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 unliganded structure, in “open” conformation, but close together in the 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.

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 tissue and is the counterpart to the protein uteroglobin from rabbit.

The higher resolution structure has extensive solvent structure, including a sulfate binding site between both the α1B2 and α2B1 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 (Kurijan, et al., J. Mol. Biol., 1986, 192, 133-154) and those determined spectroscopically (Lian, et al., Biochemistry, 1993, 32, 5809-5814; Schlitching, 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 (Haeiche, 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 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.