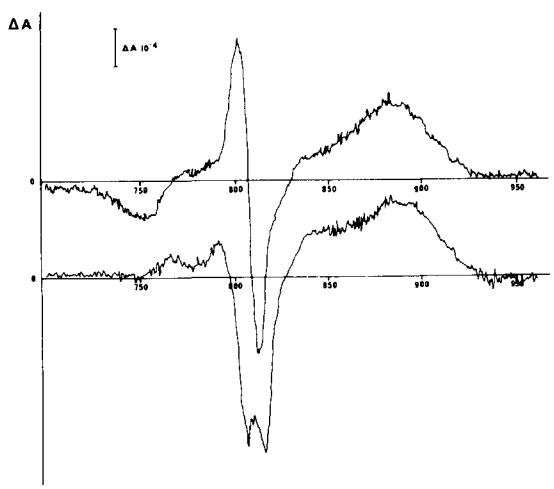

Fig. 3. Upper trace: CD spectrum of reduced LM at 77 K. 6 μM LM was suspended in 16 mM Tris-HCl/0.3 mM EDTA/0.03% LDAO.58% glycerol/10 mM ascorbate. Lower trace: calculated 'reduced minus oxidized' circular dichroism spectrum of LM at 77 K. Ordinate: differential dichroic absorption. Additions of ascorbate and ferricyanide as in Figs. 1 and 2.

the contrary, the double CD band centered near 800 nm was unchanged. Oxidation of LM at 77 K resulted in CD changes similar to those already reported for reaction center [9]: disappearance of the far-red positive CD band of P-870 and of the negative CD band at 810 nm, and strong enhancement of the positive CD band at 795 nm. These changes were reflected in the calculated CD difference spectra 'reduced minus oxidized' (Fig. 3); the intense negative band located at about 809 nm at 25 °C was split at 77 K in two distinct components at 805 and 813 nm. We note a difference in this CD spectrum of LM which consists in a weak positive band at 765 nm (see Fig. 3) which was absent for intact reaction center. This band may result from a difference in the CD bands between the oxidized and reduced state of Bphs in LM.

The Q_x band of Bph

Clayton and Yamamoto [11] have shown that at 77 K the Q_x bands of the two Bphs in purified R26 reaction center are well resolved into two peaks pointing at 534 nm (Q_{xII}) and 546 (Q_{xI}). It has been established that only the 546 nm absorbing species acts as an intermediary electron acceptor [12–16].

In the spectra of LM preparation at 77 K the Q_x bands of the two Bphs were much less resolved, as the species absorbing at the lower wavelength

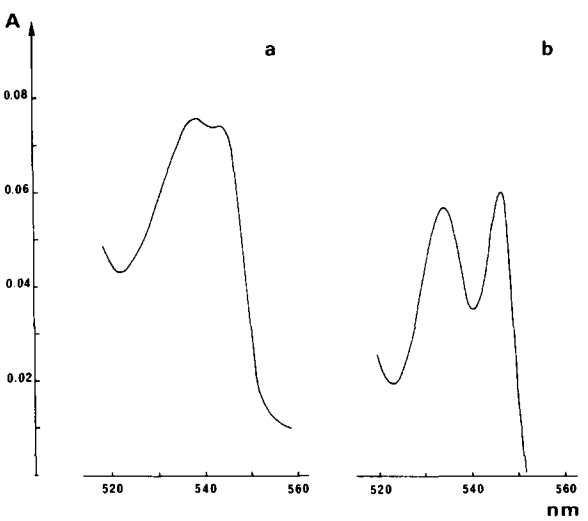

Fig. 4. Absolute absorption spectra of Bphs in the Q_x band at 77 K. (a) 2.7 μM LM, (b) 2.7 μM reaction center. Same conditions as in Fig. 1.

(Q_{xII}) shifted up to 538 nm (Fig. 4a). Similar spectral modifications were also observed by other authors when treating reaction center with SDS or chaotropic agents [17]. Besides, we observed that the Q_{xII} peaks of reaction center and LM shifted from 534 to 536 nm and from 538 to 540 nm, respectively, when potassium ferricyanide was added whereas the position of the Q_{xI} component shifted from 545 to 546 nm for both LM and reaction center. In fact, slight red-shifts of the Bph Q_y bands after light-induced charge separation have also been observed in purified R26 reaction centers in photodichroism experiments [18].

