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Crystallization and preliminary X-ray diffraction study of aldehyde reductase from a red yeast, *Sporobolomyces salmonicolor*

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Abstract

Crystals of aldehyde reductase from a red yeast, *Sporobolomyces salmonicolor*, have been grown from an ammonium sulfate solution, pH 7.0, by means of the vapor-diffusion procedure. The crystals belong to the hexagonal system, space group $P6_122$ or its enantiomorph, $P6_522$, with unit-cell dimensions of a = 72.2 and c = 320 Å. The X-ray diffraction patterns extend to at least 2 Å resolution with the use of synchrotron radiation. The crystals are stable on exposure to X-rays and suitable for high-resolution X-ray structure determination.

There is a class of monomeric NADPH-dependent oxidoreductases with molecular masses of about 35 000, which is called the aldo-keto reductase superfamily (Bohren, Bullock, Wermuth & Gabbay, 1989). This superfamily contains numerous reductases, such as aldehyde reductase (E.C. 1.1.1.2) (Bohren *et al.*, 1989), aldose reductase (E.C. 1.1.1.21) (Bohren *et al.*, 1989), prostaglandin F synthase (E.C. 1.1.1.188) (Watanabe *et al.*, 1988), 2,5-diketogluconate reductase (Anderson *et al.*, 1985), chlordecone reductase (E.C. 1.1.1.225) (Winters, Molowa & Guzelian, 1990) and so on, and these proteins show high homology in their amino-acid sequences. From the strong similarities in their amino-acid sequences, ρ-crystallin (Tomorev *et al.*, 1984) and a yeast protein encoded by the *GCY* gene (Oechsner, Magdolen & Bandlow, 1988), which do not exhibit reductase activity, are also included in this superfamily.

Since aldose and aldehyde reductases cause complications in diabetes (Kinoshita & Nishimura, 1988), the crystal structures of human and porcine aldose reductases and their aldehyde reductases have been determined by X-ray diffraction methods (El-Kabbani *et al.*, 1991, 1994; Rondeau *et al.*, 1992; Wilson, Bohren, Gabbay & Quiocho, 1992). Elucidation of the tertiary structures and reaction mechanisms of these enzymes will facilitate the design of specific inhibitors as drugs for the treatment of diabetic complications. We have found a novel aldehyde reductase in a yeast, *Sporobolomyces salmonicolor* (Katauoka *et al.*, 1992; Yamada, Shimizu, Kataoka, Sakai & Miyoshi, 1990), and succeeded in cloning the aldehyde reductase gene from this strain (unpublished data). There is quite a high homology between the amino-acid sequence of this microbial aldehyde reductase and those of aldo-keto reductase superfamily proteins. Aldehyde reductase of *S. salmonicolor* catalyzes not only the NADPH-dependent reduction of *p*-nitrobenzaldehyde and pyridine-3-aldehyde, which are typical substrates for mammalian aldehyde and aldose reductases, but also that of prochiral carbonyl compounds such as 4-chloro-3-oxobutanoate esters (Kataoka *et al.*, 1992; Yamada, Shimizu, Kataoka, Sakai & Miyoshi, 1990). Since the reduction products derived from 4-chloro-3-oxobutanoate esters are optically active alcohols, the enzyme can recognize the stereo-positions of these substrates. Furthermore, there was a difference in substrate specificity between the microbial aldehyde reductase, and mammalian aldehyde and aldose reductases (Kataoka *et al.*, 1992; Yamada *et al.*, 1990). This stereoselectivity and substrate specificity might be dependent on the tertiary structure of the enzyme, therefore elucidation of the tertiary structure of the aldehyde reductase of *S. salmonicolor* is significant for elucidation of the mechanisms underlying the stereoselectivity and substrate specificity of the enzyme. Here, we report the growth of crystals of the aldehyde reductase of *S. salmonicolor* suitable for high-resolution X-ray diffraction work and preliminary crystallographic characterization.

The enzyme was purified as described in the previous report (Yamada *et al.*, 1990). Protein solutions of concentrations of 4–10 mg ml^{-1} were prepared in the presence of 30–50% (saturated concentration) ammonium sulfate as a precipitant in 10 m*M* phosphate buffer (pH 7.0). Droplets (10 µl) of the protein solutions were pipetted onto glass slides for hanging-drop vapor-diffusion and then vapor-equilibrated with 0.5 ml of reservoir solutions in which the concentrations of the precipitant were 20–100% higher than those of the protein solutions. Crystallizations were carried out at 277 K. The best crystals were obtained when a protein solution of a concentration of 7 mg ml^{-1} containing 50% saturated ammonium sulfate at pH 7.0 was vapor-equilibrated against outer solutions with a precipitant concentration of 60%. The hexagonal–bipyramidal crystals obtained, shown in Fig. 1, had average dimensions of 0.5 × 0.5 × 0.7 mm. Crystals were mounted in glass-capillaries with a trace amount of the mother liquor. Precession

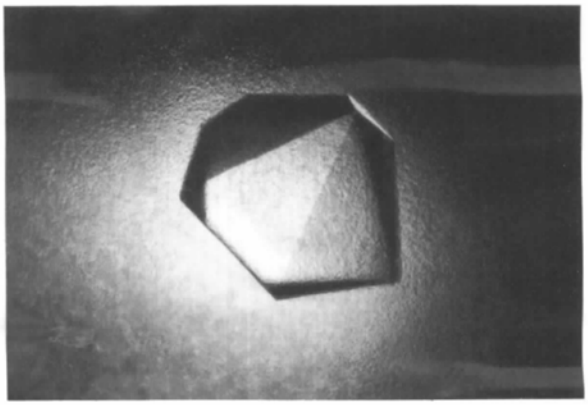

Fig. 1. A crystal of the aldehyde reductase from *Sporobolomyces salmonicolor* grown in an ammonium sulfate solution.

