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Toxicon 42 (2003) 225–228

TOXICON

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Mini-review

Peeking into a secret world of pore-forming toxins: membrane binding processes studied by surface plasmon resonance

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Accepted 1 July 2003

Abstract

Pore-formation in cell membranes is used by many toxins to kill cells. It is usually a process involving multiple steps that are difficult to analyse at the molecular level. The use of surface plasmon resonance (SPR) has only recently been introduced into the study of pore-forming toxins (PFT). It can give useful data mostly on the first steps of the pore-forming process; the binding to the lipid membranes. In particular, it can make unique contributions to our knowledge of ligand specificity and the kinetics of binding. This mini-review summarizes some recent SPR studies of PFT.

Keywords: Pore-forming toxin; Membrane binding; Surface plasmon resonance; Equinatoxin

1. Pore-forming toxins

Pore-forming toxins (PFT) possibly constitute the most widespread group of toxins found in Nature. Their great structural variety contrasts with a limited range of modes of action. The most common is formation of discrete pores in the target cell membrane by an association of monomers. This is always a multi-step process that leads from monomeric state in solution to a membrane-inserted aggregate consisting of three to even forty monomers (Gouaux, 1997). During the pore-formation, a conformational change often exposes the hydrophobic surfaces needed to penetrate the membrane and, therefore, each step may require a distinct structural conformation. In almost all cases the first step is the binding to the cell membrane. This might be non-specific or specific, when the toxin requires specific receptors, which can be either membrane lipids or proteins. Although there is a lot of biochemical data available for PFT, their mechanism of action at the molecular level is still poorly understood, with only a few well described examples. A lot of useful clues about the pore-forming mechanism can be inferred from the crystal structures of soluble (Parker et al., 1994) or most importantly oligomerised toxins in the lipid bilayer. However, only few structures of oligomerised toxins are available (Song et al., 1996). Topology and interaction with the membrane of all the other steps are studied by other biochemical and biophysical methods, mostly by using mutated variants of toxins and model lipid membranes. Excellent reviews cover these various approaches (Heyse et al., 1998; Van Geest and Lolkema, 2000; Cho et al., 2001; Heuck and Johnson, 2002).

In this mini-review we will describe the use of surface plasmon resonance (SPR), a technique that was recognised only recently as a powerful tool in studying protein or various toxin attachment to membranes or membrane receptors (Terrettaz et al., 1993; Kuziemko et al., 1996; MacKenzie et al., 1997; Cooper et al., 1998; Cho et al., 2001; Nakajima et al., 2001; Puu, 2001; Chenal et al., 2002). In the case of PFT it can give valuable information mainly about the first step of pore-formation, binding to the cell membrane.

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0041-0101/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/S0041-0101(03)00197-1

2. Surface plasmon resonance

SPR is a relatively young experimental approach that is used in studies of macromolecular interactions, such as protein–protein, protein–ligand or protein–nucleic acid (Myszka, 1997; Lakey and Raggett, 1998). In the last few years a number of papers have described its use also in protein–membrane interactions. There are number of advantages of SPR over other techniques, such as rapid and direct determination of association and dissociation rates of the binding process, the binding of proteins to the bilayer is monitored directly without any need of specific labelling of protein or lipids, and only small amounts of sample (in nM range) are required due to high sensitivity of the detection (Cho et al., 2001). In a typical SPR experiment, a lipid membrane is formed on the surface of the sensor chip and a protein solution of desired concentration is passed over. Bound protein changes the refractive index near the surface, which results in the change of the resonance angle at which surface plasmons are created and thus where no reflected light is detected. The change is expressed in resonance units and is linearly proportional to the amount of protein bound. Binding curves (sensorgrams) are used to determine association and dissociation rates by fitting the experimental data to a suitable binding model.

