A COMPARATIVE MOLECULAR MODEL OF THE Ca\textsuperscript{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 2G7.

Calmodulin (CaM) plays a pivotal role in many Ca\textsuperscript{2+}-dependent intracellular functions. It belongs to the family of proteins that exhibits a common Ca\textsuperscript{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\textsuperscript{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\textsuperscript{2+} binding sites has 5 aromatic residues. The structure is currently being analyzed with the aim of providing a detailed atomic description of the protein's mode of action.

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The structure of bovine seminal ribonuclease (RNAse BS) has been refined to a final R factor of 0.198. To solve the crystallographic structure, two crystalline modifications of RNAse G2 were used. Isomorphous replacement was employed to determine the structure of the first-type enzyme (Bacillus intermedius), space group P212121, a=114.5, b=78.9, c=33.3Å, R=0.196, 1 molecule per asymmetric unit. The second-type structure of RNAse Bi was determined by molecular replacement based on the RNase G2 model. The RNase Pb 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 R2, a=185.9, c=33.3Å, Y=119°, 2 molecules per asymmetric unit, whereas the second-type structure was determined by molecular replacement: space group P212121, a=111.4, b=69.6, c=33.5Å, 2 molecules per asymmetric unit. The 2nd-type structure of RNAse B1 was refined to 2Å resolution, R=0.21.

Crystalline complexes of RNAse Pb with guanosine-3',5'-diphosphate (pGp) (1.24Å resolution, R=0.196) and guanayl-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, 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 "recognition" region in the Pb 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 G2 and Pb were compared.

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 G2 produced by microfungi of the Asp. harzianum type have been obtained: the first belongs to space group P21, a=31.5, b=51.0, c=57.3Å, Y=92°, 2 molecules per asymmetric unit; the second to space group P21, a=30.9, b=32.2, c=49.7, Y=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 crystallographic structure of RNAse Th (Trichoderma harzianum), space group P21, a=b=59.7, c=80Å, 1 molecule per asymmetric unit, and RNase Pb, (Pen.-brevicompactum), space group I222, a=97.8, b=51.7, c=33.5Å, 1 molecule per asymmetric unit, molecular replacement based on the RNAse G2 model was used. The RNase Pb 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 B1 (Bac.-intermedius); space group R2, a=185.9, c=33.3Å, Y=119°, 2 molecules per asymmetric unit, whereas the 2nd-type structure was determined by molecular replacement: space group P212121, 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.

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The structure of bovine seminal ribonuclease (RNAse BS) (Mazzarella, Capasso, C.A. Mattia, G. Di Lorenzo and A. Zegari, Dipartimento di Chimica, Università di Napoli, Italy.

The structure of bovine seminal ribonuclease (RNAse BS) (Mazzarella, Capasso, C.A. Mattia, G. 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 b>α(1) (= 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 symmetry 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.