Quantification of the effects of melittin on liposome structure

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Abstract

An optical technique, dual-polarization interferometry, has been used to examine lipid structures at the solid/liquid interface. Changes in the lipid structures, in real time, were examined as a consequence of challenging them with a peptide (melittin) that is known to induce liposome rupture. This work suggests that it should be possible to obtain a better understanding of the detail of the melittin rupture process.

Introduction

The structure, behaviour and interactions of lipids are areas of active research. One reason behind this is the current need to develop in vitro environments that more closely resemble cell membranes. This is necessary in order to understand more clearly how the key structural elements of membrane-associated proteins influence their function. Given that a large number of protein targets for drug discovery are membrane-associated proteins, this has considerable commercial significance. Lipids are challenging, as they are able to adopt a variety of physical constructs, ranging from large vesicles with µm dimensions to planar bilayer structures of the order of 3–5 nm thick. In light of this, the straightforward measurement of the mass of lipid deposited on a surface rarely suffices in providing certainty about the structures that lipids adopt under any given condition. The use of melittin is one method used to impose the structure on lipid systems. Melittin is the active peptide in bee venom responsible for causing the membrane disruption that ultimately leads to cell death. Melittin is an amphipathic peptide known for its strong interactions with phospholipid membranes. It consists of 26 amino acids with 5–6 positive charges, making it water-soluble and yet able to associate with both natural and artificial membranes. Melittin is used as a convenient model for monitoring lipid–protein interactions and is also capable of lysing a range of lipid vesicles to produce planar bilayer structures from vesicles attached to a substrate surface [1–3]. A detailed discussion of the current understanding of the action of melittin is beyond the scope of this paper.

Materials and methods

Preparation of liposomes

Liposome preparation via hydration and sonication was carried out according to procedures found in the literature for the preparation of small (<100 nm) liposomes [4–6]. This basic preparation method was applied to all the liposomes regardless of lipid composition using lyophilized lipid powders (Avanti Polar Lipids, Birmingham, AL, U.S.A.).

Measurement methods

The liposome solutions were injected over a silicon dioxide sensor chip surface (Farfield Sensors Ltd, Manchester, U.K.) at 20 °C with a flow rate of 25 µl/min and allowed to physi-sorb on to the chip surface. The dimensions, refractive index and deposited mass of the layer structures were monitored throughout the process using a Dual Polarization Interferometer (DPI; AnaLight® Bio200, Farfield Sensors Ltd). Once the lipid layer was stable, a melittin solution (0.25 mg/ml, Sigma–Aldrich) in phosphate buffer solution (10 mM phosphate and 137 mM NaCl, pH 7.4) was injected over the sensor chip surface and the subsequent dimensional, refractive index and deposited mass changes were recorded.

Results and discussion

Liposome layer formation

It can be seen from Figure 1 that stable DOPC (dioleoylphosphatidylcholine) liposome layers are readily formed on the sensor chip surface. The liposomes appear to undergo significant distortion on the surface, eventually forming a layer that is 10–12 nm thick with a refractive index of 1.385. This refractive index value lies midway between the phosphate buffer refractive index of 1.335 and the expected refractive index value for a bilayer of 1.45. Bilayer dimensions would be expected to be of the order of 3–5 nm, so the layer dimensions are too great and the refractive index too low for the structures obtained to be true planar lipid bilayers, indicating that spontaneous liposome rupture to the bilayer on contact with the surface has not occurred. This would be expected, as the liposome is a very diffuse structure with a significant internal volume containing buffer solution.

Figure 1 shows that, after the initial liposome deposition, there is a period of 4 h (4th to 8th hour) in which the layer reduces in thickness and increases in refractive index. This
observation is consistent with the spreading out of the liposomes on the surface once the initial physisorption process is complete. There is very little mass loss during this process, which suggests that there is only minimal loss of liposomes from the surface. A range of liposome structures has been studied on the sensor chip surface. Their dynamic structural behaviour is dependent on the nature of both the polar head group, which determines the adhesion energy with the sensor chip surface, and the hydrophobic tail chain, which determines the rigidity of the liposome bilayer ‘wall’.

When the DOPC liposome layer is challenged with melittin, a dramatic rearrangement of the liposome layer is observed (Figure 2). In this experiment, the initial deposited liposome layer was of similar dimensions to those obtained in the first experiment (~18 nm with a refractive index of 1.375), but melittin was introduced before the secondary distortions seen in the first experiment occur. Immediately upon introduction of the melittin, there is a dramatic collapse and densification of the liposome layer. The layer structure immediately after the injection is 3 nm thick and has a refractive index of 1.435. These parameters are very close to those one would expect for a planar lipid bilayer (3–5 nm thick with a refractive index of 1.45). The lower refractive index suggests that the bilayer may not be complete. These observations are consistent with lysis of the liposomes by melittin to form a near-complete planar lipid bilayer on the sensor surface. It is also apparent that approximately one-half of the layer mass is lost during the melittin injection, which suggests that the upper portion of the vesicle is ‘lost’ from the surface during the liposome rupture process. A subsequent injection of melittin does not induce further major changes in the layer structure although the increase in density minimal during the second injection implies that melittin during this second injection may insert into the surface layer.
Conclusions
From the data shown here, it can be seen that real-time data on lipid structures and their behaviour when challenged with peptides can be studied in detail using dual polarization interferometry. Work is ongoing to understand the details of the melittin rupture process and to engineer a range of biologically relevant lipid structures.

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References

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