Analysis of reaction mechanism of ACCD

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ACCD (1-amino cyclopropane-1-carboxylate deaminase: E.C. 4.1.99.4) is one of the pyridoxal 5'-phosphate dependent enzymes, which catalyzes the opening reaction of cyclopropane ring and deaminates ACC to α–ketobutylate and ammonia. We have already determined the dimeric structure of ACCD from yeast *Hansenula saturnus* (yACCD) and proposed the reaction mechanism. In order to understand the reaction mechanism more in detail, we have attempted to crystallize yACCD in complex with substrate analog and also ACCD from other sources.

Pyrococcus horikoshii OT3 is hyper thermophilic archaebacteria and its entire genome sequence has been determined. PH0054 gene was assigned as ACCD and its identity of amino acid sequences with ACCD from other sources is about 30%. Particularly, the residues expected to be involved in catalytic reaction are almost preserved.

However recombinant PH0054 showed transaminase activity, but no ACCD activity. This property of PH0054 indicates there are differences in amino acid residues and structure around active site. Thus the structure analysis of PH0054 is important for studying the reaction mechanism of ACCD.

Crystals of PH0054 were grown under the condition of 0.1M Na-K Phosphate pH 6.8, and 8% 2-Propanol by hanging-drop vapor diffusion method.

X-ray diffraction data collections were carried out under cryogenic condition (100K) at the beam line BL44XU using CCD detector. Crystals were soaked into anti-freezing regents containing 15% Glycerol and cooled by flash freezing technique. Native crystals of PH0054 diffracted up to 2.5Å and belong to the space group $P3_x21$, with unit cell parameters of a=b=122.3, c=115.0 Å, and γ=120°. The data were processed with MOSFLM and scaled with SCALA (CCP4 suite). The statistics of the intensity data are present in Table 1.

Table 1. Data statistics

Wavelength (Å)	0.9701
Resolution range (Å)	40-2.5
Number of observed reflections	36,330
Unique reflections	2,786
Completeness (%)	98.7
Multiplicity	6.1
Averaged $I/\sigma(I)$	7.6
R_{meas} (%)	7.6