The Q_x band of BChl

The 600 nm absorption band should a priori be quite complex because all four BChls present in the reaction center contribute here by their Q_x transitions. In the steady 'light minus dark' difference spectrum of LM recorded at 25 °C the Q_x band was bleached with a minimum at 598 nm, whereas in the difference spectrum of reaction center the minimum was found at 602 nm (not shown). We verified by recording a difference spectrum 'chemically reduced minus oxidized' that the position of this minimum was not modified at 77 K. The contribution of this component (assigned to P-870) to the total Q_x band amounted to near 30% at 77 K in both reaction center and LM.

The absolute absorption spectrum of BChls Q_x band in reduced reaction center at 77 K was slightly asymmetric on its red side and a shoulder at 605 nm was apparent [9] (and Fig. 5a). This second component appeared well resolved from the main one (600 nm) in the first derivative spectrum. When reaction center was oxidized by potassium ferricyanide, the maximum of the BChls Q_x band was shifted to 598 nm and its intensity was reduced by 30% (Fig. 5b). In the first derivative spectrum the two spectral species 600 and 605 nm remained well resolved by the amplitude of the latter diminished by 33% as compared with the reduced state of reaction center (see Fig. 5a and b).

By recording spectra of LM under the same conditions, we ascertained that the half bandwidth of the BChls Q_x band was similar to that of the reaction center for the same absorbance. However, the shoulder on the higher wavelength side of the

