

## Crystallographic Investigation of Regulatory and Catalytic Mechanism of Human Hexokinase I.

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Abstract No. Ales1656

Beamline(s): X12C

**Introduction:** Mammalian hexokinase I governs the first step of glycolysis in brain and red blood cells. The enzyme consists of two structurally similar domains, each being homologous to yeast hexokinase. Only the C-terminal domain possesses catalytic activity, while the N-terminal domain is regulatory. The separated domains bind an inhibitor, glucose 6-phosphate (G6P), and its antagonist, phosphate. In the intact, full-length enzyme, however, G6P may not bind to the catalytic domain. Instead, catalysis may be subject to allosteric regulation by G6P-association with the N-terminal domain. Unfortunately, in all crystal structures of hexokinase I interdomain contacts are influenced by oligomerization and/or lattice contacts. Mutations of L797 and L798 in the domain interface abolish the allosteric inhibition. The structure of the mutant may be valuable for understanding the mechanism of hexokinase regulation. In addition the ATP analogue, AMP-PNP, is a potent inhibitor of many phosphotransferases. The structure of hexokinase I with bound AMP-PNP would provide information about catalytic mechanism of the enzyme.

**Methods and Materials:** Crystals for the hexokinase I mutant (L797T/L798E/T536A), the non-dimerizing mutant (G280A/R283A/G284Y), and the wild-type enzyme complexed with AMP-PNP, Mg<sup>2+</sup> and glucose, were obtained by evaporation diffusion method of hanging drops, containing 7-14% (w/v) polyethylene glycol 8000, citrate or MES buffer (pH 5.6 or 6.5), 0.1-0.2 M sodium acetate, glucose and either G6P or AMP-PNP/Mg<sup>2+</sup>. Crystals were transferred to 25% glycerol solution and flash-frozen in liquid nitrogen. The crystallographic data for the mutant (L797T/L798E/T536A) were collected to 2.8 Å resolution (Rmerge=0.07). The data for the non-dimerizing mutant (G280A/R283A/G284Y) and the wild-type enzyme co-crystallized with AMP-PNP were collected to a 2.8 and 2.9 Å with Rmerge=0.07 and 0.09, respectively. The structures are being refined with CNS-Solve using PDB entries 1HKB and 1DGK as starting models.

**Results:** The structure of the mutant (L797T/L798E/T536A) reveals conformational changes in the interdomain area that may be responsible for regulatory properties of the mutant. Two other structures of hexokinase I complexed with AMP-PNP reveal weak binding of the ligand, though its concentration was 1000-fold in excess of that used for other phosphotransferases. The weak binding of the ligand to hexokinase I suggests differences in recognition of adenine nucleotides by hexokinase relative to other ATP-dependent kinases.