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Detergent effects on the reaction center-B890 complex of *Chromatium vinosum* and the mode of bacteriochlorophyll binding as revealed from circular dichroism and nuclear magnetic resonance spectroscopy

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Additional effects of detergents have been examined on absorption and circular dichroism (CD) spectra of a reaction center (RC)-B890 complex of a photosynthetic bacterium, *Chromatium vinosum*. Anionic detergents in rather higher concentrations destroyed both B890 and RC complexes at the same time, and neutral detergents had a relatively small effect on absorption and CD spectra of the RC-B890 complexes. In contrast, zwitterionic and cationic detergents changed the absorption and CD spectra of the B890 complex at lower concentrations, when α-helical structures of the B890 polypeptides were almost conserved. In this case some amino acid side chains showed increasing molecular motions, as detected by ^{13}C-NMR of the RC-B890 complexes from ^{13}C-enriched cultures. NMR results from a reconstitution experiment which used a ^{13}C-enriched B890-polypeptide and bacteriochlorophyll *a* with natural abundant carbons implied that histidine residues may be associated with the protein environment about the bacteriochlorophyll molecules.

Introduction

Purple photosynthetic bacteria, anoxygenic facultative phototrophic bacteria, produce ATP by cyclic photophosphorylation when grown anaerobically in the light [1]. Intracytoplasmic membranes of purple photosynthetic bacteria contain one or more unique light-harvesting (or antenna) pigment-protein complexes. The antenna pigments, i.e., bacteriochlorophyll *a* and carotenoids, are bound to hydrophobic membrane polypeptides [2–7]. The non-covalent polypeptide-pigment association effects a shift of the red absorption band of BChl to longer wavelength from 770 nm to 800–890 nm. A spatial arrangement of antenna-pigment complexes around reaction

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Supplementary data to this article are deposited with and can be obtained from: Elsevier Science Publishers B.V., BBA Data Deposition, P.O. Box 1345, 1000 BH Amsterdam, The Netherlands. Reference should be made to No. BBA/DD/340/850/343. The supplementary information includes: absorption and CD spectra of the RC-B890 complex in the presence of detergents listed in Table I except for those of LDAO, SDS and Triton X-100. The figure legends for these supplementary data have been included at the end of this article.

Abbreviations: BChl, bacteriochlorophyll; B890, an antenna bacteriochlorophyll-protein complex that shows the absorption peak at 890 nm; CD, circular dichroism; DSS, sodium-2,2-dimethyl-2-silapentane-5-sulfate; LDAO, lauryldimethylamine *N*-oxide; NMR, nuclear magnetic resonance; His, histidine; RC, reaction center; TMS, tetramethylsilane; Triton X-100, α-[4-(1,1,3,3-tetramethylbutyl)phenyl]-ω-hydroxypoly(oxy-1,2-ethanediyl) (or *p-t*-octylphenylpolyoxyethylene ether).

centers in order to optimize energy transfer from the B800–850 complexes to photochemical RC's via B890 complexes [2]. With sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis, RC-B890 and B800–850 were isolated from membranes of a purple sulfur bacterium, *Chromatium vinosum*. The RC-B890 is a complex which contains both RC and B890. The B890 BChl-protein of purple photosynthetic bacteria (such as *Rhodopseudomonas sphaeroides, Rhodopseudomonas capsulata* and *Rhodospirillum rubrum*) consists of two polypeptides (called α and β chains). Interaction of BChl's with apoproteins is of vital importance in primary processes of photosynthesis. Many molecular spectroscopic investigations have been applied to explore the nature of the interactions [8]. However, no direct evidence concerning BChl-binding sites has been obtained yet. In order to understand molecular organization and the mode of BChl binding in the pigment complexes of RC-B890, we investigated effect of detergents on the absorption, circular dichroism (CD) and nuclear magnetic resonance (NMR) spectra of the isolated RC-B890 complex. In general BChl-protein complexes are destroyed by detergents. If detergents differently affect BChl spectral forms, we can obtain information of interaction modes of BChl complexes through detergent effects on absorption, CD or NMR spectra. In the course of this study, we found some detergents which predominantly worked on the B890 complex. The ^{13}C-NMR spectra of the complex isolated from a ^{13}C-enriched culture and reconstitution experiments, which used ^{13}C-enriched B890 polypeptides and BChl *a* with natural abundant carbon, also gave information on the BChl binding mode in the B890 complex.

