

Structural Basis of Pore Formation by the Bacterial Toxin Pneumolysin

The following commentary was written by Dr Clare Sansom. The original article was published in *Cell*: Sarah J. Tilley, Elena V. Orlova, Robert J.C. Gilbert, Peter W. Andrew and Helen R. Saibil (2005) Structural Basis of Pore Formation by the Bacterial Toxin Pneumolysin. *Cell*. Volume 121, Issue 2, 22 April 2005, Pages 247-256.

Many pathogenic species of bacteria secrete toxic proteins that can punch holes in the phospholipid membranes that surround cells, leading to leakage of the contents of the cell (cytolysis) and thus to cell death. Now, for the first time, electron microscopist Helen Saibil, her colleagues at Birkbeck College, Sarah Tilley and Elena Orlova, and collaborators Robert Gilbert from Oxford and Peter Andrew from Leicester, have shown this process in action.

Saibil's images, published in the April 22 issue of *Cell*¹, show the protein pneumolysin from *Streptococcus pneumoniae* forming two types of complex on the surfaces of model cell membranes: a ring-shaped "prepore" that forms first on the membrane surface and a pore in which the protein penetrates deep through the membrane to disrupt it. The transition from the prepore to the pore is accompanied by an astonishing conformational change in the protein monomer, which includes one part of the protein changing from an alpha helix into an extended beta structure.

Streptococcus pneumoniae, as its name implies, causes pneumonia; it can also cause meningitis and the ear infection otitis media. Although *S. pneumoniae* infection is rarely fatal in developed countries, it is a significant cause of death in the developing world, particularly among young children. The protein pneumolysin is released from the bacterium as a soluble monomer; it associates to form ring-shaped pores when it comes into contact with a membrane. The exact three-dimensional structure of the pneumolysin monomer is unknown, but it can be assumed to be essentially the same as that of the closely related protein perfringolysin O, found in the bacterium that causes gas gangrene, *Clostridium perfringens*. The crystal structure of perfringolysin² shows a flat molecule that consists of four distinct domains. Three of the domains - numbered 1, 2 and 4 - are arranged in a line, with domain 3 packing against domain 2. Each is largely composed of extended beta sheets (Figure 1), but domain 3 also contains two groups of alpha helices that play an important role in pore formation. But just how this soluble molecule

can form such large and destructive structures on the surface of cells has remained a mystery until now.



Figure 1

Saibil and her group incubated pneumolysin briefly at 37°C with small, spherical structures with phospholipid walls known as liposomes. The liposomes contained cholesterol, which is known to be necessary for toxin activity. Images of the preparation at a magnification of 42,000, obtained using the Tecnai F20 FEG microscope at Birkbeck College, showed the toxin forming two different ring shaped structures on the surface of the liposomes. One, known as the prepore (Figure 2a), sat on the membrane surface with only a small part of each protein monomer penetrating part way into the membrane; in the other, the pore (Figure 2b), the protein had bored an obvious hole in the membrane bilayer. Pores of different sizes were observed, and many appeared to have either about 38 or about 44 subunits.

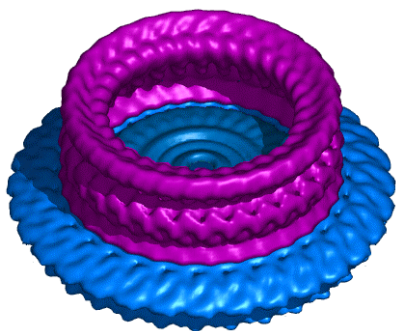


Figure 2a

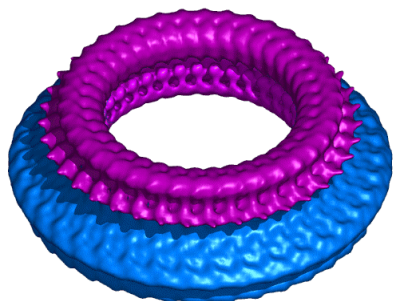


Figure 2b

Electron microscopy does not normally reproduce molecular structure at the same level of detail as, for example, X-ray crystallography, where it is possible to distinguish individual atoms. Instead, the resulting image is akin to an “envelope” of electron density that reveals the overall shape of the molecule. However, where an atomic level structure of one or more parts of the molecule or assembly studied - or, as in this case, a structure of a related molecule - is available, it can be fitted into the envelope, revealing more structural details.

