INTERACTIONS OF MACPF PROTEINS WITH LIPID MEMBRANES

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MACPF-domain proteins (Membrane Attack Complex/Perforin) are part of a large protein family, which comprises both lytic and nonlytic proteins. The MACPF domain is present in proteins in almost all domains of the tree of life except in Archaea. MACPF domain is typical for pore-forming proteins of the membrane attack complex members of complement (MAC) and perforin (PF), where it was originally identified. In lytic members, MACPF domain is necessary for insertion of protein into the membrane or for oligomerization and formation of a transmembrane pore. Several members, especially lytic ones, also contain additional domains which are necessary for binding of protein to lipid membranes. MACPF-domain proteins have different roles in producing organisms – some have important role in the immune system, others are involved in attacking prey or in pathogen invasion, however, for majority the role is still unknown.

In our work we examine the role of domain organisation of several MACPF-domain proteins on interaction with lipid membranes. Firstly, we constructed an annotated list of all MACPF-domain containing protein sequences that were available in public databases at NCBI and EBI-EMBL (as of 24.04.2011). Several MACPF-domain proteins were chosen for further studies based upon their domain organisation, predicted solubility and producing organism. Cell-free protein expression system was used for production of recombinant MACPF proteins because it enables high-throughput synthesis. Pore formation of produced proteins was assessed with measuring haemolytic activity while interaction with lipid membranes was examined by centrifugation assay. The results showed that four of tested MACPF-domain proteins bind to red blood cells and also to multilamellar vesicles composed of total lipid extract of erythrocytes; however none of the tested proteins showed haemolytic activity.