03-Crystallography of Biological Macromolecules

PS-03.05.35 HIGH-RESOLUTION X-RAY STRUCTURE REFINEMENT OF HUMAN CARBONIC ANHYDRASE I. by M.Ramanadham and K.K.Kannan*, Solid State Physics Division, Bhabha Atomic Research Centre, Trombay, Bombay 400085, India

X-ray studies on human carbonic anhydrase I, (HCAI), and its complexes with anionic inhibitors and sulfonamides, as well as metal substitution studies have been undertaken in our laboratory with a view to obtain a comprehensive picture of structure, function and drug-protein interactions of this important enzyme. The native HCAI model was refined earlier by us at 2Å resolution using photographic data. In order to enhance the precision of the native structure, refinement at 1.6Å resolution was taken up with x-ray data recorded at Photon Factory, Japan, using synchrotron radiation and imaging plates. An initial model consisting of all the 260 amino-acid residues and the zinc ion was first refined at 2Å resolution by the method of stereochemically restrained least-squares. An R-value of 0.167 was obtained at the end of the refinement, during which 195 solvent atoms were added to the model. Subsequently, the refinement was extended to 1.6Å resolution (25,122 observations). The current R-value for a model consisting of 2,230 atoms is 0.194. Corrections to some of the loop regions and solvent editing are currently underway.

PS-03.05.36 CRYSTAL STRUCTURE OF HUMAN CARBONIC ANHYDRASE I AT PH6 AND IMPLICATION TO FUNCTION. By V.S.Yadava and K.K.Kannan, Solid State Physics Division, Bhabha Atomic Research Centre, Bombay 400 094, INDIA.

Carbonic anhydrase catalyses CO_2 hydration reaction

$$CO_2 + H_2O \text{ ---- } HCO_3^- + H^+$$

The forward reaction is favoured above pH7 while the reversible reaction is favoured below pH7. The coordination geometry around Zn^{2+} is also expected to be different in the two pH regimes as per spectroscopic evidence of Co^{2+} substituted enzyme. At low pH the acidic form of the enzyme is active whereas the basic form is active at high pH. The structure of human carbonic anhydrase I at pH 8.5 has been reported at 2A (Kannan et al, 1984, *Ann. N.Y. Acad. Sci.*, 429, 49). The structure of the carbonic anhydrase I at pH6 has been determined to compare it with high pH form and also to understand the structure-function correlation. Starting with the phases calculated with the native structure without solvents, the structure has been refined by SRLS method using 14927 reflections to 2A collected at the synchrotron beam line (Kannan et al, 1989, *Photon Factory Report, Japan,* 7, 112) and model editing with FRODO on a graphics system. The current R-value is 0.184. The Zn^{2+} is at a distance of 1.98A from NE2 of HIS94, 2.08A from NE2 of HIS96 and 2.10A from ND1 of HIS119. In carbonic anhydrase II the zinc ion has same coordination at pH6 and pH8.5 (Lindahl et al, 1992, *Acta Cryst.*, B48, 281). However, the water molecule observed in carbonic anhydrase I structure at pH8.5 at the fourth coordination site of the tetrahedrally liganded metal, has not so far been found in the present structure. The results of these investigations at 1.6A resolution along with detailed comparision with the high pH structure and its significance to function will be discussed.

PS-03.05.37 STRUCTURE OF HUMAN CARBONIC ANHYDRASE I COMPLEXED WITH GOLD CYANIDE INHIBITOR: INHIBITION MECHANISM. By Vinay Kumar* and K.K. Kannan, Solid State Physics Division, Bhabha Atomic Research Centre, Bombay-400085, India.

Monovalent anions like CN^-, SH^-, Iodide, $Au(CN)_2$ inhibit Carbonic anhydrase catalyzed reversible CO_2 hydration reaction. $Au(CN)_2^-$ anion was observed to bind to the outer sphere of the metal ion (Kannan, K. K. , *Biomolecular structure, Conformation, Function and Evolution: Diffraction and Related studies*, edited by R. Srinivasan, E. Subramaniam & N. Yathindra, 1980, 1, pp. 165-181) and inhibition mechanism was not well understood earlier as two CN^- ligands of the inhibitor anion were not defined unambiguously in the Fourier maps (Eriksson et al., *Zinc enzymes* edited by I. Bertini, C. Luchinat, W. Maret & M. Zeppezauer, 1986, pp. 317-328). We have refined structure of the title complex against 2Å data (Nobs=14478) using PROLSQ and FRODO/TOM on vector general/ IRIS-4D/20 graphics. The crystallographic R-factor has improved from 28.8% for the initial model to 17.3% for the final model with good stereochemistry. The CN^- groups of the active site $Au(CN)_2^-$ anion were located in the Fourier maps during the course of refinement and were subesquently refined. Unlike other monovalent anions such as Iodide (Vinay Kumar et al., *Acta cryst.* 1987, A43, c23), $Au(CN)_2^-$ anion does not replace the Zn^{2+} bound H_2O/OH^- but binds at a different site, with the Zn^{2+} to nearest atom of $Au(CN)_2^-$ (Nitrogen) distance of 3.5Å and Nitrogen atom lone pair directed towards the positive Zn^{2+} ion ($\angle$Zn-N-C = 147°). Interactions with the enzyme is observed to marginally distort the geometry of the inhibitor anion, with N-C-Au angle deviating by 13° from the ideal value. The deviations were ascertained to be real by restoring to ideal values the $Au(CN)_2^-$ positional parameters prior to PROLSQ cycles of refinement and also as restraints were derived from the ideal value. In addition, the Zn^{2+} bound H_2O/OH^- activity linked group in the complex structure is observed to be displaced, away from the metal ion by 0.4Å (Zn^{2+}-O= 2.3Å) with a concomitant shift of 0.2Å from OG_1 atom of Thr199, resulting in a distance of 2.8Å from the N atom of the inhibitor anion. The local changes in the active site and stereochemistry of the anion may suggest that bonding electrons of N atom ($Au(CN)_2^-$) accept a possible H-bond from Zn^{2+} liganded OH^- group. The involvement of bonded electron pair in the formation of a stable H-bond has earlier been considered by Millen (*J. Mol. Structure*, 1990, 237, 1-18). The H atom of OH^- group may reorient itself in the presence of a H-bond acceptor at

the site occupied by the inhibitor N atom and may thus be released from the lock-in positon with OG_1 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.