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A resonance Raman investigation of the effect of lithium dodecyl sulfate on the B800–850 light-harvesting protein of *Rhodopseudomonas acidophila* 7750

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The effect of the detergent lithium dodecyl sulfate (LDS) on the resonance Raman spectrum of the carotenoid and bacteriochlorophyll in the B800–850 light-harvesting complex *Rhodopseudomonas acidophila* 7750 was determined. Treatment of B800–850 complex with 0.1% LDS resulted in a 3 cm^{-1} downshift of the carotenoid ν_3 Raman band, a 2 cm^{-1} upshift of the carotenoid ν_1 band, with no change in the ν_2. This indicates that LDS treatment induces a 7-*cis*-isomerization of the carotenoid and a supplementary torsion of the polyene chain around the C(8)–C(9) bond. Also, treatment of B800–850 complex with LDS resulted in several changes attributable to alterations in the nature of the binding to the protein of the acetyl and keto-carbonyls of one bacteriochlorophyll. No change was observed in the coordination of the bacteriochlorophyll central magnesium. These data indicate that the 800 nm-absorbing bacteriochlorophyll remains bound to the protein upon LDS treatment. The resonance Raman data indicate that the pronounced absorption spectral changes reported previously (Chadwick, B.W., Zhang, C., Cogdell, R.J. and Frank, H.A. (1987) Biochim. Biophys. Acta 893, 444–451) are attributable to a dramatic perturbation of the protein environment upon LDS treatment of the B800–850 complex.

The molecular interactions which regulate the in vivo spectral features of the light-harvesting system of photosynthetic organisms are not well understood. Several groups have studied B800–850 pigment-protein complex from purple photosynthetic bacteria because it is possible to obtain spectrally altered forms of this complex using different bacterial growth conditions or by chemical or physical means [1–6]. Through a comparison of the properties of the altered complexes with those of the unaltered complexes the features which control the spectral properties of the native systems can be identified.

Clayton and Clayton [6] were the first to report that the 800 nm absorbance of B800–850 complex from *Rhodobacter sphaeroides* 2.4.1 could be attenuated using lithium dodecyl sulfate (LDS). They demonstrated that following treatment with LDS, dialysis of the sample against Tris buffer containing lauryldimethylamine *N*-oxide (LDAO) reinstated the 800 nm absorption band [6]. They concluded that monomeric bacteriochlorophyll (BChl) can interact reversibly with a specific binding site

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Abbreviations: LDAO, lauryldimethylamine *N*-oxide; LDS, lithium dodecyl sulfate; BChl, bacteriochlorophyll.

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on the protein. Kramer et al. [7] extended this work and constructed a structural model for the B800–850 complex based on differences observed in the absorption, fluorescence polarization, linear dichroism and circular dichroism spectra taken from complexes prepared in LDS or LDAO. They proposed that the B800–850 minimal unit consists of regular aggregates of two BChl dimers, two BChl monomers and three carotenoid molecules noncovalently bound to two pair of apoproteins. Also, owing to a reduction in the carotenoid-to-BChl singlet–singlet energy transfer efficiency in the LDS prepared material, they concluded that the carotenoid molecules are divided into two spectrally distinct pools, one-third of the carotenoids being associated with the BChl monomers and absorbing approx. 4 nm to the red of the remaining two-thirds of the carotenoids which were presumed associated with the BChl dimers. An assumption in this work is that the preparation of the complex using LDS caused only the dislocation of the monomeric 800 nm-absorbing BChl and resulted in a complex that is structurally identical to B800–850 complex prepared in LDAO.

Chadwick et al. [6,9] have argued recently that the LDS-induced absorption changes in *Rhodopseudomonas acidophila* 7750 may be brought about by changes in protein conformation owing to solubilization of the B800–850 complex in the different detergent. Here, we present a resonance Raman study of the B800–850 complex from *Rps. acidophila* 7750 which supports this argument and addresses three specific issues: (1) the reason for the reversibility (upon dialysis in LDAO buffer) of the LDS-induced attenuation of the 800 nm absorption band; (2) whether two pools of carotenoids exist in native B800–850 complexes; (3) the reason for the 4 nm blue shift of the carotenoid absorption bands upon treatment of B800–850 complex with LDS.

