

residues amongst the 20 naturally occurring amino acids. The postulated function of this domain is to increase the water-binding capacity of the protein, thus enabling the protein to compete effectively with the multitude of inorganic cations for solvation. Turning the protein into a large polyanion of net charge -28 prevents self aggregation and keeps the protein in solution.

Rusticyanin from *Thiobacillus ferrooxidans* is a type I blue-copper protein stable and active in sulfuric acid at pH values as low as 0.2. The crystal structure of rusticyanin reveals several buried chargeable residues. The two histidine copper ligands are shielded from solvent by interactions with nearby hydrophobic residues. Some aspartic acid residues, notably Asp 88, located near the copper-binding site, are buried inside the protein. These carboxyls are postulated to be in uncharged form and will have pK_a values quite different from the values in free solution. The net effect on the protein is to shift the pH profile of stability towards the more acid range.

PR04.16.16 THE BEHAVIOR OF CONCAVALINA WITH CROSS-LINKING REAGENT IN THE ANHYDROUS ACETONITRILE. Minxie Qian, Qichen Huang and Yuojing Tang, Department of Chemistry, Peking University, Beijing 100871, P.R. CHINA

Concanavalin A is a saccharide-binding protein from Jack Bean. Well-ordered crystals were soaked in mother liquor of 1.2 M phosphate (pH 6.45) with 7.5% glutaraldehyde solution for 30 min. After washing cross-linked crystal with pure water 5 times, the crystal named as CON III, was soaked in the anhydrous acetonitrile for 5 min. The X-ray diffraction data of CON III was collected to 2.8 Å resolution. For comparison, X-ray diffraction data were subsequently collected for both native crystal (named as CON I) and two additional cross-linked crystals (named as CON II and CON IV). The CON II was only washed 5 times with pure water after cross-linked. After the CON IV was treated as CON III, it was soaked in pure water for 1 h. The crystals diffracted to 1.8 Å for CON I, 1.9 Å for CON II and 1.9 Å for CON VI, respectively. The cell dimensions of CON II and CON VI remain relatively constant with respect to the native CON I. But the cell dimensions of CON III were dramatically changed, which were decreased about 5 Å in both a axis and b axis, and was increased 1 Å in c axis. The changes of cell dimension are depended on the arrangement of molecule in the crystal. The major feature of secondary structure of Concanavalin A is that the polypeptide chain only contains several beta-strands, which parallel each other along c axis.

Several deviations in the regions of C-terminal and flexible loops between beta-strands are observed by superposition of main chain of CON III with native CON I. The segment corresponding to the four residues peptide chain (between PHE233 and ASN237) in the C-terminal adopts a distinctly different position in CON III. The maximum main-chain movement is about 14 Å at ASN237. The configurations of both CON II and CON VI are as same as the one of Native CON I. It is shown that the changed configuration of segments effected by the anhydrous acetonitrile are rapidly recovered in the pure water. The refinement of cross-linked crystals are in the process.

Analysis & Structure of Macromolecules

MS 04.17.01 THE STRUCTURE-BASED DESIGN OF THREE POTENT INHIBITORS OF TEM-1 β -LACTAMASE. Natalie C. J. Strynadka¹, Richard Martin³, Bryan Jones³, John Vederas², Michael N. G James¹, ¹MRC Group in Protein Structure and Function, Department of Biochemistry, ²Department of Chemistry, University of Alberta, Edmonton, Alberta, Canada, ³Department of Chemistry, University of Toronto, Toronto, Canada

TEM-1 β -lactamase is a wide-spread plasmid-mediated enzyme responsible for inducing antibiotic resistance in bacterial species via its ability to cleave and inactivate the beta-lactam ring in the classic penicillin and cephalosporin families of antibiotic drugs. TEM-1 is particularly notorious in the clinical setting in that newly arising mutants have shown resistance to an ever-increasing spectrum of β -lactam drugs as well as the small number of currently existing β -lactam inhibitors[1].

We have used the high-resolution structural coordinates of native TEM-1 [2], of the acyl-enzyme intermediate complex of TEM-1 and the substrate Penicillin G [2], and of the complex of TEM-1 with a large, 165 amino-acid β -lactamase inhibitory protein, BLIP [3,4], to design three novel, small-molecule inhibitors against TEM-1. The first two compounds were designed to mimic transition state intermediates in the reaction pathway. These molecules have been synthesized and analyzed kinetically for their ability to inhibit TEM-1 β -lactamase. They are shown to be highly potent both against the enzyme and bacterial cells in culture with K_i 's in the nM range. The third inhibitor is a non-peptide analog of the beta-hairpin of BLIP that was observed to inhibit the active site of TEM-1 in the TEM-1/BLIP complex[4].

