PS04.17.40 CRYSTALLOGRAPHIC EVIDENCE OF A LARGE LIGAND-INDUCED CONFORMATIONAL CHANGE BETWEEN THE TWO DOMAINS OF THE GLUTAMINE-BINDING PROTEIN. Yuh-Ju Sun[1], A-Yen. Hsin[1], Chien Ho[2], Bi-Cheng Wang[3], Chwan-Deng Hsiao[1]. Crystallographic Laboratory, Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan, 11529[1], Department of Biological Sciences Carnegie Mellon University, Pittsburgh, PA 15213, USA[2], Departments of Biochemistry and Molecular Biology University of Georgia, Athens, GA 30602-7229

The large-scale movement of rigid globular domains has been found in many proteins. Such conformational changes have been found to be responsible for specific functions, such as ligand binding, catalysis, and recognition by other biomolecules. Among these, bacterial periplasmic binding proteins involved in ligand transport across cell membrane and/or chemotaxis, can be used as probes for understanding the mechanism of the movement. The three-dimensional structures of a periplasmic binding protein from *Escherichia coli,* "glutamine-binding protein"(GlnBP) without and with ligand have been determined by X-ray crystallographic methods. They are composed of two globular domains which are held together by two short connecting hinges. The two domains are far apart in the GlnBP unliganded structure, in "open" conformation, but close together in the GlnBP-Gln liganded structure, in "close" conformation. This large conformational change between the two domains is a consequence of approximately 48° bending of small domain through the connecting hinges.

PS04.17.41 COMPARISON OF CLARA CELL PHOSPHOLIPID-BINDING PROTEIN STRUCTURES. T.C. Umland[1], S. Swaminathan[1,2], W. Furey[1,2] and M. Sax[1,2]. [1]VA Medical Center, PO Box 12055, University Dr. C, Pittsburgh, PA 15240 and [2]University of Pittsburgh, Pittsburgh, PA 15260

Crystal structures of Clara cell phospholipid-binding protein (CCPBP) have been determine for protein isolated from both human and rat. In the case of the human protein, the three-dimensional structure is known for two different crystal forms. Thus, structural comparisons between the same protein in different crystallographic environments, as well as comparisons between CCPBP from different species is possible. CCPBP is secreted by Clara cells into the distal airways of the lung. It has also been identified in other tissue and is the counterpart to the protein uteroglobin from rabbit and related species. The protein is a homodimer weighing approximately 16 kDa. It is covalently bound together in an antiparallel manner by two disulfide bonds, and a large interior cavity is formed between the two monomers. The dimer may remain intact after reduction of the disulfide bonds, and the presence or absence of these bonds control access to the interior cavity, in which ligand binding occurs. CCPBP may bind phosphatidylinositol, phosphatidylcholine, several PCB derivatives, and progesterone within this cavity. The comparison of the several known structures of CCPBP will provide insight into the mechanism of ligand binding and the differences in ligand affinity by the protein from various species.

PS04.17.42 A CRYSTALLOGRAPHIC EXAMINATION OF HEMOGLOBIN LIGAND BINDING AND ALLOSTERY. Gregory B. Vásquez,[1] Xinhua Ji,[1] Igor Pechik,[1] Michael Karavitis,[2] Herman Kwansa,[2] Enrico Bucci,[2] Clara Fronticelli,[2] and Gary L. Gilliland.[1] [1]Center for Advanced Research in Biotechnology of the University of Maryland Biotechnology Institute and the National Institute of Standards and Technology, 9600 Gudelsky Drive, Rockville, MD 20850. [2]Department of Biochemistry, University of Maryland at Baltimore, 108 N. Greene St., Baltimore, MD 21210.

