

MS04.07a.05 THE CRYSTAL STRUCTURE OF COLICIN

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The structure of the entire channel-forming bacteriocin (protein toxin) colicin Ia has been solved to a resolution of 2.4 Å by multiple isomorphous replacement. The formation of ion-permeable channels in target cell membranes is a general mechanism of cytotoxicity. The process involves secretion of a soluble protein which inserts into the plasma membrane of the target cell and forms a lethal pore. Colicins, *Escherichia coli* protein toxins, are a well-characterized example of this class of proteins. Colicin Ia crystals, comprised of approximately 80% solvent, are in spacegroup C22₁ (a=64.4 Å, b=178.6 Å, c=285.5 Å). All data sets used in the structure determination were collected from frozen crystals with a synchrotron light source (SSRL beamline 7-1). Heavy atom derivatives were obtained using mercurial soaks of engineered single-site cysteine mutants.

The structure of the 69kD colicin Ia protein reveals the structural relationships between the three distinct domains which function, respectively, to i) bind to a receptor on the outer membrane of susceptible bacteria, ii) translocate across the outer membrane through the receptor, and iii) bind to the inner membrane and form a pore in the presence of the transmembrane voltage. The domains are separated by an extraordinarily long helical coiled-coil.

MS04.07a.06 THE CRYSTAL STRUCTURE OF THE ASSEMBLY DOMAIN OF THE CARTILAGE OLIGOMERIC MATRIX PROTEIN: A PENTAMERIC COILED-COIL.

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The crystal structure of the assembly domain of the cartilage oligomeric matrix protein (COMP), a pentameric glycoprotein of the thrombospondin family found in cartilage and tendon, was determined at 2.03 Å resolution using MIRAS phasing with xenon, (CH₃)₃Pb(COOCH₃)₃ and Pr(COOCH₃)₃ further improved by solvent flattening and five-fold averaging. Self-association of COMP, as well as of at least two other extracellular matrix proteins, thrombospondins 3 and 4, is achieved through the formation of a five-stranded α -helical bundle which involves 64 N-terminal residues (20-83). The complex is further stabilized by the interchain disulphide bonds between cysteines 68 and 71. Circular dichroism measurements show that the structure of the assembly domain remains intact even at temperatures above 100°C. While the crystal structures of two-, three- and four-stranded α -helical bundles were reported before, that one of the pentameric coiled coil is novel. The origins of the extreme thermal stability, the unusual degree of oligomerization and the role of the internal hydrophobic axial cavity are the questions to be addressed in the current study. The peptides containing 64, 52 or 46 residues were produced by expression in *Escherichia coli*, but well diffracting crystals were obtained only with the 46 residues fragment (P2₁, a=38.47 Å, b=49.47 Å, c=54.98 Å and β =103.84°). The central part of the molecule which includes five heptad repeats (residues 29-65), obeys approximate five-fold symmetry, while the remaining residues at the N- and C-termini show significant deviations from that. Strong symmetry violations could explain the little success achieved in our earlier attempts to solve the structure by the molecular replacement methods with the idealized theoretical model. Fragments adjacent to the disulphide bridges are significantly disordered in the current model probably due to the partial degree of oxidation or disulphide bridge reshuffling. The long hydrophobic axial cavity in the core of the structure is regularly constricted by the rings of aliphatic side chains. Two additional constrictions are formed by the rings of methionines and glutamines. The ability of the cavity to accommodate non polar groups was successfully used for preparing the xenon derivative, but in the native structure the cavity is filled with water molecules.

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Hot Macromolecular Structures II**MS04.07b.01 CRYSTAL STRUCTURES OF A VARIETY OF CATALYTIC ANTIBODY FABS AT 2.0 Å RESOLUTION.**

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Catalytic antibodies were designed to catalyze a variety of different chemical reactions. To date, more than 100 different chemical reactions have been catalyzed with the assistance of antibodies. The catalytic efficiency of these antibodies has varied, but few have had catalytic rates comparable to enzymes. Furthermore, only a few catalytic antibody structures have been determined. Based on the crystallographic investigations described below, a comparison will be made to evaluate similarities and differences between a number of different catalytic antibodies. The structures suggest a number of different modification that can be made to improve the catalytic rates. Furthermore, by studying the mature antibody structures and comparing them to the germline antibody structures, one may be able to learn a great deal about how the immune system increases its affinity for antigen, and allow one to think differently in the design of catalytic antibodies and haptens.

We have determined the 3-dimensional crystal structure of the 48G7 Fab ester hydrolysis catalytic antibody at 1.9 Å resolution in the presence of hapten, and 2.6 Å in the absence of hapten. Very few changes are observed between the two structures. We are presently refining the structures of the germline constructs of the 48G7 antibody in an effort to understand the antibody maturation process.

