02.1–9 CRYSTALLOGRAPHIC INVESTIGATIONS OF FRAGMENT TR_2C FROM BULL TESTIS CALMODULIN. By Anders Svensson and Lennart Sjölin, Dept of Inorganic Chemistry, Chalmers University of Technology, S-412 96 Göteborg, Sweden.

Ca^{2+} activated calmodulin functions as regulator of a wide variety of metabolic processes. It activates for instance in smooth muscle contraktion the light-chain myosin kinase and thus interest has, in this frame, been focused on calmodulin as a plausible target for drugs treating hypertension. TR_2C is a fragment of calmodulin containing the C-terminus, the amino acid residues 78-148. It has been shown that this fragment acts as an equally good receptor of drugs as the complete calmodulin molecule, yet it cannot activate e.g.light-chain myosin kinase. TR_2C has been crystallized in the tetragonal space group $P4_12_12$ (or $P4_32_12$) with the cell constants A=B=52 A and C=129 A using PEG 4000 as precipitating agent at pH 4.7. Other crystal forms have been obtained at higher pH (7.4) in 40% ammoniumsulfate, but they have so far been too small to be measured by X-rays. A data collection on the tetragonal form has been initiated and the crystals diffract to at least 2.5 A resolution. The structure investigation of the complete molecule is well under way in other laboratories (c.f. Cook, Dedman, Means and Bugg, J. Mol. Biol., 255,8152, 1980) but we feel it is important to investigate the structure of a fragment of the complete molecule for detailed comparisons.

02.1–10 STRUCTURE OF MYOHEMERYTHRIN AT 1.7/1.3 Å RESOLUTION. By Steven Sheriff and Wayne A. Hendrickson, Laboratory for the Structure of Matter, Code 6030, Naval Research Laboratory, Washington, DC 20375 USA.

Myohemerythrin, isolated from the muscles of sipunculan worms, is an oxygen-binding protein. The active center in ligated hemerythrins consists of two octahedral iron atoms coordinated by protein side chains. One iron atom is bound to three histidines, the other to two and they are bridged by a glutamate, an aspartate and an oxide ion. The remaining ligand is molecular oxygen under physiological conditions, but it can be replaced by small ions such as azide in the present case. The iron-iron distance is 3.23Å. The iron-oxide ion distances average 1.79Å and the other iron-ligand distances average 2.14Å, although they vary from 2.03Å to 2.26Å. The current R value is 0.159 with an rms deviation from ideal bond lengths of 0.017Å. Application of an overall anistropic ΔB during refinement yielded large improvements in the R value and ΔF map. This anisotropy accounts for the observed fall-off in diffraction, d_{min} = 1.3Å along b and 1.7Å along a and c. The pattern of fall-off correlates with the extent of lattice contacts between molecules and the direction of helices within molecules. The polypeptide backbone consists of 4 α-helices in a left-twisted anti-parallel bundle and an N-terminal arm of non-repetitive secondary structure. Seven side chains were modeled as discretely disordered with two conformations each. There are 157 water molecules, 75 of which are at unit occupancy and several of the remainder are modeled as discretely disordered. There are three partially occupied sulfate positions, two of which are confirmed by anomalous scattering techniques. Plots of the average isotropic B for the main and side chains vs. residue number reveal the helical turns as each helix has an external side. For the 71 residues (of 118) involved in α-helices $\phi = -64.6° \pm 5.4°$ and $\psi = -41.8° \pm 6.3°$.

02.1–11 THE STRUCTURE OF ERABUTOXIN B AT 1.4A RESOLUTION. STRUCTURE/FUNCTION RELATIONS INCLUDING FUNCTIONAL IMPLICATIONS OF THE EXTENSIVE WATER STRUCTURE AND SIDE-CHAIN DISORDER IN THE REACTIVE SITE. By P.E. Bourne, P. W. R. Corfield, A. Sato and B. W. Low, Department of Biochemistry, Columbia Universith, New York, NY 10032 USA and by Janet L. Smith, Laboratory for the Structure of Matter, Naval Research Laboratory, Washington, DC 20375 USA

The structure of the postsynaptic neurotoxin erabutoxin b has been refined at 1.4A by the restrained least squares algorithm of Hendrickson and Konnert to a crystallographic R factor of 0.15A (r.m.s. deviations from ideality of bonded distances is 0.015A). At intermediate stages in refinement two amino acid residue sequence inversions became evident along the polypeptide chain. Of the 104 water molecules found per protein molecule, twenty-three may each occupy one of two alternative sites. These alternate packing modes of small clusters of water molecules in certain regions are frequently associated with alternative side-chain group orientation.

