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Structural Basis of Enzyme Adaptation to Alkaline pH

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pH is one of the key parameters that affect stability and function of proteins. The crystal structure of the vitamin B_6-dependent enzyme phosphoserine aminotransferase from the obligatory alkaliphile *Bacillus alcalophilus* has been determined at 1.08 Å resolution [1], the highest resolution for any PLP-dependent enzyme so far. The final model contains 5824 protein atoms and 949 water molecules, and has an *R*-factor of 11.7% (R_{free} = 13.9%) after anisotropic B-factor refinement with SHELX. The enzyme displays a narrow pH optimum of enzymatic activity at pH 9.0. The final structure was compared to the previously reported structure of the mesophilic phosphoserine aminotransferase from *Escherichia coli* [2] and to that of phosphoserine aminotransferase from a facultative alkaliphile, *Bacillus circulans* subsp. *alkalophilus* [3]. All three enzymes are homodimers with each monomer comprising a two-domain architecture. Despite the high structural similarity, the alkaliphilic representatives possess a set of distinctive structural features. Two residues directly interacting with pyridoxal-5'-phosphate are replaced, and an additional hydrogen bond to the O3' atom of the cofactor is present in alkaliphilic phosphoserine aminotransferases. The number of hydrogen bonds and hydrophobic interactions at the dimer interface is increased. Hydrophobic interactions between the two domains in the monomers are enhanced. Moreover, the number of negatively charged amino acid residues increases on the solvent accessible molecular surface and fewer hydrophobic residues are exposed to the solvent. Further, the total amount of ion pairs and ion networks is significantly reduced in the *Bacillus* enzymes, while the total number of hydrogen bonds is increased. The mesophilic enzyme from *Escherichia coli* contains two additional ß-strands in a surface loop with a third ß-strand being shorter in the structure. The identified structural features are proposed to be possible factors implicated in the alkaline adaptation of phosphoserine aminotransferase.

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Ultra high resolution X-Ray and neutron diffraction studies of fully deuterated Aldose Reductase show a catalytic proton pathway

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Human Aldose Reductase (AR), an enzyme in the polyol pathway belonging to the aldo-ketoreductase family, is implied in diabetic complications. X-ray electron density maps solved at very high resolution of AR complexes with different inhibitors (IDD-594, 0.66 Å; IDD-552; IDD-393; Fidarestat, 0.90 Å) show within the active site crucial protonation states; providing a good model to study the inhibition and enzymatic mechanisms. To confirm them, we have started neutron diffraction experiments. First trials based on H2O/D2O exchange, using crystals of 0.1 mm^3, showed neutron diffraction up to only ~4.5 Å. New crystallisation trials, with fully deuterated protein (EMBL,Grenoble) complexed with the inhibitor IDD-594, succeeded. X-Ray tests of these crystals at the SBC-APS achieved a resolution of 0.8 Å at 15K (refined mosaicity 0.2°) and the structure was refined using SHELX. Neutron Laue diffraction measured on LADI (ILL,Grenoble) achieved a resolution of 2.2 Å at room temperature, despite a small crystal volume of only 0.15 mm3. Larger crystals growth is under way. The subatomic resolution difference maps of the deuterated crystals suggested a proton channel between Tyr48 by Lys77, important for the catalytic reaction. The neutron density maps showed clearly the deuterium atoms in the active site region, confirming this proton channel.