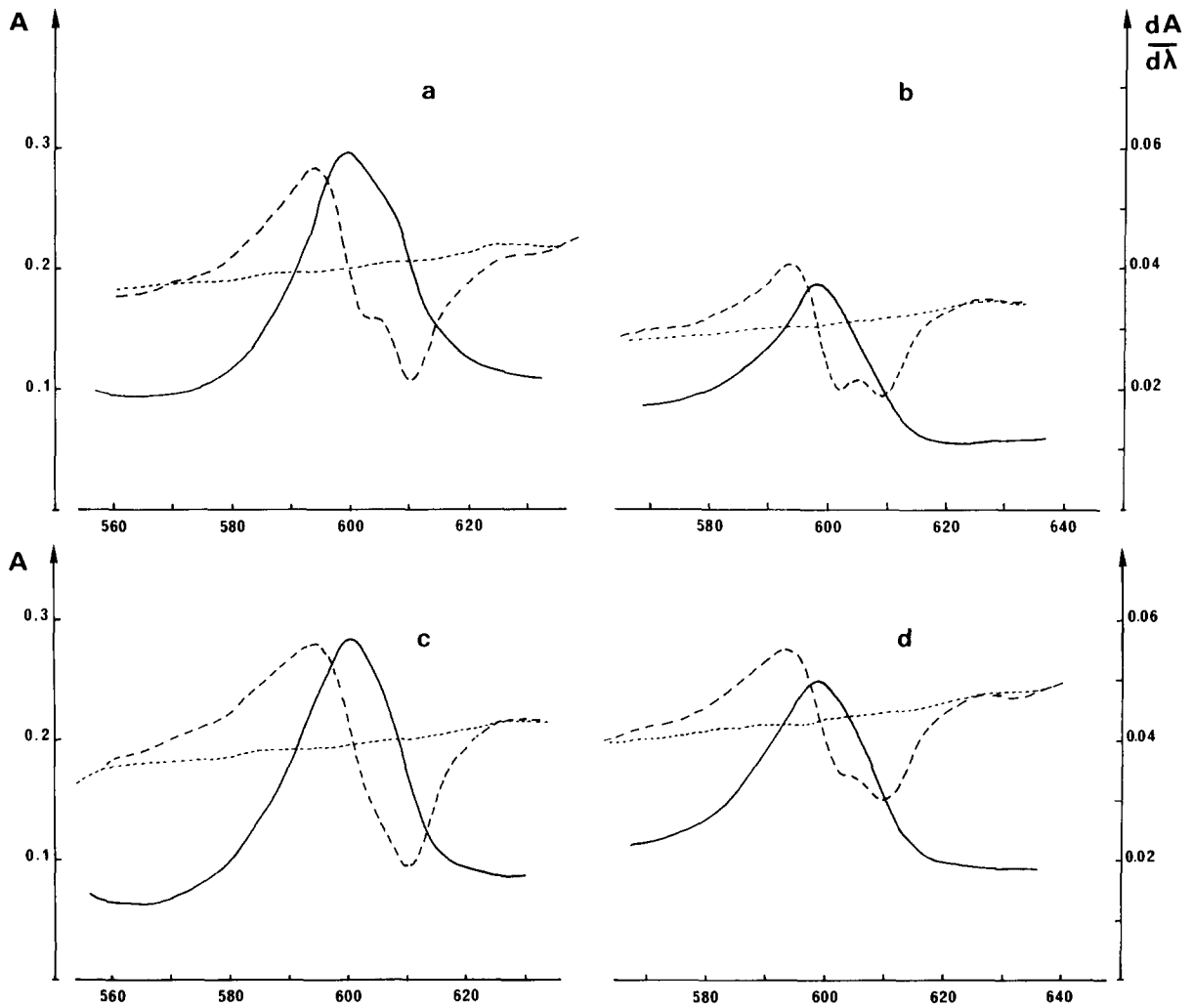

Fig. 5. Absolute (———) and first derivative (- - - - -) absorption spectra of 4.3 μM reaction center and LM (· · · · · ·) base line: (a) reduced reaction center, (b) oxidized reaction center, (c) reduced LM, (d) oxidized LM. Same conditions as in Fig. 1 and 2.

Q_x band (see Fig. 5c) was less proeminent than in the reaction center spectrum and this was confirmed in the first derivative spectrum where the 598 and 605 components were less distinct. We consider that this spectral modification is a consequence of the blue-shift of the P-870 Q_x band at 598 nm (see above). On the other hand, in the oxidized state of LM, when the contribution of P-870 vanishes, the two spectral species were better resolved (Fig. 5d).

The fact that the 605 nm absorption species was present in the reduced as well as in the oxidized state of both reaction center and LM indicated that this component did not belong to the 'special pair $(BChl)_2$' but to the remaining BChls, at variance with an initial interpretation [9].

Resonance Raman spectra

Resonance Raman spectra of LM at 30 K excited at 496.5 nm (not shown) contained contributions from spheroidene alone [2]. These spectra could not be distinguished from those of control reaction center, thus showing, in particular, that the native, specific *cis* conformation described for reaction centers of purple bacteria by Lutz et al. [3] was preserved in LM. On view of more recent

work on intact reaction centers (Szponarski, W., Lutz, M., unpublished data) the proposal made in Ref. 3 that this conformation might be a hindered di-*cis* configuration involving one methylated and one unmethylated *cis* double bond may, however, be questioned, as well as the proposal by Koyama et al. [19] of a 15-15′ mono*cis* configuration. Resonance Raman of LM at 30 K excited at 363.8 nm contained contributions from the BChls, the Bphs and speroidene (Fig. 6). In these spectra, the relative contributions from spheroidene were increased, compared to those in reaction center spectra, by factors of about 20% with respect to the BChl contribution. The relative increase cannot be ascribed to any shift of the Soret band of the porphyrins but rather to minor changes in the excitation profiles of the RR spectra of the carotenoid itself in the near ultraviolet., [20].

In addition to the change in carotenoid contribution, resonance Raman spectra of LM excited at 363.8 nm significantly differed from those of reaction centers by two other sets of features. First, bands at 185, 255, 678, 775, 876 and 1589 cm^{-1} significantly decreased relatively to other, non-carotenoid bands (Fig. 6). With the possible exception of the 255 cm^{-1} band, all of them have previously been shown [21] to arise from Bph rather than from BChl. Thus, under excitation at the top of the Soretband, the relative contribution from the Bph molecules in the resonance Raman spectra of LM is weaker than in reaction center spectra.