Materials and Methods

Materials. Chromatium vinosum was cultured anaerobically under continuous illumination with a 60 W incandescent lamp at 10 cm distance for 3 or 4 days at 30°C. The culture medium used cotnains 0.1% Na_2S, 0.2% $Na_2S_2O_3$, 0.2% $NaHCO_3$ (w/v), and inorganic salts. The RC-B890 complexes were prepared as described previously [9–11]. Intracytoplasmic membranes were solubilized by 1% SDS in a 50 mM Tris/5 mM thioglycolic acid buffer and were electrophoresed in polyacrylamide gels in the same buffer with 0.05% SDS. The upper band with pink color was cut and homogenized with the 50 mM Tris/5 mM thioglycolic acid buffer. The supernatant of the homogenized solution was concentrated with a membrane cone (Amicon Centriflo type CF25). In the final preparation the RC-B890 contained less than 0.01% SDS. The corresponding ^{13}C-enriched samples were prepared from the cells cultured in a medium with $NaH^{13}CO_3$ instead of $NaHCO_3$. The treatment with detergents was done at ambient temperature (about 20°C), and an incubation time of about 15 min was taken. For reconstitution experiments a ^{13}C-enriched B890-polypeptide from *C. vinosum* was extracted with a mixture of chloroform and methanol (1 : 1, v/v) from freeze dried ^{13}C-enriched RC-B890 complexes. The extracted polypeptide was liberated from pigments and phospholipids by gel filtration on Sephadex LH-20 (Pharmacia) in the mixture of chloroform and methanol [12]. The fractions containing the B890-polypeptide were washed with chloroform and lyophilized.

Methods. Absorption spectra were recorded on a recording spectrophotometer Uvidec-510 (Jasco), or UV-365 (Shimadzu). CD spectra were obtained on a recording circular dichrometer J-500 C with a data processor DP-500 (Jasco). For observation of a near infra-red region an S-1 type photomultiplier was used with a 10 mm quartz cell. An S-20 type photomultiplier was used with a 0.05 mm quartz cell in the ultra-violet region. For the elimination of the scattering effect the sample cells were placed just before the photomultipliers. The scanning rate of 10 nm/min and the time constant of 8 s was enough to obtain the spectra. NMR spectra were measured with a Bruker CXP-300 FT NMR spectrometer whose ^{13}C resonance frequency is 75.46 MHz. The observations were carried out under ^{1}H broad band decoupling of 2 W with internal ^{2}H lock using deuterated solvents in 10 mm sample tubes. The 30° pulse and the recycle delay of 0.5 s were normal conditions for the observation with a spectral width of 31.25 kHz. The 40000–60000 transients were accumulated. The line broadenings of 10 Hz were applied to all spectra. The chemical shifts were referred to 2,2-dimethyl-2-silapentane-5-sulfate (DSS) or tetramethylsilane (TMS).

Results

Effects of detergents on a RC-B890 complex were examined by absorption and CD spectroscopy. In Table I the detergents used in the experiments are summarized. Zwitterionic (LDAO and lauryl-*N*,*N*-dimethyl-*N*-carboxymethylbetaine) and cationic (dodecyltrimethylammonium chloride and cetyltrimethylammonium bromide) detergents were found to affect the absorption and CD spectra of B890 BChl in the RC-B890 complex. In Fig. 1 a typical example is shown for the case of LDAO. Most of the absorption intensity and the CD magnitude around 890 nm are due to the B890 BChl's, but some small contributions from RC may exist in this region as seen later. The small absorption peak and the large S-shaped CD bands around 800 nm are due to the BChl's in the RC [13–15]. It is clearly seen that 0.05% (2.2 mM) of the detergent decreased the absorption peak at 890 nm and the CD trough at 890 nm. At the concentration of 0.3% (13.1 mM) of LDAO, the absorption band around 890 nm became smaller and the CD spectra exhibited only a broad positive peak around 850 nm. This CD spectrum shows some resemblances to those of the RC complexes of photosynthetic bacteria (for example, *Rps. sphaeroides* [15], or *Rhodopseudomonas palustris* [13], or *R. rubrum* [14], which means that the signals due to B890 essentially disappeared at this concentration of the detergent. The CD spectrum in the presence of 0.3% LDAO, however, is different from those for the isolated RC's in the point that the 800 nm CD features are larger relative to the 850 nm features. Also the peak position (about 850 nm) is somewhat lower in the

