FORMATION OF PROTEIN CHANNELS IN TARGET MEMBRANES

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INTRODUCTION

Many membrane-damaging proteins act at a physical level by inserting themselves into the target lipid bilayer and generating hydrophilic transmembrane channels. The first clear documentation of this principle was made in the complement field, where it was found that self-association of the terminal five serum complement components into channel-forming C5b-9 protein complexes inserting into the target bilayer constituted the molecular principle underlying the phenomenon of immune cytolysis. Subsequently, the recognition emerged that membrane damage by channel formers occurred in a similar fashion in many other instances. The present discussion will focus on the cytolytic C5b-9 complement complex and on two prototypes of channel-forming bacterial exotoxins that we have been studying. Attention will be drawn to the numerous analogies now recognized to exist among these protein systems.

Certain features appear common to all channel-formers. The proteins are produced as water-soluble polypeptides, but they undergo an irreversible transition from a hydrophilic to an amphiphilic state after binding and insertion into the target bilayer. In their final, membrane-bound form, they thus assume properties characteristic of integral membrane proteins. This is possible because of exposure of lipid-binding, apolar surfaces on the molecules that firmly anchor them to the lipid matrix. Membrane insertion and channel formation is invariably the consequence of self-association (oligomerisation) of the protein monomers on and in the lipid bilayer. Lipids and proteins of the membrane targets, although sometimes involved in the initial binding of native toxin molecules to the cell surface, probably never themselves signifi-
cantly contribute to formation of the channel structures per se.

The initial binding of the channel-formers to the membrane surface requires specific "binder" or "acceptor" molecules in some cases but may require none in others. Examples for the former category of molecules are the C9 complement component, and the sulfhydryl-activated bacterial exotoxins such as streptolysin-O. C9 binds to membrane-bound C5b-8 "precursor" terminal complexes, whereas the sulfhydryl-activated toxins bind to membrane cholesterol. These proteins will not bind to membranes lacking the respective binder molecules. Examples for membrane insertion of proteins without the apparent requirement for specific binders are given in the case of precursor C5b6/C5b-7 complement complexes and staphylococcal α-toxin. These proteins bind to Protein-free liposomes and specific lipidic binders have also not been unequivocally identified. When specific binders are present, membrane attachment is generally very effective even at low protein molarity. In contrast, binding of proteins directly to the bilayer in the absence of a high-affinity acceptor is generally of low efficacy. For this reason, the presence of relatively high concentrations of the latter category of channel-formers as opposed to the former is usually required to generate the channels and induce cytolysis.

The protein channels may constitute a homogeneous population of structures (e.g. staphylococcal α-toxin hexamers), or they may exhibit marked heterogeneity due to the presence of varying numbers of monomeric subunits harbored in a given polymer. Examples for the latter are the channels formed by C9, which is the major channel-forming subunit of the C5b-9 complement complex, and by the sulfhydryl-activated toxins such as streptolysin-O. The process of oligomerisation directly leads to exposure of lipid-binding surfaces on the proteins due probably to changes in their conformation. The molecular basis for this unique hydrophilic-amphiphilic transition has not yet been elucidated for any single channel-former. However, structural studies at a molecular genetic level are now underway and considerable progress in this field should be made in the near future.

Although the monomeric native proteins are sensitive to proteolytic degradation, all of the oligomerized protein complexes studied to date have, fortunately, been found to be remarkably stable. They withstand not only the action of very high concentrations of non-ionic detergent and deoxycholate, but also resist destruction by proteases at neutral pH (Tranum-Jensen et al, 1978, Bhakdi and Tranum-Jensen, 1978, Füssle et al, 1981). Their isolation from target membranes is therefore usually quite simple—much more so than the isolation of native proteins from serum or from bacterial culture supernatants. All three protein channels to be discussed here were originally isolated from membranes after lysis of target erythrocytes with unpurified toxin.