**PS04.16.11 CRYSTAL STRUCTURE OF THE YEAST CELL-CYCLE CONTROL PROTEIN, $P13^{suc1}$, IN A STRAND-EXCHANGED DIMER.** N. Khazanovich[1], K. S. Bateman[1], M. Chernaia[1], M. Michalak[2], M. N. G. James[1], [1]MRC Group in Protein Structure and Function, [2]Cardiovascular Disease Research Group, Department of Biochemistry, University of Alberta, Edmonton, Alberta, Canada TOG 2H7

$P13^{suc1}$ from *S. pombe* is a member of the CDC28 kinase specific (CKS) class of cell-cycle control proteins, that includes CKS1 from *S. cerevisiae* and the human homologues CksHs1 and CksHs2. $P13^{suc1}$ participates in the regulation of $p34^{cdc2}$, a cyclin-dependent kinase controlling the $G_1$-S and the $G_2$-M transitions of the cell cycle. The CKS proteins are believed to exert their regulatory activity by binding to the kinase, in which case their function may be governed by their conformation or oligomerization state. Analysis of various assemblies of the CKS proteins, as found in different crystal forms, should help to clarify their role in cell cycle control. Previously determined X-ray structures of $p13^{suc1}$, CksHs1 and CksHs2 show that these proteins share a common fold but adopt different oligomeric states. $P13^{suc1}$ and CksHs1 were solved as monomers [1,2]. In addition, CksHs2 and $p13^{suc1}$ were observed in assemblies of strand-exchanged dimers [3,4].

We report the X-ray crystal structure of $p13^{suc1}$ to 1.95 Å resolution in space group $C222_1$. It is present in the crystals as a strand-exchanged dimer. The overall monomeric fold is preserved in each lobe of the dimer but a single ß-strand (Ile94 to Asp102) is exchanged between the central ß-sheets of each molecule.

Strand exchange, which has been observed for $p13^{suc1}$ in two different space groups, and for CksHs2, is now confirmed to bc an intrinsic feature of the CKS family. A switch between levels of assembly may serve to coordinate the function of the CKS proteins in cell cycle control.

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**PS04.16.12 STRUCTURAL COMPARISON OF TWO HIGHLY HOMOLOGOUS THERMOPHILIC BACTERIAL ALCOHOL DEHYDROGENASES.** Yakov Korkhin, Felix Frolow, Oren Bogin, Moshe Peretz, A. Joseph Kalb(Gilboa) and Yigal Burstein, Faculty of Chemistry, The Weizmann Institute of Science, Rehovot 76100, Israel.

The NADP-dependent alcohol dehydrogenases from *Thermoanaerobium brockii* (TBAD) and *Clostridium beijerinckii* (CBAD) have 75% sequence identity and yet they differ by 26°C in their inactivation temperatures ($T_{1/2}^{60min.}$= 93°C for TBAD and $T_{1/2}^{60min.}$= 67°C for CBAD). The structures of TBAD and CBAD in the holo-enzyme form have been solved and refined to a resolution of 2.5Å and 2.05Å respectively. The overall three-dimensional structures are highly homologous with $RMSD_{C\alpha}$= 0.6Å. and $RMSD_{\text{all atoms}}$= 1.0Å. Structural differences between the two enzymes are discussed in terms of their role in thermostabilization. Based on the 3D-structures, certain point mutations have been engineered and their consequences for enzyme stability have been assessed.

**PS04.16.13 STRUCTURE OF CALCIUM-FREE MANNOSE-BINDING PROTEIN** Kenneth K.-S. Ng, Shaun S. Snyder, William I. Weis, Department of Structural Biology, Stanford University, Stanford, CA 94305-5400

Calcium ions play key roles in the structures and biological activities of the carbohydrate-binding proteins known as C-type lectins. In the liver asialoglycoprotein receptor, for example, the reversible conformational change that accompanies calcium ion binding and release is integral to the protein's role as an endocytic carrier. Crystal structures of the calcium-bound form of several C-type lectins have previously been determined. We now report the structure of the calcium-free form of the carbohydrate-recognition domain of rat liver mannose-binding protein (MBP-C) and the one-calcium form of rat serum mannose-binding protein (MBP-A). The structures were solved by molecular replacement using the calcium-bound structures of MBP-C and MBP-A as search models. The loops which are involved in calcium-binding in the native state adopt different conformations when calcium ions are not present. For MBP-C, the four copies of the protein in the asymmetric unit reveal an additional range of loop conformations. Structural changes correlate with the kinetics of calcium-dependent changes in intrinsic tryptophan fluorescence.

**PS04.16.14 WHY IS *BACILLUS LICHENIFORMIS* ALPHA-AMYLASE SO THERMOSTABLE?** A. Shaw and R.Bott. Genencor International Inc., 180 Kimball Way, South San Francisco, CA94080.

*Bacillus licheniformis* is a mesophilic organism that secretes an alpha-amylase which is hyperthermostable. It is used industrially for starch liquefaction, during which the enzyme and substrate are steam jetted at 105°C or greater, with retention of 80% of its activity. This α-amylase requires calcium for activity and stability. It has been crystallized in an orthorhombic form with two molecules/asymmetric unit.

To begin to understand why a protein from a mesophile is hyperthermostable, the crystal structure of the enzyme with calcium bound has been determined, and refinement completed to 1.9 angstroms resolution.

We shall present a comparison of the *Bacillus* alpha-amylase structure with known non-thermostable alpha-amylase structures, and discuss the factors responsible for the remarkable stability of the *Bacillus* enzyme.

**PS04.16.15 ADAPTATION TO EXTREME ENVIRONMENTS: INSIGHTS FROM HALOPHILIC FERREDOXIN AND ACIDOPHILIC RUSTICYANIN.** Menachem Shoham and Dong Zhao, Case Western Reserve University School of Medicine, Department of Biochemistry, Cleveland, Ohio 44106-4935.

What causes a protein to withstand extremes in pH or to remain soluble in saturated salt? Some insights can be gained by comparing the crystal structures of extremophilic proteins with those of other members of the same family.

The crystal structure of a halophilic 2Fe-2S ferredoxin from *Haloarcula marismortui* suggests two mechanisms for keeping this protein soluble and active at the saturated salt solution prevailing in the cytosol of this archaebacterium: 1. *halophilic substitution* of polar uncharged surface residues by aspartic and glutamic acid; 2. *halophilic addition* of an extra domain consisting of two amphipathic helices and intervening loops. This domain is inserted in between the two antiparallel β strands 1 and 2 instead of the β hairpin found at this position in plant-type 2Fe-2S ferredoxins. The surface of this domain is entirely made up of 15 carboxylates. Glutamic and aspartic acid are known to be the best water-binding

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-