

X-RAY STRUCTURE OF APO-2,5-DI-KETO-D-GLUCONIC ACID REDUCTASE A.

Gulsah Sanli, T. Somasundaram, Michael Blaber: Florida State University, Chemistry, 104-MBB-4380 FSU, Tallahassee, Florida 32306-4380

A 1.9 Å resolution x-ray structure of recombinant *Corynebacterium* 2,5-diketo-D-gluconic acid reductase A (2,5-DKGR A) was determined by molecular replacement using the complex structure of the same enzyme with NADPH cofactor as the search model. This enzyme catalyzes the NADPH-dependent stereo-specific reduction of 2,5-diketo-D-gluconate (2,5-DKG) to 2-keto-L-gulonate, a precursor in the industrial production of vitamin C. 2,5-DKGR A belongs to the aldo-keto reductase superfamily and shares the common TIM barrel fold. The superimposed structures of apoenzyme and NADPH:enzyme complex reveal significant conformational changes around the cofactor binding region formed by residues 22-28, 46-52 and 107-114. All of the active site residues are displaced in their positions by 1.03-6.46 Å. Residues important for catalysis or cofactor binding, such as His-108, Trp-109, Arg-238, Ser-233, have poorly defined density in the apo form and appear to be somewhat disordered. Additionally, the last 15 residues at the C-terminal, which form one side of the substrate binding pocket, have no defined electron density in the apo-form. The results show that besides providing a hydride ion for catalytic reduction, the binding of NADPH cofactor to 2,5-DKGR A appears to order the structure to a catalytically competent conformation.