

W0129

Conformational Flexibility of UDP-GlcNAc Pyrophosphorylase. C.R. Sides, J.J. Wilson, J. Sakon, Univ. of Arkansas, Fayetteville, AR 72701 USA.

Protein dynamics has been an area dominated by NMR studies. UDP-*N*-acetylglucosamine, a precursor for glycosylation, is the product of a reaction catalyzed by UDPGlcNAc pyrophosphorylase (AGX1). AGX belongs to the SGC superfamily that is made up of various nucleotide-sugar binding proteins. We obtained a series of human apo-AGX1 structures with the best one in space group $P2_1$ ($a=80$, $b=75$, $c=87$, and $\beta=92^\circ$) at 1.9 Å resolution with $R=22\%$. AGX1 consists of N-terminal and C-terminal domains that are both involved in substrate binding. Molecules in one asymmetric unit as well as in different crystal forms adopt different orientations of the N-terminal domain with respect to the C-terminal domain. Comparison with previously determined holo-AGX1 shows an even larger conformational change of the N-terminal domain with respect to the C-terminal domain. Interestingly, the N-terminal domain rotates about a point rather than about an axis. Hinge type motion allows the substrate to bind to the otherwise inaccessible active site. Refinement of apo-AGX1 also shows an alternate conformation of His352 and that Cys251 in both monomers and Cys431 in one monomer are oxidized to adopt a sulfenate form. The complex with transition state analogues also identifies residues interacting with the transition state.