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 CONCANAVALIN A WITH CROSS-LINKING REAGENT IN THE ANHYDROUS ACETONITRILE. Minxie Qian, Qichen Huang and Yuoqi 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% gluteraldehyde 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 2.8Å 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 1.8Å for CON I, 1.9Å for CON II and 1.9Å 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. Strynadka[1], Richard Martin[3], Bryan Jones[3], John Vederas[2], Michael N. G James[1], [1]MRC Group in Protein Structure and Function, Department of Biochemistry, [2]Department of Chemistry, University of Alberta, Edmonton, Alberta, Canada, [3]Department 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 particularily 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 resoluiton. The details of the design strategy and the resulting kinetic and strucutral observations will be discussed.

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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 Chak[2]. [1]Institute of Molecular Biology, Academia Sinica, Taipei Taiwan 11529, ROC, [2] Institute of Biochemistry, National Yang-Ming University, Taipei, Taiwan, ROC

E group colicins (from El 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-