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Screening of detergents for solubilization, purification and crystallization of membrane proteins: a case study on succinate:ubiquinone oxidoreductase from *Escherichia coli*

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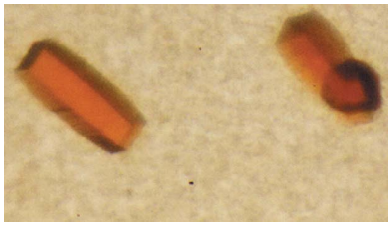


Succinate:ubiquinone oxidoreductase (SQR) was solubilized and purified from *Escherichia coli* inner membranes using several different detergents. The number of phospholipid molecules bound to the SQR molecule varied greatly depending on the detergent combination that was used for the solubilization and purification. Crystallization conditions were screened for SQR that had been solubilized and purified using 2.5%(*w*/*v*) sucrose monolaurate and 0.5%(*w*/*v*) Lubrol PX, respectively, and two different crystal forms were obtained in the presence of detergent mixtures composed of *n*-alkyl-oligoethylene glycol monoether and *n*-alkyl-maltoside. Crystallization took place before detergent phase separation occurred and the type of detergent mixture affected the crystal form.

1. Introduction

Membrane proteins consist of one or more hydrophobic regions that are buried in the membrane as well as hydrophilic regions that contain charged or polar residues that are exposed to water. For crystallization, membrane proteins are usually solubilized from the membranes using a detergent and then purified in the presence of a detergent. The membrane proteins thus prepared are water-soluble protein–detergent complexes in which the membrane-anchored hydrophobic portions are covered with amphiphilic detergent molecules. Crystallization of membrane proteins has been carried out using these protein–detergent complexes. Since the successful crystallization of bacteriorhodopsin (Michel & Oesterheld, 1980) and porin (Garavito & Rosenbusch, 1980) in 1980, many membrane proteins have been crystallized and there are currently 167 unique structures (http://blanco.biomol.uci.edu/Membrane_Proteins_xtal.html) in the Protein Data Bank. However, the crystallization of membrane proteins is still a difficult task and the quality of the crystals obtained has often been insufficient for X-ray diffraction studies. One of the obstacles in the crystallization of membrane proteins is that detergents suitable for solubilization, purification and crystallization must be found by trial and error.

Succinate:ubiquinone oxidoreductase (SQR) is a member of the citric acid cycle and catalyzes the oxidation of succinate to fumarate in conjunction with the reduction of ubiquinone to ubiquinol during aerobic respiration. The enzyme from *Escherichia coli* inner membranes consists of four subunits with five prosthetic groups: one covalently bound FAD, three Fe–S clusters and one haem *b*. The flavoprotein subunit (70 kDa) and Fe–S-containing subunit (30 kDa) are hydrophilic and contain all of the prosthetic groups except for the haem *b*, which is contained in two smaller membrane-anchoring subunits (14 and 13 kDa). As *E. coli* SQR can easily be purified in large quantities according to an established method (Kita *et al.*, 1989) and the X-ray structure has already been determined by Yankovskaya *et al.* (2003) at 2.6 Å resolution, the enzyme appears to be suitable for studies of membrane-protein crystallization. In this work, the phospholipid contents of *E. coli* SQR preparations obtained after solubilization and purification using different detergents were analyzed. Screening of crystallization conditions was performed for

SQR that was prepared using sucrose monolaurate and Lubrol PX for solubilization and purification, respectively, and two new crystal forms were obtained in the presence of detergent mixtures composed of *n*-alkyl-oligoethylene glycol monoether and *n*-alkyl-maltoside.