TABLE I
LIST OF DETERGENTS USED IN THE EXPERIMENTS
$\mathbf{R} = CH_3(CH_2)_{11}$

Chemical name	Structural formula
Anionic	
Sodium dodecyl sulfate (SDS)	$\mathbf{R}\text{–}O\text{–}SO_3^- \cdot Na^+$
Sodium dodecylbenzene sulfonate	$\mathbf{R}\text{–}C_6H_4\text{–}SO_3^- \cdot Na^+$
Cationic	
Dodecyltrimethylammonium chloride	$\mathbf{R}\text{–}N^+(CH_3)_2\text{–}CH_3 \cdot Cl^-$
Cetyltrimethylammonium bromide	$CH_3(CH_2)_{15}N^+(CH_3)_2\text{–}CH_3 \cdot Br^-$
Zwitterionic	
Lauryl-*N*,*N*-dimethylamine *N*-oxide (LDAO)	$\mathbf{R}\text{–}N^+(CH_3)_2\text{–}O^-$
Lauryl-*N*,*N*-dimethyl-*N*-carboxymethylbetaine	$\mathbf{R}\text{–}N^+(CH_3)_2\text{–}CH_2CO^-$
Nonionic	
α-[4{1,1,3,3-Tetramethylbutyl)phenyl]-ω-hydroxypoly(oxy-1,2-ethanediyl) (Triton X-100)	$CH_3\cdot C(CH_3)_2\text{–}CH_2\text{–}C_6H_4\text{–}(OCH_2CH_2)_nOH$; $n = 90$ or 10
Dodecyloctaoxyethylene ether	$\mathbf{R}(OCH_2CH_2)_8OH$

wavelength. These spectral features cannot be explained in the present stage, but this may come from the contribution from B890 modified by the detergent. The 780 nm absorption peak growing on addition of the detergent is due to BChl's freed from the B890 protein complex. This precluded the comparison of the absorption spectrum in the presence of 0.3% LDAO to that for an isolated RC of *C. vinosum* [16], but its light minus dark spectra showed about 20% of photobleaching at 880 nm (not shown). In the CD spectra the monomeric BChl gave negligible signals around 780 nm because of a relatively small CD magnitude of a monomeric BChl [8]. When another zwitterionic detergent lauryl-*N*,*N*-dimethyl-*N*-carboxymethylbetaine was used, the B890 was also selectively destroyed at the concentration of 0.2% (figure in Supplementary Material). Cationic detergents dodecyltrimethylammonium chloride and cetyltrimethylammonium bromide also gave similar results (figures in Supplementary Material).

An anionic detergent, SDS, affected the absorption and CD spectra of the RC-B890 complex in fairly different way from the zwitterionic or cationic ones. Fig. 2 shows the addition effect of SDS on the absorption and CD spectra of the RC-B890 complex. Though addition of 0.1% LDAO essentially diminished the 890 nm CD trough, SDS did not change the CD spectrum of the RC-B890 complex at the concentration of 0.1%. However, at higher concentrations of the detergent, all CD troughs and peaks decreased proportionally to each other. Another anionic detergent, sodium dodecylbenzene sulfonate, worked somewhat differently from SDS (figure in Supplementary Material). Thus, this detergent induced

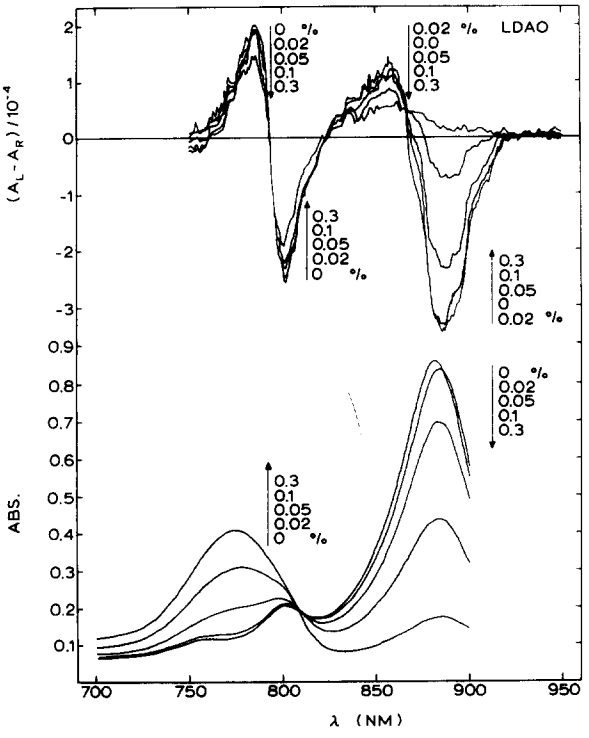

Fig. 1. The near infra-red absorption (below) and circular dichroism (above) spectra of the RC-B890 complex of *C. vinosum* in 50 mM Tris with 5 mM thioglycolic acid buffer in the absence and the presence of lauryldimethylamine *N*-oxide (LDAO) at ambient temperature. The concentration of the detergent is expressed by volume to volume.

