03-Crystallization of Biological Macromolecules

This supports a mechanism based on 1,2 hydride transfer driven by the electric field of the metal cations and the polarization of the O-O bonds. The substitution of residues close to, but not at, the active site leads to interesting effects. The E186Q mutation removes a charge near a cation site(4). The mutant enzyme has low activity with Mg** or Co**, It is fully active with Mn**, and its pH optimum is shifted down by about 1.5 pH units. The X-ray structure of the mutant protein was done in the presence of xynase and either Mg** or Mn**. The E186Q-Mn** structure is essentially identical to the wild type, but in the E186Q-Mg** complex, major changes at the active site affects the second metal binding site, but not substrate binding. These changes are clearly related to the presence of the engineered side group of Q186. Amimiation may stimulate promotion of E186 in the wild type enzyme, leading at low pH to the same conformation change as seen in the E186Q-Mg** structure.


MS-03.04.07 ENGINEERING METAL SPECIFICITY IN GLUCOXYLASE (XYLOSE) ISOMERASE. By Joel Jania, Laboratoire de Biochimie Structuelle, CNRS et Université Paris-Sud, 91405-Orsay, France

XYLOXYL anomer (XI) convert xylose to xylulose and glucose to fructose. It is used by the food industry to prepare isoglucose, xylitol that is sweeter than pure glucose, and usually called glucose isomerase. Its activity requires divalent cations (Mg**, Co** or Mn**) Protein engineering was carried out on XI from the bacterium Actinobacillus niger, in a collective effort led by Prof. S. Wodak (Free University, Brussels) and Plant Genetic Systems (Gent and Brussels, Belgium). Results led among other things to a better understanding of the catalytic role of metal. The X-ray structure of a trigonal crystal form containing the whole 17K2 tetramer in its asymmetric unit was solved in Orsay using synchrotron radiation from LURE-DCI. The model was refined to a R factor of 0.15 at 2.2 Å resolution(11), and further X-ray studies were done on a series of complexes of wild type and mutant enzymes with substrates, linear and cyclic inhibitors, and a variety of cations. Twenty structures with comparable resolution and refinement statistics are now available for Actinobacillus niger XI(11).

Each XI subunit carries two catalytic sites 3 Å apart. Sugar substrates bind to both catalytic through their keto and hydroxyl oxygens. Metal binding maintains the sugar in an open conformation and plays an essential part in isomerization. While significant activity is retained when potential base catalysts are removed by site directed mutagenesis, mutating metal ligand residues at either site deeply perturbs catalysis(11).


Green plate like crystals, reaching sizes of about 0.5x0.5x1mm and suited for high resolution studies, have been obtained for catalase HPII from E.Coli. Crystals with the same morphology have also been produced for mutants N201H (brown crystals) and N201A. These crystals appeared in a few days with the hanging-drop vapor diffusion method at room temperature and using PEG 3350 and LiCl as precipitants at pH 9.0.

Crystals from native HPII dried at least to 2.2Å and are quite stable to X-ray radiation. A complete data set (98% of the unique reflections at 2.8Å) has been collected using an Image Plate (MARESEARCH) area detector. In spite of the molecular weight differences (84 KDa and 57KDa per subunit for HPII and BLC respectively), the initial analysis of the electron density maps shows that the molecular structure has a high similarity with the tetrameric bound light catalase (BLC). The structure determination will be presented.