A second system under investigation is the sulfide oxidation antibody catalyzed reaction. This Fab structure has been determined at 1.7 Å resolution in the presence of hapten, and 2.2 Å resolution in the absence of hapten. Similar to the ester hydrolysis Fab structure, very few changes are observed between the apo and hapten bound forms. Interestingly, the antibody binding site appears to be primarily an entropic trap for the two substrate molecules that combine to form product. Based on the structure determination, modification of hapten design and antibody mutagenesis are in progress to improve the catalytic efficiency of the antibody reactions.

A third system under investigation is the aminoacylation antibody catalyzed reaction. The structure has been determined at 2.6 Å resolution. Of all of the catalytic antibodies, this antibody is one of the fastest antibody catalyzed reactions to date.

MS04.07b.02 CRYSTAL STRUCTURE OF THE GUANINE NUCLEOTIDE DISSOCIATION INHIBITOR (GDI) DETERMINED AT 1.81 Å RESOLUTION.

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The crystal structure of the bovine α -isoform of guanine nucleotide dissociation inhibitor (GDI) has been determined to a resolution of 1.8 Å. GDI functions in the general recycling of Rab proteins that are involved in regulation of membrane vesicular traffic. The structure of GDI consists of two major domains. The large domain (I) is folded like a cylinder composed of four β -sheets. The topology and three dimensional structure of domain I surprisingly resemble those of mono-oxygenases and oxidases. Although GDI structure has the similar groove found in those enzymes for FAD binding, and even the sequence remnant Gly-x-Gly structurally aligned well with the corresponding sequence Gly-x-Gly-x-x-Gly for nucleotide binding in those enzymes, no bound ligand was observed in this groove of GDI structure. The smaller domain (II) of GDI contains only α -helices and forms a V-shaped structure with domain I. The three dimensional structure of GDI has distinct regions corresponding to the sequence conserved regions (SCRs) that are common to the *choroideraemia* (CHM) gene product functioning to deliver Rab to catalytic subunits of Rab geranylgeranyltransferase II. The distribution of

these conserved regions suggests that the folding of CHM and GDI may be similar. Site-directed mutagenesis of a region defined by two of the SCRs which forms a compact structure at the apex of domain I reveals a critical role for this region in the binding of Rab proteins. The crystal structure of GDI has been determined using MIRAS method and refined to crystallographic R-value of 19.8% and R-free 27.5% for a total of 430 residues of the molecule in the asymmetric unit.

MS04.07b.03 THE STRUCTURE OF EF-Tu-EF-Ts COMPLEX SUGGESTS A MECHANISM FOR NUCLEOTIDE RELEASE. Takemasa Kawashima, Carmen Berthet-Colominas, Michael Wulff*, Stephen Cusack and Reuben Leberman, EMBL Grenoble Outstation B.P. 156, 38042 Grenoble Cedex, France, *European Synchrotron Radiation Facility, B.P. 220, 38043 Grenoble Cedex, France.

The crystal structure of the bacterial elongation factor complex EF-Tu-EF-Ts from *Escherichia coli* has been determined at 2.5 Å resolution. It reveals a typical guanine nucleotide binding protein in interaction with its guanine nucleotide release factor. The complex is a tetramer where two EF-Ts form a tight dimer and each EF-Tu binds essentially to one EF-Ts, with barely any contact between EF-Tu, such that the complex is best described by the formula EF-Tu-(EF-Ts)₂-EF-Tu. Comparison of EF-Tu-GDP and EF-Tu-EF-Ts shows that the conformational change between the two structures mainly alters the magnesium binding site, by disrupting the coordination of the ion. This suggests a molecular mechanism by which EF-Ts releases the GDP bound to EF-Tu where the affinity of EF-Tu for GDP is lowered by the loss of the magnesium ion.

Kawashima, T., Berthet-Colominas, C., Wulff, M., Cusack, S. and Leberman, R. *Nature* 379, 511-518 (1996).

MS04.07b.04 GAMMA-FIBRINOGEN: CRYSTAL STRUCTURE OF A 30 KDA C-TERMINUS FRAGMENT AT 2.1 Å RESOLUTION. V.C. Yee, K.P. Pratt, H.C. Cote, I. Le Trong, D.W. Chung, R.E. Stenkamp, and D.C. Teller, Departments of Biochemistry and Biological Structure, University of Washington, Seattle, WA, 98195

The crystal structure of a 30 kDa carboxyl terminus fragment of the fibrinogen gamma chain has been determined using MIR phases to 2.5 Å resolution, and refined against diffraction data to 2.1 Å resolution. Fibrinogen is the central structural protein in the blood coagulation process. Cleavage of fibrinogen by the serine protease thrombin yields fibrin, which spontaneously polymerizes to form a clot matrix. Subsequent covalent crosslinking by factor XIIIa, a transglutaminase, produces a mechanically and proteolytically stable blood clot.