2. Methods

2.1. Expression and preparation of membranes

A *Bam*HI fragment (*sdhCDAB*) was inserted into pLC339 vector as described by Kita *et al.* (1989). The plasmid was introduced into *E. coli* K12 strain ST4785/pGS133, which lacks the cytochrome *bo* operon. Cells were grown aerobically at 310 K in a 10 l jar fermentor containing LB medium (Miller, 1972) and kanamycin (50 mg l^{-1}) under vigorous agitation and aeration. The addition of kanamycin was essential for the overproduction of *E. coli* SQR, which was at least sixfold higher than in the wild-type strain. The cells were harvested in the late exponential phase of growth and washed in 50 m*M* Tris–HCl buffer pH 7.4 containing 3 m*M* EDTA and 0.1 m*M* PMSF. The typical yield was about 200 g of wet cells from 10 l of culture. Membrane vesicles were prepared from freshly grown cells (200 g) suspended in 500 ml 50 m*M* Tris–HCl buffer pH 7.4 containing 20 m*M* EDTA and a protease-inhibitor cocktail (Sigma) by EDTA/lysozyme treatment followed by disruption with a French press (Yamato *et al.*, 1975). After the removal of any unbroken cells by low-speed centrifugation, membranes were pelleted by ultracentrifugation at 200 000*g* for 2 h at 277 K. The pellet was suspended in 600 ml buffer solution (50 m*M* Tris–HCl pH 7.4 and 10 m*M* EDTA) and the suspension (25 ml) loaded onto buffer (50 ml) containing 44%(*w*/*v*) sucrose was centrifuged at 200 000*g* for 2 h in a Hitachi P45AT fixed-angle rotor. The reddish-brown coloured band of membranes which formed in the middle of the ultracentrifugation tube was separated from the white pellet. The membrane fraction was diluted four times with the buffer and then precipitated by centrifugation at 200 000*g* for 2 h. The pellet was resuspended in a minimum amount of buffer (~80 ml) containing 10%(*w*/*v*) sucrose.

2.2. Estimation of SQR concentration

Since the absorbances at 280 nm (A_{280}s) of the detergents and chemicals in the buffer solutions used in this study were small, the concentration of the *E. coli* SQR was estimated using the calculated molar extinction coefficient at 280 nm (ε_{280} = 129 440), giving A_{280} = 10.6 for a pure SQR solution at 10 mg ml^{-1}. The ε_{280} value was calculated using $\varepsilon_{280} = 5690n_x + 1280n_y$ (Edelhoch, 1967), where 5690 and 1280 are the molar absorption coefficients at 280 nm of tryptophan and tyrosine and n_x and n_y are the number of tryptophan and tyrosine residues in *E. coli* SQR, respectively.

2.3. Assay for phospholipid content

The phospholipid content was assayed using the Fiske–SubbaRow method (Bartlett, 1959). A suitable quantity of the membrane suspension or purified SQR preparation was mixed with 0.5 ml 10 *N* H_2SO_4 in a clean glass test tube and heated in an oven at 423–433 K for more than 3 h. Organic compounds were dehydrated and decomposed to carbon by the H_2SO_4 and the inorganic phosphorus was liberated from the phospholipid. After the addition of several drops of 30% H_2O_2, the solution was again heated (423–433 K) for at least 1.5 h. 4.6 ml 0.22%(*w*/*v*) ammonium molybdate and 0.2 ml of the Fiske–SubbaRow reagent (Bartlett, 1959) were added to the solution, mixed thoroughly and heated for 7 min in a boiling water bath. The colourless solution turned blue and the absorbance at 830 nm (A_{830}) was measured. The concentration of the phosphorus liberated from the phospholipid was estimated from the A_{830} values of standard solutions containing an inorganic phosphate compound of known concentration. For simplicity, we assumed that the phospholipid contained only one P atom unless described otherwise. The A_{830} given by 0.02 µmol PO_4^{3-} is about 0.2 and thus 1 mg of SQR (0.008 µmol) with one bound phospholipid molecule produces an A_{830} of about 0.08. The concentration of the phospholipid in the membrane suspension prepared as described above was about 20 m*M*.

