02.1-8 CRYSTAL STRUCTURE OF Cd,Zn METALLOTHIONEIN AT 1.9A RESOLUTION. By S.A. Collett and C.D. Stout, Department of Molecular Biology, Research Institute of Scripps Clinic, La Jolla, CA 92037.

Cd,Zn metallothionein, isoform II, as isolated from rat liver contains 5 moles Cd^{2+} and 2 moles Zn^{2+} per mole of protein (MW 6500 daltons, 61 amino-acids, 20 cysteines). The protein crystallizes in space group $P4_12_12$, a=b=30.9A, c=120.1A, with one molecule per asymmetric unit. The structure was solved using the anomalous scattering effects from Cd and partially refined at 2.3A resolution (W.F. Furey, et al, Science, 1986, 231, 704-710). A new data set to 1.9A resolution has been collected from one crystal maintained at 4°C; for 21272 total observations the average I/σ is 13.7; I/σ for data in the shell 2.1-1.9A is 4.2; for 6704 independent reflections in point group 422 Rsymm(I) is 4.6%. The structure is being refined by constrained least squares. The model has been rebuilt using residue-deleted 2Fo-Fc maps. Structure factor calculations have included a model for the solvent contribution and Cd anomalous scattering. Refinement is still in progress; the current model has R=0.27 for 455 atoms and 6426 reflections to 1.9A with rms deviations from ideality of 0.04A for bond distances. At present 41 water molecules have been included in the model. The refinement has confirmed all 20 of the cysteine thiolate to metal bonds of the original model, and revealed details of the protein stereochemistry, metal ligation and solvent structure. The structure is being compared with the solution structure of Cd_7 metallothionein from rabbit liver derived by two-dimensional NMR methods (W. Braun, et al, J. Mol. Biol., 1986, 187, 125-129). The crystal and solution structures exhibit significant differences, but there may be a stereochemically allowed path by which they could interconvert. Supported by NIH grant GM-36535.

02.1-9 IMPROVED PURIFICATION, CRYSTALLIZATION, AND X-RAY STUDY OF HYDROGENASE OF SULFATE REDUCING BACTERIUM. By Y.Higuchi, N.Yasuoka, M.Kakudo Himeji Institute of Technology, Y.Katsube Institute For Protein Research, Osaka University, T. Yagi Shizuoka University, and H.Inokuchi Institute for Molecular Science

Hydrogenase is an enzyme which catalyzes reversible dehydrogenation of molecular hydrogen, and is found in some species of bacteria. Almost all the hydrogenases so far isolated and characterized are iron-sulfur proteins, but flavin, nickel, copper and selenium are detected in some hydrogenases.

The hydrogenase(hydrogen:ferricytochrome c3 oxidoreductase, EC 1.12.2.1) of the sulfate reducing bacteria is functioning bidirectionally, i.e., to produce dihydrogen and to discard excess electrons released from lactate and pyruvate to effect substrate level phosphorylation and to oxidize dihydrogen to supply electrons to the sulfate reducing system, thus regulating the bacterial energy metabolism.

The hydrogenase solubilized from the particulate fraction from Desulfovibrio vulgaris Miyazaki F(IAM 12604) has been crystallized. Although the solubilized hydrogenase purified by the previous method revealed a single band upon disc electrophoresis, it could not be crystallized. The apparently homogeneous hydrogenase has been separated into three components of similar molecular weights by high performance liquid chromatography on DEAE-Toyopearl. Each hydrogenase component was successfully crystallized by means of vapor diffusion method with polyethylene glycol or 2-methyl-2,4-pentanediol as a precipitating agent. Only small needle-like crystals were obtained in one or two crystallization trials out of ten without seeding. The best crystals were obtained from the solution of each component(P1, P2 and P3) by sitting drop vapor diffusion method using 2-methyl-2,4-pentanediol and polyethylene glycol 1000 seeded with crushed microcrystals. The crystals were obtained either by cross-seeding(i.e., introducing the microcrystals from P1 to the crystallization droplets of P2 or P3, or vice versa) or self-seeding.

Precession photographs show that the crystals of each component belong to the same space group $P2_12_12_1$ with slightly different unit cell dimensions. Those of P1 crystal is a=102.1(1), b=126.8(3) and c=66.9(1) A. The unit cell volume of $8.66X10^6$ A^3 suggests that it contains one molecule/asymmetric unit (V_m=2.43). P2 and P3 were crystallized with the cell edges of a=99.6(1), b=126.8(3) and c=66.9(1) A. The crystals diffract more than 2.5 A in still photographs. We have collected native data up to 4 A resolution for P1 crystals on a Rigaku four-circle diffractometer. We are continuing the x-ray work to elucidate the crystal and molecular structure of hydrogenase. Since the structure of cytochrome c3 from D. vulgaris Miyazaki F has been refined, the three dimensional structural study of hydrogenase may throw light on the mechanism of the electron transfer between the two proteins. Structure analysis of hydrogenase is in progress.

02.1-10 THE STRUCTURE OF THE NEURAMINIDASE GLYCOPROTEIN OF INFLUENZA VIRUS (TOKYO/3/67) AT 2.4 A RESOLUTION. By J.N. Varghese (1), P.M. Colman (1), W.G. Laver (2), and R.G. Webster (3). (1) C.S.I.R.O. Division of Protein Chemistry, Parkville, Australia. (2) John Curtin Medical School, Australian National University, Canberra, Australia. (3) Department of Virology and Molecular Biology, St.Jude Children's Research Hospital, Memphis, Tennessee, U.S.A.

The structure of the Tokyo/3/67 strain of neuraminidase, previously determined (Varghese, Laver and Colman, Nature 303:35-40,1983) to 2.9 A resolution, is being refined to 2.4 A resolution with the addition of higher resolution synchrotron X-ray data. Carbohydrate structure of the four glycosylated sites on the subunit have been modeled. The site at residue 200 is found to cover part of the surface of a neighbouring subunit in the tetramer, while the other three carbohyrdates extend out into the solvent, one of which is an important crystal contact. Crystals soaked in sialic acid reveal details of the binding of sialic acid in the active site pocket in difference fourier maps. The 3D structure of a single site mutation of a Lysine to Glutamic Acid at residue 368 of Tokyo/3/67 has also been determined at 3 A resolution. The mutant was raised by growing virus in the presence of a neutralising monoclonal antibody.