Application Note # 157

Detection of Toxin Induced Pore Formation in Membranes

Pore forming toxins are a protein family which interacts with the cell membrane causing hemolytic pores that are lethal for their target cells. In water the proteins are firmly folded but upon interaction with the cell membrane lipids, they become oligomeric integral membrane structures. The binding of toxins depends on the membrane structure.

Multi-Parametric Surface Plasmon Resonance (MP-SPR) is a highly sensitive and label-free method used to study surface changes. Lipid vesicle layers were spread on a sensor surface and four sea anemone actinoporins interactions on lipids were measured. Sticholysin I and II had clearly higher binding and pore forming ability when compared to other two actinoporins. Cholesterol in the lipid structure was found to increase the binding of equinatoxin II and fragaceatoxin C.

Introduction

Biomolecular interactions are routinely measured in the field of drug discovery and biosensor development. Surface Plasmon Resonance (SPR) is a well-established method utilized to measure binding affinity and kinetics. Comprehensive Multi-Parametric Surface Plasmon Resonance (MP-SPR) instruments can perform measurements in a wide angular range (40-78 degrees) and at more than one wavelength, thus making MP-SPR an excellent tool to measure interactions on lipids, membrane extracts and living cells.

The optical setup of the MP-SPR instruments enables measurement of multiple optical parameters simultaneously. Cross-correlation of the parameters allows simple in-line characterization of the interfering bulk signal, using the PureKinetics™ feature, and enables for real-time correction of the bulk effect. This is extremely useful when altering buffers are used or when an optimal reference surface does not exist, such as during molecular interactions with lipid layers or materials.

Materials and methods

Lipid vesicles were attached to carboxymethyl dextran coated sensor slides after the surface was cleaned using the detergent 3-[(3-Cholamidopropyl)dimethyl-ammonio]-1-propanesulfonate, "CHAPS"). Model lipid systems contained 1,2–dioleoyl–sn-glycero–3–phosphocholine (DOPC) and sphingomyelin (SM) with and without cholesterol (Chol). Lipid vesicles were applied to the dextran surface (0.5 mM lipid concentration) for 12 minutes. Unbound lipids were rinsed using 50 mM NaOH. Bovine serum albumin (BSA) was used as a blocking molecule to prevent any non-specific binding to the substrate. Running buffer was 10 mM Tris, 140 mM NaCl, pH 7.4.

Equinatoxin II (EqtII), fragaceatoxin C (FraC), sticholysins I, and II (StnI and StnII) produced by three different sea anemone species were studied (Figure 1). Actinoporins (4.0 µM) were applied for 10 minutes on the lipid surface and the bound mass (ng/cm²) was calculated. The sensor surface was regenerated using CHAPS.

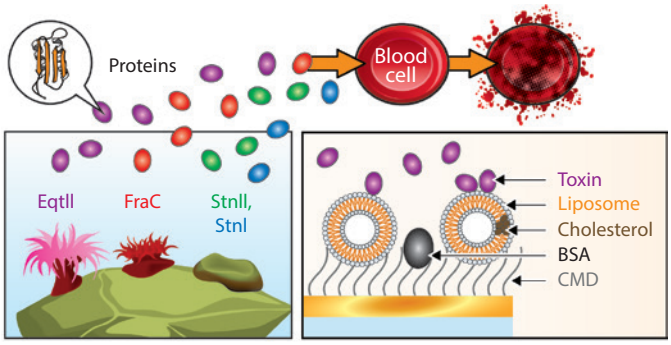

Figure 1. Sea anemone actinoporins (pore forming toxins) bind to the lipid membrane causing cell death. Equinatoxin II (EqtII), fragaceatoxin C (FraC), sticholysin II (StnII) and sticholysin I (StnI) binding to lipid vesicles were measured using MP-SPR.

Results and discussion

The selected actinoporins have a very similar molecular structure. However, pore-forming activity is known to be different. Based on the MP-SPR measurements, binding of StnII and StnI to DOPC:SM (4:1) lipid vesicles was more than three times higher than binding of FraC and EqtII actinoporins (Figure 2 A). When the membrane contained cholesterol (DOPC:SM:Chol, 1:1:1), binding of FraC and EqtII was clearly increased (Figure 2 B). MP-SPR results were in good agreement with isothermal titration calorimetry (ITC) results (Figure 3).

Pore-forming ability of actinoporins was linked to affinity on the lipid structure. Differences were caused by a sequence variability along the molecule's first 30 N–terminal residues.