2.4. Solubilization and purification of SQR

In this study, two new crystal forms of *E. coli* SQR were obtained using a preparation that was solubilized from the membranes using 2.5%(*w*/*v*) sucrose monolaurate (CMC = 0.4 m*M*; Dojindo) and purified in the presence of 0.5%(*w*/*v*) Lubrol PX (Nacalai Tesque). Lubrol PX, which is a cheaper detergent than sucrose monolaurate, is a mixture of C_nH_{2n+1}-$(OCH_2CH_2)_m$-OH (abbreviated as C_nE_m) with different-length hydrocarbon and ethylene glycol chains and is virtually the same detergent as THESIT used by Yankovskaya *et al.* (2003). The membrane suspension prepared from 100 g of *E. coli* cells was diluted with buffer solution (20 m*M* Tris–HCl pH 7.4, 10 m*M* $MgCl_2$ and 2 m*M* sodium malonate) to give a phospholipid concentration of 4 m*M* and a freshly prepared 25%(*w*/*v*) sucrose monolaurate solution (477 m*M*) was stirred into the suspension until a final concentration of 2.5%(*w*/*v*) was achieved. After stirring for 1 h at 277 K, the solution was centrifuged at 200 000*g* for 1 h. The clear reddish-brown supernatant containing the solubilized SQR was applied onto a column of GE Healthcare DEAE Sepharose FF (500 ml bed volume) equilibrated with buffer *A* [20 m*M* Tris–HCl pH 7.4, 2 m*M* sodium malonate and 0.5%(*w*/*v*) Lubrol PX]. After washing the column with 2000 ml buffer *A*, SQR was eluted with 4000 ml of buffer *A* containing a linear gradient of 0.0–0.3 *M* NaCl. Fractions containing SQR (A_{412}/A_{280} > 0.5; Fig. 1, lane 1) were pooled. Solid polyethylene glycol 3350 (PEG 3350) was gradually added (30 g per 100 ml) to the pooled fraction containing about

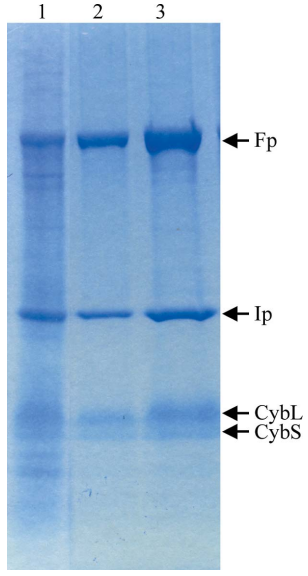


Figure 1
A 12% SDS–PAGE gel stained with Coomassie Brilliant Blue showing the purity of the purified *E. coli* SQR. Lane 1, an eluted fraction from DEAE Sepharose FF; lane 2, an eluted fraction from Source 15Q; lane 3, the purified SQR after sucrose density-gradient ultracentrifugation. Hydrophilic (Fp and Ip) and hydrophobic (CybL and CybS) subunits are shown by arrows.

Table 1
Average number of phospholipid molecules bound to purified SQR.

The *E. coli* SQR was solubilized using 0.1% (1.9 m*M*), 1.0% (19 m*M*), 2.5% (48 m*M*) and 4.0% (76 m*M*) sucrose monolaurate from membranes with different phospholipid concentrations and purified in the presence of 0.5%(*w*/*v*) Lubrol PX. Values in parentheses are the molar ratios of SML to phospholipid in the membrane suspension.

SML† concentration (m*M*)	Phospholipid concentration in membrane suspension (m*M*)	Average No. of phospholipids per SQR monomer‡§
1.9 (4.8)	0.4	11
19 (0.95)	20	10
19 (4.8)	4.0	8
48 (3.0)	12	6
48 (12)¶	4.0	6
48 (24)	2.0	6
76 (3.8)	20	6
76 (19)	4.0	6

† Sucrose monolaurate (CMC 0.4 m*M*). ‡ The concentration of the purified SQR was estimated by A_{280} (A_{280} = 10.6 for 10 mg ml^{-1} SQR solution). § It was assumed that the phospholipid contained only one P atom. ¶ Crystallization was performed for SQR solubilized under this condition.

530 mg SQR and the pellet obtained by centrifugation was dissolved again in buffer *A*. Residual impurities were successfully removed using a GE Healthcare Source 15Q column (70 ml bed volume). Fractions with an $A_{412}/A_{280} > 0.6$ obtained by elution with 1000 ml of a linear gradient of 0–0.3 *M* NaCl were pooled (Fig. 1, lane 2). The purified SQR (200 mg) was then subjected to sucrose density-gradient ultracentrifugation. The pooled SQR was precipitated by adding solid PEG 3350 and dissolved again in a minimum amount of buffer *A* (∼4 ml). The SQR solution (2 ml) was carefully loaded onto buffer *A* (∼73 ml) containing a 6–40%(*w*/*v*) sucrose-density gradient and centrifuged at 200 000*g* overnight in a Hitachi P45AT fixed-angle rotor. The SQR, which focused as a deep reddish-coloured sharp band in the middle of the gradient (Fig. 1, lane 3), was separated from the broad pale-reddish upper half in approximately 95% yield, precipitated by PEG 3350 and stored at 193 K for subsequent use. Sodium malonate, an inhibitor of SQR, was added to all of the purification and crystallization steps in order to stabilize the enzyme.