Second, the carbonyl stretching region (1620–1750 cm^{-1}, Fig. 7) of LM largely differed from that of reaction center (untreated samples): bands at 1708 and 1680 cm^{-1} were weakened (using the set of skeletal bands of BChl as a reference) and assumed apparent downshifts, while bands at 1660, 1640 and 1635 cm^{-1} were enhanced and shifted. These spectral differences were not primarily due to possible differences in proportions of the P-870$^+$ and P-870 species in the samples inasmuch as they were also observed in

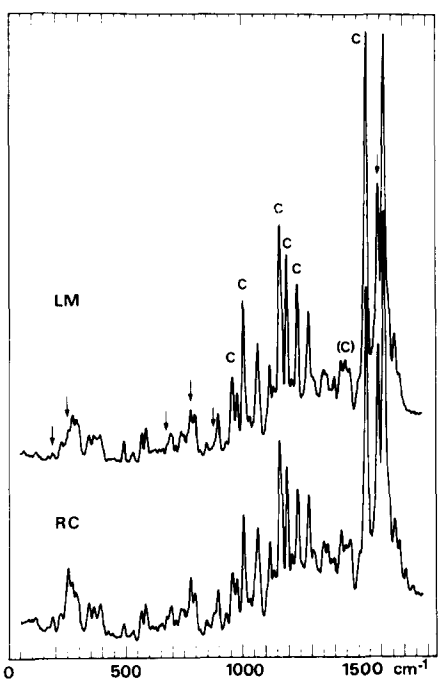

Fig. 6. Resonance Raman spectra of reaction center (RC) and LM at 15 K. Excitation: 363.8 nm; spectral resolution at 1000 cm^{-1}: 8.5 cm^{-1}; summation of two scans. C indicates RR bands from spheroidene. Arrows point at RC bands which are weakened in LM spectra. These bands most likely arise from Bph (see text).

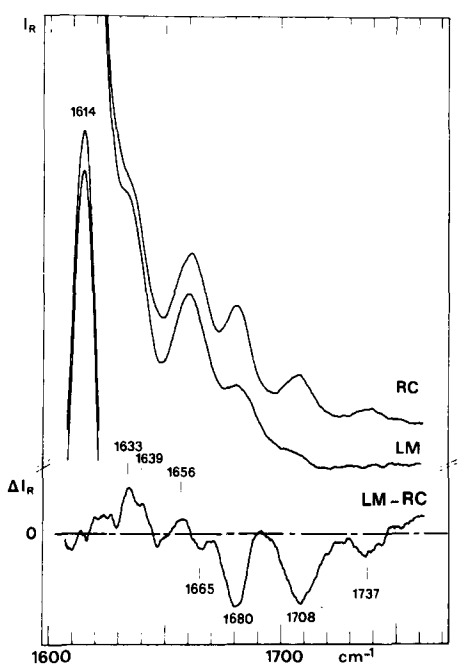

Fig. 7. Carbonyl stretching region of RR spectra of reaction center (RC) and LM, at 15 K. Excitation: 363.8 nm; spectral resolution: 8 cm^{-1}. Summation of four scans (RC) and six scans (LM) (LM-RC): difference between RC and LM spectra normalized on the intensity of the 1614 cm^{-1} band (methine bridges stretching). The zero line is approximate.

ferricyanide-treated LM (not shown). Bands of this spectral region are expected to arise from 12 unequivalent vibrators, namely the 9 C = 0 and 2 C = 0 groups of the six porphyrin pigments. More precisely, the 1680 cm^{-1} cluster and the 1705 cm^{-1} band of reaction centers must arise from 9-keto carbonyls protein bound and free from intermolecular bonding, respectively. Bands of the 1660 cm^{-1} cluster should arise from both free 2-acetyl carbonyls and protein-bound 9-keto groups. The lower frequency components should primarily arise from protein-bound 2-acetyl carbonyls [4,7,21].

