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Reaction Centers from a New Halophilic Purple Nonsulfur Photosynthetic Bacterium *Rhodospirillum sodomense*

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Both reaction centers containing and lacking cytochrome *c* were obtained from a new halophilic photosynthetic bacterium *Rhodospirillum (R.) sodomense*. The cytochrome *c* has a very low reduction potential not to be reduced by Na-ascorbate. The special pair bands in the both reaction centers exists at around 850 nm, some 20 nm to the blue of that of a nonhalophilic species, *R. rubrum*.

A new species of halophilic anoxygenic purple nonsulfur bacterium named *Rhodospirillum (R.) sodomense* has been isolated from water/sediment of the Dead Sea.[1] Growth was best at 12% of NaCl. Cells contained bacteriochlorophyll *a* (BChl *a*) and carotenoids of the spirilloxanthin series. Absorption spectra revealed the presence of a B875 (light-harvesting I), but no B800/B850 (light-harvesting II) photopigment complex.[1] Photoenergy conversion to electrochemical one takes place in reaction centers of photosynthetic organisms associated with electron transport initiated by light energy. The reaction centers are membrane proteins which carry out photoinduced electron transfers across membranes. In purple bacteria photoenergy can be continuously acquired by cyclic electron transfers through the reaction centers. Some species have cytochrome subunit bound to the reaction centers and others have not. The role of cytochrome in the re-reduction of the oxidized reaction center is still controversial problem. The presence or absence of bound cytochrome *c* in the reaction center and the oxidation-reduction properties of the cytochrome *c* is a very crucial issue. We have tried to characterize the photosynthetic reaction center of *R. sodomense* in comparison with that of the nonhalophilic purple bacterium, *R. rubrum*.[2] The reaction centers of *R. sodomense* have been isolated with bound cytochrome *c* in contrast to those from *R. rubrum*. The special pair band position exists at 846.5 nm, some 20 nm to the blue of that of *R. rubrum*. Reaction centers without cytochromes have been also isolated.

Cultivation methods were previously described in references.[1,3,4] Chromatophores were prepared by the sonication method as described previously.[5,6,8] Reaction centers were isolated from the whole chromatophore membrane by a N,N-dimethyldodecylamine N-oxide (LDAO) treatment followed by DEAE-Toyopearl column chromatography.[8] A chromatophore suspension with an absorbance of 30-40 at 875 nm was incubated in the dark at 0 ^{o}C in the presence of 0.25% LDAO for 1 h, followed by ultracentrifugation at 100000 x g. The dispersed ultracentrifugation pellets were treated again in the same conditions for some preparations. The

supernatant liquid containing crude reaction centers was dialyzed against 20 mM Tris-HCl with 0.05% LDAO at pH 8.5, and then chromatographed on a column of DEAE-Toyopearl previously equilibrated in 20 mM Tris-HCl/0.05% LDAO (pH 8.5), at 4 ^{0}C. *R. sodomense* reaction centers were first washed by 100 mM NaCl buffer with 3 times column volumes and then were eluted by the same buffer with a 100-300 mM NaCl gradient. Final purification was obtained using a 7.5 mm x 300 mm Gel chromatography-HPLC column (TSKgel G3000PW) with 20 mM Tris-HCl (pH 8.5)/0.05% LDAO. Absorption spectra were recorded on a Shimadzu UV-3100 recording spectrophotometer. Circular dichroism (CD) and magnetic circular dichroism (MCD) spectra were obtained on a recording circular dichrometer, Jasco J-500 C equipped with an electric magnet of 13.5 T in conjunction with a Jasco DP-500 data processor.

From 10 g of *R. sodomense* cell paste (250 A_{875} x volume in ml of chromatophores), 4 A_{800} x volume in ml of reaction center was isolated. The reaction centers can be isolated both with and without bound cytochrome *c* as shown by the presence and absence of a 400 nm peak in the absorption spectra of Fig. 1a (solid and dashed lines). One time LDAO treatment yielded reaction centers with cytochrome *c*, while two time treatments gave ones without cytochrome *c*. Both reaction centers containing and lacking cytochrome *c* gave a similar elution profile on the Gel chromatography with one main elution peak (Fig. 1b), excluding the possibility that the reaction center with cytochrome *c* is a mixture of cytochrome *c* and the reaction center lacking cytochrome *c*.

