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Crystallization and preliminary analysis of bovine adenosine deaminase

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Adenosine deaminase (ADA) from bovine intestine was crystallized with purine riboside by vapour diffusion using ammonium sulfate as precipitant. The crystals are tetragonal and have unit-cell parameters $a = b = 80.03$, $c = 141.68$ Å. They belong to space group $P4_12_12$ or $P4_32_12$ and diffract to at least 2.0 Å resolution. The structure is being solved by molecular replacement.

1. Introduction

Adenosine deaminase (ADA, E.C. 3.5.4.4) catalyzes the irreversible deamination of adenosine or deoxyadenosine to inosine or deoxyinosine, respectively. ADA is one of the enzymes involved in the purine-metabolism pathway and is found in most mammalian tissues. It is indispensable to the upkeep of a competent immune system, since heritable deficiency of ADA is associated with severe combined immunodeficiency disease (SCID; Martin & Gelfand, 1981). It recently has been shown to be involved in T-cell activation (Kameoka *et al.*, 1993). Both enzyme-replacement therapy (Levy *et al.*, 1988) and gene therapy (Marshall, 1995) have been used to treat victims of this rare disease. Bovine ADA derivatized with polyethylene glycol (Hershfield *et al.*, 1987) has been administered to replace deficient human ADA.

Gene sequences have been reported for human ADA (Wiginton *et al.*, 1984), mouse ADA (Yeung *et al.*, 1985) and *E. coli* ADA (Chang *et al.*, 1991). Recently, the primary structure of bovine ADA has been reported (Kelly *et al.*, 1996). The homology of three mammalian enzymes is very high. The sequences of human and mouse ADA are 83% identical, those of bovine and mouse ADA are 85% identical, and those of human and bovine ADA are 91% identical. In particular, the amino-acid residues around the active site are highly conserved. Only one active-site residue is different between human ADA and mouse ADA, and all the residues around the active site are identical in human ADA and bovine ADA.

The crystal structure of mouse ADA was first reported in 1991 (Wilson *et al.*, 1991). All X-ray studies on ADA have been carried out using mouse ADA only (Sharff *et al.*, 1992; Wilson & Quiocho, 1993; Sideraki, Mohamedali *et al.*, 1996; Sideraki, Wilson *et al.*, 1996). Although bovine ADA is more closely related to human ADA, with which it is functionally interchangeable, the crystal structure of bovine ADA has not yet been reported. In this paper, the crystallization of bovine ADA with the inhibitor purine riboside is described.

2. Methods and results

2.1. Crystallization

ADA from bovine intestine was purchased from Boehringer–Mannheim Biochemicals and purine riboside was purchased from Sigma. The protein solution, which was used with no further purification for crystallization, was 20 mg ml^{-1} protein in 50 m*M* Tris–HCl buffer at pH 7.5 with 10 m*M* purine riboside. All crystallization trials were carried out using the hanging-drop method of vapour diffusion by mixing 2 µl of bovine ADA protein solution with 2 µl of the reservoir solution and equili-

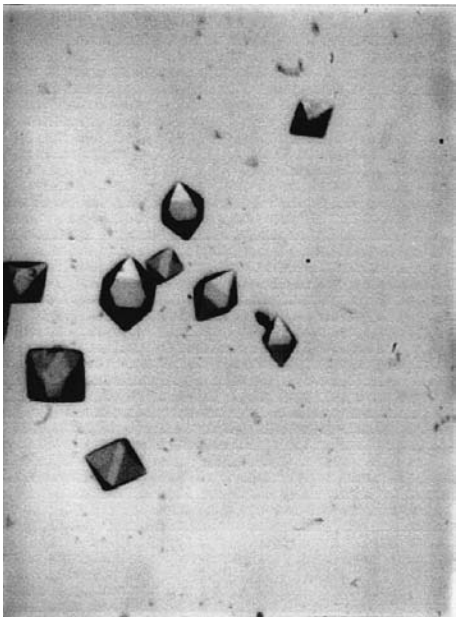

Figure 1
Crystals of bovine adenosine deaminase complexed with purine riboside.