Cells of *Rps. acidophila* 7750 were grown as previously described [2]. The cells were harvested by centrifugation at 15 000 × *g*. Broken cells were prepared by French pressure disruption according to Cogdell et al. [2]. The B800–850 complex was isolated from *Rps. acidophila* 7750 as described in Ref. 9. B800–850 complex at an absorbance of 1 cm^{-1} measured at 857 nm (approx. 8 μM) in 10 mM Tris buffer (pH 8.0) containing approx. 0.07% LDAO was treated with lithium dodecyl sulfate (LDS) by adding 10% aqueous solution of LDS (final LDS concentration was 0.1%). Resonance Raman spectra were recorded using the spectrometer set-up described previously [10].

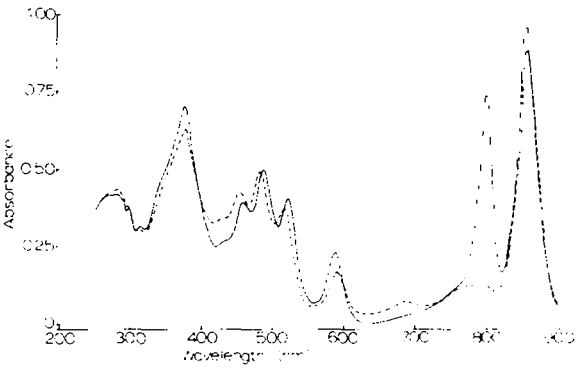

Fig. 1. The effects of LDS on the absorption spectrum of *Rps. acidophila* 7750. The solid line represents the spectrum taken before LDS addition. The dotted line represents the spectrum taken approx. 2 h after addition of 0.1% LDS. The spectra were not normalized and were taken from samples having identical protein concentrations.

Fig. 1 shows the effect on the absorption spectrum of adding 0.1% LDS to the B800–850 complex isolated from *Rps. acidophila* 7750. In this paper we focus specifically on the observations that: (1) the BChl monomer Q_y band near 800 nm has been reduced to less than 15% of its original intensity (overnight dialysis of the LDS-treated B800–850 complex against 20 mM Tris buffer (pH 8.0) to remove the LDS followed by addition of LDAO (final concentration of 0.3%) resulted in an approx. 70% recovery of the intensity of the 800 nm absorption band and a reduction of the intensity of the small broad peak at 770 nm); and (2) the carotenoid absorption band have been blue-shifted by 4 nm.

Fig. 2 displays resonance Raman spectra (488 nm excitation) of the carotenoid of B800–850 (type I) antenna of *Rps. acidophila* 7750 before and after LDS treatment. The spectrum of the untreated material (Fig. 2.2) is very similar to the those of the spirilloxanthin series in antenna complexes previously published [11,12]. Furthermore, this spectrum exhibits features characteristic of an all-*trans* configuration of the carotenoid [11–13].

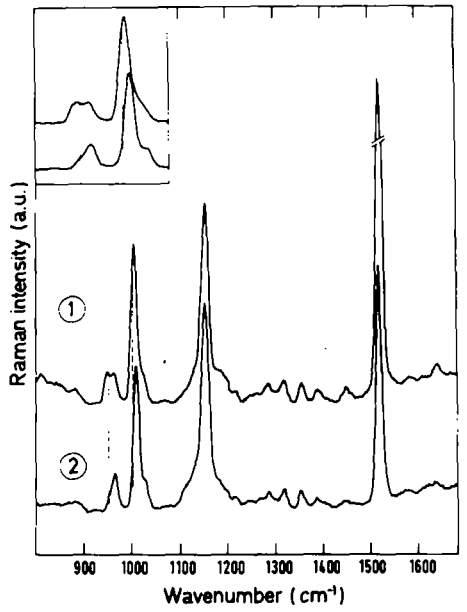

Fig. 2. Resonance Raman spectra (800–1700 cm^{-1}) of the carotenoids in untreated B850–800 complexes of *Rps. acidophila* (2) and in B850–800 complexes after LDS treatment (1). Excitation wavelength = 488 nm; $T = 30$ K. Inset: id. 900–1050 cm^{-1} spectral range.

However, the presence of a strong 960 cm^{-1} band has been assigned to an out-of-plane distortion of the end of the conjugated chain of the molecule [12].

