SHORT COMMUNICATIONS

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Crystals of bovine heart ubiquinol–cytochrome *c* reductase diffracting X-rays up to 2.8 Å resolution at 276 K

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Abstract

Bovine heart ubiquinol–cytochrome *c* reductase stabilized with sucrose monocaplate was crystallized with polyethylene glycol as the precipitant at 277 K. X-rays diffracted by the crystal were detected up to 2.8 Å resolution at 266 K, without using a synchrotron source. The space group and cell dimensions are $P6_1$ or $P6_5$ and $a = b = 128.5$ and $c = 715.7$ Å, respectively.

1. Introduction

Ubiquinol–cytochrome *c* reductase (E.C. 1.10.2.2) is one of three electron-transfer complexes which pump protons in the respiratory chain of aerobic organisms (Hatefi, 1985). Solving the three-dimensional structure of this enzyme at atomic resolution has long been the most important subject in the elucidation of the reaction mechanism of this enzyme. Recently four research groups have been trying to crystallize this enzyme (Yu *et al.*, 1996; Lee *et al.*, 1995; Berry *et al.*, 1995; Kawamoto *et al.*, 1994).

2. Methods and results

Ubiquinol–cytochrome *c* reductase was purified from bovine heart muscle with the method of Rieske (Rieske, 1967). The purified preparation which contains cholate as the solubilizing agent was washed with 50 m*M* Tris–HCl buffer pH 8.0 containing 660 m*M* sucrose and 0.6% sucrose monocaplate for several times on an Amicon diaflow apparatus equipped with a membrane filter with the pore size of 200 kDa for exchanging the cholate with sucrose monocaplate. The medium was then exchanged with 40 m*M* potassium phosphate buffer pH 6.5 containing 660 m*M* sucrose and 0.5% sucrose monocaplate on the diaflow apparatus. At the final exchange, the protein solution was concentrated to 20–25%(*w*/*v*) to form microcrystals without forming amorphous material. The microcrystals thus obtained were collected by centrifugation and dissolved in a minimal volume of 40 m*M* potassium phosphate buffer pH 6.5 containing 660 m*M* sucrose and 0.5% sucrose monocaplate. The dissolved crystals were dialyzed against the same medium for 5 h for complete exchange of the buffer system. A minimal amount of polyethylene glycol (PEG 4000, Sigma), 1.5–2.1%(*w*/*v*) depending on the preparation, was added to the dialyzed preparation at the protein concentration of 9%(*w*/*v*). The protein solution was kept at 277 K for 1–2 weeks to grow the crystals large enough for X-ray diffraction experiments.

Fig. 1 shows hexagonal column crystals of ubiquinol–cytochrome *c* reductase obtained by the present method. X-ray diffraction of the crystals placed in a quartz capillary 1 mm in diameter was measured with an imaging-plate detector (Rigaku, R-AXIS IIC) on a rotating-anode X-ray generator (Rigaku RU-200) at 276 K. The detector-to-crystal distance was 100 mm. The temperature of the capillary was controlled by a cryostat, FTS system (Air Jet Model Tc-84, Storm Ridge, New York). X-ray diffraction was detected up to 2.8 Å (Fig. 2) after exposing the single crystal for 30 min. The diffraction power of the crystals was not weakened after the X-ray exposure in different angle for 24 h. Unfortunately, the large cell dimensions resulted in incompletely resolved diffraction spots. The diffraction quality was not improved significantly by using the synchrotron source at the Photon Factory, Tsukuba, Japan. The space group and cell dimensions are determined from the well resolved diffraction spots obtained at the Photon Factory to be $P6_1$ or $P6_5$ and $a = b = 128.5$ and $c = 715.7$ Å, respectively. A reasonable value of 3.9 $Å^3\,Da^{-1}$ is obtained for V_m for the asymmetric unit containing two minimal catalytic units corresponding to 500 kDa. The calculated solvent content is 69% (Matthews, 1968). The X-ray diffraction quality depends very much on the temperature. At 281 K the highest resolution was only 6 Å, suggesting a large amount of damage to the protein in the crystal by the X-rays, as in the case for other crystals reported so far.

Fig. 1. Crystals of bovine heart ubiquinol–cytochrome *c* reductase. The overall dimensions of the area in each photograph represent 0.8 × 1.2 mm. The concentration of PEG 4000 was 1.92%.

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The enzyme preparations used for crystallization gave 11 bands in the sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) analysis as reported previously (Schägger *et al.*, 1986). Recrystallization did not affect these 11 bands, in contrast to the deletion of one of the subunits in the SDS–PAGE pattern as has been reported (Yu *et al.*, 1994). The microcrystallization at the final step of our purification procedure, as described above, significantly improves the purity, homogeneity and stability of the purified preparation, as given above, all of which seem critical for the crystal growth. In fact, no modification of the absorbance spectrum of the preparation stored at 273 K (or on ice) was detectable for 50 d or longer.

Bovine heart cytochrome *c* oxidase is crystallizable to provide X-ray diffraction up to 2.5 Å or higher, when decyl maltoside is used as the reagent for stabilizing the enzyme in the aqueous solution (Tsukihara *et al.*, 1995). Any modification for the detergent structure decreases the X-ray diffraction quality or even eliminates the crystallizability of the enzyme preparation, suggesting that crystals with high diffraction quality have high specificity for the detergent structure. Sucrose monolaurate also gave a crystal of beef heart ubiquinol–cytochrome *c* reductase with an X-ray diffraction quality at the same level as that of the preparation stabilized with sucrose monocaplate, though the reproducibility was slightly lower. Furthermore, the crystals with X-ray diffraction quality at the 3 Å resolution level have been obtained from two other groups using different detergents (Yu *et al.*, 1996; Lee *et al.*, 1995). Thus, these results suggests that the detergents which provide crystals which diffract to 2.8–3.0 Å may not be the best detergents for the crystallization. In other words, design and synthesis of new detergents is indispensable in the improvement of the crystallization conditions of beef heart ubiquinol–cytochrome *c* reductase.

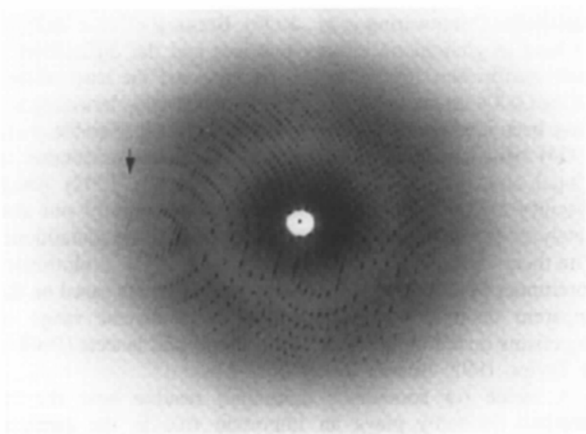

Fig. 2. X-ray diffraction pattern of a single crystal of bovine heart ubiquinol–cytochrome *c* reductase. An oscillation photograph taken at an oscillation angle of 1° without using X-ray beam of synchrotron radiation, as described in the text. The arrow denotes the diffraction spots at 2.8 Å resolution.

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