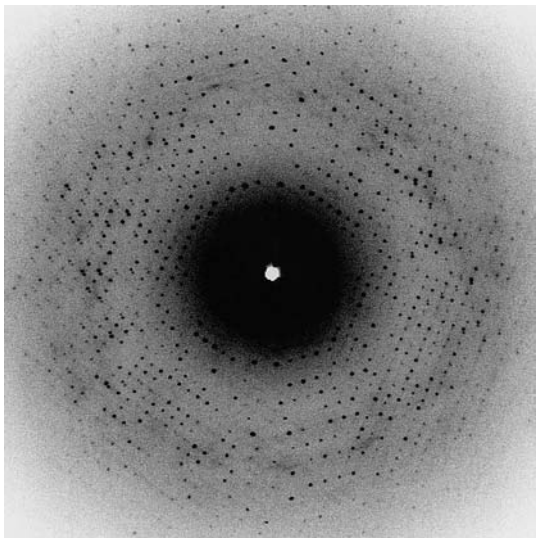

Figure 2
A diffraction image of bovine adenosine deaminase complexed with purine riboside.

brating the drops over the reservoir at room temperature. The initial trials were performed using grid sceening based on the crystallization conditions for mouse ADA: 15%(*w*/*v*) polyethylene glycol 6000, 0.1 *M* citrate buffer pH 4.2 (Wilson *et al.*, 1988). However, these experiments did not yield any crystals, only amorphous aggregation. Additional trials were performed using a commercially available sparse-matrix screening kit from Hampton Research (Jancarik & Kim, 1991). Small crystals of the complex with purine riboside were obtained from the experiments designed on the basis of results from the screening. The crystals of bovine ADA complexed with purine riboside used for data collection were grown against reservoirs containing 2.0–2.2 *M* ammonium sulfate, 2%(*v*/*v*) polyethylene glycol 400 in 0.1 *M* HEPES buffer at pH 7.5. The crystals grow to maximum dimensions of approximately 0.3 × 0.2 × 0.15 mm in two weeks (Fig. 1).

2.2. Data collection

The X-ray diffraction data were collected at room temperature on a Rigaku R-AXIS IIc imaging-plate system using Cu $K\alpha$ radiation from a Rigaku RU200 rotating-anode generator operated at 40 kV and 100 mA. The crystal-to-detector distance was 100 mm and the oscillation range was 2°. A limited number of reflections were observed to approximately 2.0 Å resolution (Fig. 2).

A total of 126487 observed reflections were scaled and reduced to yield a data set containing 18097 unique reflections with an $R_{\rm merge}$ of 7.8%. The data set was 93.0% complete to 2.37 Å resolution and 91.3% complete to 2.25 Å resolution, with the data in the 2.3–2.25 Å resolution shell being 81.4% complete. The average $I/\sigma(I)$ value in this shell was 1.37.

The crystal class was determined to be tetragonal, space group $P4_12_12$ or $P4_32_12$, with unit-cell parameters $a = b = 80.03$, $c = 141.68$ Å. It is quite different from the crystals of mouse ADA, which are in space group $C2$ with unit-cell parameters $a = 102.36$, $b = 94.11$, $c = 72.93$ Å, $\beta = 127.2°$. A V_m value of 2.77 Å^3 Da^{-1} is consistent with the presence of one molecule (41 kDa) per asymmetric unit and corresponds to a solvent content of 55.6%. This value is in the range of values tabulated by Matthews (1968). The structure of mouse ADA (Wilson *et al.*, 1991; PDB code 1add) will be used as a search model for structure solution using molecular replacement because of the high homology of its sequence (Yeung *et al.*, 1985) with bovine ADA (Kelly *et al.*, 1996).

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