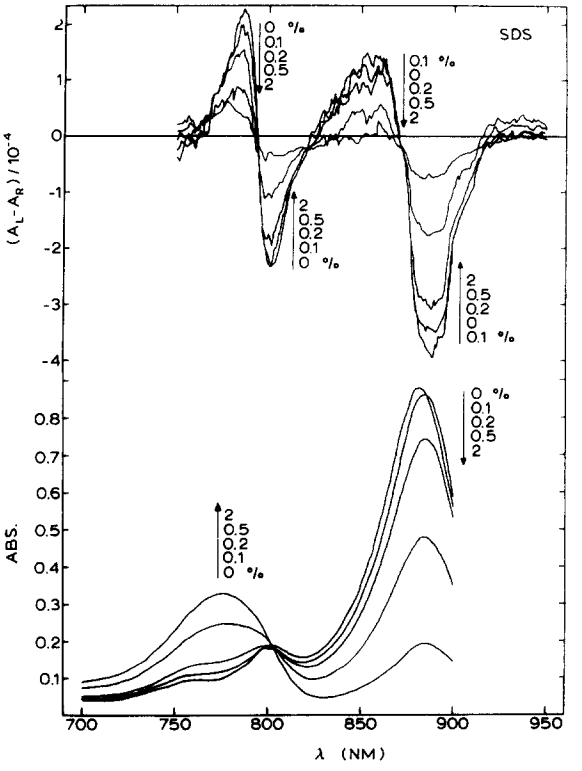

Fig. 2. The near infra-red absorption (below) and circular dichroism (above) spectra of the RC-B890 complex of *C. vinosum* in 50 mM Tris with 5 mM thioglycolic acid buffer in the absence and the presence of sodium dodecyl sulfate (SDS) at ambient temperature. The concentration of detergent is expressed by volume to volume.

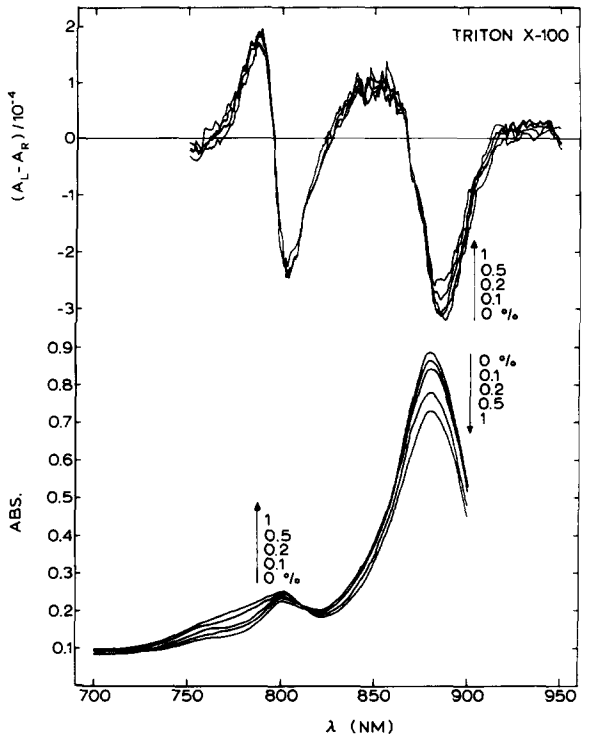

Fig. 3. The near infra-red absorption (below) and circular dichroism (above) spectra of the RC-B890 complex of *C. vinosum* in 50 mM Tris with 5 mM thioglycolic acid buffer in the absence and the presence of α-[4-(1,1,3,3-tetramethylbutyl)phenyl]-ω-hydroxypoly(oxy-1,2-ethanediyl) (Triton X-100) at ambient temperature. The concentration of the detergent is expressed by volume to volume.

