the site occupied by the inhibitor N atom and may thus be released from the lock-in positon with OG1 atom of Thr199 as observed in the active form of the native enzyme (Merz, K.M., *J. Mol. Biol.*, 1990, 214, 799-802). This may sterically inhibit the binding of the substrate CO_2 molecule.

PS-03.05.38 CRYSTAL STRUCTURE DETERMINATION OF THE FLAVIN DOMAIN OF CORN NITRATE REDUCTASEBy Guoguang Lu†, Wilbur Campbell¶, Ylva Lindqvist† and Gunter Schneider†, † Department of Molecular Biology,Swedish University of Agricultural Sciences, Box 590, BMC, 751 24 Uppsala, Sweden,¶ Plant Biotechnology Group and Department of Biological Sciences,Michigan Technological University, Houghton, MI49931, USA

Nitrate reductase (NR, EC 1.6.6.1) of higher plants catalyzes the two-electron reduction of nitrate to nitrite using NADH as the electorn donor. This reaction is the first step in the reduction from nitrate to ammonium, via which higher plants assimilate nitrogen from soil. NR is a homodimer where each subunit is composed of an approximately 100kD polypeptide and three cofactors, FAD, Fe-heme and Mo-pterin. The subunit can be divided into three functional domains, each of which contains one co-factor.

Crystals of the flavin domain of corn nitrate reductase expressed in *E coli*. have been obtained at room temperature using 0.1M TES pH=8.0 and 0.54-0.64M sodium citrate as precipitant. Crystals belong to the rhombohedral space group R3 with cell dimensions a=b=145.4Å, c=47.5Å, a=b=90°, g=120°. All crystals are seriously twinned and a method has been developped to reduce the intensity data from such a twin crystal to a dataset corresponding to a single crystal. Diffraction datasets of native crystals were collected on a Xentronix area dectector to 3.2Å resolution with Rmerge=8%, and at the synchrotron radiation source at Darsbury by film to 2.5Å resolution. We are presently trying to solve the structure by molecular replacement using a model of Ferredoxin Reductase. Application of the rotation and translation functions gave an initial solution that is presently refined.

PS-03.05.39 Crystal structures of active site mutants of T4 endonuclease V : a pyrimidine-dimer-specific excision repair enzyme, K. Morikawa[1], M. Ariyoshi[1], K. Katayanagi[1], T. Doi[1], D. Vassylyev[1], E. Ohtsuka[2], [1]Protein Engineering Research Institute, 6-2-3 Furuedai, Suita, Osaka 565, and [2]Faculty of Pharmaceutical Sciences, Hokkaido University Sapporo, Hokkaido 060, Japan

Irradiation with ultraviolet light causes the formation of pyrimidine-dimers in DNA which is lethal and mutagenic *in vivo*. Bacteriophage T4 endonuclease V (T4 endoV) is an enzyme responsible for the first step of the excision repair in bacteriophage T4-infected *E. coli* cells. This enzyme has two distinct catalytic activities, a pyrimidine-dimer glycosylase and an apurinic/apyrimidinic endonuclease, although it is a rather small protein consisting of 137 residues. The crystal structure of the wild type enzyme was refined at 1.45 A resolution. The melecule is formed by a single compact domain which consists of three α-helices, five reverse turns and loops. In conjunction with results from site-directed mutagenesis experiments, the refined structure allows the identification of the catalytic center for the glycosylase which consists of Glu23 and surrounding basic residues, such as Arg 3, Arg 22 and Arg 26. The crystal structures of three active site mutants, E23Q, E23D, and R3Q, were also determined at atomic resolution. The results suggest that Glu23 is directly involved in the glycosylation catalysis, and that Arg3 has an important role for substrate binding.

PS-03.05.40

A COMPARISON OF THE ACTIVE SITE COPPER SUBSTITUTED LIVER ALCOHOL DEHYDROGENASE AND TWO CONFORMATIONS OF NATIVE Zn-LADH AT 1.8 AND 1.9 Å RESOLUTION. By Salam Al-Karadaghi and Eila Cedergren-Zeppezauer, Department of Structural Chemistry, Arrhenius Laboratories of Natural Sciences, Stockholm University, 10691 STOCKHOLM, Sweden.

Horse liver alcohol dehydrogenase (alcohol:NAD^+ oxidoreductase, EC 1.1.1.1) is a zinc enzyme that catalyzes the transfer of two electrons and one proton between primary or secondary alcohols and NAD^+. The enzyme is a dimer of two subunits, each consisting of 374 amino acids divided into two domains. The physico-chemical properties of LADH have been extensively studied and several crystal forms of the protein have been obtained. High resolution data was collected at the Hamburg synchrotron of two conformational states of the protein (Al-Karadaghi et al., Acta Cryst. Section D, 1993, submitted and Cedergren-Zeppezauer, Al-Karadaghi, Lamzin, Dauter and Wilson, in preparation). We present here a detailed comparison of the refined, closed structures of native Zn-LADH in complex with NADH and the inhibitor DMSO (Zn-ERD) at 1.8 Å, the similar complex of copper substituted LADH (Cu-ERD) (Al-Karadaghi et al., 1993, PROTEINS, submitted) and an open conformation of Zn-LADH to 1.9 Å resolution without cofactor or inhibitor bound.

The active site Zn(II) ion of LADH , essential for binding of the substrate, is coordinated to two sulfur atoms (from cysteins), one nitrogen (from histidine) and the oxygen of water in the open conformation (in which no cofactor is bound). The Zn-coordination sphere in both enzyme conformations have distorted tetrahedral geometry. Only small differences could be observed for the metal ligand parameters in spite of the fact that in the Zn-ERD complex the water molecule has been exchanged by DMSO, the nicotinamid ring of NADH is at van der Waals distance to the metal and a large domain movement has influenced secondary structure elements delivering ligands to the zinc ion.

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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.