Recent concerns about blood-borne pathogens, such as HIV and hepatitis, have accelerated studies of hemoglobin for the purpose of developing blood substitutes as an alternative to transfusions. Hemoglobin is probably one of the best studied allosteric proteins, but the mechanisms of its allosteric transformations (deoxy → oxy or T → R) are still elusive. In this study, two recombinant, mutant T-state hemoglobins (ßC112G and ß(C93A,C112G)) at 2.0 and 1.8 Å resolution, respectively, are reported along with the three-dimensional structure of R-state carbonmonoxy-hemoglobin at 2.2 Å resolution. The structures have been solved by difference Fourier techniques followed by least-squares refinement. The mutant ßC112G is partially oxygenated with oxygen bound to both α-hemes. The oxygen molecule associated with the α_1 heme has full occupancy while the molecule associated with the α_2 heme is partially occupied. The results of the comparison of this partially ligated, T-state hemoglobin with the structures of ß(C93A,C112G) and deoxyhemoglobin (Fronticelli, *et al., J. Biol. Chem.*, 1994, **269**, 23965-23969) indicate significant changes in the heme geometry and the position of the F-helix in the α_1 subunit. In the partially ligated α_2 subunit, only deviations in the positions of the iron and proximal histidine are observed. The R-state carbonmonoxyhemoglobin extends the structure from 2.7 Å (Baldwin, *J. Mol. Biol.* , 1980, **136**, 103-128) to 2.2 Å resolution. The higher resolution structure has extensive solvent structure, including a sulfate binding site between both the $\alpha_1\beta_2$ and the $\alpha_2\beta_1$ subunits, and has Fe-C-O angles of 125° and 162° for the α and ß subunits, respectively, that differ from the angles previously reported in the lower resolution crystallographic study (Baldwin, 1980), however are nearer to the angles determined for myoglobin crystallographically (Kurijan, *et al., J. Mol. Biol.*, 1986, **192**, 133-154) and those determined spectroscopically (Lian, *et al., Biochemistry*, 1993, **32**, 5809-5814; Schlichting, *et al., Nature*, 1994, **371**, 808-812).

PS04.17.43 WHAT IS THE STRUCTURAL BASIS FOR THE COUPLING OF Ca^{2+} AND PEPTIDE BINDING BY CALMODULIN? S. Weigand, S. Mirzoeva, L. Shuvalova, T. Lukas, D.M. Watterson, W.F. Anderson. Mol. Pharm. & Biol. Chem., Northwestern University Medical School, Chicago, IL 60611, USA

The goal of this research is to determine the structural basis of the observed enhancement of Ca^{2+} binding by calmodulin (CaM) in the presence of CaM binding structures. Calmodulin is a Ca^{2+} modulated signal transducing protein that activates many important eukaryotic enzymes. It is known that in the presence of CaM binding structures, the Ca^{2+} binding affinity of CaM is enhanced (Haiech, J., et al. 1991. *J. Biol. Chem.* **266**: 3427-31). The structural basis of this enhancement is not known. Glutamate 84 of CaM appears to be an interface contact residue in a CaM:peptide complex (Meador, W.E., et al. 1992. *Science* **257**: 1251-5). A charge reversal mutant of CaM, E84K-CaM, has altered biological activity, but still binds peptide. However, initial data indicate that the Ca^{2+} binding activity of the E84K-CaM:peptide complex is diminished.

In order to address the structural basis for the Ca^{2+} binding enhancement, it is important to compare high resolution structures (< 2.0 Å) of CaM and E84K-CaM in different binding states.

Suitable resolution structures exist of the uncomplexed CaM (Chattopadhyaya, R., et al 1992. *J. Mol. Biol.* **288**: 1177-92, Ban, C., et al. 1993. *PDB*:1osa). However, a high resolution structure of CaM complexed with RS20, a peptide corresponding to the CaM recognition sequence of smooth muscle/non-muscle myosin light chain kinase, is needed. Also, the structure of E84K-CaM:RS20 complex has not yet been determined. We have collected high resolution data, and are determining the structures of both the wild type CaM:RS20 complex (1.73 Å) and the E84K-CaM:RS20 complex (1.90 Å). Once these structures are determined, we believe they will offer insight into the basis for the coupling of CaM's Ca^{2+} binding and peptide binding activities.

(Supported in part by NIH grants GM30861 and T32-GM08320.)

PS04.17.44 DOMAIN INTERACTIONS IN CRYSTALLINS. G. Wright[1], B. Norledge[1], H. Driessen[1], C. Slingsby[1], R. Kroone[2], E. Mayr[3], S. Trinkl[3], A. Basak[1]. [1]Department of Crystallography, Birkbeck College, Malet St. London, UK. [2]Department of Molecular Biology, University of Nijmegen, The Netherlands. [3]Biophysics Institute, University of Regensburg, Germany

Transparency and refraction of eye lenses are dependent on the spatial organisation of the lens α-, β– and γ-crystallin proteins. Random aggregation and phase separation cause sharp discontinuities in the index of refraction leading to light scattering and cataract. The oligomeric β-crystallins and monomeric γ-crystallins form a superfamily of proteins and are an excellent example of how domain swapping can create dimers from monomers as a result of conformational differences in domain linkers. The 21 kDa γ-crystallin family has two branches: the ubiquitous, highly conserved γS, and the more variable branch comprised of at least six members γA - γF in mammals. A predicted model of γS-crystallin shows that it differs from other γ-crystallins mainly in the interface region between domains. In bovine lens γB and γD phase separate at low temperature whereas γE separates at body temperature and consequently is implicated in cold cataract. Our previous crystallographic studies on a single domain of γB show how sequence extensions effect domain interactions.