In order to decide whether the changes observed in the carbonyl stretching region affected carbonyls of BPh or of BChl, or of both, we examined the same regions of spectra excited in the 529–547 nm range of the Q_x bands of Bph. In these conditions, the resonance Raman scattering of these two molecules indeed is selectively enhanced with respect to that of the BChls [3]. Although excited in x-polarized transitions, these spectra contained contributions from the conjugated carbonyls of the Bphs [21] (Fig. 8). Due to the splitting of the Q_x bands of reaction center and LM at low temperature (Fig. 4), it is possible to excite resonance Raman spectra of each of the two Bph molecules more or less independently [18]. The C = 0 stretching regions of resonance Raman spectra of LM and reaction center excited at 537 and 545 nm were very similar (Fig. 8). The main features of reaction center spectra at 1627 cm^{-1} (537 nm excitation), 1633, 1678 and 1705 cm^{-1} (545 nm) indeed were present in LM spectra, with essentially the same relative intensities. Due to the weakness of the 1655–1660 and 1702–1705 cm^{-1} bands of the 537 nm spectra, it was difficult to decide whether they were shifted for LM compared to reacton center. Nevertheless, considering the amplitudes of the variations observed in the

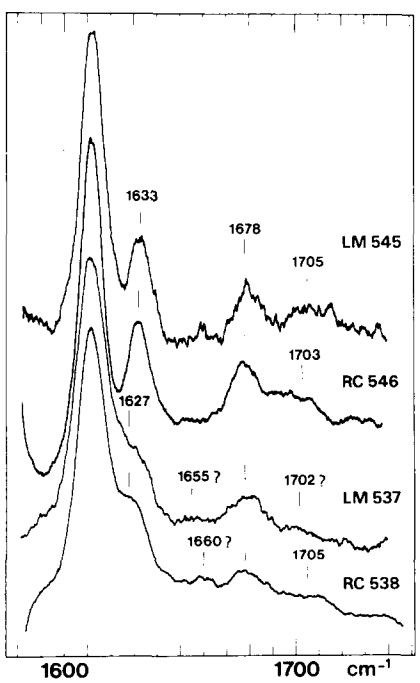

Fig. 8. Carbonyl stretching regions of RR spectra of bacteriopheophytins present in reaction center (RC) and LM (537, 538, 545, 546): excitation wavelengths (nm); temperature 20 K, resolution 5 cm^{-1}. Summation of eight scans (RC) and three scans (LM).

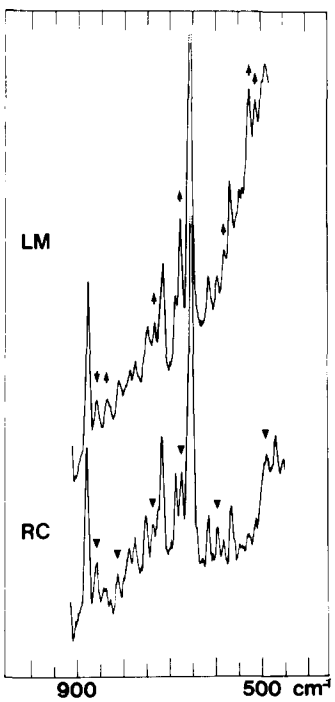

Fig. 9. Medium frequency regions of RR spectra of bacteriopheophytins present in reaction center (RC) and LM at 24 K. Excitation wavelength: 545 nm. Resolution at 700 cm^{-1} 5.5 cm^{-1}. Single scans. In the RC spectrum black triangles indicate bands absent in RR spectra of Bph in vitro in the same conditions of excitation (see text). In the LM spectrum arrows indicate RC bands which are significantly enhanced or weakened in the LM spectrum. The frequency scale is inverted compared to the preceding figures.

spectra excited at 363.8 nm, it could be safely concluded that most of the latter variations concerned the BChl carbonyls, the only possible exception being the slight downshift of the 1660 cm^{-1} band, which might be due to Bph (537 nm).

Resonance Raman spectra of the LM unit excited in the Q_x band of the Bphs (529–547 nm) presented relative intensity differences compared to reaction center spectra. These differences most probably were not all simply related to the upshift of the 535 nm electronic band, inasmuch as they also occurred in spectra excited at 545–547 nm. In particular, these variations concerned bands which are specifically active for reaction centers in these conditions of excitation, and which are absent from resonance Raman spectra of Bph in any other environment [22]. For example, the additional 675 cm^{-1} band was about 2 -fold stronger in LM spectra excited in the 529–547 nm range than in reaction center spectra (Fig. 9).

