

IMPROVED UNDERSTANDING OF LIPID MEMBRANES THROUGH COMBINED QCM-D AND EIS MEASUREMENTS

A current trend in the field of sensing is to use instruments in which several detection principles can be combined. Methods that combine structural and functional investigations of biomolecules are very valuable to improve the understanding of biomolecular functions. It is of interest to determine if there is an interaction at all and, if so, whether the properties of the lipid membrane are altered by this interaction. One field in which this approach is valuable is the study of protein and peptide interactions with lipid membranes.

INTRODUCTION

Biological membranes have many important functions, and membrane damage is fatal to cells and bacteria. Therefore, a common mechanism of toxins is to disrupt the membrane or interfere with the transport of ions across the membrane. Typically, antibacterial peptides incorporate into bacterial membranes and destroy the ion gradient between the inside and the outside of the bacterium.

APPROACH AND EXPERIMENTAL SETUP

This application note reviews two examples of experiments where QCM-D and Electrical Impedance Spectroscopy (EIS) were combined. The first example deals with the disruption of a lipid membrane through the catalytic action of the enzyme PLA2, found in insect and snake venom. PLA2 cleaves phospholipids into the corresponding lysolipids and fatty acids by catalyzing the hydrolysis of a phosphoester bond in the lipid molecule. This cleavage disrupts the membrane (Figure 1). The second example demonstrates the insertion of trans-membrane pores in a model membrane by the addition of the peptide Gramicidin D (Figure 2).

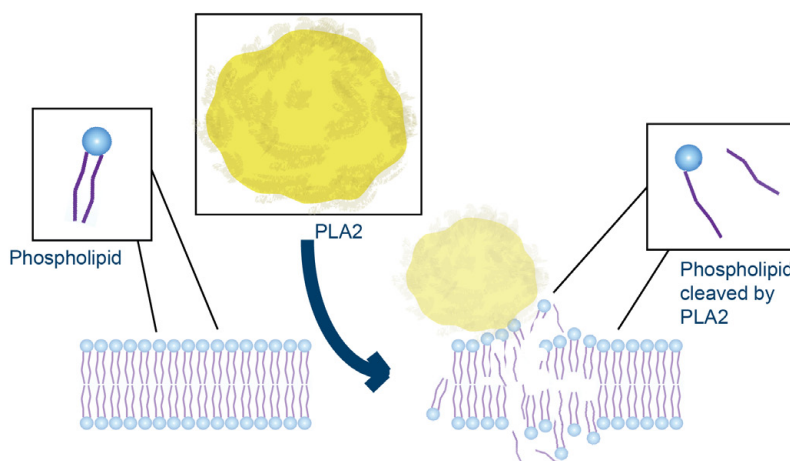


FIGURE 1 Illustration of the enzyme PLA2 and its effect on a lipid membrane.

The combination of QCM-D and EIS is especially attractive in these examples; the effect on the membrane can be sensitively detected by the electrochemical method, whereas the strength of the QCM-D method lies in its ability to characterize the membrane's viscoelastic (soft or rigid) properties.

RESULTS AND DISCUSSION

The formation of good quality lipid membranes was first ascertained by both QCM-D and EIS. The formation of a lipid bilayer on the QCM-D sensor was detected in real-time by both frequency and dissipation shifts and by single frequency impedance spectroscopy, as demonstrated in Figure 3. Bilayer formation follows the typical pathway via the adsorption of a

critical mass of liposomes, which eventually ruptures and forms an extended lipid bilayer on the surface with the characteristic QCM-D responses $\Delta f = -26$ Hz and $\Delta D < 0.5$, indicating that a layer a few nanometers thick (typically 5 nm) and rigid as indicated by the low ΔD has formed on the sensor surface. Note that bilayer formation as monitored by QCM-D is already complete long before the impedance amplitude stabilizes. A plausible explanation for this observation is that the fusion and rupture of adsorbed liposomes is followed by a slower annealing process, resulting from increased ordering of the lipid molecules in the lipid membrane and improved electrical sealing of the membrane. The first example, involving membrane disruption, is shown in Figure 1, where the action of the enzyme immediately alters the electrical properties of the membrane, as evi-

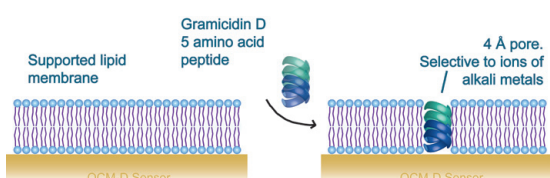


FIGURE 2. Bode plots showing the insertion of Gramicidin D as a function of peptide concentration, redrawn after [1] with permission from the authors.

denced by the sharp decrease in the impedance amplitude (Figure 3). By QCM-D, the process is first characterized by mass uptake, followed by mass removal only after the system has been rinsed with buffer.

In the second example, impedance spectra were recorded at different stages of an experiment involving a supported lipid membrane and the pore-forming peptide Gramicidin D, as schematically depicted in Figure 2. As expected, the resistivity of the membrane decreased after exposure to Gramicidin D (Figure 4). The insertion of the peptides is seen in the EIS spectra, while no changes are observed in the QCM-D signals (data not shown). This means that this modification of the resistivity is not linked to modification of the mechanical properties of the membrane, but most likely to the insertion of active Gramicidin D [1].

