02.1-18 A COMPARATIVE MOLECULAR MODEL OF THE Ca^{2+} SENSITIVE REGULATORY PROTEIN CALMODULIN BASED ON TURKEY SKELETAL TROPONIN-C. By Natalie C.J. Strynadka and Michael N.G. James, Medical Research Council of Canada Group in Protein Structure and Function, Department of Biochemistry, University of Alberta, Edmonton, Alberta, Canada T6G 2H7.

Calmodulin (CaM) plays a pivotal role in many Ca^{2+}-dependent intracellular functions. It belongs to the family of proteins that exhibits a common Ca^{2+}-binding structural motif of helix-loop-helix which includes troponin-C (TnC) and parvalbumin. Based on the high sequence identity between CaM and TnC (46%) and with the knowledge of the detailed 3-D structure of TnC, we have constructed an atomic model of CaM. The TnC structure that was used was the one proposed for the 4 Ca^{2+} ion form of TnC (Herzberg et al., J. Biol. Chem. 261, 2638 [1986]). The side chains of TnC were replaced by the homologous residues of CaM using the computer program MUTATE (R. Read). The 2 most significant deletions are the loss of the 11 residue N-terminal helix and a 3 residue Lys-Gly-Lys deletion from the interdomain helix. This latter deletion causes the relative orientation of the N- and C-terminal domains to change by 60° from that of TnC. In order to relieve unacceptably close van der Waals contacts and to correct the geometry at Pro66, 750 steps of conjugate gradient energy minimization, using GROMOS (van Gunsteren), were done on the modelled CaM. In our model, each of the Ca^{2+} binding sites has 5 ligands from the protein and one coordinating water molecule, in agreement with a previous prediction (Herzberg & James, Biochemistry 24, 5298 [1985]). The side chain of Tyr99 is accessible to solvent and stacks against the side chain of Gln135. Tyr138 makes close contacts with Phe89 and Phe141. Regions of conformational flexibility in CaM and TnC are suggested by the differences in certain interhelix angles of these two proteins (Babu et al., Nature 315, 37 [1985]). Hydrophobic patches between helices B and C, and between F and G, suggest the binding sites for the trifluoperazine type drugs.

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02.1-19 THE CRYSTAL STRUCTURE OF MANGANESE SUPEROXIDE DISMUTASE FROM BACILLUS STEAROTHERMOPHILUS. By M.W. Parker and C.C.F. Blake, Laboratory of Molecular Biophysics, University of Oxford, England.

The superoxide dismutases (SOD, EC 1.15.1.1) are a class of metalloproteins containing either copper and zinc or manganese or iron. The enzyme catalyses the disproportionation of superoxide to water and peroxide. The crystal structure of manganese superoxide dismutase from Bacillus stearothermophilus has been solved to a resolution of 2.4Å by the methods of isomorphous and molecular replacement. The crystallographic R-factor after eight cycles of Hendrickson-Konnert refinement is 0.26 and the r.m.s. deviation from ideal bond lengths is 0.025Å. The tertiary fold of the protein exhibits a striking resemblance to iron-containing SODs but bears no resemblance to the copper/zinc-containing SODs. The manganese is tetrahedrally surrounded by three histidines and an aspartate. The active site is lined by an array of aromatic residues. The structure is currently being analysed with the aim of providing a detailed atomic description of the protein's mode of action.

02.1-20 HUMAN CARBONIC ANHYDRASE I - IODIDE COMPLEX: STRUCTURE AND INHIBITION MECHANISM. By Vinay Kumar, Padma Satyamurthy and K.K. Kannan, Neutron Physics Division, Bhabha Atomic Research Centre, Bombay 400 085, India.

Iodide like other anions (CN^-, SH^- etc) is a competitive inhibitor of HCO_3^- reaction and an uncompetitive inhibitor of CO_2 hydration reaction of carbonic anhydrase isozymes. Crystals of Human carbonic anhydrase I isozyme (HCAI) were soaked in a solution of 0.2M NH_4I in 2.5M $(NH_4)_2SO_4$ pH ~ 8.5. Three-dimensional intensity data for HCAI-I^- crystals was collected to a resolution of 2.5Å on an Arndt-Wonacott oscillation camera. Data processing was done on a Scandig-3 microdensitometer controlled by a PDP 11/34 computer (P.K. Pal et al., Int. Sum. School on Cryst. Comp., 1983, Kyoto Japan) followed by 3-dimensional scaling. 48159 reflections were scaled to get 10150 unique reflections. The overall R-factor on F_{obs} was 11.7%. Phases were determined from the refined structure of HCAI (K.K. Kannan et al., Ann. New York Academy of Sciences, 1984, 429, 49-60) with 197 solvent molecules included. (2Fo-Fc) and (Fo-Fc) Fourier maps were computed wherein the Iodide (I^-) position was located and included in the structure. This structure was refined using the restrained least squares (J.H. Konnert, Acta Cryst., 1976, A32, 614-617) method and model building interactive graphics. The initial R-factor was 30.3%. The R-factor after 12 cycles of refinement and one model fitting on a Vector General 3400 graphics system using Frodo (T.A. Jones, J. Appl. Cryst., 1978, 11, 268) programme is 20%. RMS delta and sigma values for covalent bond distances are 0.014 and 0.020, for planar torsion angles 2.5° and 15.0° and for main chain bonded thermal values (B) is 1.3Å² and 1.00Å² respectively. Refined occupancy and B values of I^- ion are 69% and 11.4Å². I^- is inhibiting the enzyme by replacing the catalytically important solvent molecule and is at a distance of 2.57Å from Zn^{2+} ion (Fig. 1). There seem to be two more low occupancy I^- sites with

respective occupancies and B values of 45%, 23.7Å² and 27%, 12.2Å² bound in regions of the molecule other than the active site. Also, there is an undefined large peak in the electron density map near SG of Cys 212 residue.