Finally, a very weak band, which is generally present around 1735–1740 cm^{-1} in resonance Raman spectra of reaction centers excited at 363.8 nm [21], faded out in LM spectra (Fig. 7). This band, occasionally observed in resonance Raman spectra of BChl in vitro [21], may possibly arise from stretching of ester carbonyl(s) of BChl or Bph molecule(s), free from intermolecular bonding, but nevertheless drawn into resonance by the reaction center structure and not by that of LM.

Discussion

Changes in electronic properties of the pigments bound in reaction center upon treatment with SDS necessarily result directly or indirectly from changes in the environmental interactions assumed by the pigments. As we have shown in a preceding paper [1], these modifications can be attributed mostly to the denaturing action of SDS rather than to the removal of the H subunit.

The remarkable change in the infrared region of the absorption spectrum of BChls in LM with respect to that of intact reaction center is the blue shift of P-870 absorption maximum by about 20 nm (270 cm^{-1}), at normal and low temperature, and a broadening of the bandwidth of about 35% at 77 K. The broadening of the Q_y band of the donor bacteriochlorophyll (870 nm at 77 K) may be due to inhomogeneity of the LM preparation [1] or to a modified mixing of charge transfer states [18,23,24]. The influence of glycerol and ubiquinone on the Q_y band of the donor in LM but not in the reaction center suggests a better accessibility of the donor in LM. This fits in with conclusions made in a preceding paper [1].

The resonance Raman spectra of BChl indicate such environmental changes. The conjugated carbonyls of BChl and Bph constitute key groups in anchoring these pigments to the reaction center protein [4,7]. Differences observed in the C = 0 stretching region between resonance Raman spectra of reaction centers and LM essentially arise from BChl molecules. They reveal two types of structural changes in LM compared to reaction center. The first one, corresponding to the vanishing of the 1705 cm^{-1} band in ferricyanide-treated as well as untreated samples, consists of the binding of at least one 9 C = 0 group (which is free in reaction center) to additional site(s) in LM. Although this site(s) may well be proteic and although their use in LM may result from a conformational change it is tempting to propose that free 9 C = 0 group(s) became accessible to small polar molecules, e.g., water when the H subunit is removed. The additional contribution to resonance Raman spectra from these newly bonded 9 C = 0 groups is likely to occur in the 1660 cm^{-1} band [4]. The relative intensity of the 1700 cm^{-1} band was also decreased when chemically reducing P-870^+ in R26 reaction centers [4]. This can be taken as an indication that these groups may belong to primary donor BChls.

The other spectral variations occuring in the ν(C = 0) region of RR spectra most probably reflect conformational changes in the protein rather than changes in binding sites of other carbonyls.

Indeed, these spectral variations essentially consists of relative intensity variations rather than frequency shifts and hence are most likely to arise from changes in conjugation of bound C = 0 groups with the main π electron system of the dihydrophorbin rings. Such changes in conjugation must result from changes in orientations of the carbonyls with respect to the mean molecular plane, induced by conformational changes of the protein to which they are bound.

These structural changes have minimal effects

on the pigments ground-states, as far as they induce only minimal changes in the remainder regions of the resonance Raman spectra of the BChls and Bphs. The absorption and CD spectra of LM recorded at room and low temperature have shown that, contrary to the 870 nm electronic transition, the 810 nm transition has the same wavelength position and bandwith in LM as in intact reaction centers. This result indicates that unlike suggested by other authors [18,25,26] the 810 nm band cannot be ascribed to the higher excitonic component of the P-870 dimer but rather belongs to the BChls absorbing at 800 nm as was already concluded by Shuvalov et al. [27].

Moreover, the fact that changes in the redox state of the donor induce in LM the same absorbance variations around 790–810 nm as in reaction centers suggests that the environment of these BChls is not altered in LM.