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photographs were taken at room temperature with a Huber precession/rotation camera, using graphite-monochromatized Cu $K\alpha$ radiation generated by an M18X rotating-anode X-ray generator (MAC Science Co., Ltd, Tokyo) operated at 50 kV and 90 mA, with a fine-focus filament. The crystal-to-film distance was set at 100 mm. The X-ray diffraction pattern was recorded on a 125 × 125 mm imaging plate (Fuji Photo Film Co., Ltd), which was digitized at 50 μm intervals with an R-AXIS DS read-out system (Rigaku Co., Ltd, Tokyo). The precession photographs (Fig. 2) showed that the crystals belong to the hexagonal system. The Laue symmetry and the systematic absences of reflections indicated that the space group is $P6_122$ or its enantiomorph, $P6_522$. The unit-cell dimensions determined from the precession photographs were refined to be a = 72.2 and c = 320 Å by a least-squares fit of diffraction spots recorded on an imaging plate with a DIP100 rotation camera system (MAC Science Co., Ltd, Tokyo). Assuming one or two molecules per asymmetric unit, the value for the crystal volume per unit molecular mass, V_m (Matthews, 1968), was calculated to be 3.5 or 1.7 Å^3 Da^{-1}, respectively, using a molecular mass of 35 kDa. The crystals were of good quality and stable on exposure to X-rays. Preliminary work involving synchrotron radiation at 2.5 GeV at the BL-6A beamline of the Photon Factory, the National Laboratory for High Energy Physics, showed that the crystals diffract X-rays to at least 2 Å resolution on an imaging plate equipped with Sakabe's Weissenberg camera (Sakabe, 1991). This indicates that the present crystals are suitable for high-resolution X-ray structure determination. The collection of data for the native crystals is in progress, which will be used for the molecular-replacement method, using the structure of the human or porcine enzyme as a search model (El-Kabbani, 1994).

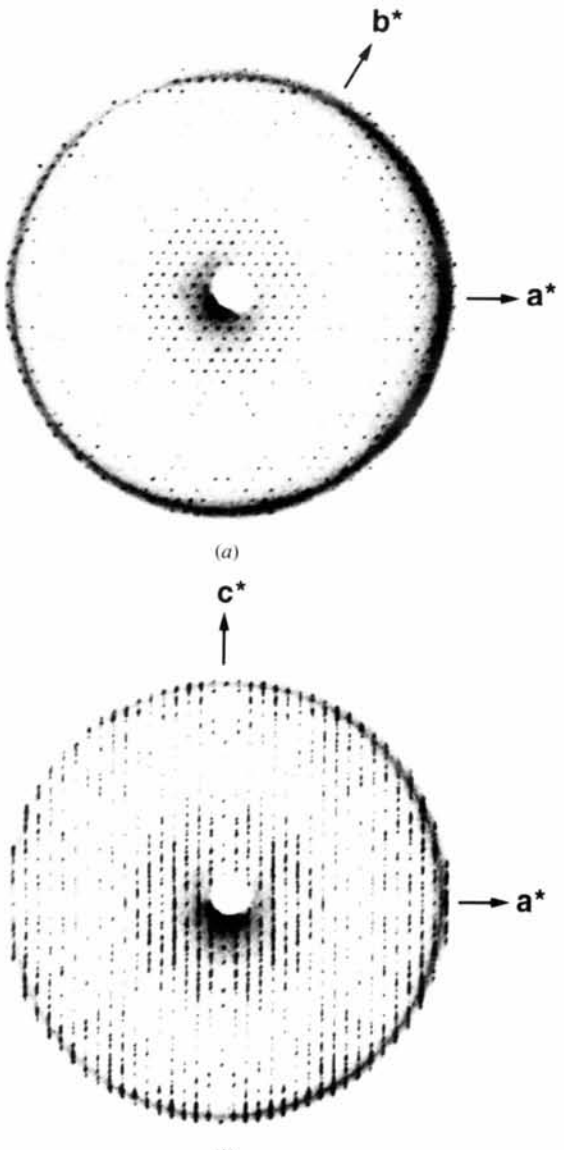

Fig. 2. X-ray precession photographs of crystals of aldehyde reductase from *Sporobolomyces salmonicolor* recorded on 125 × 125 mm imaging plates; (*a*) *hk*0 and (*b*) *h*0*l*. The conditions were: crystal-to-film distance, 100 mm; precession angle, 12.4° (= 3.6 Å resolution); and exposure time, 12 h.

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