Fitting the crystal structure of perfringolysin O into the electron density envelope of pneumolysin in the prepore proved to be relatively straightforward. A piece of density similar in shape to the first three domains of the monomer could be easily recognised. The fourth domain could then be fitted into the remaining density by twisting it about a thin connecting region and moving it by about 1.5 nm, pushing the tip of this domain into the upper part of the membrane. This part of the molecule includes a loop rich in the large, aromatic amino acid, tryptophan, and previous biochemical studies had suggested that this loop would interact with the membrane³.

However, it was strikingly clear from the original images that the formation of the pore from the

prepore is accompanied by a dramatic change in the protein monomer conformation. Domains 1 and 4 of the monomer could be clearly recognised, but with the molecule doubled over into an arch, it was only possible to fit domains 2 and 3 into the density if each was split into two parts. Figure 3 shows the perfringolysin molecule “docked” into the electron density map of the pore.

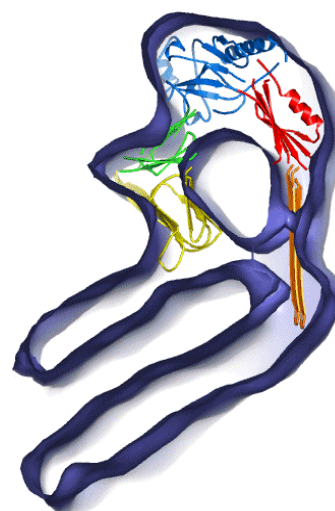


Figure 3

The cartoon shown in Figure 4 illustrates the change in conformation as monomers associate into the prepore and then into the pore form.

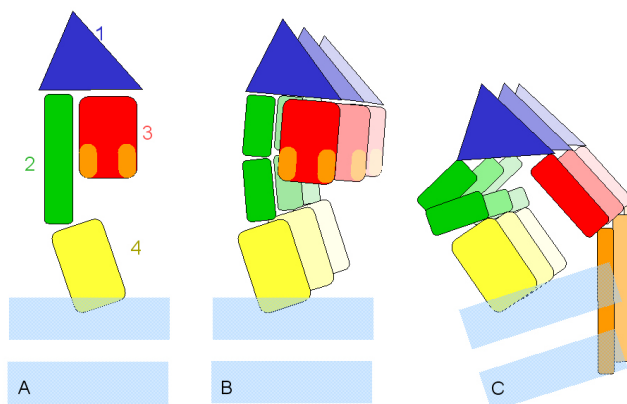


Figure 4

The most dramatic changes occur between the prepore, shown in B and the pore in C. During pore formation, domain 2 bends in half, “squashing” the molecule to bring domains 1 and 4 closer together. Most dramatically, the helices on each side of domain 3 re-fold into extended “hairpin” structures,

each now composed of two extended beta strands. They modelled this structural transition based on the results of spectroscopic studies^{4,5} and the observed electron density was entirely consistent with the earlier results.

The hairpin structures penetrate into and through the membrane, forming the pore from a continuous cylinder of beta strands known as a beta barrel. These are very common protein structures, but what marks this one out is its size. The outer membranes of bacteria such as *E. coli* contain proteins called porins that allow nutrients to pass into the bacterial cell and toxins to be removed. A typical porin contains only about 16 strands but is still larger than most of the beta barrels found in nature. Pneumolysin pores contain either 152 or 176 strands, four from each monomer, and are 25nm or more in diameter. It is not surprising that punching a hole of this size into a cell membrane will have devastating consequences for the cell.

A transition from one of the two main types of regular structure often found in proteins (alpha) into other (beta), as is observed here, is extremely rare.

And there are other examples where the consequences are equally devastating. A change from alpha to beta structure is associated with the transition of the prion protein from its normal structure into the pathogenic form that gives rise to diseases such as BSE.

Understanding the mechanism of pore formation must be the first step to developing methods of disrupting it. It may be that the insights gained from Saibil's pioneering work will lead to the development of inhibitors of pore formation as novel agents against these dangerous bacteria.

References

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