We will report on crystallographic studies of engineered crystalline. Complete γS-crystallin has resisted crystallization but the isolated N and C-terminal domains have. The C-terminal domain of βB2-crystallin has also been crystallized as well as a mutant γB crystallin with a γE linker. These studies will aid in defining the role of linkers, extensions, and surface hydrophobic patches in determining domain interactions in crystalline.

PS04.17.45 STRUCTURAL AND KINETIC ANALYSIS OF CD4 MUTANTS THAT ARE DEFECTIVE IN HIV BINDING. Hao Wu[1], David G. Myszka[2], Susan W. Tendian[3], Christie G. Brouillette[3], Ray W. Sweet[2], Irwin M. Chaiken[2], Wayne A. Hendrickson[1,2], [1]Department of Biochemistry and Molecular Biophysics, Columbia University, 630 West 168th Street, New York, NY 10032, [2]SmithKline Beecham Pharmaceuticals, King of Prussia, PA, 19406, [3]Southern Research Institute, Birmingham, Alabama, 35205

It is well established by equilibrium binding studies that CD4 interacts with gpl20 in the range of nanomolar affinity. Little is known, however, about the mechanism of this interaction. In this report, we analyzed the native and several mutant forms of the HIV-binding fragment (D1D2) of CD4 using a combination of kinetic, structural and thermodynamic approach. Our real-time binding kinetic data from BIAcore measurements showed that the affinity decrease in HIV-binding defective mutants is mainly due to the decrease in association rate. The predominant alteration of association-rate by neutral mutations may invariantly suggest conformational adaptation in this and many other protein-protein interactions.

PS04.17.46 SHORT HYDROGEN BONDS IN A PROTEIN RECEPTOR-PHOSPHATE COMPLEX: EVIDENCE FROM STRUCTURES REFINED AT 1 Å RESOLUTION. Zhongmin Wang*, Hartmut Luecke†, and Florante A. Quiocho*,‡. *Structural and Computational Biology and Molecular Biophysics Program, ‡Howard Hughes Medical Institute and Department of Biochemistry, Baylor College of Medicine, Houston, Texas 77030 and †Stanford Synchrotron Radiation Laboratory, Stanford University, Stanford, CA 94309

The protein receptor (a phosphate-binding protein or PBP with a mass of 33,000 daltons) serves as an initial and extremely specific component of bacterial active transport. Fully consistent with the stringent specificity of PBP, the initial 1.7 Å structure of the complex shows that the completely dehydrated and sequestered phosphate forms 12 hydrogen bonds (11 with donor groups and 1 with an acceptor group) in addition to one salt link with a guanidinium group. The distance of the hydrogen bond between the donor phosphate O4 oxygen and the acceptor group (an oxygen of carboxylate of Asp 56) is 2.45 Å. This short hydrogen bond has also been observed in the 1.9 Å structure of a fully active mutant PBP in which the donor group Thr141 was replaced by an acceptor Asp residue and in several high resolution structures of other PBP mutants. Moreover, the Asp141 substitution further introduced another short hydrogen bond (2.50 Å) between the phosphate O2 oxygen and an Asp141. In order to cement these findings of short hydrogen bonds, as well as obtain an atomic structure, excellent synchrotron data for the wild-type and the Asp141 mutant PBP were collected to resolutions of 0.98 Å and 1.00 Å, respectively. Using SHELXL-93, full matrix refinement of both structures with hydrogen atoms and anisotropic B-factor for non-hydrogen atoms against the ultra high resolution data confirmed the existence of the short hydrogen bonds. These short hydrogen bonds in the PBP-phosphate complexes could be classified as low barrier hydrogen bonds with energies ranging from 12 to 24 kcal/mole [1,2,3]. Nevertheless, the K_d values of 1 - 10 μM for the complexes do not reflect these high energy hydrogen bonds.

We thank Dr. George M. Sheldrick for providing the program SHELXL and helpful advice.

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