2.5. Detergent exchange

Detergent exchange was performed at 277 K as follows. After purification in the presence of Lubrol PX, the SQR precipitated by PEG 3350 was dissolved in 20 m*M* Tris–HCl buffer pH 7.4 containing 2 m*M* sodium malonate, 200 m*M* KCl and 1% of the detergent of choice. The A_{280} of the SQR solution was set to about 10. After incubation for 20 min on ice, the enzyme was precipitated by adding a 1.4-fold volume of 40%(*w*/*w*) PEG 3350 when the type of detergent exchanged was an *n*-alkyl-oligoethylene glycol monoether (C_nE_m). The pellet obtained by centrifugation was again dissolved in the same buffer and the enzyme was precipitated by PEG 3350 after a 20 min incubation period. This procedure was repeated several times. Exchanges to *n*-alkyl-glucosides (C_nG) and *n*-alkyl-maltosides (C_nM) were performed similarly, but the enzyme was precipitated by adding a 1.2-fold volume of 4.0 *M* ammonium sulfate. Since ammonium sulfate can cause phase separation of a solution containing C_nE_m and PEG can cause phase separation of a solution containing C_nG or C_nM, an appropriate choice of precipitants should be made in order to avoid the denaturation of the enzyme caused by phase separation. The completeness of detergent exchange was checked by thin-layer chromatography (Reiss-Husson, 1992).

Table 2
Average number of phospholipid molecules bound to purified SQR.

The *E. coli* SQR was solubilized and purified using different combinations of detergents. The concentration of phospholipids in the membranes was 4 m*M*, assuming that the phospholipid contained only one P atom.

Detergent used for			
Solubilization (2.5%)	Purification (0.5%)	Average No. of phospholipids per SQR monomer†	Crystallization‡
LDAO§	LDAO	∼100	Failed
DOC¶	LDAO	∼100	Failed
DOC	SMC††	19	Failed
SMC	SMC	16	Failed
SML‡‡	SML	6	Successful
SML	Lubrol PX§§	6	Successful
Lubrol PX	Lubrol PX	24	Failed
Lubrol PX (4%)	Lubrol PX	6	Successful

† The concentration of the purified SQR was estimated by A_{280} (A_{280} = 10.6 for 10 mg ml^{-1} SQR solution). ‡ Results of crystallization trials according to the optimized procedure described in the text. § *N*,*N*-Dimethyldodecylamine-*N*-oxide (CMC 1.4 m*M*, 0.03%). ¶ Deoxycholic acid (CMC 5 m*M*, 0.2%). †† Sucrose monocaprate (CMC 2.5 m*M*, 0.13%). ‡‡ Sucrose monolaurate (CMC 0.4 m*M*, 0.02%). §§ C_nH_{2n+1}-$(OCH_2CH_2)_m$-OH (n = 12, 14; m = 9.5).

2.6. Crystallization and X-ray diffraction experiments

Crystallization conditions were screened by the hanging-drop vapour-diffusion technique using 96-well CrystalClear Strips (Hampton Research). The SQR was dissolved in 10 m*M* Tris–HCl pH 7.6 buffer containing 2 m*M* sodium malonate and the detergent of choice (A_{280} = 30) and centrifuged for 20 min at 20 000*g* to remove any undissolved material. To set up the hanging-drop vapour diffusion, a droplet made up of 0.5 µl SQR solution and 0.5 µl reservoir solution was incubated over a well containing 100 µl reservoir solution. Initial screening was carried out at 277 and 293 K using the commercially available crystallization kits Crystal Screen (Jancarik & Kim, 1991) and Crystal Screen II from Hampton Research.

X-ray diffraction experiments were performed using the synchrotron beamlines BL44XU at SPring-8 (Harima, Japan), BL-5A at PF and NW12A at PF-AR (Tsukuba, Japan). For X-ray diffraction experiments at 100 K, crystals were transferred to reservoir solution supplemented with 15% glycerol and then frozen by rapid submergence in liquid nitrogen. The data were processed and scaled using programs from the *HKL*-2000 suite (Otwinowski & Minor, 1997).