Fibrinogen is a disulfide-crosslinked dimer of heterotrimers, and contains two copies each of the alpha, beta, and gamma chains. A recombinant 30 kDa C-terminus fragment of the gamma chain containing the principle factor XIIIa crosslinking site, the calcium-binding site, the platelet receptor recognition domain, part of the polymerization surface, and one of the sites which accelerate t-PA activation of plasminogen, has been the focus of our crystallographic studies. This fragment crystallizes in space group P2₁ and its structure has been determined using MIR phases to 2.5 Å resolution. The model has been refined against 2.1 Å resolution diffraction data to give R_{cryst}=15.5% and R_{free}=22.7%.

This first structure of a large fibrinogen fragment provides fresh insight into the polymerization and crosslinking events important during blood clot formation. In other work, we have determined several crystal structures of factor XIII in various forms. Our structural characterization of both enzyme and substrate extends our understanding of the transglutaminase reaction, and provides information that will be helpful in continuing studies toward obtaining the structure of an enzyme-substrate complex.

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MS04.07b.05 STRUCTURAL BASIS OF CALCIUM INDUCED E-CADHERIN RIGIDIFICATION AND DIMERIZATION

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The cadherins mediate cell adhesion and play a fundamental role in normal development. They typically consist of five tandemly repeated extracellular domains, a single membrane spanning segment, and a cytoplasmic region. The N-terminal extracellular domains mediate cell-cell contact while the cytoplasmic region interacts with the cytoskeleton through the catenins. Cadherins are defined by their calcium dependence: removal of calcium abolishes adhesive activity and renders cadherins vulnerable to proteases. The two N-terminal domains of epithelial cadherin (E-cadherin) have been expressed in *E. coli* and crystallized from 11 mg/mL solutions by the hanging-drop vapour-diffusion method in the presence of Ca²⁺. The well solution consisted of 1.2M Ammonium sulphate, 30 mM CaCl₂, 100mM Tris buffer pH 9.0. Plate-like crystal clusters of dimensions 0.6 x 0.4 x .02 mm grow within 2 days in space group C2 (a=122.0, b=80.5, c=73.2 Å, β=114.5°. The structure was solved (to 2.0 Å resolution) using a combination of multiwavelength anomalous diffraction (MAD) phasing and real space averaging techniques. The structure reveals a two-fold symmetric dimer, each molecule of which binds a contiguous array of three bridged calcium ions. Each molecule of the dimer is composed of two 7-stranded β-barrels connected by a 10 residue linker. Not only do the bound calcium ions linearize and rigidify the molecule, but they promote dimerization. The E-cadherin dual domain structure defines the role played by calcium in the cadherin mediated formation and maintenance of solid tissues.

MS04.07b.06 A THREEFOLD SYMMETRIC β-PRISM FOLD WITHOUT INTERNAL SEQUENCE HOMOLOGY IN THE STRUCTURE OF THE TWO-CHAIN LECTIN, JACALIN. M. Vijayan, K. Sekar, R. Banerjee, S.K. Mahanta, A. Suroliya and R. Sankaranarayanan, Molecular Biophysics Unit, Indian Institute of Science, Bangalore - 560012, INDIA.

Jacalin, a tetrameric lectin from the seeds of jackfruit (*Artocarpus integrifolia*) specific to the tumor associated T-antigenic disaccharide, contains a 133 residue long α-chain and a 20 residue long β-chain in the subunit. The protein does not exhibit sequence homology with any other protein of known structure and hence its structure with bound methyl-α-galactose was determined by the multiple isomorphous replacement method and refined to R=17.5% for 20,822 reflections at 2.43 Å resolution. The crystal asymmetric unit contains two half-tetramers, each with 222 symmetry. Each subunit essentially consists of two Greek keys and one Greek key-like motif, arranged in a threefold symmetric fashion. The β-chain forms part of the Greek key-like motif and plays an important role in subunit association. Unlike in the case of vitelline membrane outer layer protein-I, the threefold symmetry is not reflected in the sequence. Furthermore, the three Greek keys in the former are connected by disulfide bridges while in Jacalin the threefold symmetric structure is stabilized entirely by non-covalent interactions. The structure of Jacalin presents the first observation of a β-prism fold in a lectin. Two of the loops at one end of the prism constitute the carbohydrate binding site which shows novel features including the involvement of a N-terminal amino group generated by post-translational modification.