Purification and Crystallization of the Reaction Center from the Thermophilic Purple Sulfur Bacterium *Chromatium tepidum*

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Synopsis. The reaction center of *Chromatium tepidum* was crystallized by the vapor diffusion method using octyl β-D-glucoside and polyethylene glycol-4000 (PEG) as a detergent and a precipitant, respectively. Good crystals were obtained at reservoir PEG concentrations between 20—27.5% for a PEG concentration in protein solution of 8%.

The reaction center is the key protein in the primary process of photosynthesis and conducts an ultrafast charge separation across membranes by using light energy, which initiates the energy conversion reaction chain. For the elucidation of this fascinating function, structural information is crucial. Reaction centers from two purple nonsulfur bacteria have been crystallized and the structures have been analyzed by the X-ray diffraction method.[1—6] Until now, no reaction center from a purple sulfur bacterium has been crystallized. *Chromatium (C.) tepidum* is a novel purple sulfur bacterium with thermophilic properties.[7—10] It grows optimally at 50 °C. The purified reaction centers from *C. tepidum* are of two types; one with attached cytochromes, and the other without attached cytochromes.[11] The function of the attached cytochromes should be of importance in the reduction of the photooxidized reaction centers, which makes cyclic reactions feasible. In this regard, a structural basis for the function of the cytochromes is fundamental. Furthermore, the purified reaction center has thermostability up to 55 °C.[9] The structural basis for this thermostability would be very interesting. Hence as an initial approach to the elucidation of the structure of the reaction center, it was isolated to crystallization purity from the purple sulfur bacterium with an aim to clarify the correlation of its structures and functional and thermostable origins.

Experimental

C. tepidum was cultivated as reported previously.[7,8] Purification of the reaction center was conducted with the modified method described in our previous paper.[11] Cell paste (30 g wet weight) was dispersed in 100 ml of 20 mM Tris-HCl buffer(pH 8.5) with 0.05% LDAO. The mixture was sonicated for 2 min×10 times at 4 °C. The chromatophore membranes were then dispersed in a buffer at a constant concentration (OD_{850} of 50) and treated with 0.25% LDAO for 45 min at 40 °C. The ultracentrifugation pellet was dispersed again[12] in the same buffer at an OD_{850} of 50 and treated with 0.25% LDAO for 30 min at 40 °C. Following ultracentrifugration, the supernatant was purified by DEAE column chromatography. Crystallization was performed by the vapor diffusion method with octyl β-D-glucoside (OG) and polyethylene glycol-4000 (PEG) as a detergent and a precipitant, respectively. The crystallization conditions were similar to those used for *Rhodobacter sphaeroides* R-26.[4,5] Phosphate buffer (15 mM, pH 7.0) with NaCl, 0.1% NaN_3, and 1.0 mM EDTA was used for both the protein and reservoir solutions. The concentrations of NaCl were 0.36 M and 0.6 M for the protein and reservoir solutions, respectively (1 M=1 mol dm^{-3}). The concentrations of the protein solutions were 10—20 mg ml^{-1}.

OG was purchased from Wako Chemicals Co., Ltd., and *N,N*-dimethyldodecylamine *N*-oxide (LDAO) was a kind gift from Kao Co., Ltd.

Results and Discussion

The reproducible preparation of membrane proteins is rather difficult because the detergent treatment is often very tricky. We have improved the reproducibility of the detergent treatment by sonication and successive treatment with the detergent. This successive treatment improved the reproducibility of the preparation. As described in a previous paper,[11] *C. tepidum* yields two types of reaction centers; one with attached cytochromes and the other without cytochromes. Treatment of the chromatophores with LDAO two times, followed by DEAE-column chromatography with 20 mM Tris-HCl buffer (pH 8.5) including 0.05% LDAO yielded first the reaction centers with attached cytochromes at NaCl concentrations of 125—135 mM. This was the reaction center type we tried to crystallize. Reaction centers without attached cytochromes were eluted next at NaCl concentrations of 135—145 mM.