3. Results and discussion

3.1. Phospholipid content of purified SQR

Since the major constituent (∼80% in weight) of the *E. coli* inner membranes is phospholipids (Tanford, 1980), the phospholipid content is a good indicator of the amount of lipid bound to the purified SQR. After solubilization from the membranes using 2.5%(*w*/*v*) sucrose monolaurate and purification in the presence of 0.5%(*w*/*v*) Lubrol PX as described in §2, the concentration of the phosphorus liberated from the phospholipid molecules bound to the purified SQR was measured. Assuming that the phospholipid molecules bound to the purified SQR contained one P atom and that the A_{280} of the 10 mg ml^{-1} SQR solution was 10.6, six bound phospholipid molecules per SQR monomer were detected. The phospholipid contents of SQR prepared under different solubilization conditions were also analyzed. Solubilization with 2.5%(*w*/*v*) or 4.0%(*w*/*v*) sucrose monolaurate gave a value of six regardless of the molar ratio between the detergent and phospholipid in the membrane suspension, whereas the phospholipid content increased to 11 on a decrease in the sucrose monolaurate concentration (Table 1). Therefore, these

six phospholipid molecules seem to be more tightly bound to the SQR. We also performed solubilization and purification using other commercially available detergents (Table 2). *N*,*N*-Dimethyldodecylamine-*N*-oxide (Fluka) and deoxycholic acid (Wako) produced SQR preparations containing ∼100 phospholipid molecules. However, the phospholipid content was reduced to 19 when purification was performed in the presence of 0.5%(*w*/*v*) sucrose monocaprate (Dojindo). Accordingly, most of the phospholipid molecules were loosely bound to the SQR and were removed by the action of 0.5%(*w*/*v*) sucrose monocaprate. On the other hand, 2.5%(*w*/*v*) and 4%(*w*/*v*) Lubrol PX gave phospholipid contents of 24 and six, respectively. In the X-ray structure of *E. coli* SQR solubilized using 4% THESIT, which is virtually the same detergent as Lubrol PX, two well ordered phospholipid molecules, phosphatidylethanolamine and cardiolipin, were found per SQR monomer (Yankovskaya *et al.*, 2003). Since phosphatidylthanolamine and cardiolipin contain one and two P atoms, respectively, there are three P atoms per SQR monomer in the X-ray structure. The disagreement over the number of P atoms probably arises from two causes. Firstly, since the concentration of the SQR was estimated from the calculated molar extinction coefficient in this study, the number of P atoms determined are relative rather than absolute values. Secondly, there may be additional disordered phospholipid molecules which are not detected by X-ray analysis.

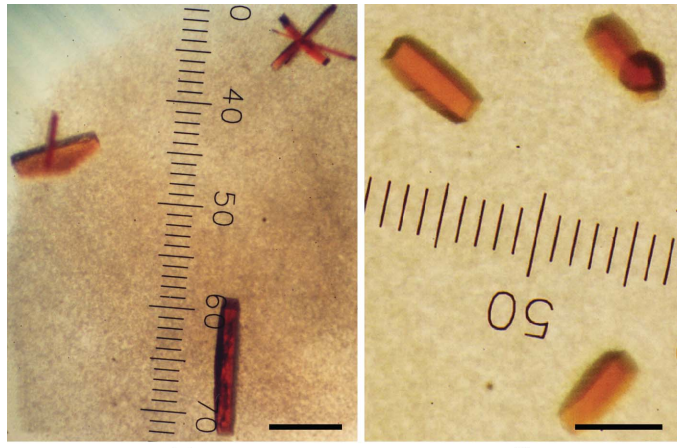
Since the Fiske–SubbaRow method only gives an average phospholipid content, it is not known whether or not the purified SQR was uniform in the number of bound phospholipid molecules. Following the Source 15Q column, the purified SQR with six bound phospholipid molecules was subjected to sucrose density-gradient ultracentrifugation at 200 000*g* overnight. After centrifugation, most of the SQR (about 95%) was focused in a sharp deep-reddish band which formed in the middle of the gradient, with a small amount of the enzyme (about 4%) spread broadly at the upper part of the gradient. The phospholipid content of the SQR in the sharp band was six, but in the broad band the phospholipid content was in the range 10–15 depending on the position of the gradient. This result indicates that the uniformity in the phospholipid content was improved by sucrose density-gradient ultracentrifugation.

