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