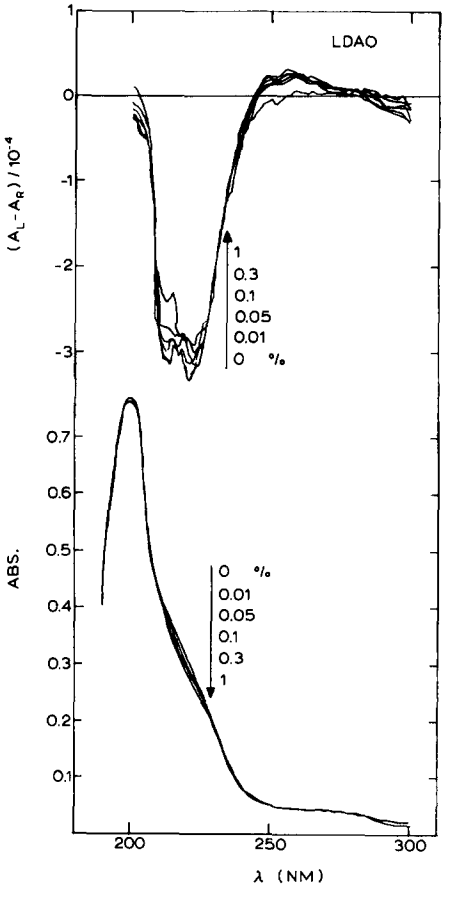

Fig. 4. The ultraviolet absorption (below) and circular dichroism (above) spectra of the RC-B890 complex of *C. vinosum* in 50 mM Tris with 5 mM thioglycolic acid buffer in the absence and the presence of lauryldimethylamine *N*-oxide (LDAO).

rather small changes (less than 20%) to all bands, except for 860 nm peak (which decreased by half) in the concentration of 0.5%.

In clear contrast to the detergent effects described above, neutral detergents (Triton X-100 and dodecyloctaoxyethylene ether) worked differently on RC-B890. Fig. 3 shows the absorption and CD spectra of the RC-B890 complex in the presence of Triton X-100 as a typical example of the neutral detergents. Addition of Triton X-100 at concentrations below 0.2% gave no change on the spectra of B890 complex. Addition of higher concentrations of Triton X-100 decreased the 890-nm trough slightly. Thus the neutral detergent does not significantly affect the absorption and CD spectra of the RC-B890 complex.

Fig. 4 shows effects of added LDAO on the absorption and CD spectra of RC-B890 in the ultraviolet region. The addition of LDAO up to 1.0% shows essentially no effect. Similarly, SDS and Triton X-100 (up to 2%) did not alter the absorption and CD spectra of RC-B890 in the ultraviolet region (not shown). Thus the detergents used caused no change in the secondary structures of the RC-B890 complexes.

Since the absorption and CD experiments revealed that LDAO destroyed the B890 complex structure, the mode of BChl binding with polypeptides in B890 was investigated by ^{13}C-NMR spectra. Figs. 5 and 6 show ^{13}C-NMR spectra of a ^{13}C-enriched RC-B890 complex in the absence and presence of LDAO and Triton X-100, respectively. The addition of LDAO induced the

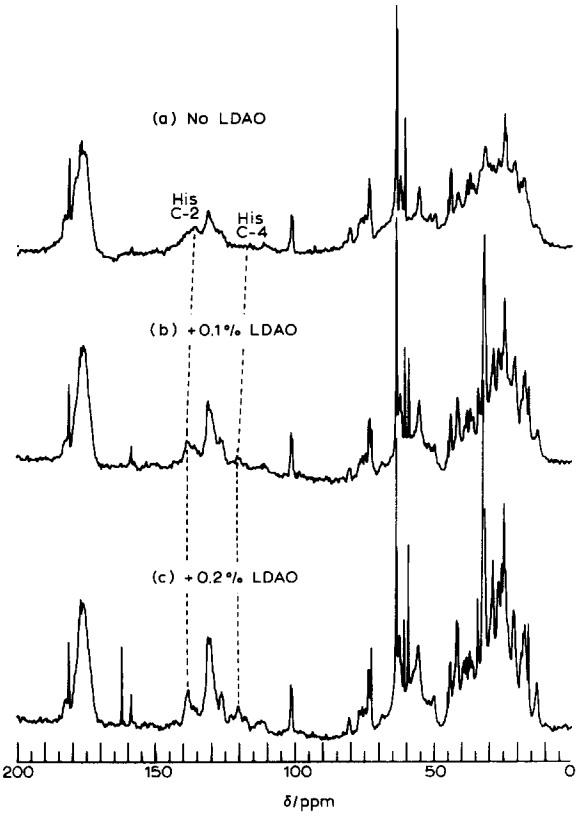

Fig. 5. The 75.46 MHz ^{13}C-NMR spectra of ^{13}C-enriched RC-B890 in 10 mM phosphate $^{2}H_2O$ buffer in the absence and presence of LDAO at 300 K. The absorbance of the sample was 20 at 890 nm in 1 cm optical cell. The detergent concentration is expressed by volume to volume. The spectrum was obtained under ^{1}H broad band decoupling of 2 W with ^{2}H lock using deuterated solvents in 10 mm sample tubes. The spectral width was 31.25 kHz. The accumulation times was 60000. The line broadening of 10 Hz was applied to the spectrum. The chemical shifts are referred to sodium-2,2-dimethyl-2-silapentane-5-sulfate (DSS). The data are plotted in proportion to the absolute intensity.