For crystallization, the detergent and the buffer were changed from LDAO–Tris-HCl buffer (pH 8.5) to 0.8% OG–phosphate buffer (pH 7.0) by DEAE column chromatography. The purity of the reaction center as judged by the absorption ratio of A_{280}/A_{800} was also increased by this process.

The reaction center preparations of *C. tepidum* with purity indexes (OD_{280}/OD_{800}) of 1.7—1.9 were selected for crystallization at 4 °C in OG by using PEG as a precipitant. In the crystallization of *C. tepidum* reaction centers, 1,2,3-heptanetriol (HT), a small amphillic molecule, induced denaturation of the protein. This is peculiar to this reaction center of a purple sulfur bacterium, and is in contrast to the fact that HT worked to aid crystallization of reaction centers from other purple nonsulfur bacteria.[13—15]

Table 1 summarizes the correlation of the time course of the protein solution and reservoir PEG concentrations in the crystallization of the reaction centers. It

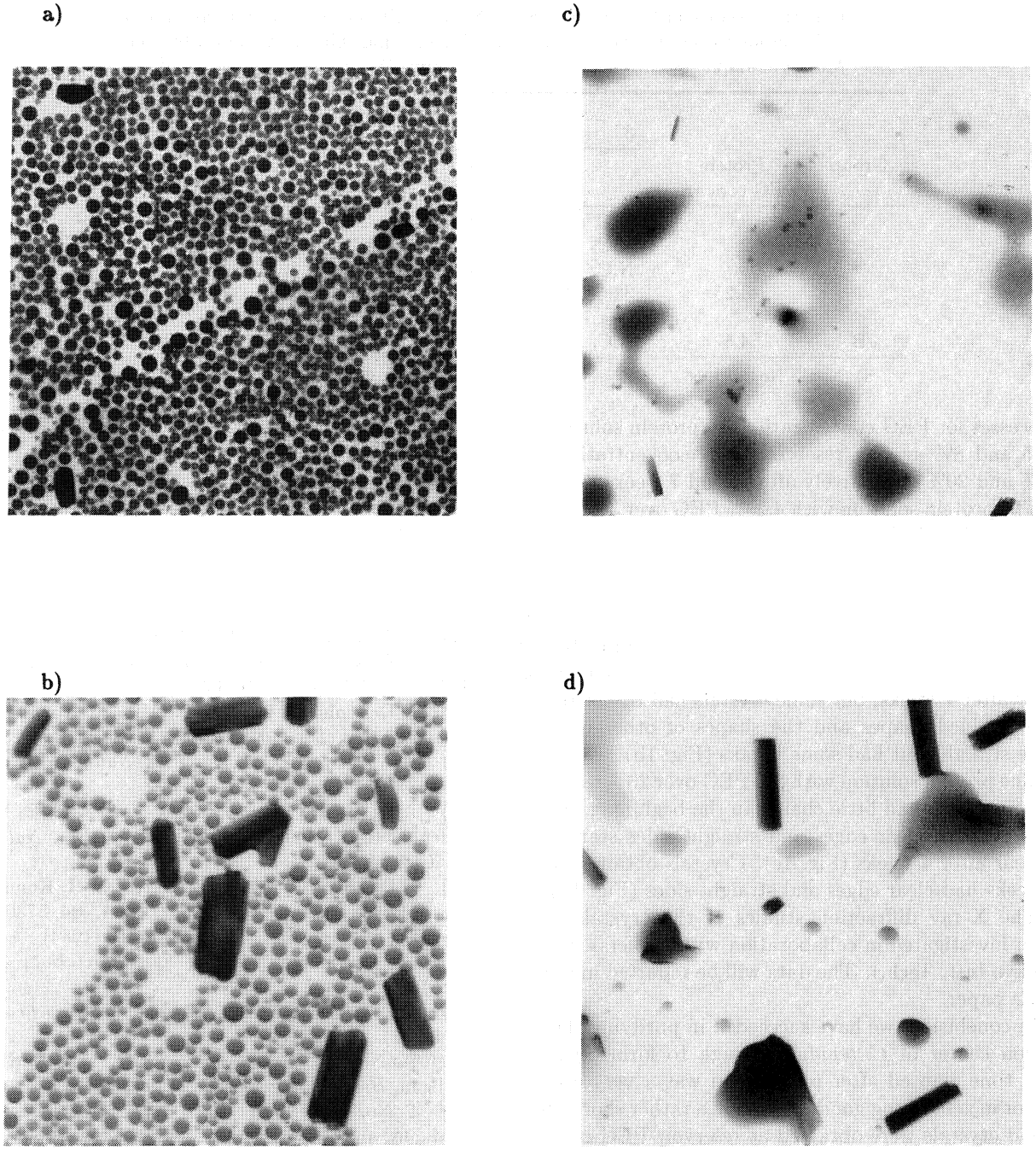

Fig. 1. Photographs of crystals of reaction centers of *Chromatium tepidum* formed at 4.25% protein solution PEG over 30% reservoir solution PEG after 4 weeks (a) and 7 weeks (b), and for 8% protein solution PEG over 20% reservoir solution PEG after 4 weeks (c) and 7 weeks (The biggest crystal has 0.3 mm length) (d).

also shows the effect of the amount of time in liquid nitrogen after purification. The maximum added PEG concentration allowed without yielding precipitation depended critically on the time elapsed after purification of the reaction centers. Thus, the reaction centers just after purification did not yield precipitate in PEG concentrations up to 8% (group A), however after more than one month at liquid nitrogen temperature, reaction centers formed precipitate in PEG concentrations as low as 4.5% (group B). The time required to begin crystallization and the numbers of crystals grown were severely dependent on the time elapsed after purification. Furthermore, the quality of the crystals was lower in group B than in group A. Hence, the effects of the protein solution and reservoir PEG concentrations on the crystallization were compared specifically for the reaction centers just after purification (i.e. group A). Figures 1a, 1b, 1c, and 1d show the crystal growing

