Poster Presentations

[MS5-P14] Structural Studies of C-reactive Protein.

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C-reactive protein (CRP) is an evolutionarily conserved innate immune protein and a member of the ancient pentraxin family. Human CRP contains an arrangement of five identical non-covalently bound subunits, each with a calcium dependent ligand binding site and an effector binding site located on opposite sides of the molecule. All five protomers of CRP are arranged symmetrically around a central pore, consisting of 206 amino acids folded into two antiparallel β-sheets with a flattened jellyroll topology [1]. Phosphocholine (PC) is the main principal ligand for CRP, expressed on the surface of damaged cell membranes; they are widely distributed in lipopolysaccharides of bacteria and other microorganisms. The binding of PC is mediated by a phosphate-calcium interaction within the calcium binding site of CRP. Opposite to the ligand binding site is the effector face where the complement protein C1q is thought to interact with CRP [2]. C1q is a 400kDa protein and responsible for the activation of the classical complement pathway. Composed of 3 different polypeptide chains, A, B and C, there are a total of 18 subunits displaying overall appearance of a bouquet of flowers. Each chain comprises of a collagen like region of around 81 amino residues, followed by a globular head region (GHR) of around 135 residues [3]. Site directed mutagenesis studies involving C1q and CRP suggest that the interaction between the two proteins occurs via the GHR [3, 4]. The role of CRP within the human body includes the binding of microbial antigens and damaged cells, opsonising particles for phagocytosis and regulation of the inflammatory response by the induction of cytokine synthesis and activation of complement. CRP levels within the human body rise rapidly in response to inflammation, infection or tissue damage and is commonly used as a clinical biomarker, being an effective indicator for the severity of inflammation within an individual [5]. In addition, it is believed that elevated baseline levels (>1mg/L) can be a strong indicator for the development of conditions such as heart disease [6]. Although stable under physiological conditions, CRP is thought to dissociate into individual subunits, which are speculated to be either monomeric CRP (mCRP) or membrane bound CRP [7]. We have successfully dissociated CRP into mCRP in vitro, and separated out both proteins by size exclusion chromatography. We have also produced C1q GHR from intact C1q using established procedures [8]. The aim of this study is to characterise the possible interaction between the GHR of C1q and CRP isoforms and the effector mechanisms which underlie the activation of the complement pathway.

[1] Shrive, A. K. et al, (1996) Nature Structural Biology, 3, 346-354.
[2] Thompson, D. et al, (1999) Structure, 7, 169-177.
[3] Gaboriaud, D. et al, (2003) The Journal of Biological Chemistry, 278, 46974-46982.
[4] Agrawal, A. et al, (2001) The Journal of Immunology, 166, 3998-4004.
[5] Yudkin, J. S. et al, (2000) Atherosclerosis, 148, 209-214.
[6] Pepys & Hirschfield (2003) The Journal of Clinical Investigation, 111, 1805-1812.
[7] Eisenhardt, S. U. et al, (2009) Circulation Research, 105, 128-137.
[8] Paques, E. P. et al, (1979) Journal of Physiological Chemistry, 360, 177-183.

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