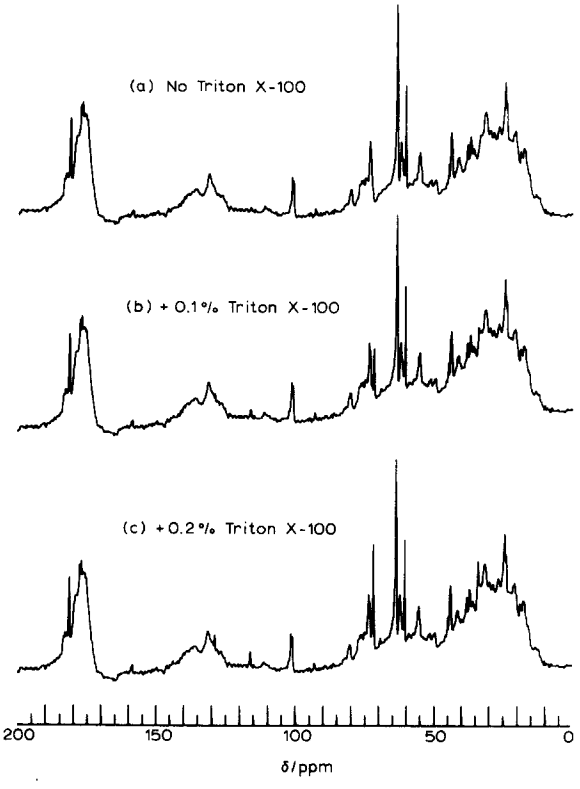

Fig. 6. The 75.46 MHz ^{13}C-NMR spectra of ^{13}C-enriched RC-B890 in 10 mM phosphate $^{2}H_2O$ buffer in the absence and presence of Triton X-100 at 300 K. The other experimental conditions were same as those in Fig. 5.

band narrowing, while Triton X-100 did not make signals sharp. NMR band narrowing indicates fast local molecular motions of amino acid residues in the B890 proteins. The band narrowing is especially prominent in the aliphatic region (0–50 ppm). It should be noted that some signals (25, 32 and 60 ppm) attributable to LDAO are visible in the spectra, though the RC-B890 complex is ^{13}C-enriched and LDAO has natural abundant carbons. Significant changes were seen in the signals which may be attributable to His C-2 (136–138 ppm) and C-4 carbons (118–120 ppm). The signals around 138 ppm became significantly sharp when LDAO was added up to 0.2%. This signal was not the main peak in the absence of LDAO. Further interactions of BChl-polypeptide were explored from reconstitution experiments which used only the chloroform/methanol extracted B890-polypeptide and BChl *a*. Fig. 7 shows the ^{13}C-NMR spectra of the ^{13}C-enriched B890-polypeptide in the absence and the presence of BChl *a* with carbons in natural abundance.

