PS04.16.07 PRELIMINARY CRYSTALLOGRAPHIC STUDIES OF TRIOSEPHOSPHATE ISOMERASE FROM THE HYPERTHERMOPHILIC ARCHAEON *PYROCOCCUS WOESEI*. Graeme S. Bell, Rupert J.M. Russell, David W. Hough, Michael J. Danson and Garry L. Taylor. School of Biology and Biochemistry, University of Bath, Bath, BA2 7AY, U.K.

Structural studies on triosephosphate isomerase (TIM), isolated from *P. woesei*, are being carried out to further our knowledge of archaeal enzymes. In addition, as *P. woesei* exhibits an optimal growth temperature of 100°C, elucidation of enzymes from this organism will hopefully highlight structural features which could confer hyperthermostability.

The 8-stranded α/β barrel, first discovered in chicken TIM, is the most frequently occurring motif found in proteins , and is a common structural scaffold for enzymes which perform a diverse range of functions. For this reason it is a good model enzyme to select for comparative study ; there are now six known 3-D structures of TIM from both bacterial and eukaryal sources (both mesophilic and thermophilic), however, there is no data yet on the structure of archaeal TIMs.

The gene of *P. woesei* TIM has been cloned and sequenced and the enzyme has been overexpressed in *Escherichia coli*, with subsequent purification to homogeneity. (Prof. Reinhardt Hensel, University of Essen - private communication). The derived protein sequence, comprising 224 residues, is the shortest TIM sequence known yet. In addition, TIM from *P. woesei* has been found to exist as a homotetramer of 100kDa, contrary to all known bacterial and eukaryal TIMs, which are homodimeric.

To extend this work further, crystallisation studies have been carried out on the recombinant protein, and preliminary crystallographic data will also be presented.

PS04.16.08 THE EFFECT OF DENATURANTS ON PROTEIN MOBILITY. Jennifer L. H. Dunbar & Gregory K. Farber, Department of Biochemistry and Molecular Biology, Pennsylvania State University, University Park, PA 16802

Although chemical denaturants of proteins are frequently used in folding studies, their exact mechanism of action is still unknown. It is uncertain whether these denaturants act directly through binding to induce structural changes or whether they mediate unfolding indirectly through the solvent[1,2,3]. Spectroscopic techniques such as circular dichroism provide useful information on the overall effects of adding a denaturant to a protein solution, but they do not yield exact structural details of the denaturant's interaction with the protein and solvent. Knowledge of these finer points of denaturation is essential in gaining a deeper understanding of both early events in denaturation and the variation proteins exhibit in their relative susceptibilities to denaturants.

We have used x-ray crystallography as a tool to probe changes in the structure of ribonuclease A in the presence of guanidinium. Ribonuclease A provides an excellent model because it has been extensively studied in terms of both its structure and folding. Four guanidiniums are observed in the denaturant structure, of which three form a cluster. The most important change noticed is a reduction in the mobility of the protein when guanidinium is added. Similar effects are seen in an analogous study of dihydrofolate reductase in the presence of urea.

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PS04.16.09 CRYSTAL STRUCTURE AND THERMODYNAMICS STABILITY OF TYR TO PHE MUTANTS OF HUMAN LYSOZYME. [1]S. Fujii, [1]Y. Yamagata, [1]Y. Sumikawa, [2]K. Takano, [2]M. Kubota, [2]K. Yutani, [1]Faculty of Pharmaceutical Sciences, Osaka University, [2]Institute for Protein Research, Osaka University

In order to clarify the contribution of hydroxy group of tyrosine residues to the conformational stability of human lysozyme, six Tyr mutants (TYR->PHE; Y20F, Y38F, Y45F, Y54F, Y63F and Y124F) were constructed. The thermodynamic parameters for the denaturation were investigated using DSC and the crystal structures were solved at high resolution (resolution=1.8Å; R-factor=0.159-0.178). Small structural arrangements were observed locally around the mutation sites. The most striking change of hydration structure was found in the Y38F protein, which presents a decreased value in ΔG for the denaturation. The OH group of Y38 of wild protein doesn't adopt the hydrogen bonding with any side chain. Two heavily hold water molecules are lost by the removal of OH group of Y38. In the case of Y54F protein which presents a most large decreasing of the denaturation ΔG, the removal of OH group causes the breakdown of the hydrogen bonds to OD2 of D67 and the bridged water as expected. The hydration structure around the mutation site is maintained, but the thermal parameters, B-factors of water molecules and aromatic moiety of F54 are larger than that of the wild protein. Then we expected that the removal of tyrosyl hydroxy group in Y54F mutant doesn't cause the drastic change of water structure but cause the subtle destabilities of structural motion, large B-factor' s, of the water molecules and aromatic ring moiety. We estimate the structural characteristics such as ASA value and cavity volume, and analyze the correlations to the thermodynamic properties.

PS04.16.10 HEAVY CROSS-LINKED PROTEIN CRYSTALS AND ITS APPLICATION. Qichen Huang, Minxie Qian, Zhimin Wang and Youqi Tang, Department of Chemistry, Peking University, Beijing 100871, P.R.China

Crystals of lysozyme, concanavalin A, and trichosanthin, were heavy crosslinked at gluaraldahyde (up to 10.0%, 50 days), the crystals become very stable even subsequently solvent substitution by denaturant, organic solvent, non- aqueous solution contain some organic probe compounds (which are insoluble in water), water, and back soaking of water or mother liquor, respectively. Although their some cell dimensions have much changed (up to 7%), they keep original space group and still diffract X-ray to high resolution and can be used to crystallographic studies for differenet special purposes, for example, to determin the partially defolding structures, to understand the behavior the structural water on the protein bound, to find the binding sites of antibody or drugs. This method may also be used to search heavy atom derivatives, to study time-resolved structures of protein reaction dynamics. Some selected crystals data collection conditions are listed as follows. LYS-I (lysozyme, in 2.0M guanidine hydrochloride solution) has collected data to 2.1 Å resolution, LYS-II (in 3.0M guanidine hydrochloride), 2.2Å, LYS-III(in 90% v/v aceto- nitril and water), 1.9 Å, LYS-IV(in 95% acetonitril), 1.9 Å, LYS-V(in anhydrous acetonitrile), 2.5 Å, LYS-VI (in pure water), 1.9 Å, and LYS-VII (back soaking from aceto- nitrile to water) 2.0Å, for comparing. CON-I(concanavalin Å in anhydrous acetonitrile), 2.5 Å, CON-II(in water), 1.9 Å, CON-III (back soaking from acetonitrile to water), 1.9 Å, CON-IV (in mother liquor), 1.8 Å for comparing. TCS-I (trichosanthin, in anhydrous acetonitrile), 2.3 Å, TCS-II (in acetonitrile solution contain organic fluorine compoud as probe for serching the bind sites of antibody), 2.5 Å, TCS-III (in mother liquor) 1.9 Å, for comparing. Some data set refinements had been carried. In CON-I, the big change (more than 14Å) of C-terminer four residues peptide chain, and the direction reversing of one loop are observed. Now, further refinements of all other data set are in progress.