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Crystallization and preliminary X-ray analysis of L-serine 3-dehydrogenase complexed with NAD(P)$^+$ from *Pyrobaculum calidifontis*

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Introduction

L-Serine dehydrogenase (L-SerDH; EC 1.1.1.276). catalyzes the dehydrogenation of L-serine in the presence of NAD(P)$^+$. This enzyme catalyzes dehydrogenation at the β-carbon (C3) position of L-serine. The suspected product of the reaction is 2-aminomalonate semialdehyde, which nonenzymatically decomposes into 2-aminoacetaldehyde and CO_2.

Currently, no L-SerDH has been so far reported in archaea, the third domain of life, or in hyperthermophiles. Within the genomic sequence of an aerobic hyperthermophilic archaeon, *Pyrobaculum calidifontis*, we found a gene (open reading frame identification number Pcal_0699) whose predicted amino acid sequence exhibits 36% identity with that of *P. aeruginosa* L-SerDH. Moreover, we succeeded in the expression of the gene in *E. coli* and confirmed that the gene product surely exhibits L-SerDH activity [1]. The *P. calidifontis* enzyme is probably the most thermostable L-SerDH described to date, which makes this enzyme potentially useful for stereospecific synthesis of L-serine and measurement of L-serine in foods and in a variety of other environments. The structure information of this thermostable L-SerDH may be available for the development of its application. In the present study, we describe the crystallization and preliminary X-ray analysis of *P. calidifontis* L-SerDH, as well as the expression of the gene in *E. coli*, as a first step in the structural analysis of L-SerDHs from archaeal strains.

Materials and Methods

Initial screening for crystallization was carried out with CrystalScreen and CrystalScreen 2 at 293 K using the sitting-drop vapor diffusion method.

The NAD(P)$^+$-dependent L-SerDH crystal was flash-cooled in liquid nitrogen at 100 K. Diffraction data were collected at 1.57 Å resolution using monochromated radiation of wavelength 1.0 Å and an ADSC CCD detector system on the NE3A beamline at the Photon Factory, Tsukuba, Japan. The oscillation angle per image was set to 1°. The crystal-to-detector distance was 184 mm. The data were processed using *HKL*-2000.

Results and Discussion

The diffraction-quality crystal (maximum dimensions of 0.6 × 0.2 × 0.1 mm; Fig. 1) was obtained within two weeks using a reservoir solution composed of 1.9-2.0 *M* ammonium sulfate, 100 m*M* acetate buffer (pH 4.5).

The crystals belonged to the monoclinic space group *C*2. The R_{merge} value in the highest resolution shell (5.9%) indicates that the crystals are diffracting far better than 1.57 Å (Fig. 2). Assuming one protein molecule in the asymmetric unit, the crystal volume per enzyme mass (V_M) and the solvent content were calculated to be 3.0 Å^3 Da^{-1} and 59.8%, respectively, which are within the frequently observed ranges for protein crystals.

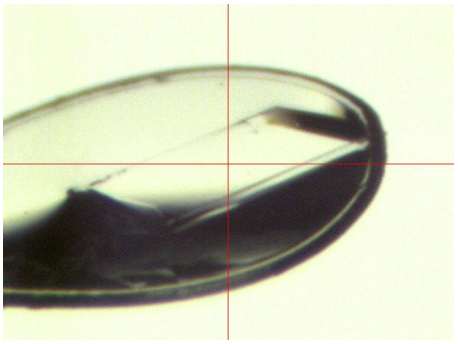

Figure1 Monoclinic crystal of *P. calidifontis* L-SerDH. Maximum dimensions of the crystal are 0.6 × 0.2 × 0.1 mm.

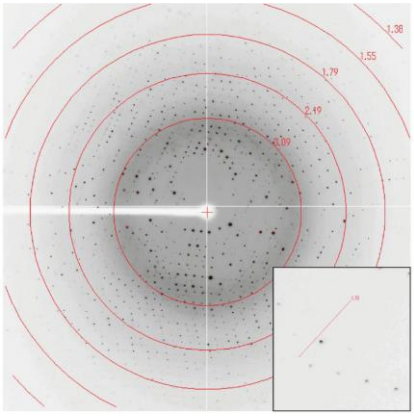

Figure2 X-ray diffraction of a *P. calidifontis* L-SerDH crystal. The high-resolution area is enlarged (insert).

References

[1] K. Yoneda *et al*., *Acta Crystallographica Section F.* (2013) **F69**, 134-136.

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