Three near-infrared absorption peaks are observed in the purified reaction centers. The peaks at 846.5, 800, and 751 nm are due to absorbance from special pair BChl *a*, mostly accessory BChl *a*, and bacteriopheophytin *a* (BPh *a*), respectively. The 846.5 nm absorption band is shifted about 20 nm towards the blue of that in *R. rubrum*. This may imply that the special pair is oriented in a special way that affects its absorbance and could be due to its halophilic nature. In the visible portion of the spectrum the 595 nm band is due to the Q_X

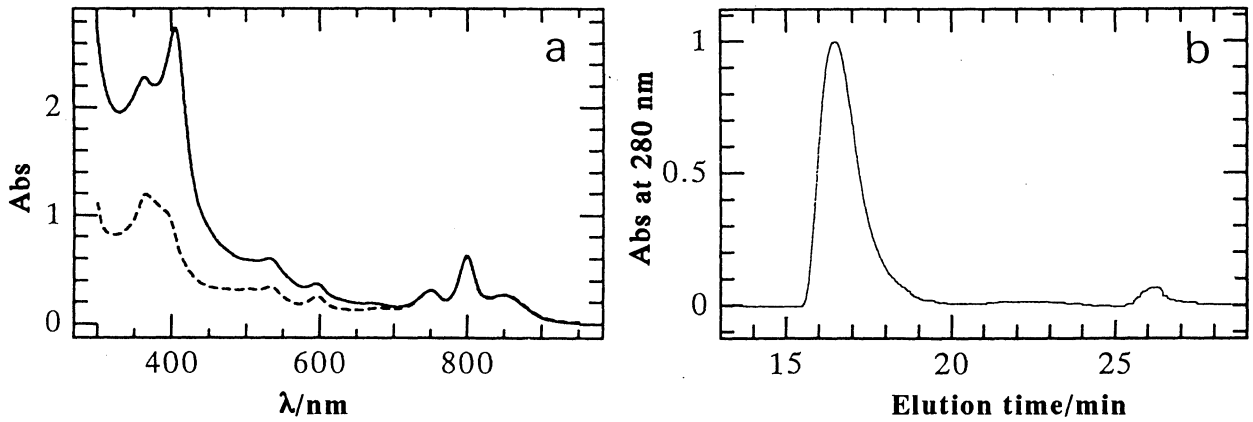

Fig. 1. Visible absorption spectra of *R. sodomense* reaction centers in 20 mM Tris-HCl/0.05% LDAO (pH 8.5). Solid line, with a bound cytochrome *c*; dotted line, without a bound cytochrome *c* (a). The HPLC elution profile for the reaction centers on a Gel chromatography with 20 mM Tris-HCl (pH 8.5)/0.05% LDAO in 0.4 ml/min (b).

transition of BChl *a* and the 531 nm band has contributions from the cytochrome *c*, carotenoids, and the Q_X transition of BPh *a*. The peak at 400 nm is due to the cytochrome *c* and the 366 nm band can be attributed to the Soret bands for BChl *a* and BPh *a*. The cytochrome-free reaction centers have a very similar absorption spectrum

to that of the cytochrome-containing ones, with the principle difference being the absence in the former of the characteristic cytochrome absorption bands and some broad absorption tail in the near UV region. In addition, small shifts in the wavelengths of the near infrared absorption bands are observed in the two samples. The peak of the reaction center photoactive BChl *a* is 846.5 nm in the cytochrome-containing samples and 849.5 nm in the cytochrome-free reaction centers. The shift induced by the presence of cytochrome *c* is a 3 nm blue shift which is opposite in direction from that observed in the thermophilic purple bacterium, *Chromatium (C.) tepidum* which gave also both types of reaction centers.[8] No effect of the cytochrome *c* was observed on the positions of the 800 nm accessory BChl *a* and the 751 nm BPh *a* absorption bands.