According to photoselection experiments [18] the formation of the radical pair P^+Q^- in reaction center would elicit the development of a new, differently oriented band at shorter wavelength than 800 nm, a blue shift and the bleaching of the 805 nm band. These absorbance changes seem also present in the low temperature CD difference spectra of P minus P^+ in both LM and reaction center.

The light-induced absorbance changes have indicated that the maximum of the Q_x absorption of P-870 pointed at 602 nm in reaction center, whereas it was blue shifted to 598 nm in LM. The P-870 Q_x band contributed for 30% to the overall absorption at 600 nm, a fact which was already observed by Slooten [28] in AUT-reaction center particles of *Rps. sphaeroides* at 20 °C; he concluded to the occurence of two spectral species, one absorbing at 602 nm and the other at 595 nm. The first was attributed to P-870 and the second to the remaining BChls. This analysis is in good agreement with the results described above for reaction center and LM; P-870 was shown to contribute at 602 nm in reaction center and 598 nm in LM at 77 K. However besides this P-870 absorption the remaining spectral species attributed to the other BChls was observed as a composite band at 77 K, with similar features in LM and reaction center.

Absorbance, as well as LD and CD studies [11,18,25,26,10] on purified *Rps. sphaeroides* reaction center have suggested that the two Bphs molecules are in very weak (or in no) excitonic interactions. Therefore, the two different absorption Q_x bands, i.e. 534 nm (Q_{xII}) and 545 nm (Q_{xI}), should reflect different environments in the protein core for the two Bphs; this could also explain the existence of two components in the Q_y band of Bph evidenced in photoselection studies [18].

In LM we observed that the Bph Q_{yII} band is shifted to 538 nm, whereas the 546 nm component is unperturbed. At the same time, the Bphs Q_y absorption band became broader especially on its blue side (see Fig. 2 left); this may be due to a change in the shape of the Q_y absorption band of the Bph_{II} component. Resonance Raman spectra excited in the Soret band and common to the six porphyrins, as well as those excited in the Q_x bands of the Bphs, indicate that the structural rearrangement resulting in the above changes of electronic spectra of the Bphs must be small. Indeed, the binding states and conformations of the conjugated carbonyls are unaffected by the SDS treatment and removal of the H subunit, except perhaps one (keto ?) group of Bph (537 nm).

Moreover, only relative intensity changes can be observed for skeletal bands of the Bphs. These changes may well correspond to the alterations in electronic structures discussed above. Indeed, most of the Raman spectral changes affect bands which are additional to resonance Raman spectra of Bphs in vitro excited in the same conditions [21]. It has been shown that these additional bands correspond to resonance modes, e.g. C = O stretching which are normally uncoupled to x-polarized electronic transitions, but active on y-polarized transitions. This phenomenon has bee tentatively ascribed to reorientation in the reaction center of the Q_x transitions of the two Bphs molecules due either to weak excitonic interactions between the two Q_x transitions of these molecules, or to other unspecified electronic environmental interactions (e.g., conjugated π system, or charged groups in the vicinity of the molecules) [21].

Arguments in favor of the first hypothesis are that both Bph molecules appear affected in a very similary way, and that in excitation conditions where resonance should occur on a single component of the Q_x transition (e.g., 545 nm, Fig. 8) contribution from the two Bphs molecules occur in

the Raman spectra (e.g., four carbonyls group appear active under 545 nm excitation). Additional data also supporting the first hypothesis are given by resonance Raman spectra of the LM unit: although the 546 nm component appears unaffected by the treatment, RR spectra excited at its red edge indeed present perturbations in the relative intensities of the additional skeletal bands, such as the 670 cm^{-1} one (Fig. 9).

From the present spectroscopic data altogether, we can conclude that the proteic environment of Bphs in LM unit is little affected relative to reaction center. The fact that the specific conformation of spheroidene is preserved in LM, means that the SDS treatment does not appreciably alter the binding site of the carotenoid. On the contrary, a perturbation of the donor BChls is evident in the absorption and CD spectra of LM and is likely due to sizable environmental alterations of these chromophores after SDS treatment, probably including a change in coordination state of conjugated ketonic carbonyl group(s).

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