Visualizing Protonation States in Serine Hydroxymethyltransferase with Neutron Crystallography

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Serine hydroxymethyltransferase (SHMT) is a pyridoxal-5'-phosphate (PLP) dependent enzyme that catalyzes the tetrahydrofolate (THF)-dependent cleavage of L-Ser to form glycine and 5,10-methylene-THF. This reaction is significant for its role in the biosynthesis of thymidine and purines, as well as the methyl group of methionine by providing single carbon units to one-carbon metabolism. Human mitochondrial SHMT (hSHMT2) is overexpressed in a multitude of cancers and is acknowledged as a significant target for anti- cancer therapeutics. Here, we present a 2.3 Å joint X-ray/neutron (XN) structure of the homodimeric SHMT from *Thermus thermophilus* (*Tth*SHMT), whose active site is conserved compared to that of hSHTM2, in the open conformation depicting the PLP cofactor covalently bound to the catalytic lysine in the internal aldimine state and a sulfate ion occupying the substrate binding site. In addition, a second joint XN structure obtained by soaking a *Tth*SHMT crystal with L-Ser revealed the substrate bound at the entrance of the active site in a solvent-exposed shallow pocket in a pre-Michaelis complex while the sulfate anion continues to block the active site. We further tracked the substrate through the active site by obtaining an X-ray structure of a pseudo-Michaelis complex by soaking a *Tth*SHMT crystal with D-Ser, a non-reactive substrate enantiomer. Nuclear density maps revealed the positions of hydrogen atoms and provided the ability to accurately assign the protonation states for the amino acid residues, L-Ser substrate, and the PLP cofactor. By direct observation of the locations of hydrogen atoms and tracking substrate positions, our study provides unique atomic-level understanding of the SHMT active site that sheds new light on the enzyme's catalytic mechanism and can be employed to advance the design of anticancer drugs targeting hSHMT2.