LDS induces dramatic changes in the resonance Raman spectrum of the carotenoid (Fig. 2.1). There is a ν_1 band upshift of (ν C=C) 2 cm^{-1} (from 1517 to 1519 cm^{-1}), a ν_3 downshift of 3 cm^{-1} (from 1005 to 1002 cm^{-1}), and the appearance of a new band at 950 cm^{-1} (see Table I). The fact that ν_1 upshifts only weakly allows the conclusion that no central isomerization occurs during LDS treatment. An upshift of a few wavenumbers is consistent only with *cis*-isomerization at the end of the conjugated polyene chain [14,15]. 9-*cis*-Isomerization of β-carotene induces a 5 cm^{-1} upshift of the ν_1 and a 2–3 cm^{-1} upshift of the ν_3 band. In contrast, 7-*cis*-isomerization induces a downshift of the ν_3 band of 3 cm^{-1} and a 1–2 cm^{-1} upshift of the ν_1 band [14] without any remarkable change in the frequency of the ν_2 band. Resonance Raman spectra of LDS-treated antenna complexes are, thus consistent with a 7-*cis*-isomerization of the carotenoid. Also, the 950 cm^{-1} has been ascribed to out-of-plane modes of the chain hydrogens [15,16]. These modes are not totally symmetric in the C2h point group and are very weak in resonance with the $^1B \leftarrow {}^1A$ transition. They are known to gain intensity when the molecule assumes a lower molecular symmetry. LDS treatment thus induces a supplementary torsion of the polyene chain: 950 cm^{-1} arises from out-of-plane wagging of C(7)H and C(8)H bonds [14]. Such a distortion of the polyene chain around the C(8)–C(9) bond could likely induce a resonance Raman spectrum resembling that of a 7-*cis*-carotenoid. However, it has recently been published [17] that relaxation of the end-chain twisting of spheroidene bound to bacterial reaction centers does not induce a change in frequency of any resonance Raman band. Thus, we must conclude that LDS treatment induces both a 7-*cis*-isomerization of the carotenoid and a distortion of the polyene chain at the same level near C(7)–C(8) carbons.

Because these spectral modifications are observed at any excitation wavelength in the 441 nm to 488 nm range, and are not accompanied by a broadening of the resonance Raman bands (data not shown), it is possible to conclude that, upon LDS treatment, carotenoids bound to the B800–850 complex of *Rps. acidophila* 7750 behave as a single pool. The reason for the LDS-induced absorption spectral shift of the carotenoid bands is also clear. The addition of LDS to the pigment-protein complex results in a protein conformational change which cause isomerization of the carotenoid to a form that absorbs 4 nm to the blue of the carotenoid in untreated complex. It is un-

TABLE I

POSITIONS OF RESONANCE RAMAN BANDS

Values are expressed in cm^{-1}.

Carotenoid		Bacteriochlorophyll		
untreated	treated	untreated	treated	assignment
–	950	1614	1614	
960	960	1630	1630	acetyl C=O bound
1005	1002	1640	–	acetyl C=O bound
1150	1150	–	1662	acetyl C=O free
1517	1519	1672	1669	bound keto C=O
		–	1677	bound keto C=O
		1701	–	free keto C=O

likely that LDS could directly affect the carotenoid structure without altering the structure of the protein to which it is bound.

