PS04.02.26  IDENTIFICATION OF Ca2+ IONS BOUND TO PROTEINS USING AN X-RAY ANOMALOUS DISPERSION TECHNIQUE. K. Hamada1, Y. Hata2, H. Miyatake1, T. Fujii1, F. Amada3, K. Fukuyama3, Interdisciplinary Faculty of Science and Engineering, Shimane University, Matsue, 690 Japan, 1Institute for Chemical Research, Kyoto University, Uji, Kyoto, 611 Japan, 3Department of Biology, Osaka University, Toyonaka, Osaka, 560 Japan

Identification of Ca2+ ions bound to moderately large proteins by an X-ray anomalous dispersion technique seems to be possible using a high-energy synchrotron source and a sensitive imaging plate. With three kinds of proteins, Serratia protease from Serratia sp E-15 (Hamada, K. et al., 1995, J. Biochem., in press), alkaline protease from Pseudomonas aeruginosa IPO3080 (Miyatake, H. et al., 1995, J. Biochem., 118, 474) and Arthromyces ramossus peroxidase (Kunishima, N. et al., 1995, J. Mol. Biol., 235, 331), the identification of Ca2+ ions in their crystals were carried out by the anomalous technique. The X-ray wavelengths used for data collection were 1.283 Å (synchrotron radiation) for Serratia protease, 1.488 Å (synchrotron radiation) for Arthromyces ramossus peroxidase and 1.542 Å (Cu-Kα) for alkaline protease. The determination of Ca2+ binding sites was performed by Bijvoet difference Fourier maps calculated with coefficients of {F(•) + F(•-)}exp(iφ(•)) for the alkaline protease. In the Serratia protease, each of five Ca2+ ions was located between two neighbors of loops in the β-sheet coil formed by the repeated sequence of GGXGXDXBX by solvent flattening using this envelope. The new electron density map calculated at 3.0Å was clear enough to locate the side chain atoms and even carbonyl oxygen atoms. The structure of hydrogenase is now in under refinement using the program XPLOR. The R-value was reduced to 0.30 from the initial model (0.510) by only positional refinement. The structure comparison with NiFe hydrogenase from the different strain, Desulfovibrio gigas will be discussed.


Human carbonic anhydrase, a zinc metalloenzyme, catalyses the reversible hydration of carbon dioxide. The proposed catalytic mechanism comprises a proton shuttle through a hydrogen bond network among residues T199, E106 (Kannan et al., FEBS LETTS, 73: 115-119, 1977). To verify this proposal we have undertaken site-directed mutagenesis studies of human carbonic anhydrase I (HCAI). Total RNA was prepared from an HEL cell line and used to prepare cDNA using AMV reverse transcriptase and an hCAI specific primer. The cDNA was amplified by PCR and cloned into the expression vector pET-3a. The gene was induced by IPTG, and the protein purified by affinity chromatography was found to be as active as the RBC HCAI. DNA sequencing revealed that the cloned gene differed from the published RBC hCAI sequence (Barlow, J.H., Nucl. Acid. Res, 15:2386, 1987) with two start codons (ATG) and five other base changes. At the protein level there are two N-terminus methionines and two mutations V311 and V218A. Site-directed mutagenesis (T199V) of this gene was done by a PCR technique using mutated oligonucleotides and confirmed by gene sequencing. Specific activity of the purified mutant protein was assayed and found to be 2% of the wild type. The mutant protein is being crystallized. The recombinant protein crystallized in thick walled glass capillary tubes (Kannan et al., JMB, 63:601-604,1972) are of a different morphology compared to the RBC HCAI. The structure of the recombinant protein and its comparison to the RBC HCAI will be reported.

PS04.02.29  MECHANISM OF Fe(III)-ZN(II) PURPLE ACID PHOSPHATASE BASED ON CRYSTAL STRUCTURES. T. Klabe1, N. Sträßer2, H. Witzel2, B. Krebs3 1Anorganisch-Chemisches Institut, Westfälische Wilhelms-Universität, Wilhelm-Klemm-Strasse 8, D48149 Münster, Germany, 2Institut für Biochemie, Westfälische, Wilhelms-Universität, Wilhelm-Klemm-Strasse 2, D48149 Münster, Germany

Purple acid phosphatases (PAPs) containing a dinuclear Fe(III)-Me(II) center (where Me can be Fe or Zn) in their active sites catalyze the hydrolysis of activated phosphoric acid esters and anhydrides, like ATP, at a pH range from 4 to 7. All mammalian PAPs are highly metalloenzymes with a molecular mass of approximately 35 kDa containing an Fe(III)-Fe(II) center in the active site. In contrast, the most intensively studied plant enzyme from kidney bean (kbPAP) is a homodimeric Fe(III)-Zn(II) metalloprotein of molecular mass 111 kDa. X-ray structures of kbPAP complexed with phosphate, the product of the reaction, and with tungstate, a strong inhibitor of the phosphatase activity, were determined at 2.7 and 3.0 Å resolution, respectively. Furthermore the resolution of the unliganded enzyme, recently solved at 2.9 Å [1] could be extended to 2.65 Å with completely new data. In the inhibitor complex as well as in the product complex, the oxyoanion bonds in a bidentate bridging mode to the two metal ions, replacing two of the presumed solvent ligands present in the unliganded enzyme form. All three struc-