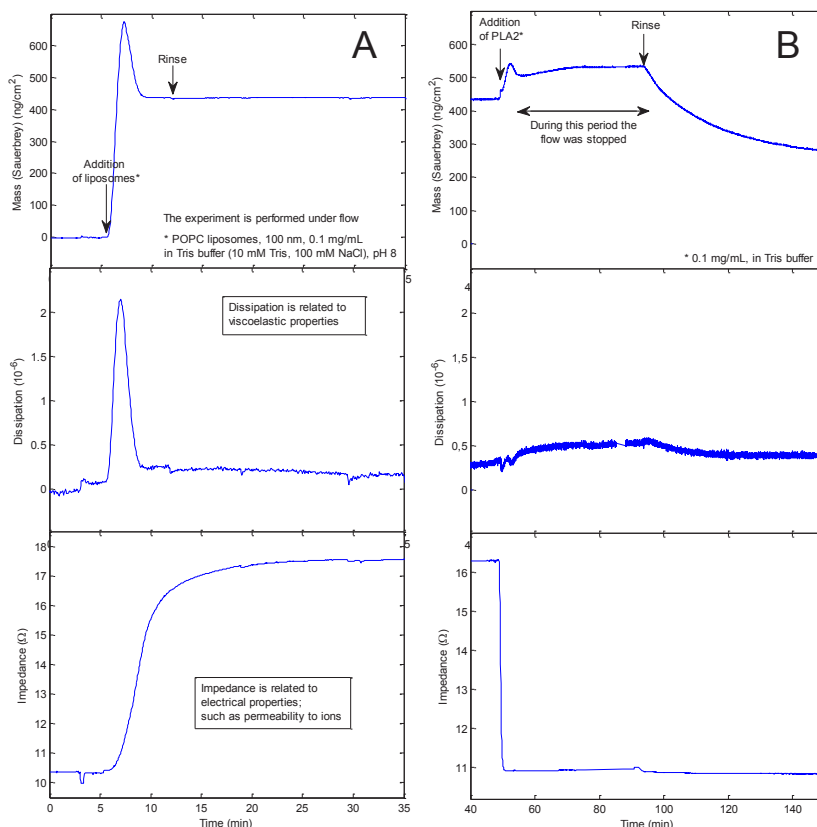
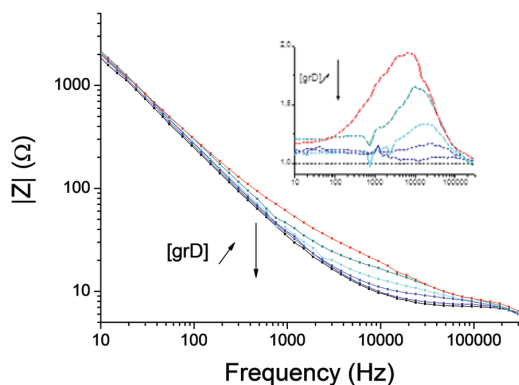


FIGURE 3 (A) QCM-D and EIS results obtained for the formation of a supported lipid membrane on the SiO₂-coated sensor surface in real-time. Figure adopted after [1] with permission from the authors and Reproduced by permission of The Royal Society of Chemistry. (B) The effect of addition of the enzyme PLA₂ to the lipid membrane. Courtesy of E. Briand.

FIGURE 4. Bode plots showing the insertion of Gramicidin D as a function of peptide concentration, redrawn after [1] with permission from the authors and reproduced by permission of The Royal Society of Chemistry. In the inset, spectra of the modulus of impedance are normalized against the modulus recorded on the sensor.

REFERENCES AND SUGGESTED FURTHER READING

[1] Elisabeth Briand, Michael Zäch, Sofia Svedhem, Bengt Kasemo and Sarunas Petronis. Combined QCM-D and EIS study of supported lipid bilayer formation and interaction with pore-forming peptides. *Analyst*, 2010, 135, 343–350

Examples of combined QCM-D and electrochemistry performed in chronoamperometric mode:

- Grieshaber, D., et al, all authors, *Swelling and Contraction of Ferrocyanide-Containing Polyelectrolyte Multilayers upon Application of an Electric Potential*, *Langmuir*, 24 (23), 13668-13676 (2008).
- Björn Wickman, Henrik Grönbeck, Per Hanarp, and Bengt Kasemo, *Corrosion Induced Degradation of Pt/C Model Electrodes Measured with Electrochemical Quartz Crystal Microbalance*. *Journal of The Electrochemical Society*, 157 (4) (2010). (Also see Application note 27).
- Barry D. Fleming, Slavica Praporski, Alan M. Bond, and Lisandra L. Martin*, *Electrochemical Quartz Crystal Microbalance study of azurin adsorption onto an alkanethiol self-assembled monolayer on gold*. *Langmuir*, 24, 323-327 (2008).

CONCLUSIONS

The combination of QCM-D and electrical impedance spectroscopy is a powerful tool to investigate structure-function relationships of proteins and peptides interacting with lipid membranes. In particular the function of ion channels can be followed while at the same time monitoring the viscoelastic properties of the lipid membrane.

ACKNOWLEDGEMENT

Dr. Elisabeth Briand is acknowledged for valuable input during the preparation of this note.

Q-Sense equipment available for similar studies...

E1 or E4 together with the ECHEM module and a potentiostat.