X-Ray Crystallographic Study of Thermostable Aspartate Aminotransferase

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Aspartate aminotransferase (AspAT), catalyzes a reversible transamination reaction between the dicarboxylic α-amino and α-keto acids. AspATs from many species were classified into aminotransferase subgroup I, which was further subdivided into subgroups Ia and Ib. A number of X-ray crystallographic studies on AspATs of subgroup Ia have been performed to elucidate the structure, function and catalytic mechanism. However, neither the X-ray structure of AspAT in subgroup Ib nor that of thermostable AspAT has yet been determined.

The PLP-type AspAT from *Thermus thetmophilus* HB8 (tAspAT) was over-produced, purified and crystallized by vapor diffusion using ammonium phosphate as precipitant at pH 4.3, with cell dimensions of a = 124.3 Å, b = 113.6Å, and c = 61.62 Å. There is one dimer in the asymmetric unit, and approximately 52% of the crystal volume is occupied by solvent. The X-ray diffraction data set was collected to 1.8Å resolution on the BL41XU station, using an X-ray beam of wavelength 0.7Å and R-AXIS IV camera at 100K. All data were processed and scaled using the program PROCESS modified for BL41XU (T. Higashi, Rigaku, Akishima), as is shown in Table 1.

Table 1

Crystal size	0.5×0.2×0.1
Exposure time (s/sheet)	90.0
Total rotation angle	81.0
No. of IP	27
No. of reflection (~1.8)	
Total	159800
Independent	75560
Completeness (%)	94.3
Rmerge	8.88
Space group	$P2_12_12_1$

The model structure of tAspAT, which had been determined by MIR method at 3.0 Å resolution, was refined by simulated annealing and energy minimization with twofold non-crystallographic symmetry restraints using the program XPLOR. Refinement and rebuilding was alternated until no further improvement in structure and statistics was apparent. At this stage, the restraint on twofold non-crystallographic symmetry was removed. The resolution was progressively increased to 1.8Å and after several rounds of refinement and manual rebuilding, R_{factor} and R_{free} were reduced to 25.7% and 27.5%, respectively. Further refinement including water molecules is in progress.