03-Crystallography of Biological Macromolecules

As much different from that of DNA I in the complex. The 350 water positions were assigned on reasonable atomic contacts against protein and other water molecules, and about 150 water molecules have small B values than 50. 198 and 199 water molecules have more than one hydrogen bond, to actin and DNA I, respectively.

Consideration of atomic distance, B value and octahedral coordination, three peaks were assigned as Cαβγ. One of them is Cαβγ binding to ATP of actin and the other two locate in the DNA I region. One of them located at the same position as Site A in the paper described by Suck.

There are two actin-DNA I contact regions and three interaction contact regions. The contact region containing actin 41(G)-44(V) A DNA I 85(T)-67(V) has live solvent bridges between two molecules. As rather weak, the region near to 106(L)-112(K), 112(H)-116(R) and 370(D)-374(F) forms main contact region and a special contact region between 191(1)-195(6) and 193(I)-286(1) and 374(P). Both regions have two water bridges between adjacent actin molecules.

PS-03.08.11 Studies of Myosin Light Chain Tertiary Structure. By L. Chung*, D.R. Brown, W. Huang, P.M. Hunter & F.M.G. Cummi, School of Physics, The University of New South Wales, P.O. Box 1, Kensington, N.S.W. 2033, Australia. #Pathology Department, The University of Sydney, N.S.W., 2000, Australia.

The aim of this project is to produce a model system that facilitates the crystallographic study of the control of myosin force generation by myosin light chains. The regulatory light chain of myosin (LC2) is responsible for the regulation of force generation in smooth and non-muscle cells. It binds one mole of calcium per mole LC2, and it is activated by phosphorylation. Sequence homology suggests that it is part of the superfamily of calcium binding regulatory proteins that includes calmodulin (CaM), and troponin C (TnC) (Collins, 1991 J. Muscle Res. Cell Motil. 12: 3-27). Our model system consists of an LC2-synthetic peptide complex that will be suitable for X-ray crystallographic studies. In the long term, we expect to determine the atomic structures of the LC2-peptide complex.

Computer modelling has been used to predict a structural model for the interaction of myosin LC2 with heavy chain (HC). An alpha-helical model of myosin heavy chain was initially built based on a predicted LC2 binding region (Mitchell et al., 1989 J. Mol. Biol. 208: 199-205). The LC2 portion of the myosin HC sequence was predicted by homology from the binding sequence of myosin light chain kinase domain of LC2 and CaM. By means of known sequence alignment, a 16 amino acid region of myosin HC for the LC2 binding site was found in the best sequence alignment. This sequence segment differs from previous studies. From homology, a model of LC2 was subsequently built by using the X-ray structure of CaM. On the basis of the CaM-peptide complex structure, which has been recently determined (McDermott, 1996 Science 275: 1253-5), the model of LC2-HC complex was ultimately made. In this model, the hydrophobic cores of both the N- and C-terminal domains of LC2 interact with the myosin HC hydrophobic residues. N-terminal charges fall close to LC2 negative charges. Hence, the predicted binding sequence was supported by modelling the LC2-HC complex.

To verify the results of modelling, biotinylated mutant light chain was mixed with one of a series of 15 residue peptides, that spanned 50 amino acid region of the myosin heavy chain sequence surrounding the predicted binding site. The peptides differed in sequence by a frame shift of two amino acids. An ELISA-type assay allowed a hierarchy of binding efficiencies to be determined for each of the nineteen 15 residue peptides. From this study, the most likely site for LC2 binding on the myosin heavy chain was determined. We are currently crystallising the LC2-peptide complex.

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03.09 - Receptor and Signal Transducing Proteins

PS-03.09.01 MECHANISM OF THE HYDROLYSIS OF Ras p21.GTP.Mg(H2O)2. A. Preisinger,* Institut für Mineralogie, Kristallographie und Strukturchemie, Technische Universität, Vienna, Austria. A. Reyer, A. Bauer Research Institute of Molecular Pathology, Vienna, Austria.

The intracellular structure of Ras p21 is stabilized by a great number of intramolecular hydrogen bonds. The structure includes six stranded beta sheets, two alpha helices and ten loops (H-Ras p21 GppNHp[H2O]2); Symmetry P3121. PDB Dataset 3P21 (E. F. Pai, U. Krüger, G. A. Petsko, N. S. Gody, W. Kabsch, A. Wittbrodt, EMBL, 1990, 9, 2351-2359).

Part of the Ras p21 molecule forms a globin-like fold used as GTP binding site. The tripod shape of GppNHp[H2O]2 is held in place by six hydrogen bonds of main chain NH of residues 13 to 18 (loop 1: G15-F16, V14, alpha helix: Gly15-Lys16-Ser17-D318). Two more hydrogen bonds are formed by Lys11 N, another two by Thr35 (loop 2) and Gly30 (loop 4). In this crystal structure the conformation of loop 4 is strongly determined by two intermolecular hydrogen bonds between Gln61 (loop 4) and Asp33* (loop 2 asterisk) of a neighbor molecule and vice versa because of symmetry. Furthermore the OH group of Tyr33* of this neighbor molecule forms an intermolecular hydrogen bond to O2 of the gamma phosphate group.

In Ras p21 GDP.Mg(H2O)2 (Symmetry P6121; PDB Dataset 1Q21) (Tong, A. M. Foy, M. V. Milhman, S. H. Kim, J. Mol. Biol. 1991, 217, 503-516) loop 2 and loop 4 have different conformations compared to Ras p21 GppNHp[H2O]211 and Asp33 (and p35 (loop 2)) and Glu61 (loop 4) form different intermolecular hydrogen bonds.

This water molecules located in the neighborhood of GTP.Mg in necessary for the hydrolysis of GTP.H2O2 to GDP.Mg.H2O (W175, W189, W191, PDB Dataset 2P21).


Hydrolization starts with the movement of the gamma phosphate atom through the plane formed by the three oxygens in the direction of W175 (inversion) (J. Feuerstein, R. S. Goodby, M. R. Webb, J. Biol. Chem. 1989, 264, 6185-6191) leading to a transition state where a rotation transfer from W175 to O2 gamma over Glu61 takes place.

A detailed analysis of the observed conformational changes of loop 2 and loop 4 and the conversion of intermolecular hydrogen bonds to intramolecular ones during hydrolysis will be given.

The importance of the highly conserved residues, especially Gly12, for the hydrolysis will also be discussed.

In vivo the rate of hydrolysis is strongly increased by GAP (GTPase activating protein). We will present a model for possible interaction of GAP by hydrogen bonds with Ras p21 into different region of GAP, one "binding domain" and one "docking domain".