Crystallization and preliminary X-ray analysis of NAD(P)H-dependent carbonyl reductase specifically expressed in the thyroidectomized chicken fatty liver

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1. Introduction

In 2003, Shibata et al. have demonstrated that the functionally unknown 29 kDa protein is specifically expressed in thyroidectomized (Tx) chicken fatty liver. On the basis of genome information of the chicken (Gallus gallus), we recently identified the gene encoding the protein. From comparison of the amino-acid sequence of this protein with those of the homologues whose crystal structures have been determined to date, we found that the protein exhibits the highest identity (36%) with a CR from fruit-fly Drosophila melanogaster (PDB entry 1sny; Sgraja et al., 2004) and contains the two consensus sequences (S-YXXXK, GXXXGXXG) of the SDR family. Moreover, we succeeded in the expression of the gene in E. coli and confirmed that the gene product surely exhibits CR activity [1]. Since the enzyme shows rather low sequence identities with CRs from human (33%; Tanaka et al., 2005), mouse (30%; Tanaka et al., 1996), and pig (27%; Tanaka et al., 2008), it is of interest to examine structural differences among insect, chicken, and mammalian CRs. The physiological function of the CR in Tx chicken fatty liver is currently unknown. The enzyme probably contribute to metabolism of accumulated ketone body derived from fatty acid. Structural analysis of the enzyme may provide important information about its physiological substrate and function. In this study, we describe crystallization and preliminary X-ray analysis of NAD(P)H-dependent CR which is specifically expressed in the fatty liver of thyroidectomized chicken.

2. Experiment

Initial screening for crystallization was carried out with Index (Hampton Research, USA) at 293 K using the sitting-drop vapor diffusion method.

The NAD(P)H-dependent CR crystal was flash-cooled in liquid nitrogen at 100 K. Diffraction data were collected at 1.98 Å resolution using monochromated radiation of wavelength 1.0 Å and an ADSC CCD detector system on the 5A beamline at the Photon Factory, Tsukuba, Japan. The oscillation angle per image was set to 1°. The crystal-to-detector distance was 249 mm. The data were processed using HKL-2000.

3. Results and Discussion

The diffraction-quality crystal (maximum dimensions of 0.6 × 0.3 × 0.05 mm; Fig. 1) was obtained within two weeks using a reservoir solution composed of 23% PEG 5000MME, 100 mM Bis-Tris buffer (pH 6.5).

The crystals belonged to the trigonal space group P3_21 (Fig. 2). Assuming two protein molecules in the asymmetric unit, the crystal volume per enzyme mass (V_M) and the solvent content were calculated to be 2.57 Å^3 Da^-1 and 51.7%, respectively, which are within the frequently observed ranges for protein crystals.

Fig.1 Trigonal crystal of Tx chicken fatty liver CR. Maximum dimensions of the crystal are 0.6 × 0.3 × 0.05 mm.

Fig.2 X-ray diffraction of a Tx chicken fatty liver CR.

Reference

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