

## Poster Presentation

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*Promiscuous substrate catalysis in tagatose-bisphosphate aldolase.*

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Tagatose-1,6-bisphosphate (TBP) from *Streptococcus pyogenes* offers a fascinating opportunity to probe promiscuous substrate turnover in an enzyme. TBP aldolase can cleave, apart from its own substrate TBP, three other bisphosphorylated D-hexoses including fructose-1,6-bisphosphate (FBP). These four sugars are diastereoisomers and differ in stereochemistry at carbon 3 and at carbon 4 with respect to the configuration of their hydroxyl groups. We have determined high resolution structures of the native enzyme in complex with natural substrates, FBP and TBP, and two competitive inhibitors. Since the TBP aldolase crystals are catalytically active, covalent reaction intermediates of TBP aldolase in complex with substrates were trapped under acidic conditions to minimize turnover. High resolution structural analysis revealed a snapshot of both substrates covalently trapped in the active site as Schiff bases undergoing C-C bond cleavage. The structural data pointed to Glu164, by virtue of hydrogen bonding to the substrate C4-OH, as the active site residue responsible for the proton abstraction at the C4-OH that initiates substrate cleavage. The isosteric mutant enzyme Glu164Gln, virtually devoid of activity, supported this interpretation. The structures of the Glu164Gln mutant in complex with TBP, FBP and two competitive inhibitors were solved at high resolution and corroborated the expected C4-OH hydrogen bonding of ligands with the carboxamide amide of Gln164, indicating retention of catalytically competent active site architecture upon mutation. The structural studies were underpinned with pH-profile studies that support Glu164 as the residue responsible for nonspecific substrate cleavage catalytic mechanism. Furthermore the pH-profile of the Glu164Gln was significantly different from that of the native enzyme corroborating Glu164 acting as the conjugate base for proton abstraction. The enzyme thus uses the same catalytic mechanism to cleave both diastereoisomers FBP and TBP, while promiscuous substrate recognition appears to be a function of subtle differences in the active site architecture when compared to the active site of the highly specific FBP aldolase from rabbit muscle.

**[1]** *Structure of a class I tagatose-1,6-bisphosphate aldolase: investigation into an apparent loss of stereospecificity*, LowKam et al., *J Biol Chem.* 2010 Jul 2;285(27):21143-52

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