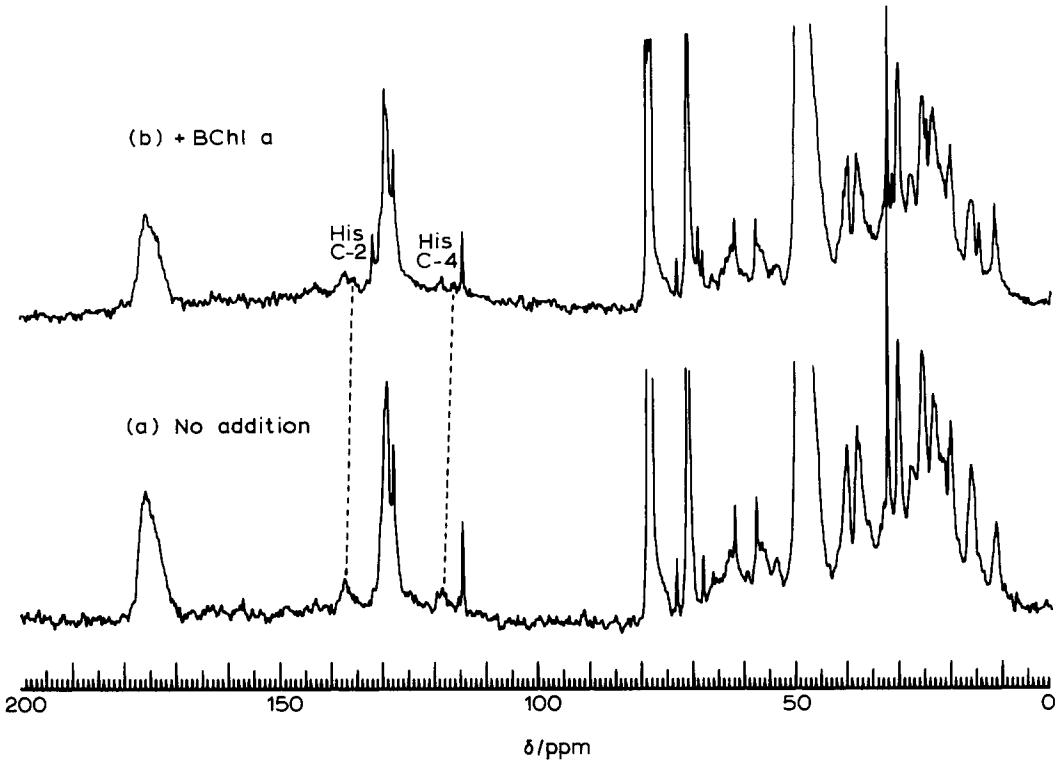

Fig. 7. The 75.46 MHz ^{13}C-NMR spectra of a ^{13}C-enriched B890-polypeptide in chloroform-d_1/methanol-d_4 (1 : 1, v/v) solution in the absence and presence of BChl *a* at 300 K. The concentration of the polypeptide was 1.5 mM, and the molar ratio of the polypeptide to BChl *a* was about 1 : 10. The chemical shifts are referred to external tetramethylsilane (TMS). The data are plotted in proportion to the absolute intensity. The other experimental conditions were similar to those in Fig. 5.

Discussion

RC-B890 is a complex which contains both RC and B890. The near-infrared band of BChl *a* has been assigned to the Q_y transition [17,18]. Although origins of near-infrared CD have not been clarified enough, it is clear at least that BChl–BChl and protein–BChl interactions are essential for the presence of CD in the near-infrared region [18]. If these essential structures are destroyed, they are keenly reflected on CD spectra. Hence, a CD spectrum is a critical measure of the presence of the native BChl complex.

As described in the Results section, LDAO, lauryl-*N*,*N*-dimethyl-*N*-carboxymethylbetaine, cetyltrimethylammonium bromide and dodecyltrimethylammonium chloride (see Table I) selectively destroyed the B890-complex structure. SDS destroyed both B890 and RC proteins. Triton X-100 and dodecyloctaoxyethylene ether did not perturb the native state of the RC-B890 complex. Selective actions of detergents should be noted. Thus zwitterionic and cationic detergents selectively destroyed the B890 complex. The anionic detergent, SDS, destroyed both B890 and RC complexes at the same time. Neutral detergents had a relatively small effect on the RC-B890 complex. Therefore, zwitterionic and cationic detergents should be suitable for the isolation of the RC from the RC-B890 complex.

The amino acid sequences of the B890 polypeptides have been determined recently for various purple photosynthetic bacteria [3–7,19–21]. It has been shown that the amino terminal region of the B890 α-polypeptide so far studied is positively charged, while that in the β-polypeptide is negatively charged. Ionic interactions between these chains are suggested to stabilize the B890 complex

[12]. Although the sequence has not been determined completely for *C. vinosum*, the homology in the sequence was found in the amino terminal region among the B890 polypeptides of the purple photosynthetic bacteria [12]. From these facts the disruption of the ionic interaction between α- and β-polypeptides by cationic and zwitterionic detergents is considered to be an important factor for the selective action of detergents.