Fig. 3 displays resonance Raman spectra of BChl (363.8 nm excitation) in LDS-treated and untreated B800–850 complex from *Rps. acidophila* 7750 (1550–1750 cm^{-1} region). In this region a strong band at 1615 cm^{-1} is present, arising from the methine bridge stretching modes of the molecule. This band is sensitive to the number of external ligands bound to the Mg, being located around 1600 cm^{-1} when two external ligands are bound to this atom or around 1615 cm^{-1} when the central Mg binds only a single external ligand [11,18]. Other bands arising from the conjugated C=O group of the molecule, 2-acetyl C=O and 9-keto are also observed. Typically a 2-acetyl C=O of BChl vibrates at 1660 cm^{-1} when free from intermolecular interactions and its frequency shifts down to 1630 cm^{-1} when engaged in hydrogen bonding [19]. The keto group vibrates at 1700 cm^{-1} when free from intermolecular interactions, its frequency shifting down to 1660 cm^{-1} when engaged in hydrogen bonding [19,20]. In resonance Raman spectra of untreated antenna the following features are present: (i) a strong band at 1616 cm^{-1} indicating that Mg of BChls in the antenna are five-coordinated, which is usual in antenna from purple bacteria [21]; (ii) two shoulders at 1630 cm^{-1} and at 1640 cm^{-1} arising from bound acetyl C–O groups; (iii) at 1672 cm^{-1} band at a 1700 cm^{-1} band. 1672 cm^{-1} is slightly too high a frequency to be arising from an acetyl group free from binding (1660 cm^{-1}). This band can be attributed to keto groups engaged in intermolecular intermolecular interactions, whereas the 1700 cm^{-1} one arises from free from intermolecular interactions keto C=O groups. This spectrum slightly differs from previously publishes resonance Raman spectra from B800–850 complexes from *Rps. acidophila* [22]. In particular, a 1700 cm^{-1} band is present in these spectra, which was lacking in the previously published spectra. However, the latter were resonance Raman spectra from type-II B800–850 complexes [2] extracted from strain TL1. This differs substantially in its absorbance spectrum from the type-I complex studied here. Type-I complexes have a smaller 800 nm band than 850 nm band, whereas type-II complexes have a larger 800 nm band than 850 nm band [2].

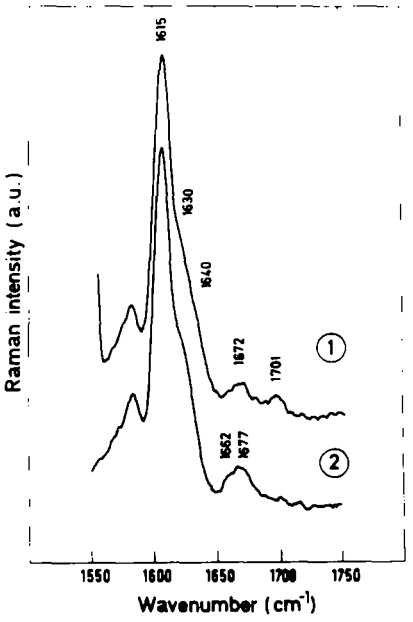

Fig. 3. Resonance Raman spectra (1550–1750 cm^{-1}) of the BChls at Søret resonance in untreated B850–800 complexes of *Rps. acidophila* (1) and in B850–800 complexes after LDS treatment (2). Excitation wavelength = 363.8 nm; T = 30 K.

In the resonance Raman spectrum of the LDS-treated material, simple modifications occur at the level of the 1640 cm^{-1} and 1701 cm^{-1} bands which are absent in resonance Raman spectra of the treated material. They are replaced by two components at 1662 and 1667 cm^{-1}. 1662 cm^{-1} is the frequency of an unbound acetyl group and 1677 cm^{-1} matches with an hydrogen bonded keto C=O group. Thus, we are able to conclude that there is a breakage of the hydrogen bond engaged by the 1640 cm^{-1} vibrating acetyl C=O and formation of a new hydrogen bond on one keto group of one BChl of the B850–800 complexes. LDS-treatment thus affects one population of BChl (two C=O vibrators), most likely the 800 nm-absorbing one. The fact that no differences appear in the 1000–1500 cm^{-1} region of resonance Raman spectra (data not shown) and in particular in the frequency and width of the 1614 cm^{-1} band (Fig. 3) indicates clearly that this molecule is coordinated in a similar manner before and after LDS treatment. This explains why

the LDS treatment is 70% reversible upon dialysis. The major part of the BChl absorbing at 800 nm remains bound to the protein via its central magnesium after LDS treatment. The lack of 100% reversibility can probably be attributed to the loss of BChl which becomes more exposed during or after a protein conformational change. A change in the interactions of both BChl C=O groups in consistent with, and indicative of, a dramatic perturbation of the protein environment.

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Note added in proof

The assignments of the 960 and 970 cm^{-1} modes of *all-trans* β-carotene made in Ref. 15 have to be inverted (Tasumi, M., personal communication). Hence the out-of-plane distortion of the B850-800 band carotenoid induced by LDS treatment should essentially concern the C_{12}-C_{13} and/or $C_{12'}$-$C_{13'}$ bands.

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