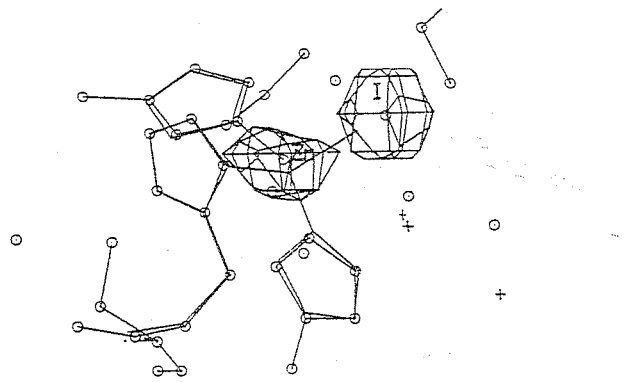

FIG. 1. Zn^{2+} AND I^- IN THE ACTIVE SITE OF HCAI

02.1-21 STRUCTURAL FLEXIBILITY IN RIBONUCLEASES. By L. Mazzarella, S.Capasso, C.A. Mattia, G. Di Lorenzo and A. Zagari, Dipartimento di Chimica, Università di Napoli, Italy.

The structure of bovine seminal ribonuclease (RNAse BS) (Mazzarella, Capasso, Di Lorenzo, Mattia *Gazz.Chim.It.* in press), a dimeric covalent protein strictly homologous to the bovine pancreatic enzyme (RNAse A), has been refined at 2 Å resolution to a final R factor of 0.205 based on 14300 reflections with $I > \sigma(I)$ (≈ 80 % of the total). The final model includes all the non-hydrogen atoms of the two subunits, related by a local twofold axis, 6 sulphate anions and 135 water molecules. The outstanding features of the model are: a) the sixteen-membered cycle linking the two subunits and involving residues Cys30 and Cys31 of the two chains; b) the two active sites formed by residues belonging to different chains. The twofold simmetry is well preserved throughout the structure, but marked deviations are observed for the hinge peptide (residues 15-21) and the external loop (65-72). In the last case the differences are propagated to the two active sites : in one subunit the aspartate 121 is hydrogen bonded to His 119 , whereas in the other subunit it is bonded to the main chain NH group of lysine 66. In both subunits the side chain group of His 119 has been treated as a mixture of two different specific conformations. The structural features in the region of the active sites permitted us to implement the widely accepted mechanism of action of ribonucleases.

02.1-22 THREE-DIMENSIONAL STRUCTURE OF GUANYL-SPECIFIC RIBONUCLEASES FROM MICROORGANISMS AT HIGH RESOLUTION. By B.K. Vainshtein, A.G. Pavlovsky, K.M. Polyakov, S.N. Borisnova, B.V. Strokopytov, A.A. Vagin, Institute of Crystallography, USSR Academy of Sciences, Moscow, USSR.

The crystal structure of guanyl-specific ribonucleases - enzymes catalyzing cleavage of a single-stranded RNA at 3'-phosphodiester bonds of guanyl nucleotides has been studied by X-ray diffraction. Two crystalline modifications of RNase C_2 produced by microfungi of the Asp.-clavatus type have been obtained: the first belongs to space group $P2_1$, a=31.5, b=51.0, c=57.3 Å, γ=92°, 2 molecules per asymmetric unit; the second to space group $P2_1$, a=30.9, b=32.2, c=49.7, γ=116°, 1 molecule per asymmetric unit. Isomorphous replacement was used to solve the structure of the 1st type, whereas the structure of the 2nd type was solved by molecular replacement method and refined to 1.35Å, R=0.198. To solve the crystal homologue structure of RNase Th_1 (Trichoderma harzianum), space group $P3_221$, a=b=55.7, c=80Å, 1 molecule per asymmetric unit, and RNase Pb_1 (Pen.-brevicompactum), space group I222, a=97.8, b=51.7, c=33.9Å, 1 molecule per asymmetric unit, molecular replacement based on the RNase C_2 model was used. The RNase Pb_1 structure was refined at a resolution of 1.75Å, R=0.167.

Isomorphous replacement method was employed to determine the 1st-type structure of the bacterial RNase Bi (Bac.-intermedius); space group B2, a=114.5, b=78.9, c=33.3Å, γ=119°, 2 molecules per asymmetric unit, whereas the 2nd-type structure was determined by molecular replacement: space group $P2_12_12_1$, a=111.4, b=69.6, c=33.5Å, 2 molecules per asymmetric unit. The 2nd-type structure of RNase Bi was refined to 2Å resolution, R=0.21.

Crystalline complexes of RNase Pb_1 with guanosine-3',5'-diphosphate (pGp) (1.24Å resolution, R=0.196) and guanylyl-3',5'-uridine phosphonate analogue were obtained and studied. As a result, a hypothesis was advanced to account for the high specificity of ribonucleases of this type to guanyl bases.

The data on the structure of the RNase Pb_1 complex with pGp gave an insight into the structure of the active site of RNase Bi; in the active site of RNase Bi a region was discovered hypothetically responsible for the recognition of the guanyl base. A comparison of this region with a "recognizing" region in the Pb_1 ribonuclease revealed their close similarity and allowed these two structures to be superimposed. As a result, the nucleotide (pGp) was located in the active site of RNase Bi, thus a "theoretical" complex of this enzyme with a nucleotide was obtained.

The structures of RNase C_2 and Pb_1 were compared.