Garcia-Linares et al. (2016) further studied how tryptophan residues of sticholysins II affect pore formation activity. MP-SPR was exploited to study natural and mutant sticholysin IIs binding on membranes with different lipid compositions.

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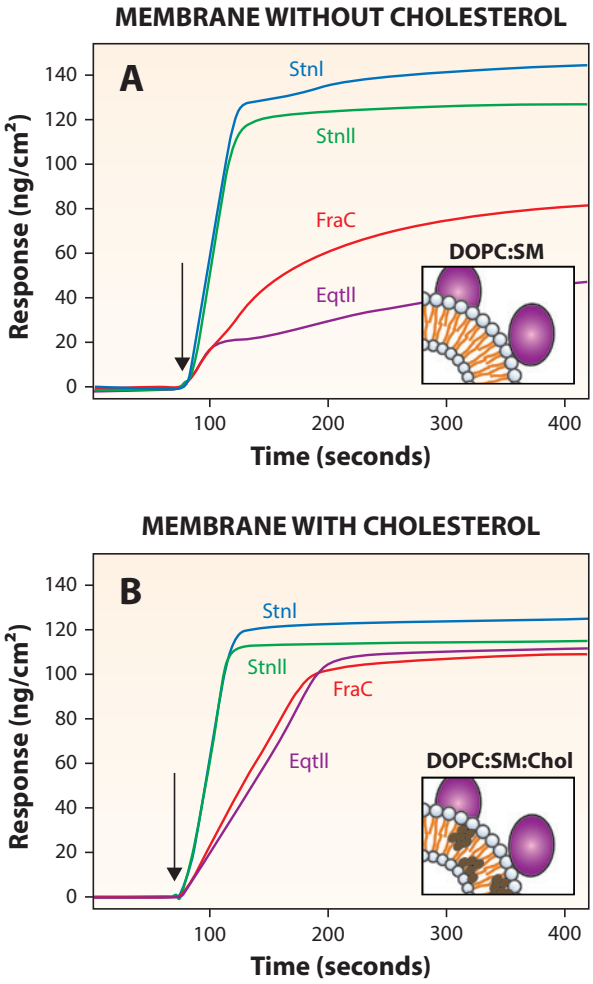

Figure 2. Actinoporins **sticholysin I** and **sticholysin II** bound clearly stronger on DOPC:SM (4:1) liposomes when compared to **equinatoxin II**, **fragaceatoxin C** (A). However, **EqtII** and **FraC** binding was increased when lipid composition contained cholesterol (B). Mass values (ng/cm²) are the average from two measurements. DOPC = 1,2–dioleoyl–sn-glycero–3–phosphocholine and SP = sphingomyelin.

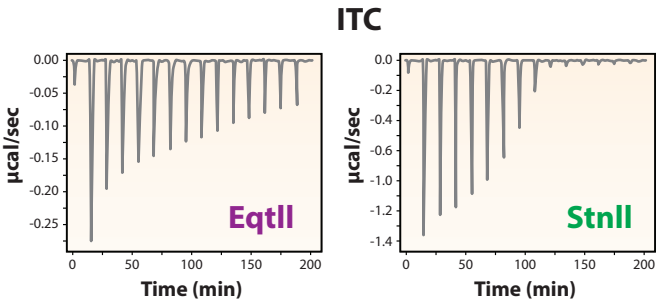

Figure 3. Binding of the **StnII** and **EqtII** to DOPC:SM vesicles were studied using Isothermal Titration Calorimetry (ITC). ITC results were in good agreement with the MP-SPR results.

Conclusions

MP-SPR provides valuable information on interaction mechanisms of toxins. Interactions of small or large molecular weight toxins can be measured in real-time and label-free. Additionally to membrane studies, molecular interactions with target molecule, biomaterial, and living cells can be studied using the MP-SPR method.

Before interaction measurement, the conformation of the lipids can be confirmed using thickness and refractive index information provided by MP-SPR. See how thickness and refractive index were determined for lipid bilayer and vesicle layer using MP-SPR (Application Note #139).

Original publication

Garcia-Linares et al. Biochemistry, 2016, 55 (48), pp 6630–6641
DOI: 10.1021/acs.biochem.6b01007

References

Garcia-Linares et al. Biochemistry, 2016, 55 (46), pp 6406–6420
DOI: 10.1021/acs.biochem.6b00935

Recommended instrumentation for reference assay experiments

MP-SPR Navi™ 200 OTSO, 210A VASA or 220A NAALI

Sensor surfaces: Au, SiO_2, CMD

Software: MP-SPR Navi™ Control, DataViewer, LayerSolver and TraceDrawer™ for MP-SPR Navi™

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