There are two main approaches to immobilise membranes on the surface of the sensor chip. In the first approach, intact vesicles are captured. Lipid membranes of the vesicles contain minor amounts of a ligand that allows affinity binding to the antibody immobilised on the surface of the chip. Such approach was used by MacKenzie et al., 1997, where they incorporated small amount of lipopolysaccharide in the artificial liposomes to allow the capture on sensor chip via immobilised anti-LPS monoclonal antibody. In the second approach, lipid bilayers or monolayers are formed spontaneously on the surface of the chemically modified chip. Two commercially available chips (Biacore) are the most popular. Hydrophobic association sensor chip (HPA) contains long-chain alkanethiol molecules covalently attached to the gold surface. Vesicles are adsorbed onto a surface forming a supported lipid monolayer. On the other hand, L1 chip allows formation of the lipid bilayers. Its surface contains dextran matrix modified with hydrophobic anchors enabling capture of vesicles that fuse and subsequently form a bilayer. In such a way tethered membranes of various lipid composition can be prepared. The importance of lipid properties such as headgroup composition, charge, fluidity, etc. for the binding can then easily be checked.

3. Equinatoxin binding

A variety of the second approach was used in studying membrane interactions of equinatoxin II (EqtII), PFT from the sea anemone *Actinia equina* (Hong et al., 2002). EqtII belongs to actinoporins, a group of toxins with the unique mechanism of action (Anderluh and Maček, 2002). EqtII possesses an exposed aromatic cluster on the bottom of the molecule that was shown to be involved in the binding to the membrane (Hong et al., 2002). The N-terminal amphipatic helix was also shown to be positioned in the lipid bilayer upon binding (Anderluh et al., 1999) and it also participates in the final pore formation by creating the walls of the final pore (Malovrh et al., 2003). EqtII binding was studied on a tethered membrane in order to get insight into the role of these two parts during initial attachment.

Bilayer was tethered on the gold chip by thiolipids (Lang et al., 1994; Terrettaz et al., 2002). Such approach provides a well-defined system, where both leaflets of the bilayer are fluid (Fig. 1A). Gold chip (black line) is first covered with β-mercaptoethanol (grey spheres) and than partially with thiolipids (black spheres) that contain a short tether with a thiol group for the covalent attachment to the chip (Lang et al., 1994). An overview of a single experiment is presented in Fig. 1B. A bilayer is spontaneously formed, when a solution of small unilamellar vesicles (SUV) is passed over the chip. The resulting bilayer was washed few times with water before the actual binding experiment was done. Afterwards, the chip was regenerated by a few injections of SDS that removes the tethered lipids, but not the thiolipids. New experiment can than be performed by repeating the injection of SUV.

By the use of various mutants we were able to show that binding of EqtII proceeds in two steps. The experimental traces obtained were fitted to a two-step binding model (Fig. 1C). The first step represents the attachment of the toxin to the membrane by an aromatic cluster on the surface of the molecule. The residence time of the toxin in the membrane was changed when crucial tryptophans were mutated to phenylalanines. The resulting 14-fold higher overall K_D was mostly caused by changes in the dissociation rates of this first stage. In the second step, the N-terminal helix is displaced from the molecule and inserted deeper into the membrane. This step of the binding was resolved by a double cysteine mutant, which had two cysteines engineered in such a way that in the oxidised state the helix was fixed to the body of the molecule, thus preventing insertion into the membrane. When comparing the binding of both forms, reduced and oxidised, a two-fold increase in the dissociation rate from the second step was observed in the oxidised form, clearly indicating the role of the helix on the second step of the binding.

A two-step binding with associated conformational change was observed also in the case of diphtheria toxin T-domain at acidic pH. The first step represented low affinity binding followed by a conformational change that resulted in a high affinity interaction with a slow dissociation rate. It was possible for the first time to obtain the quantitive values of the kinetics of T-domain binding to the membranes (Chenal et al., 2002).

