

PS04.07.09 THREE DIMENSIONAL STRUCTURE OF HUMAN C-REACTIVE PROTEIN. Trevor J. Greenhough*, Graham M.T. Cheetham¹, David Holden, Dean A.A. Myles, William G. Turnell^{1,2}, John E. Volanakis³, Mark B. Pepys², Anne C. Bloomer¹ and Annette K. Shrive, Dept. of Physics, Keele University, Keele, Staffs, ST5 5BG, UK. ¹MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, UK. ²Immunological Medicine Unit, Dept. of Medicine, Royal Postgraduate Medical School, Hammersmith Hospital, London W12 0NN, UK. ³Division of Clinical Immunology and Rheumatology, Dept. of Medicine, University of Alabama in Birmingham, Birmingham, Alabama 35294 USA, * and CCLRC Daresbury Laboratory, Warrington WA4 4AD, UK

Human C-reactive protein (CRP), first discovered in 1930 and the subject of intense clinical interest ever since, is a trace plasma protein that is expressed rapidly and dramatically as part of the acute phase response to infection or injury. The structure contains a remarkable crystal contact, where the Ca binding loop including Glu147 from one protomer (Type II) coordinates into the Ca site of a (Type I) protomer in a symmetry related pentamer, revealing the probable mode of binding of the principal ligand phosphocholine (PC) and providing information concerning conformational changes associated with calcium binding. The Glu147-Phe146 dipeptide from this loosely associated 140-150 loop mimics phosphate-choline binding, mediated through calcium and a hydrophobic pocket centered on Phe66, in the accepting Type I protomer, with Glu81 suitably positioned to interact with the choline group. The movement of the loop results in the loss of calcium in the donating Type II protomer where large concerted movements of the structure, involving residues 43-48, 67-72 and 85-91, are seen. A striking structural cleft, on the pentameric face opposite to the PC binding site, suggests an important functional role, perhaps in complement activation. There are significant conformational differences from SAP, both at the tertiary and molecular levels. The structure provides insights into the molecular mechanisms by which this highly conserved plasma protein, for which no polymorphism or deficiency state is known, may exert its biological role.

PS04.07.10 CRYSTAL STRUCTURE OF THE 29KDa SUB-UNIT OF THE RECOMBINANT PLATELET-ACTIVATING FACTOR ACETYLHYDROLASE. Y. S. Ho[†], Z. Dauter[‡], Keizo Inoue[†], Z. S. Derewenda[†]. [†]Department of Molecular Physiology & Biological Physics, University of Virginia, [‡]EMBL-Hamburg, Germany, [‡]Department of Health Chemistry, Faculty of Pharmaceutical Sciences, The University of Tokyo.

Platelet-activating factor (PAF) is a potent phospholipid mediator involved in many physiological events. The enzyme that is responsible for breaking down PAF exists both intracellularly in cells and extracellularly in the blood plasma. PAF-acetylhydrolase (PAF-AH) hydrolyses the acetyl moiety of PAF at the *sn*-2 position. PAF-AH differs from other phospholipase A₂ (PLA₂) in three aspects. First, it does not require Ca²⁺ ions for its activation. Second, it differs from all known PLA₂ in that it is serine dependent, however, it differs from other serine hydrolases in that it does not have the consensus GX₂SXG sequence. Third, unlike PLA₂ which cleave the ester bond at the *sn*-2 position of phospholipids regardless of its length, PAF-AH has a specificity for acyl moiety not longer than C₄. The intracellular form has been isolated from bovine brain, the protein is a heterotrimeric enzyme consisting of a 29- 30- and 45kDa subunits. The 29kDa subunit has been successfully isolated and crystallized.

The structure was solved by MIR and optimized anomalous scattering methods based on three derivatives at 2.2Å resolution. The model has an R-factor of 24.5% and refinement process using

XPLOR is underway. The general fold of the enzyme belongs to the α/β family with five parallel β -sheets in the center and the helices packed on both sides of the β -sheets. It has a unique fold that is different from all other α/β hydrolases and it is the first characterized PLA₂ that is serine dependent. Gel filtration column chromatography of the enzyme showed that it forms a homodimer. From the model, the active site is found to be buried at the interface between the homodimer. The active site residues consist of S47, D192 and H195. Experiments are underway to compare the differences between PAH-AH and PLA₂ regarding the specificity of PAF-AH for short chain acetyl moiety. The model will be refined further to 1.7Å using, data collected from beamline BW7B at DESY by cryogenic method.

PS04.07.11 A LEFT-HANDED β -HELIX REVEALED BY THE CRYSTAL STRUCTURE OF A CARBONIC ANHYDRASE FROM AN ARCHAEON. C. Kisker, H. Schindelin, D. C. Rees, Division of Chemistry and Chemical Engineering, 147-75CH, California Institute of Technology, Pasadena, CA 91125, USA

Carbonic anhydrases are Zn²⁺ containing enzymes catalyzing the reversible hydration of CO₂. These metalloenzymes are among the fastest enzymes described so far having turnover numbers of up to 10⁶ s⁻¹. Until recently, two classes of carbonic anhydrases have been recognized: the "eukaryotic" class including seven isozymes from various higher vertebrates and two isozymes from *Chlamydomonas reinhardtii* and the "prokaryotic" class represented by chloroplast carbonic anhydrases and two bacterial enzymes. A carbonic anhydrase (CAM) that exhibits no significant sequence similarity to known carbonic anhydrases has been characterized from the thermophilic archaeon *Methanosarcina thermophila*. The crystal structure of this enzyme [1] revealed that it mainly consists of a left-handed parallel β -helix, a domain motif that has been observed for the first time in this enzyme and in the recently determined structure of UDP-N-acetylglucosamine acyltransferase [2]. The β -helix in CAM consists of seven complete turns and each turn contains three short β -strands. As a consequence, the β -helix contains three parallel β -sheets that are essentially flat. This fold is of particular interest since it contains only left-handed cross-over connections between the parallel β -strands, which have so far been very infrequently observed. The active form of the enzyme is a trimer with three zinc-containing active sites, each located at the interface between two monomers. While the arrangement of active site groups differs between this enzyme and the carbonic anhydrases from higher vertebrates, there are structural similarities in the zinc coordination environment, suggestive of convergent evolution dictated by the chemical requirements for catalysis of the same reaction. Based on sequence similarities, the structure of this enzyme is the prototype of a new class of carbonic anhydrases with representatives in all three phylogenetic domains of life.

[1] C. Kisker, H. Schindelin, B. E. Alber, J. G. Ferry and D. C. Rees, *EMBO J.*, in press.

[2] C. R. H. Raetz and S. L. Roderick, *Science* **270**, 997-1000 (1995).