The structures of each of the enzyme-inhibitor complexes has been solved and refined to 1.7 Å resolution. The details of the design strategy and the resulting kinetic and structural observations will be discussed.

1. Blazquez, J. *et al. Antimicrob. Agents. Chemother.* 37, 2059-67 (1993)
2. Strynadka, N. C. J., *et al. Nature* 359, 700-705 (1992)
3. Strynadka, N. C. J., *et al., Nature* 368, 657-660. (1994)
4. Strynadka, N. C. J., *et al., Nature:Structural Biology (in press)*

MS04.17.02 THE CRYSTAL STRUCTURE OF THE ImmE7 PROTEIN SUGGESTS A POSSIBLE COLICIN INTERACTING SURFACE. Hanna S. Yuan,¹ Martin K. Safo,¹ Tze-Ping Ko,¹ Wen-Yen Ku, Shih-Yang Hsieh,² and Kin-Fu Chak². ¹Institute of Molecular Biology, Academia Sinica, Taipei Taiwan 11529, ROC, ² Institute of Biochemistry, National Yang-Ming University, Taipei, Taiwan, ROC

E group colicins (from E1 to E9) are plasmid-borne antibiotic-like bacteriocins which are active against sensitive *Escherichia coli* and closely related coliform bacteria. Immediately after production, colicin forms a complex with its coordinately produced immunity protein (ImmE) in order to neutralize its toxicity toward the host cell. The immunity proteins can only completely protect a cell from the action of their cognate colicin despite their high sequence similarity. The mechanism for the specific protein-protein interaction between colicins and immunity proteins, and the inhibition of toxicity incurred after the formation of Col/Imm complex have not been explained.

We have determined the crystal structure of the immunity protein of colicin E7 (ImmE7) at 1.8 Å resolution. This is the first X-ray structure determined in the superfamily of colicin immunity proteins. Two heavy-atom derivatives were used in solving the structure by multiple isomorphous replacement method. The current model comprises 85 residues (3 to 87), and 75 water mole-

cules, yielding an R-factor of 18.7 % for 7363 reflections between 6.0-1.8 Å. ImmE7 protein contains four antiparallel α -helices and based on the genetic, kinetic and our structural data, a region rich in acidic residues is identified. Presumably this area is involved in the specific protein-protein interactions. We suggest that all the DNase-type immunity proteins, as well as colicins, must share a "homologous-structural framework" and the specific interaction is relied upon how well these two proteins' charged residues match on the interaction surface. These model can be described as a key and lock analogy, that not only the key must fit into a lock (homologous framework), but also the teeth of the key need to completely match the pitches of the lock, thus leading to the specific immunity of the colicin.

MS04.17.03 P69 PERTACTIN: LRR, PRR AND EFFICACIOUS VACCINE. Paul Emsley, Ian Charles, Neil F. Fairweather, & Neil W. Isaacs. Department of Chemistry, University of Glasgow, Glasgow G12 8QQ, UK.

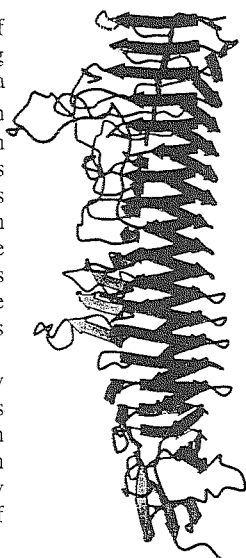
Bordetella pertussis is the bacterial agent causing the respiratory disease known as whooping cough. In the course of the disease, *B. pertussis* binds to the pulmonary epithelial cells. One of these adhesins has been identified as pertactin. Pertactin is an Arg-Gly-Asp (RGD) sequence-containing protein. Such sequences has been known to mediate the interaction of a range of extracellular proteins to a family of cell surface adhesion receptors called integrins. The nature of the interaction of such proteins and integrins is of importance to cell attachment and mobility. Recent human trials of acellular vaccines containing pertactin show considerable efficacy (1).