Figure 2 shows the CD (a) and MCD (b) spectra for the cytochrome-containing reaction center from *R. sodomense* in the near-infrared region in the presence of Na-ascorbate. Except for the short wavelength shift of the special pair absorbance, the spectral features in the CD and MCD spectra are similar to those of *C. tepidum*.[6] A couplet-type CD was observed in the Q_X region of BChl *a* (580-680 nm) (data not shown). The reaction center lacking cytochrome *c* exhibited similar CD and MCD spectra in the near infrared region (data not shown).

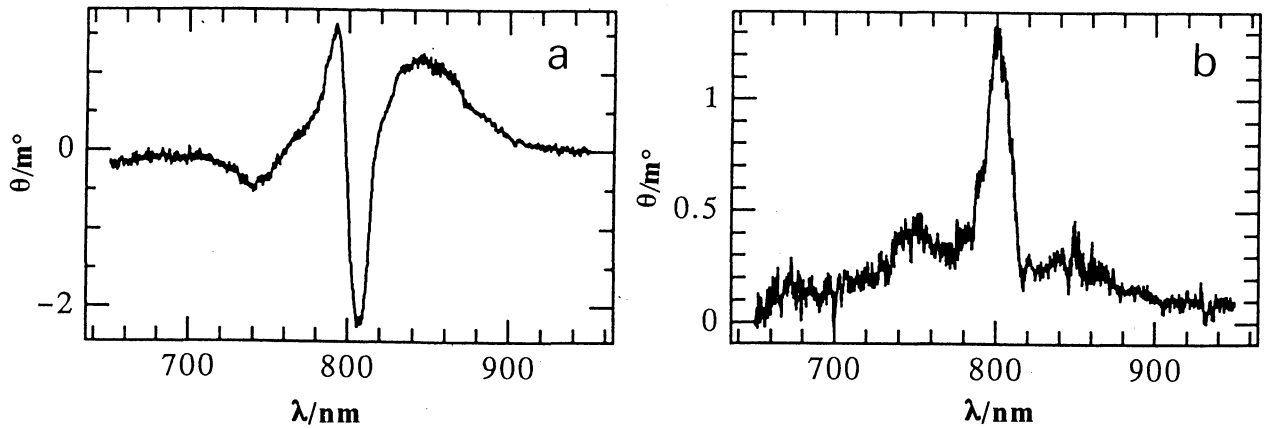

Fig. 2. Near-infrared circular dichroism (a) and magnetic circular dichroism (b) spectra for *R. sodomense* reaction centers containing a bound cytochrome *c* in the presence of Na-ascorbate in a 20 mM Tris-HCl buffer with 0.05% LDAO at pH 8.5.

Figure 3 shows the MCD spectra in the visible region for cytochrome-containing and cytochrome-free reaction centers from *R. sodomense* in the presence of Na-ascorbate in the Tris-HCl buffer at pH 8.5. From the comparison of the MCD spectra for the reaction center of *C. tepidum*[6] the 535 and 595 nm negative MCD bands for the reaction center lacking cytochrome *c* can be attributed to BPh *a* and BChl *a* transitions and the relative magnitude of the bands (1: 2) is proportional to the amount of pigments present in the reaction center. As seen in the MCD spectra for *C. tepidum*[6] the complex feature in the MCD for the reaction center with cytochrome *c* is derived from the overlapped contribution of MCD of the bound cytochrome *c* . The difference spectrum (Fig. 3b) clearly shows the MCD contribution from the bound cytochrome *c* from the similarities in the band positions and overall profiles to those for cytochrome *c*.[7] The very intriguing point is that the MCD band shape corresponds to that for the oxidized state. This means that the bound cytochrome *c* in the *R. sodomense* reaction center exists in an oxidized state in the presence of Na-ascorbate, thus has a low reduction potential. The reaction center from *C. tepidum* also has attached cytochromes, however it has cytochromes which can be reduced by Na-ascorbate.[8] Existence of a bound cytochrome *c* with a low reduction potential is unique for this halophilic purple bacterium,