Table 1. Ranges of Reservoir Solution Polyethylene Glycol (PEG) Concentrations to Form Crystals for Respective Protein Solution PEG Concentrations and Lapse of Time for Reaction Centers Just after Purification (Group A) and after One Month at Liquid Nitrogen Temperature (Group B)

Group	Protein PEG concn/%	Time/week 3	4	5	7	16
		Reservoir PEG concentration to form crystals/%				
A	8	N.C.[a)]	20—25	20—25	20—25	
	7	N.C.	20—27.5	20—27.5	20—27.5	
	5	N.C.	25—30	25—30	22.5—32.5	
	4.25	32.5—35	30—35	27.5—30	22.5—30	
B	4.5	N.C.	N.C.	N.C.	N.C.	20—28

a) N. C.: No crystals.

processes for PEG concentrations in protein solution of 4.25 and 8% against reservoir PEG concentrations of 30% and 20% respectively after 4 and 7 weeks.

The protein solution with 4.25% PEG over 30% reservoir solution PEG yielded small oil drops after 3 weeks. This may be attributable to the large PEG concentration difference between the protein and reservoir solutions. Some small nuclei or small crystals started to appear at 4 weeks (Fig. 1a). These crystals had distorted shapes and the sides were not straight. Many crystals grew after 7 weeks, but some crystals had rather round or combined shapes and the shapes of other crystals were straight but had some breaks (Fig. 1b).

The protein solution with 8% PEG over 20% reservoir solution PEG had little change in the beginning. Small crystals with clear edges and straight sides started to appear after 4 weeks (Fig. 1c). Crystals obtained after 7 weeks had clear edges and straight sides (Fig. 1d).

The X-ray diffraction studies of the crystals have been investigated in collaboration with Proferssor Miki (Tokyo Inst. Tech.). The data will be reported in a separate paper.

In conclusion, we have succeeded in purifying the reaction center of *C. tepidum* enough to form crystals. The time elapsed after purification was a very crucial factor in generating good crystals in a rather short time. Good crystals were obtained at reservoir PEG concentrations between 20—27.5% and a PEG concentration in protein solution of 8%.

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