The significance of these results to inter-molecular packing, intra-molecular non-covalent bond structure and to proposed binding modes in the reactive site will be discussed.

02.1–12 REFINED STRUCTURE AND ELECTROCHEMICAL BEHAVIOR OF CYTOCHROME c_3. By Y. Higuchi, N. Yasuoka, M. Kakudo and K. Niki*, Institute for Protein Research, Osaka University, Yamadaoka 3-2, Suita, Osaka 565 and *Department of Electrochemistry, Faculty of Engineering, Yokohama National University, Tokiwadai, Hodogaya-ku, Yokohama 240, JAPAN.

Some cytochromes have been reported to carry two or more heme groups within a single polypeptide chain. Among them the structural studies of cytochrome c_3 (Haser et al. (1979) Nature 282, 806; Higuchi et al. (1981) J. Biochem. 89, 1659) and cytochrome c_7 (Haser et al. (1979) J. Mol. Biol. 130, 97) have been carried out. It is interesting whether heme-heme interactions are observed in these multi-heme cytochromes. Here we wish to report the refined structure of cytochrome c_3 from Desulfovibrio vulgaris Miyazaki F (IAM 12604), and to describe the electrochemical behavior in terms of the structure including a plausible assignment of the observed microscopic redox potentials to each heme.

The structure was solved by the MIR method and refined by the restrained parameter least squares procedure of Hendrickson and Konnert (Higuchi et al. (1984) J. Mol. Biol. 172, 109). The isotropic temperature factors of individual atoms were refined and 47 water molecules located on the difference map were incorporated into the refinement. The crystallographic R factor is 0.176 for 9907 significant reflections in the 6.0 to 1.8 A shell.

The electrochemical behaviors of cytochrome c_3 have been studied by the pulse

polarography. The macroscopic formal potentials of the four consecutive one electron reversible electrode reactions have been evaluated by a least squares fit to the analytical equation for the overall electrode process. The obtained values of the macroscopic formal potentials of cytochrome c_3 in 0.03 M phosphate buffer at pH 7.4 are as follows: $E_1^{0'}$ = -0.240, $E_2^{0'}$ = -0.297, $E_3^{0'}$ = -0.315 and $E_4^{0'}$ = -0.357 V. If the four redox sites are equivalent and non-interacting the spacing between macroscopic potentials are: $E_{1,2}^{0'}$ = -0.0252 $\Delta E_{2,3}^{0'}$ = -0.00208 and $\Delta E_{3,4}^{0'}$ = -0.0252 V. The observed value of $\Delta E_{2,3}^{0'}$ = -0.018 V implies that the two mid-value sites are almost equivalent and are allosterically interacting. The rest two hemes are supposed to be non-equivalent and non-interacting.

An inspection of the structure and the environment of heme groups led us to assume the model for the electrochemical behavior. (1) Heme 3 and 4 are equivalent and interacting. (2) Heme 1 and 2 are non-equivalent and non-interacting. This model enables us to deduce the intrinsic (microscopic) potentials to be -0.243, -0.316, -0.299 and -0.351 V. The plausible assignment to each heme is Heme 2, 3, 4 and 1, respectively. Heme 3 and 4 would have an attractive interaction through Phe-20. Other physico-chemical properties will be well understood in terms of the refined structure.

02.1–13 RIBONUCLEASE-A: X-RAY STUDIES OF PROTEIN-NUCLEOTIDE INTERACTIONS. By N.Borkakoti, D.S.Moss and R.A.Palmer.

The results of X-ray analyses of three ribonuclease-A-nucleotide complexes, at 2.3 Å, are reported. A modified purine mononucleotide, 8-oxo-guanosine 2'-phosphate in a syn conformation, binds at the pyrimidine-binding site of the catalytic cleft. Solvent molecules are expelled from the active site due to inhibitor binding. The positions of the side-chains of the active-site residues Gln-11, His-12 and Thr-45 are well defined and do not alter on inhibitor binding. The mobility of Lys-41 is greatly reduced in the protein-nucleotide complexes and the terminal amino group interacts directly with the 2'-phosphate group of the nucleotides. In the complex of the enzyme with a modified pyrimidine, cytidine-N(3)-oxide 2'-phosphate, His-119 is stabilised in the minor site of the native protein, while in the protein-purine derivative the imidazole group is located in the major site. Inhibitor binding induces movements in the side-chains of Lys-7 and Lys-66 which also modify the conformation of the active-site cleft of ribonuclease A.