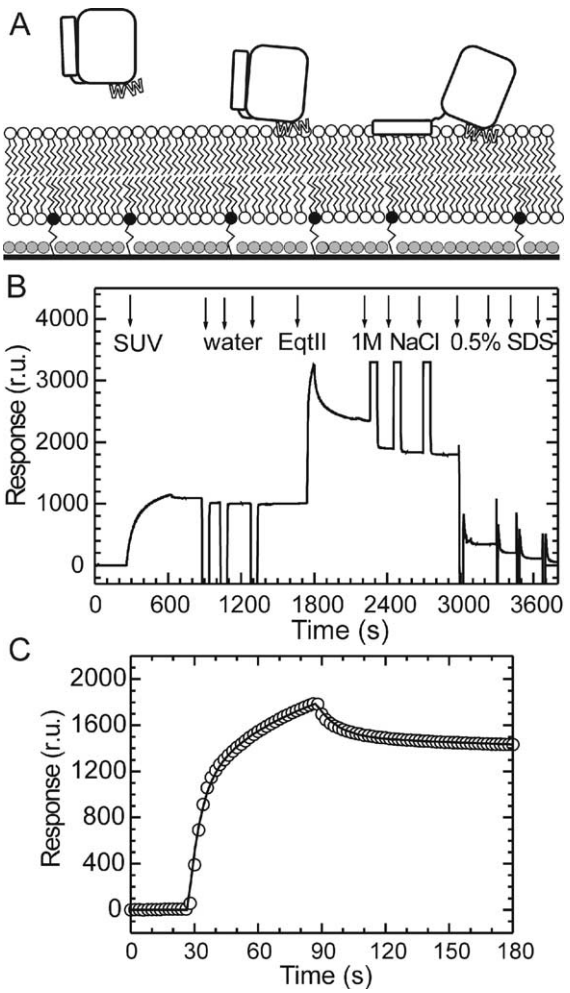

Fig. 1. Overview of EqtII binding study using tethered lipid bilayers. (A) Overview of the tethered lipid bilayer model system. See the text for the details. (B) Overview of the single experiment. (C) Binding of equinatoxin to DOPC/SM 1/1 (mol/mol) tethered membranes. DOPC/SM 1/1 membranes were used because EqtII showed high permeabilising activity on liposomes of such lipid composition (Belmonte et al., 1993). Experimental points (circles) are shown together with the fit (line) obtained from the two-state binding model. The calculated K_D was 0.75×10^{-8} M (Hong et al., 2002) (adapted from Hong et al., 2002 with permission).

4. Binding of small pore-forming peptides

The specificity of the binding and mode of action of small membrane-damaging peptides melittin and magainin was also studied recently (Mozsolits et al., 2001; Papo and Shai, 2003). Different steps during their action were detected for the first time. Sensorgrams were fitted to a two-step binding model, where the first step represented the actual binding and the second step deeper insertion. By comparing the binding to lipid bilayers and monolayers some further interesting insights into melittin and magainin molecular action were obtained (Papo and Shai, 2003). Melittin affinity for monolayers was reduced in comparison to bilayers, as there was no inner leaflet available for the insertion and subsequent creation of functional pores. The binding of magainin was not influenced by the lipid system used as it acts via the so-called 'carpet' mechanism, where the peptide is localized on the membrane surface only. It was, therefore, possible to differentiate between the two general mechanisms of membrane damage: pore-formation and detergent-like effect (carpet mechanism) by using SPR.

Although the use of SPR is not so widespread in PFT studies, it is clear that this technique can give important insights in the molecular mechanism of PFT action. In particular, binding specificity and, perhaps uniquely, kinetic parameters can be obtained easily. Finally, since the initial steps of pore-formation do not cause changes in membrane conductivity or lysis it is a significant tool to resolve these silent stages.

Acknowledgements

A.G. and P.M. acknowledge the financial support of the Slovenian Ministry of Education, Science and Sport. A.G. was a long-term FEBS fellow during EqtII studies. J.H.L acknowledges the support of the BBSRC and the Wellcome Trust. The thiolipids were a kind gift from Horst Vogel.

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