Poster Presentation

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Structural features and low-temperature adaptation of aspartate racemase

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Aspartate racemase (AspR) catalyzes the interconversion between L- and D-aspartates without PLP. The only crystal structure of the PLP-independent amino-acid racemase is now available from a hyperthermophilic archaeon. To elucidate structural features and low-temperature adaptation of the racemase group, we determined the crystal structures of AspR from Lactobacillus sakei NBRC 15893 (LsAspR), which works in the low-to-medium temperature range, and for comparison AspR from Thermococcus litoralis DSM 5473 (TlAspR), which has the maximum activity at 95 °C. LsAspR and TlAspR weree crystallized at 20 °C by the sitting-drop vapour-diffusion method using a precipitant solution of 25% (v/v) PEG-MME 550, 5% (v/v) 2-propanol and 0.1 M sodium acetate pH 4.8 and a precipitant solution of 24% (w/v) PEG1500, 0.2 M L-proline and 0.1 M HEPES pH 7.5, respectively. The structures of LsAspR and TlAspR were determined by molecular replacement and refined at 2.6 Å resolution (R=23.8%, Rfree = 31.6%) and 2.0 Å resolution (R=18.7%, Rfree = 25.0%), respectively. Both LsAspR and TlAspR molecules are homodimers with molecular two-fold axis. The subunit of each enzyme molecule comprises the N-terminal and C-terminal domains. The molecule is formed mainly by intersubunit interactions between the N-terminal α-helices and intersubunit hydrogen-bonds between the N-terminal β-sheets in the dimer interface. The active-site cleft exists between both the domains. The spatial arrangement of the strictly conserved cystein residues in the cleft reveals the Cys residues involved in the enzymatic catalysis. A structural comparison of LsAspR and TlAspR reveals structural factors probably involved in thermostability of AspR. The molecular volume, intersubunit interaction, and the number of ion pairs suggest that the LsAspR molecule is more loose than that of TlAspR.

Keywords: aspartate racemase, low-temperature adaptation, crystal structure