Perforin – Pathology, Biochemistry and Interactions

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Cytotoxic lymphocytes T and Natural killer cells play a significant role in the human immune system. They are indispensable in the elimination of malignant cells and cells infected with the intracellular pathogens. In order to eliminate their targets, killer cells release protein perforin (PFN), which interacts with the membranes in pH- and calcium- dependent manner, oligomerizes into a ring-like structures and forms a pore. These pores damage the integrity of cellular membranes and enable the entry of granzymes – proapoptotic proteases into the cellular interior. In humans and mice, mutations of PFN gene lead to fatal immune disorders, but biochemical characteristics of mutants are still to be elucidated.

It is not much known about mechanisms of PFN pore formation, its pH dependent activity and interactions with ions. The main drawback in analyses of PFN biochemistry is its unavailability for the experiments, since it is hard to produce active recombinant form. We have successfully developed technique for production of the active mouse PFN. We confirmed its presence in the isolates with Western Blot and hemolytic experiments. Protein isolate is able to lyse the erythrocytes only in presence of low concentrations of calcium – hallmark of PFN activity.

Results of ion induced lysis of red blood cells and surface plasmon resonance (SPR) show that some other divalent ions can interact with PFN, but only Ca^{2+} is required for the hemolytic activity due to the ionic specificity of the protein. With the *in silico* simulation of protein dynamics in the presence of calcium we confirmed that this ion stabilizes PFN structure. On the basis of PFN-membrane simulation we identified the amino acids required for initial protein-membrane interactions, mutated in some immune disorders. Results will collectively help to generate mutants in order to investigate mechanism of PFN pore formation.