The X-ray crystal structure of pertactin has been solved to 2.5 Å using the MIRAS method. The structure is a 18 stranded β -helix mostly L-shaped in cross section (2) with 2 proline-rich regions (PRRs). The number of residues per turn varies between 18 and 25, but is typically 23. Pertactin has Leucine Rich Repeat (LRR) features, as does pectate lyase (2). There are several loops protruding from the helix. Two of these are extended proline-rich regions ((GGPXX)₆) and -(PPQ)₅.

The former loop is located directly after the RGD in the sequence, and is thought to mediate the interaction with epithelial cells, the latter is an immunodominant region. Pertactin may be a potential model for the structure of bacterial hemagglutinin (FHA).

(1) Science 269, 307 (1995).

(2) M. D. Yoder, S. E. Lietzke, and F. Jurnak, *Structure* 1, 241 - 251 (1993).



MS04.17.04 THE 1.0 Å RESOLUTION REFINED STRUCTURE OF MATING PHEROMONE Er-1 FROM EUPLOTES RAIKOVI. Daniel H. Anderson, Manfred S. Weiss, David Eisenberg, Molecular Biology Institute and Department of Chemistry and Biochemistry, University of California, Los Angeles. Los Angeles, California, 90095-1570, USA

Er-1 is one of a family of protein pheromones that modulate self-non-self recognition for cell aggregation and mating in the 1-cell ciliated protozoan *Euplotes raikovi*. We summarize the course and results of the refinement of the Er-1 model against atomic resolution X-ray data. The model was refined with anisotropic thermal parameters, to a R-factor of 12.92%, and includes 4 disor-

dered sidechains, 22 water molecules, a disordered ethanol, and "riding" hydrogen atoms. There is almost no net libration of the molecule, but the sidechains vibrate relative to the backbone. Post-refinement analysis of the model revealed that this dense crystal is perfused by hydrogen bonding networks of solvent and protein atoms. The ends of helices are capped by hydrogen bonding to solvent and symmetry related molecules. Water molecules mediate almost all of the interhelical hydrogen bonding, and many of the lattice interactions. Indirect evidence is presented that motions at the sites of discrete disorder may be correlated, and that protonation of acidic sidechains may switch the conformations.

MS04.17.05 THE SOLVENT STRUCTURE OF CONCANAVALIN A ANALYSED AT 2 Å AND THEN 0.94 Å RESOLUTION. T. Gleichmann, A. Deacon, S. Trapani and J. R. Helliwell, Department of Chemistry, University of Manchester M13 9PL, UK

Our investigation deals with the reliability and verification of water molecules in proteins found by X-ray crystallography at both room temperature (to 2 Å resolution) and low temperature (0.94 Å resolution). At room temperature several 2 Å coordinate sets of concanavalin A were used as test cases to determine the number of conserved sites i.e. within the solvent structures of different crystal packing arrangements of the sugar free and two distinct sugar bound forms of the protein. Within the sugar free crystal form it was found that three waters in the sugar binding site are conserved in a number of derivatives (Ni, Co, Mn, Cd substituted concanavalin A) and match the positions of three sugar-oxygen atoms (from the sugar bound crystal structures). Overall in the three different crystal packings a large number of solvent sites are conserved; within a distance range of 0 to 1.2 Å, 73 water sites (49%) in a mannoside subunit and 80 water sites (54%) in a glucoside subunit are conserved compared to the cobalt-substituted sugar free concanavalin A subunit (at 1.6 Å, the best room temperature crystal structure coordinate set we have). In order to assess methods' aspects of the reliability and precision of the water structure we also compared two Co-concanavalin A structures at room temperature.

One was obtained with Laue data (at 2 Å) and the other was with the 1.6 Å monochromatic data set. A total of 150 waters were found from the Laue refinement of which 119 agreed with the monochromatic refinement (including the three receptor binding site waters). Finally by use of flash freezing, a short wavelength intense SR beam (CHESS) and a CCD area detector a resolution of 0.94 Å has been achieved for the sugar free crystal form (this is a record for a protein of 25 kDa molecular weight, to our knowledge!). This X ray data set comprises 117000 unique reflections (75.4% complete). In this low-temperature refined structure (R-factor, SHELXL93, 13.1%) the number of detected solvent sites has increased from 147 to 290 and the quality of the electron density greatly enhanced.