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Crystal structure of substrate and AMPPNP bound propionate kinase from *Salmonella typhimurium*: Substrate specificity and phosphate transfer mechanism

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Propionate kinase reversibly transfers phosphoryl group from propionyl phosphate to ADP in the final step of non-oxidative catabolism of L-threonine to propionate. There are contrasting views on the phosphoryl transfer mechanism of propionate kinase. Here we report X-ray crystal structures of propionate and nucleotide analog (AMPPNP) bound *Salmonella typhimurium* propionate kinase at 1.8-2.2 Å resolutions. Although the mode of the nucleotide binding is comparable to those of other members of ASKHA superfamily, propionate is bound at a distinct site, deeper in the hydrophobic pocket defining the active site. The role of Ala88, earlier proposed to be the residue determining substrate specificity, was examined by determining the crystal structures of propionate bound Ala88 mutants A88V and A88G. Kinetic analysis and structural data are consistent with a significant role of Ala88 in substrate specificity determination. In the structure of StTdcD A88V-AMPPNP-Propionate complex, AMPPNP was cleaved to AMP and PNP either due to an unreported catalytic activity of the enzyme or due to radiation damage. The released PNP probably reacted with propionate forming propionyl-pyrophosphate, supporting direct in-line transfer mechanism. Phosphoryl transfer reaction is likely to occur via an associative SN2-like transition state. The proximity of strictly conserved His175 and Arg236 to carboxyl of propionate and γ-phosphate of ATP suggests their involvement in catalysis. Moreover, ligand binding does not induce global domain movement as reported in some other members of ASKHA superfamily. However, the active site pocket defining residues Arg86, Asp143 and Pro116-Leu117-His118 segment are also likely to contribute to substrate specificity.

Biography

Subashini Mathivanan is a PhD candidate at the Indian Institute of Science, Bangalore, India. Her research expertise is on protein crystallography, emphasized on structural and functional characterization of *Salmonella typhimurium* propionate kinase and *Photorhabdus luminescens* oxalate decarboxylase.

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