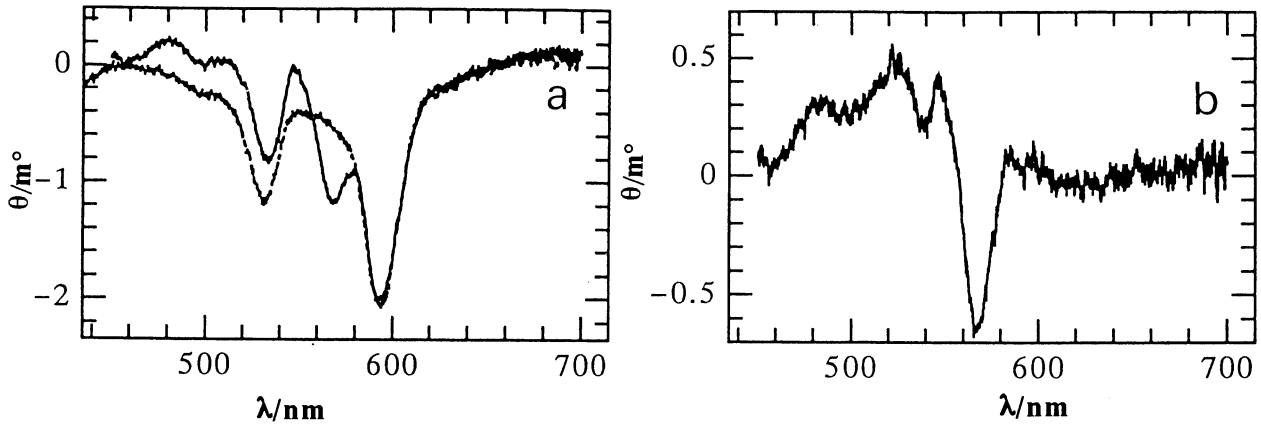

Fig. 3. Visible magnetic circular dichroism spectra of cytochrome-containing (solid line) and cytochrome-free reaction centers (dotted line) of *R. sodomense* normalized at 595 nm (a), and the difference spectrum between the two (b) in a 20 mM Tris-HCl buffer with 0.05% LDAO at pH 8.5.

and may have some physiological meanings for photosynthesis in this organism.

In conclusion the halophilic nonsulfur purple bacterium *R. sodomense* contains a photosynthetic reaction center with the bound cytochrome *c* which exists in the oxidized state in the presence of Na-ascorbate, and its special pair absorbs at 846.5 nm, some 20 nm blue relative to that of *R. rubrum*.

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References

1) E. E. Mack, L. Mandelco, C. R. Woese, and M. T. Madigan, *Arch. Microbiol.*, in press.
2) H. Noël, M. van der Rest, and G. Gingras, *Biochim. Biophys. Acta*, **275**, 219 (1972).
3) J. M. Ortega and P. Mathis, *Biochemistry*, **32**, 1141 (1993).
4) M. T. Madigan, *Int. J. Syst. Bacteriol.*, **36**, 222 (1986).
5) M. Ohta, T. Nozawa, M. Hatano, H. Hayashi, M. Tasumi, and K. Shimada, *Biochim. Biophys. Acta*, **1011**, 33 (1989).
6) T. Nozawa, M. Mimuro, M. Kobayashi, and H. Tanaka, *Chem. Lett.*, **1990**, 2125.
7) L. Vickery, T. Nozawa, and K. Sauer, *J. Am. Chem. Soc.*, **98**, 351 (1976).
8) T. Nozawa, J. T. Trost, T. Fukada, M. Hatano, J. D. McManus, and R. E. Blankenship, *Biochim. Biophys. Acta*, **894**, 468 (1987).

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