Influence of the triterpenoid saponin Quil A on mixed monolayers of phosphatidylycholine and cholesterol

T. Paepenmüller and C.C. Müller-Goymann

Institute für Pharmazeutische Technologie, Technische Universität Braunschweig, Mendelsohnstr. 1, D-38106 Braunschweig, Germany

Materials and Methods

Materials

The triterpenoid saponin Quil A (QA) was supplied by Accurate Chemical, Westbury, USA. Cholesterol (Chol) was purchased from Sigma Aldrich, Taufkirchen, Germany.

Egg-yolk phosphatidylycholine was supplied by Fluka (Buchs, Switzerland). Water used for the study was of ultra pure quality.

For QA a molecular weight of \( M_w = 2000 \) g mol\(^{-1} \) was assumed.

Methods

Lipids were dissolved in chloroform to obtain a concentration of 5 mg ml\(^{-1} \). Both pure and mixed solutions (25:75, 50:50, 75:25 PC:Chol by volume) were prepared.

The concentration of the aqueous QA subphase was 1·10\(^{-4} \) mg\( \cdot \)ml\(^{-1} \) or 1·10\(^{-5} \) mg\( \cdot \)ml\(^{-1} \).

Compression isothermes

For the monolayer preparation 50 µl of the lipid solution were transferred on an aqueous or Quil A containing subphase using a microlitre syringe with reproducibility adaptor (Hamilton, Bonaduz, Switzerland).

After evaporating the solvent for 15 minutes the monolayer was compressed using a NIMA 611 LB-trough (NIMA, Coventry, Great Britain) equilibrated at 20 °C with a barrier speed of 10 cm\(^2\)\( \cdot \)min\(^{-1} \). The corresponding surface pressure was recorded against the time.

Transfer experiments

For the transfer experiments a monolayer was prepared from 25 µl of the lipid solution spread on an aqueous subphase.

After the evaporation it was precompressed to 10 mN m\(^{-1} \) and transferred to a QA containing subphase. The surface pressure was recorded against time.

Results

A compression of a QA solution with a concentration of 1·10\(^{-4} \) mg ml\(^{-1} \) yields isotherms according to expanded monolayers (figure 1). The isotherms show no indication of a film collapse. At higher surface pressures QA molecules might be dragged back from the interface into the subphase. In contrast the 10-fold lower concentrated samples show no change in surface pressure upon compression. The QA concentration of the air/liquid interface might be too low to result in a compressible monolayer. Both solutions are far below the CMC of an aqueous QA solution. The latter was found to be 0.5 mg ml\(^{-1} \) at 20°C (Wilhelmy plate).

The influence of the subphase type on the monolayers was studied with a QA concentration of 1·10\(^{-5} \) mg ml\(^{-1} \). Figure 2 shows the interaction of QA with the pure lipid monolayers. For the monolayer of pure Chol the incorporation of QA from the subphase expands the monolayer. The increase of area per molecule at high surface pressures indicates that QA molecules remain within the monolayer even at high pressures, instead of being expelled again.

QA also expands the monolayer of PC, although not to the same extent as for Chol. The small increase in area at higher surface pressures hints at a partial exclusion of QA from the monolayer, although a given amount of QA molecules might remain within the monolayer. According to the isotherms neither PC nor Chol molecules are solubilised in the subphase.

For the mixed monolayers (figure 3) the effect of the QA subphase is similar to the isotherms measured with the pure Chol monolayer. It is assumed that this effect is due to Cholesterol molecules anchoring the QA molecules in the monolayer.

The transfer of a Chol monolayer on a subphase containing 1·10\(^{-5} \) mg ml\(^{-1} \) QA results in a sudden increase of surface pressure (figure 4). This increase is concentration dependent as a 10-fold lower concentrated subphase shows a slower increase. The resulting graph shows the shape of a typical langmuir adsorption isotherm.

Conclusion

An influence of Quil A on monolayers of phosphatidylycholine and cholesterol could be shown in this study. It emerged that the strongest effect was discovered in conjunction with cholesterol containing monolayers.

In context within the ISCOM characterisation this is an evidence that mainly cholesterol is responsible for the incorporation of QA into these colloidal structures.

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References


Introduction

Immune-stimulating complexes (ISCOMs) are colloidal lipiddic structures particularly qualified as potent drug delivery systems for vaccine formulations. Morphologically they feature a unique cage-like structure and form spontaneously in an aqueous pseudoternary system of phospholipid, cholesterol and quillaja saponin A (Quil A).

Beside the ISCOM matrices other colloidal structures can be found in this system, mainly elongated or ring-like associates [1,2].

Quil A is the responsible agent determining both the formation of the colloidal structures and the stimulating effect on the immune system. To elucidate the underlying mechanisms of the ISCOM formation, we have focused on the influence of Quil A on the lipids or ring-like associates [1,2].

Morphologically they feature a unique cage-like structure which is an evidence that mainly cholesterol is responsible for the formation of the colloidal structures and the stimulating effect on the immune system.

Results

A compression of a QA solution with a concentration of 1·10\(^{-4} \) mg ml\(^{-1} \) yields isotherms according to expanded monolayers (figure 1). The isotherms show no indication of a film collapse. At higher surface pressures QA molecules might be dragged back from the interface into the subphase. In contrast the 10-fold lower concentrated samples show no change in surface pressure upon compression. The QA concentration of the air/liquid interface might be too low to result in a compressible monolayer. Both solutions are far below the CMC of an aqueous QA solution. The latter was found to be 0.5 mg ml\(^{-1} \) at 20°C (Wilhelmy plate).

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