02.1–14 CRYSTALLIZATION OF 3α,20β–HYDROXYSTEROID DEHYDROGEANSE FROM STREPTOMYCES HYDROGENANS. By Paula M. D. Fitzgerald, William L. Duax and John S. Punzi, Medical Foundation of Buffalo, Inc., Buffalo, New York, USA, 14203, and James C. Orr, Memorial University, St. Johns, Newfoundland, Canada, A1B 3V6.

3α,20β–Hydroxysteroid dehydrogenase is a NADH dependent oxidoreductase isolated from Streptomyces hydrogenans. Its steroid substrates include certain androstanes and pregnanes; both the 3- and 20-positions have been shown to be reactive. The enzyme is a tetramer with apparently identical subunits of M_r=25,000. We have obtained three crystalline modifications of this enzyme, all grown from concentrated phosphate or ammonium sulfate buffer solutions, pH between 6.2 and 8.7. Hexagonal bipyramid crystals grow when no coenzyme is added to the crystallization medium, tetragonal bipyramid crystals grow in the presence of NADH and hexagonal rods grow the the presence of NAD^+.

The hexagonal bipyramid crystals have been characterized crystallographically; they have the symmetry of space group $P6_422$ (or its enantiomorph, $P6_222$), with unit cell dimensions a=127.3Å, c=112.2Å. Volume and density considerations imply that the crystallographic asymmetric unit contains two monomers, and therefore that the tetramer possesses a twofold axis of symmetry that is coincident with a crystallographic twofold symmetry element. The fact that different crystal forms are obtained in the presence and absence of coenzyme may be taken as evidence that the molecule undergoes a conformational change upon binding of coenzyme.

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02.1–15 p-HYDROXYBENZOATE HYDROXYLASE FROM PSEUDOMONAS FLUORESCENCE. By J.M.v.d.Laan,H.Schreuder, R.K.Wieringa,W.G.H.Hol,J.Drenth,Dept. of Chemical Physics,University of Groningen,Nyenborgh 16,9747AG Groningen,The Netherlands.

p-Hydroxybenzoate hydroxylase is a yellow, NADPH dependant,flavine containing enzyme with a molecular weight of 44299 dalton. The enzyme belongs to the class of external monooxygenases. The active site catalyses two reactions :

1) $NADPH + H^+ + E\text{-}FAD.POHB \rightarrow E\text{-}FADH_2.POHB + NADP^+$

2) $O_2 + E\text{-}FADH_2.POHB \rightarrow E\text{-}FAD + 3,4\ DOHB + H_2O$

POHB is the substrate p-hydroxybenzoate and 3,4 DOHB is the product of the reaction : 3,4 dihydroxybenzoate. p-Hydroxybenzoate hydroxylase was crystallized as the oxydized enzyme-substrate complex (E-FAD.POHB).(Drenth et al. JBC (1975) 250,5268). With these crystals the three dimensional structure could be determined at a resolution of .25 nm (Wieringa et al. J.Mol.Biol (1979) 131,55).Further progress in the study of the enzymatic mechanism was seriously hampered by the bad reproducibility of the crystallisation. Improvement of the purification of the enzyme revealed a charge heterogeneity . On a DEAE-sepharose Cl-6B anion exchange column we could separate up to five fractions of p-hydroxybenzoate hydroxylase. Determination of the molecular weight of the various protein fractions from the anion exchanger with size exclusion HPLC showed that only one fraction contained pure dimers(2x44299). Crystallization experiments with the different protein fractions revealed that good crystals exclusively could be obtained from the protein fraction that contained dimers.In this way the enzyme-substrate complex could also be crystallized in the presence of NADPH,$NADP^+$ and the NADPH fragment ATP-ribose.

With crystals grown in a medium from which the substrate was deleted we were able to collect a data set up to .35 nm. We expect that this structure will indicate the conformational changes in the enzyme on substrate binding.