3.2. Crystallization

After solubilization and purification using 2.5%(*w*/*v*) sucrose monolaurate and 0.5%(*w*/*v*) Lubrol PX, respectively, the purified SQR dissolved in 10 m*M* Tris–HCl buffer pH 7.4 containing 2 m*M* sodium malonate and 0.5%(*w*/*v*) Lubrol PX (50 mg ml^{-1} protein) was subjected to a crystallization trial using commercially available screening kits. Aggregates of small crystals were observed in a large amount of amorphous precipitate using several reservoir solutions containing PEG as a precipitant. An attempt was made to optimize the crystallization conditions by using PEGs with different molecular weights and by varying the PEG concentration, the pH and the temperature. However, none of the conditions investigated improved the results. Since Lubrol PX is a heterogeneous detergent, the detergent was exchanged for commercially available synthetic homogeneous $C_{12}E_8$ and $C_{10}E_8$ (Fluka) and the screening was carried out again. However, no single crystals other than aggregates of small crystals were observed. Additive Screen kits (Hampton Research) were used to examine the effects of adding various compounds but also failed. Single crystals of *E. coli* SQR were finally obtained when either *n*-dodecyl-β-D-maltoside ($C_{12}M$, Dojindo) or *n*-decyl-β-D-maltoside ($C_{10}M$, Dojindo) was used as an additive, but their optimum quantities varied subtly from experiment to experiment.

To attain reproducible crystallization, the Lubrol PX in the purified SQR was exchanged for detergent mixtures composed of $C_{12}E_8$ and $C_{12}M$ in ratios of 10:0, 9:1, 8:2 and 7:3 (by weight). Exchange to detergent mixtures with higher $C_{12}M$ content denatured the SQR and the addition of PEG 3350 caused phase separation of the SQR solutions, in which the SQR was concentrated in the phase enriched with the detergent. After detergent exchange, the enzyme was dissolved in 10 m*M* Tris–HCl pH 7.4 and 2 m*M* sodium malonate solution containing $C_{12}E_8$ and $C_{12}M$ in the prescribed ratio with a total concentration of 0.2%(*w*/*v*) and optimization of the crystallization conditions was repeated. Plate-shaped deep-red crystals with typical dimensions of 0.2 × 0.1 × 0.02 mm (Fig. 2*a*) appeared reproducibly within 3 d from reservoir solution composed of 16%(*w*/*v*) PEG 3350, 100 m*M* Tris–HCl pH 8.0, 2 m*M* sodium malonate and 200 m*M* KCl at 293 K when the ratio of $C_{12}E_8$ and $C_{12}M$ was 8:2 (by weight).

Since it has been noted that phase separation plays a major role in the crystallization of membrane proteins (Garavito & Picot, 1990; Reiss-Husson, 1992), the concentrations of PEG 3350 that caused phase separation of solutions containing $C_{12}E_8$ and $C_{12}M$ with a total concentration of 0.2% were examined at 293 K. The 10:0 solution, which contained only 0.2%(*w*/*v*) $C_{12}E_8$ in 100 m*M* Tris–HCl pH 8.0, 2 m*M* sodium malonate and 200 m*M* KCl, separated into two phases at 28%(*w*/*v*) PEG 3350. However, as the ratio of $C_{12}M$ increased, the concentration of PEG 3350 required gradually decreased to 23% and finally to 7%(*w*/*v*) PEG 3350 at 8:2 and 0:10, respectively. Therefore, the crystallization of *E. coli* SQR seems to take place using the 8:2 mixed detergent because the PEG 3350 concentrations necessary for crystallization and phase separation are closer to each other at 8:2 than those observed for the other mixed detergents with lower $C_{12}M$ contents. Interestingly, crystals of the same quality were also obtained at 7.5:2.5, but microcrystals formed only occasionally at 8.5:1.5. Crystals of the same crystal form were also obtained under a similar crystallization condition (20% PEG 3350, 100 m*M* Tris–HCl pH 8.2, 2 m*M* malonate and 200 m*M* KCl at 293 K) using an 8:2 mixture of $C_{12}E_8$ and $C_{10}M$. X-ray diffraction experiments were performed under liquid-nitrogen-cooled conditions at 100 K using synchrotron radiation. Although fresh crystals diffracted to better than 3 Å resolution, the diffraction limits rapidly reduced to lower than 4 Å resolution owing to radiation damage. Analysis of the symmetry and systematic absences of diffraction patterns indicated that the crystals belonged to the monoclinic space group $P2_1$, with unit-cell para-



