MS04.07a.05 THE CRYSTAL STRUCTURE OF COLICIN IA. Michael Wiener, Douglas Freymann, Partho Ghosh, Robert Stroud. Department of Biochemistry & Biophysics, University of California, San Francisco, 94143-0448.

The structure of the entire channel-forming bacteriocin (protein toxin) colicin Ia has been solved to a resolution of 2.4Å 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 $C222_1$ (a=64.4Å, b=178.6Å, c=285.5Å). 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. Vladimir Malas Ekevich*, Vladimir Efimov, Richard Kammerer and Jürgen Engel, *Department of Structural Biology and Department of Biophysical Chemistry, Biozentrum, University of Basel, Basel, Switzerland

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, $(CH_3)_3Pb(COOCH_3)_3$ and $Pr(COOCH_3)_3$ 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 ($P2_1$, 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. Raymond C. Stevens, Chemistry Department, University of California, Berkeley 94720

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 it's affinity for antigen, and allow one to think differently in the design of catalytic antibodies and hapten.

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. Ke Zeng, Isabelle Schalk, Shih-Kwang Wu, Enrico A. Stura, Jeanne Madison, M. Huang, Tandon Anurag, W. E. Balch and Ian A. Wilson, Departments of Molecular Biology and Cell Biology, The Scripps Research Institute, 10666 N. Torrey Pines Rd., La Jolla, CA 92037

The crystal structure of the bovine a-isoform of guanine nucleotide dissociation inhibitor (GDI) has been determined to a resolution of 1.8Å. 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 ß-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 geranylgenranyltransferase II. The distribution of