Application Note

Binding of the membrane fusion protein IM30 to supported lipid membranes

Protein/lipid interactions are involved in many biological processes. Studying protein binding to membrane surfaces is crucial for understanding the mechanisms and consequences of such events. Here, QCM was used to follow binding of an unusual membrane fusion protein to planar lipid membranes, employing the qCell T system.

Summary

Binding of proteins to a lipid membrane can be governed by electrostatic and/or hydrophobic interactions. Additionally, in case of oligomeric proteins the protein structure can alter during and/or after membrane binding. Here, we present data showing that IM30, a large ring-shaped oligomer, can bind to a net uncharged lipid bilayer and that binding is enhanced in presence of anionic lipids. IM30 binding to membrane surfaces is accompanied by a significant change in damping, indicating that the protein forms a viscoelastic layer on either membrane, in agreement with binding as a ring.

Background

Successful formation of planar lipid membranes on substrates by spreading of liposomes critically depends on the liposomal lipid composition. QCM is the method of choice to follow this process and to ensure sufficient surface coverage. Furthermore, this method allows following the kinetics of protein binding to a formed lipid membrane, employing the change in oscillator frequency of the quartz crystal to which the membrane is attached. In parallel, the viscoelastic properties of the attached mass can be determined by monitoring changes in damping of the oscillations, potentially yielding information about structural properties of the bound protein. Here, we have used QCM-D to follow binding of the membrane fusion protein IM30 to lipid bilayers. IM30, a protein conserved in chloroplasts and cyanobacteria, is membrane active and can trigger fusion of certain lipid bilayers [1].

Method

Liposomes, sized employing a tip sonifier, were made of DOPC and DOPG (4:1) at a total lipid concentration of 5 mg/ml in 10 mM Tris pH 8.0, 100 mM NaCl, 5 mM $CaCl_2$ (= liposome buffer, composed as described in [2]). Pure DOPC liposomes were prepared accordingly. Liposomes were diluted to 0.2 mg/ml for the experiment. IM30 was used at a concentration of 4 µM (monomer concentration) in 50 mM Hepes/KOH, pH 7.6 (= Hepes buffer). The IM30 monomer concentration was converted to a ring concentration, assuming an average protomer number of 52 per ring [3].

The QCM measurement typically involved the following steps, with alternating sequences of pumping (100 µl/min) and incubation:

1. "Hepes buffer": pumping 300 s
2. Liposome solution: pumping 120 s
3. "Hepes buffer": pumping 300 s
4. Protein solution: pumping 90 s

After each step, the measurement was continued until a stable signal was obtained.

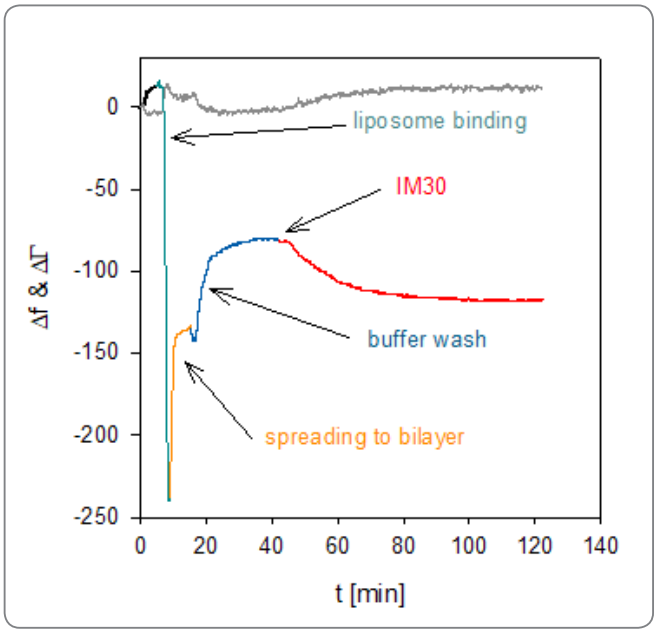

Figure 1. Example data set showing liposome spreading (4:1 DOPC/DOPG) and IM30 binding (0.8 µM) at 25°C.

