

02. STRUCTURAL MOLECULAR BIOLOGY

02.1-9 CRYSTALLOGRAPHIC INVESTIGATIONS OF FRAGMENT TR₂C FROM BULL TESTIS CALMODULIN. By Anders Svennsson and Lennart Sjölin, Dept of Inorganic Chemistry, Chalmers University of Technology, S-412 96 Göteborg, Sweden.

Ca²⁺ activated calmodulin functions as regulator of a wide variety of metabolic processes. It activates for instance in smooth muscle contraction the light-chain myosin kinase and thus interest has, in this frame, been focused on calmodulin as a plausible target for drugs treating hypertension. TR₂C 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. TR₂C has been crystallized in the tetragonal space group P4₁2₁2 (or P4₃ 2₁2) with the cell constants A=B=52 Å and C=129 Å 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 Å 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 3.23 Å. The iron-oxide ion distances average 1.79 Å and the other iron-ligand distances average 2.14 Å, although they vary from 2.03 Å to 2.26 Å. The current R value is 0.159 with an rms deviation from ideal bond lengths of 0.017 Å. Application of an overall anisotropic AB during refinement yielded large improvements in the R value and ΔF map. This anisotropy accounts for the observed fall-off in diffraction, d_{min} = 1.3 Å along b and 1.7 Å 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 φ = -64.6° ± 5.4° and ψ = -41.8° ± 6.3°.

02.1-11 THE STRUCTURE OF ERABUTOXIN B AT 1.4 Å 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 University, 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 1.4 Å by the restrained least squares algorithm of Hendrickson and Konnert to a crystallographic R factor of 0.15 Å (r.m.s. deviations from ideality of bonded distances is 0.015 Å). 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 ELECTRO-CHEMICAL BEHAVIOR OF CYTOCHROME c₃. 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 c₃ (Haser et al. (1979) Nature 282, 806; Higuchi et al. (1981) J. Biochem. 89, 1659) and cytochrome c₇ (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 c₃ 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 Å shell.

The electrochemical behaviors of cytochrome c₃ have been studied by the pulse