03-Crystallography of Biological Macromolecules

The geometry of Cu-ERD could be described as trigonal bipyramidal with one vacancy. The Cu(II) ion is located in the plane between the three strongly bound protein ligands S_2N. The oxygen of DMSO is at a 3.2 Å distance from the copper. This arrangement resembles blue copper proteins with Type I metal sites. The visible spectrum and EPR characteristics are similar although the strongly bound ligand set differs compared to azurin or plastocyanin, which is N_2S, and the weakly interacting ligand is either a carbonyl oxygen or a methionine sulfur.

PS-03.05.41 THE STRUCTURE OF PHOSPHORIBOSYLAMINO-IMIDAZOLESUCCINOCARBOXAMIDE SYNTHASE FROM THE YEAST *SACCHAROMYCES CEREVISIAE* AT 3 Å RESOLUTION. By V.M.Levdikov, A.I.Grebenko, V.V.Barynin, W.R.Melik-Adamyan, Institute of Crystallography Academy of Sciences of Russia, Leninsky pr. 59, Moscow 1117333, Russia.

Phosphoribosylaminoimidazolesuccinocarboxamide synthase (EC 6.3.2.6) from the yeast *Saccharomyces cerevisiae* is a monomeric enzyme catalyzing one step in the purine biosynthesis pathway. Crystals of the enzyme which diffract to at least 2.0 Å were obtained by the vapor diffusion method (Grebenko, A.I. et al. J.Mol.Biol., 1992, **228**, 298-299). Crystals belong to the space group $P2_12_12_1$, with unit cell dimensions a = 62.3 Å, b = 63.5 Å and c = 80.9 Å with one molecule in the asymmetric unit. Native crystal diffraction data at 2.5 Å resolution, and four derivatives data at 3.0 Å resolution from crystals soaked in 5 mM $KAu(CN)_2$, 2.5 mM $Cs_3UO_2(CNS)_5$, 1mM $Na_2Pd(NO_2)_4$ and 2mM mersalyl were collected using a SYNTEX-P2₁ diffractometer.

The initial phases to 3.0 Å resolution were derived from the multiple isomorphous replacement method with program complex BLANK (Vagin,A.A. unpublished). Several cycles of solvent flattening with negative density truncation were applied to produce a map in which 70% of the 306 residues chain could be traced. A partial model was built using FRODO. Refinement of the partial structure by Hendrickson-Konnert method is in progress. We intend to present our most current model of the phosphoribosylaminoimidazolesuccinocarboxamide synthase at the conference.

03.06 – Protein–Saccharide Interaction

MS-03.06.01 ATOMIC INTERACTIONS BETWEEN CARBOHYDRATES AND PROTEINS. By Florante A. Quiocho, Howard Hughes Medical Institutes and Baylor College of Medicine, Houston, TX 77030.

In recent years our laboratory has been engaged in the structure-function studies of five proteins that bind carbohydrates — three bacterial periplasmic receptors for the active transport of and chemotaxis toward carbohydrates (monosaccharides and linear and cyclic oligosaccharides), one antibody against bacterial cell surface polysaccharide O-antigen determinant and aldose reductase. While high resolution x-ray crystallography is our primary experimental approach in these studies, we have also utilized site-directed mutagenesis, rapid kinetics, calorimetry, low angle x-ray scattering, and theoretical techniques. Common recurring features of the atomic interactions between proteins and carbohydrates will be presented in light of the crystallographic analysis of these and other proteins. Time permitting, other features of protein-carbohydrate interactions obtained by way of the other techniques will also be presented.

MS-03.06.02 CONCANAVALIN A AND ITS INTERACTION WITH SACCHARIDES

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The crystal structures of the complexes of concanavalin A with methyl α-D-mannopyranoside (space group $P2_12_12_1$, cell dimensions a=123.7, b=128.62, c=67.17 Å) and methyl α-D-glucopyranoside (space group $I2_13$, cell dimension a=167.8 Å) have been determined and refined at 2 Å resolution. Saccharide-free concanavalin A (space group I222, cell dimensions a=88.7 Å, b=86.5, Å c=62.5 Å) has also been refined at 2 Å resolution. A cadmium-substituted form of the saccharide-free protein has been refined at 2 Å resolution and a cobalt-substituted form at 1.6 Å resolution. In the solution of the $I2_13$ crystal structure the replacement of the native metal ions by cadmium ions was critical

This work builds on the structural studies of concanavalin A initiated in the 1970's by various groups. In particular these studies described a *cis* peptide between Ala 207 and Asp 208. Asp 208 is required to stabilise the Ca^{2+} binding site. We have determined the interaction of concanavalin A with saccharides at the atomic level. The results presented are a development of our initial studies on the mannoside complex at 2.9 Å resolution.

The steric requirements for sugar binding in both the mannoside and glucoside cases are particularly mediated by residues Tyr 12, Tyr 100, Asp 208 and Arg 228 as well as Asn 14 and Leu 99. Saccharide is bound to the protein by direct hydrogen bonds involving OH-3, OH-4, O5 and OH-6 and by extensive van der Waals contacts. On binding of saccharide, several water molecules leave the site and Tyr 12 and Tyr 100 reorient. Binding of both saccharides is the same except for van der Waals contacts between the axial O2 of the mannoside and the protein which cannot occur in the case of the equatorial O2 of the glucoside.