Results

Employing the above introduced time scheme, formation of a supported lipid bilayer (SLB) was successfully monitored by QCM-D (Fig. 1). Only in presence of divalent cations in the "liposome buffer" spreading of DOPG-containing liposomes was observed. While not necessary for spreading, the same buffer was used in case of pure DOPC liposomes in order to keep the experimental conditions identical.

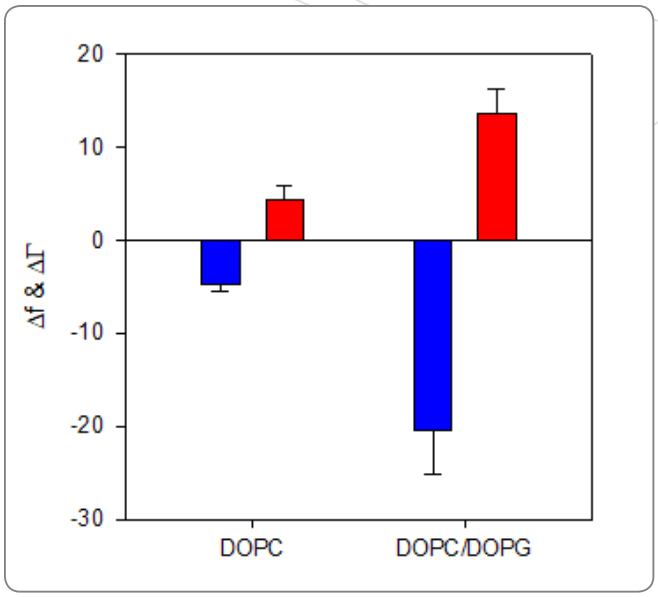

Figure 2. Changes in frequency shift (blue) and damping (red) upon IM30 binding. Number of replicates: n=4 (DOPC), n=5 (DOPC/DOPG). Error bars correspond to the standard error of the mean.

The change in frequency observed upon formation of the SLB was -93 ± 5 Hz for DOPC/DOPG (n = 5) and -97 ± 6 Hz for DOPC (n = 4), and thus similar for the two membranes. In contrast, the damping changed only slightly by 1-2 Hz upon bilayer formation. The small change in damping is in agreement with formation of a rigid layer [4], and thus the mass-deposit can be calculated according to the Sauerbrey-equation, yielding for Δf = -95 Hz a mass surface density of 420 ng/cm². This is in perfect agreement with values found in the literature [5]. Note that this mass corresponds not only to the dry weight of the lipids but also includes the hydration shell between support and lipid bilayer [5]. Loading the cell with about 0.8 µM IM30 rings lead to an increase of both, the frequency shift and damping (Fig. 1). However, as the change in frequency and damping was stronger in case of DOPG-containing membranes, IM30 binding was clearly enhanced in presence of anionic lipids (Fig. 2). Furthermore, the ratio between the frequency shift and the damping shift was similar for both types of lipid membranes (ratio for DOPC: -1.7 ± 0.2, for DOPG: -1.5 ± 0.4). As a thumb-rule, if the damping signal is higher than about 10% of the frequency signal, the layer formed on the surface cannot be treated as rigid. Thus, we can infer that a significant part of the bound IM30 had formed a viscoelastic layer and not a rigid compact layer on the membrane surfaces.

Conclusion

By using QCM-D, binding of a large homo-oligomeric protein ring to lipid bilayers was followed. The importance of anionic lipids for IM30 binding to lipid membranes was shown. Information about the viscoelastic properties of the bound protein were inferred by comparing the change in frequency and the change in damping. The significant change in damping upon IM30 binding indicates that the absorbed mass was not tightly coupled to the surface (as e.g. in case of pure lipid membranes). This suggest that IM30 had bound as a ring, as already indicated by electron microscopy [3]. A more detailed interpretation has to await determination of absolute mass bound to the surface.

Reference

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