(*a*) (*b*)

Figure 2
Crystals of *E. coli* SQR obtained using (*a*) 0.16% $C_{12}E_8$ and 0.04% $C_{12}M$ and (*b*) 0.16% $C_{10}E_8$ and 0.04% $C_{10}M$. The scale bar represents 0.2 mm.

Table 3
Statistics of data collection and processing.

Values in parentheses are for the highest resolution shell.

Wavelength (Å)	1.000
Space group	$P4_122$ (or $P4_322$)
Unit-cell parameters	
a (Å)	121.8
b (Å)	121.8
c (Å)	633.4
Solvent content† (%)	62
Resolution range (Å)	40.0–2.9 (3.04–2.9)
No. of reflections	880368
Unique reflections	86980
Completeness (%)	82.0 (85.6)
$R_{\rm merge}$‡ (%)	8.2 (49.9)
$I/\sigma(I)$	17.1 (3.7)

† Assuming the presence of three molecules in the asymmetric unit. ‡ $R_{\rm merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$.

meters $a = 121.3$, $b = 186.3$, $c = 216.4$ Å, $\beta = 90.6^\circ$. Assuming six SQR molecules (120 kDa × 6) per asymmetric unit, the $V_{\rm M}$ value (Matthews, 1968) is calculated to be 3.4 Å^3 Da^{-1} with an estimated solvent content of 64%, which is comparable to that found in previous work (69%) by Yankovskaya *et al.* (2003).

In contrast, enzyme for which the detergent was exchanged from Lubrol PX to an 8:2 mixture of $C_{10}E_8$ and either $C_{12}M$ or $C_{10}M$ crystallized in a different crystal form at 293 K from a reservoir solution composed of 20% PEG 3350, 100 m*M* Tris–HCl pH 8.0, 2 m*M* sodium malonate and 200 m*M* KCl (Fig. 2*b*). Diffraction patterns were recorded on the BL44XU beamline of SPring-8 using a DIP6040 detector at a wavelength of 0.9 Å. The crystals belonged to the tetragonal space group $P4_122$ (or $P4_322$), with unit-cell parameters $a = b = 121.8$, $c = 633.4$ Å. Three SQR molecules per asymmetric unit gave a $V_{\rm M}$ value of 3.3 Å^3 Da^{-1} and an estimated solvent content of 62%. A total of 880 368 observed reflections were merged to 86 980 unique reflections in the 40.0–2.9 Å resolution range. The data-collection and processing statistics are summarized in Table 3. An attempt to solve the structure using the molecular-replacement method with the *MOLREP* program (Navaza, 1994) as implemented in the *CCP*4 package (Collaborative Computational Project, Number 4, 1994) was carried out using the refined coordinates of *E. coli* SQR (PDB code 1nek). A solution with a trimer structure, which was consistent with the previous work of Yankovskaya *et al.* (2003), was obtained in space group $P4_322$. The trimer model was subsequently subjected to rigid-body refinement and gave an *R* factor of 39%. However, further refinement is not straightforward: the phospholipid molecules were not located currently because the electron densities of the membrane-anchoring hydrophobic regions were faint.

In conclusion, we show that the phospholipid content of purified SQR depends greatly on the detergent used for solubilization and purification and that the enzyme with the fewest bound phospholipid molecules was successfully crystallized in two crystal forms using mixtures of C_nE_m and C_nM. The reaction centre of photosystem II has also been crystallized using detergent mixtures and the role of detergent mixtures in crystallization has been discussed by Rukhman *et al.* (2000). On the basis of the knowledge obtained in this study, we have succeeded in the crystallization of two membrane proteins, quinol:fumarate reductase from *Ascaris suum* mitochondria and recombinant cyanide-insensitive alternative oxidase from *Trypanosoma brucei*, using different mixtures of detergents (details will be published elsewhere).

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