Crystal structure analysis of isopropylmalate isomerase by MAD method

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Microorganism and plant biosynthesize all amino acids that are used at their protein synthesis. Leucine biosynthesis is carried out with three steps. In the first step, 2-oxoisovaleric acid and acetyl-CoA are condensed into 2-isopropylmalic acid by isopropylmalate synthase (EC 4.1.3.12). The second step is the interconversion between 2-isopropylmalate and 3-isopropylmalate. At the third step, 3-isopropylmalate is changed to 2-oxoisocaproic acid by isopropylmalate dehydrogenase (EC 1.1.1.85, IPMDH). Isopropylmalate isomerase (EC 4.2.1.33, IPMI) catalyzes the second pathway-specific reaction, and it is composed of two different subunits that are coded by two genes of the leucine operon. In this study, we determine the crystal structure of IPMI in order to reveal the reaction mechanism of the leucine biosynthesis.

We have successfully obtained crystals of Se-Met IPMI small subunit from *Pyrococcus horikoshii* by hanging-drop vapour-diffusion method.

X-ray diffraction data measurements were carried out under cryogenic condition (100K) using flash cooling technique on the BL41XU station of SPring-8. The MAD data was collected up to 2.15Å resolution using MAR-CCD detector. The diffraction data were processed with the program MOSFLM for integration, and the program SCALA (CCP4 suite) for merging and scaling. The data processing statistics are given in Table 1. The crystals belong to the hexagonal system; the space group was determined to be $P6_x22$ with unit cell parameters $a = b = 96.1$ Å, and $c = 153.7$ Å. The asymmetric unit contains two molecules of IPMI small subunit, corresponding to the V_M value of $2.77 \text{Å}^3\text{Da}^{-1}$. Searching for the coordinates of Se atoms is now in progress.

Table 1. Data processing statistics

	peak	edge	remote
Wavelength (Å)	0.97913	0.97936	0.97012
Resolution range (Å)	30.8-2.15(2.27-2.15)	29.1-2.15(2.27-2.15)	30.8-2.15(2.27-2.15)
Observed reflections	583845	584168	593222
Unique reflections	23539	23544	23542
Completeness (%)	100.0(100.0)	100.0(100.0)	100.0(100.0)
Redundancy	10.6(10.8)	10.6(10.8)	10.6(10.8)
$I/(I)$	8.1(2.7)	8.0(2.6)	7.9(2.5)
R_{mean} (%)	7.9(31.1)	8.0(32.0)	8.2(32.8)
$R_{\lambda\text{merge}}$ (%)	4.0	4.6	-