
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 peripheral 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 peripheral 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, and close together in the GlnBP liganded structure, in "close" conformation. This large conformational change between the two domains is a consequence of approximately 45° bending of small domain within the connecting hinges.

PS04.17.41 COMPARISON OF CLARA CELL PHOSPHOLIPID-BINDING PROTEIN STRUCTURES. T.C. Umland1, S. Swaminathan1, W. Furey2, and M. Saxl2. IVA Medical Center, PO Box 12055, University Dr. C, Pittsburgh, PA 15240 and University of Pittsburgh, Pittsburgh, PA 15260.

Crystal structures of Clara cell phospholipid-binding protein (CCPBP) have been determined 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 urooglobin from rabbit and related species. The protein is a homodimer weighing approximately 16 kDa. It is covalently bound together in an anti-parallel 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 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. Vasques1, Xinhuo Ji1, Igor Pechk1, Michael Karavitis2, Herman Kwans2, Enrico Bucci2, Clara Fronticelli2, and Gary L. Gilliland1. 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. 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 (8C112G and 8C93A,112G) 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 8C112G is partially oxygenated with oxygen bound to both α-hemes. The oxygen molecule associated with the α2 hemoglobin is partially occupied. The results of the comparison of this partially ligated, T-state hemoglobin with the structures of 8C93A,112G 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 α1 subunit. In the partially ligated α2 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 αβ2 and the αβ1 subunits, and has Fe-C-O angles of 125° and 162° for the α and β 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 (Kurjan, 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).


The goal of this research is to determine the structural basis of the observed enhancement of Ca2+ binding by calmodulin (CaM) in the presence of CaM binding structures. Calmodulin is a Ca2+-modulated signal transducing protein that activates many important eukaryotic enzymes. It is known that in the presence of CaM binding structures, the Ca2+ binding affinity of CaM is enhanced (Haeil, 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-S). A charge reversal mutant of CaM, E84K-CaM, has altered biological activity, but still binds peptide. However, initial data indicate that the Ca2+-binding activity of the E84K-CaM peptide complex is diminished.

In order to address the structural basis for the Ca2+ binding enhancement, it is important to compare high resolution structures (< 2.0 Å) of CaM and E84K-CaM in different binding states.