Because of high molecular weights and restricted molecular motions, BChl-proteins in solution could not give high-resolution (narrow banded) ^{13}C-NMR signals [22]. However, in the presence of LDAO the ^{13}C-NMR signals became much sharper (Fig. 5). Furthermore, ^{13}C-NMR signals show essentially no line narrowing on addition of Triton X-100 (Fig. 6). Since CD results have shown that LDAO destroyed the B890 chromophore structure, but Triton X-100 did not, the observed signal narrowing can be ascribed to the free local molecular motions of the B890 polypeptides acquired by the addition of LDAO. The isolated RC-B890 may have some aggregation structures. The disaggregation into smaller units will be induced on addition of detergents. However, the disaggregation alone cannot make the NMR line narrowing, which was clear from the NMR results with Triton X-100 (Fig. 6), because Triton X-100 induced disaggregation [23]. As mentioned before, the 220 nm CD showed no change when LDAO was added (Fig. 4), the secondary structures of the B890 polypeptides remain unaffected. Hence, the band narrowing does not imply the loss of stiffness of the main polypeptide chain, but the acquired flexibility of the amino acid residues in the B890 proteins. This would be caused probably by the destruction of strong interactions between the B890 polypeptides and BChl, and/or between the B890 polypeptides themselves.

The narrowing of NMR signals on addition of LDAO is also observed in the aromatic region. In the presence of 0.2% LDAO, the peaks were observed from 100–140 ppm. The peaks at 138 and 120 ppm are in the chemical shift range ascribable to His C-2 and C-4 carbons, respectively. These peaks were not clearly observed in the absence of LDAO. The 138 ppm was shoulder of the broad peak around 136 ppm and the 120 ppm signals was invisible in the absence of LDAO. Therefore the appearance of 138 and 120 ppm signal in the presence of LDAO indicates the free local molecular motions of His residues on addition of LDAO. Though His carbons were not only aromatic carbons that acquire free local molecular motions, the changes were consistent with the destruction of BChl-His interactions which were suggested in several papers [12,19,20].

The BChl-His interactions were also suggested from reconstitution experiments using the extracted ^{13}C-enriched B890-polypeptide and BChl *a* with natural abundant carbons. Thus the two signals which can be assigned to His C-2 (137 ppm) and C-4 (118 ppm) showed high-field shift when BChl *a* was added to the polypeptide (Fig. 7). This may suggest interactions of BChl *a* with a His residue in the B890-polypeptide, and the ring current of BChl caused the high-field shift of His carbon signals. Although these NMR data were observed in the organic solvent system (chloroform/methanol) where BChl Q_y absorption maximum was at 800 nm (not shown), it was observed that the polypeptide retains α-helical structures in this organic solvent system as indicated from the CD spectra in the ultraviolet region (not shown). Therefore, it is suggested that the α-helical structure is important for the BChl and polypeptide interactions. The α-helical structure which was shown from the CD data in Fig. 4, in the native B890 is also considered to be an essential factor for the BChl-polypeptide interaction.

In conclusion, selective actions of detergents on an isolated RC-B890 complex of *C. vinosum* were detected from near-infrared absorption and CD spectra. The detergent effects on the ^{13}C-NMR coupled with reconstitution experiments using a ^{13}C-enriched B890-polypeptide and BChl *a* with natural abundant carbons, suggested the presence of BChl-His interactions in the B890 complex.

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FIGURE LEGENDS FOR SUPPLEMENTARY MATERIALS

Fig. S-1. The near-infrared absorption (below) and circular dichroism (above) spectra of the RC-B890 complex of *C. vinosum* in 50 mM Tris with 5 mM thioglycolic acid buffer in the absence and the presence of lauryldimethylcarboxybetaine at ambient temperature. The concentration of the detergent is expressed by volume to volume.

Fig. S-2. The near-infrared absorption (below) and circular dichroism (above) spectra of the RC-B890 complex of *C. vinosum* in 50 mM Tris with 5 mM thioglycolic acid buffer in the absence and the presence of dodecyltrimethylammonium chloride at ambient temperature. The concentration of detergent is expressed by volume to volume.

Fig. S-3. The near-infrared absorption (below) and circular dichroism (above) spectra of the RC-B890 complex of *C. vinosum* in 50 mM Tris with 5 mM thioglycolic acid buffer in the absence and the presence of cetyltrimethylammonium bromide at ambient temperature. The concentration of the detergent is expressed by volume to volume.

Fig. S-4. The near-infrared absorption (below) and circular dichroism (above) spectra of the RC-B890 complex of *C. vinosum* in 50 mM Tris with 5 mM thioglycolic acid buffer in the absence and the presence of sodium dodecylbenzenesulfonate at ambient temperature. The concentration of the detergent is expressed by volume to volume.

Fig. S-5. The near infrared-absorption (blow) and circular dichroism (above) spectra of the RC-B890 complex of *C. vinosum* in 50 mM Tris with 5 mM thioglycolic acid buffer in the absence and the presence of dodecyl octaoxyethylene ether at ambient temperature. The concentration of the detergent is expressed by volume to volume.