Formation of Supported Lipid / Protein Bilayers by Surface Induced Vesicle Fusion

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SUMMARY: For certain applications in biosensor technology it is necessary to coat the sensor surface with a reconstituted lipid / protein membrane. Here a new technique is presented which, in contrast to the Langmuir-Blodgett- technique, allows the self assembly of supported bilayers from the aqueous phase by surface induced vesicle fusion. This paper is focussed on the formation process but gives also a detailed characterization of the physical properties of the resulting supported bilayers, as well as an analysis of the stability of these membranes for various supports.

In previous studies it was shown that under appropriate conditions lipids can be deposited onto solid supports from liposome suspensions (1). We could furthermore show that it is possible to incorporate proteins like MHC-molecules (Major Histo Compatibility) and that these molecules maintain their biological activity (2).

Ellipsometry is a surface sensitive technique which can be used to measure the optical density and thickness of interfacial layers with a resolution down to some Å.

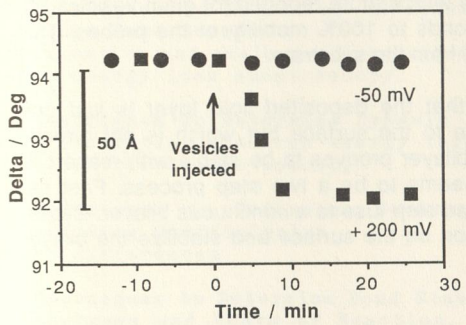

Fig.1 Change of the phase retardation of the reflected light from a gold surface incubated with a sonicated DMPC vesicle suspension in PBS (1mg/ml). A 1500Å Au layer was prepared on a glass support by vapor deposition. The surface potential (measured with a vibrating electrode) was -50mV. In a parallel experiment it was altered by Ar-plasma sputtering to +200 mV

When a freshly Ar-sputtered gold surface is incubated with lipid vesicles after a few minutes a interfacial layer with the optical properties of a lipid bilayer (refractive index n=1.55, Thickness d≈45Å) builds up (Fig1). The appearent higher thickness may result from adherent vesicles. No deposition was observed, when the freshly prepared Au surface was not plasma sputtered.

Measurements using Surface Plasmon Spectroscopy revealed that the kinetics of the deposition of the interfacial layer is determined by at least two time constants. This might reflect two basic steps in the surface induced fusion process.

Fluorescence microscopy shows, that the lipid layer which is formed on a quartz slide is homogeneous over several mm after the vesicles are washed off. For these experiments 1% of a fluorescent lipid probe (NBD-DPPE Avanti) was added to the lipid.

Pattern FRAP (Fluorescence Recovery after Photolysis) was used to determine the lateral mobility and the phase behavior of this lipid layer.

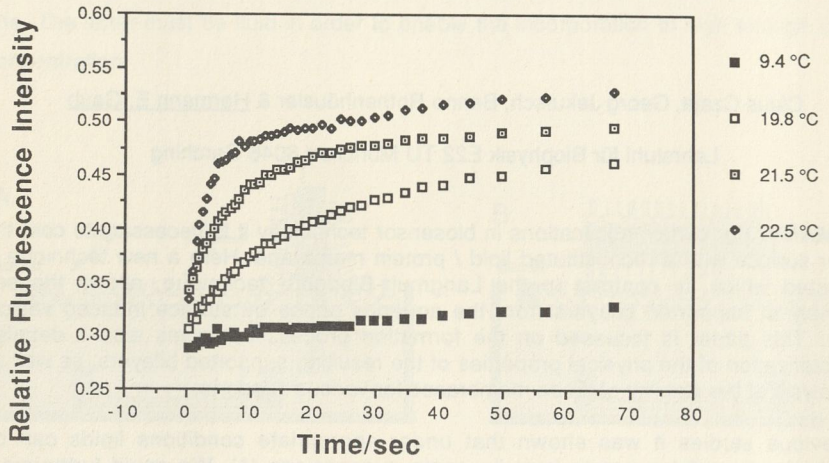

Fig2. Redistribution of a fluorescent lipid probe in a supported DMPC bilayer on quartz for various temperatures after photobleaching (5µm grid spacing).

The lipid layer shows long range lateral mobility and exhibits a fluid/solid phase transition at T=22C which occurs close to the temperature reported for giant vesicles (3). The recovery reaches 50 % which corresponds to 100% mobility of the probes. This means that the lipid layer is totally decoupled from the substrate.

All facts together lead to the conclusion that the deposited lipid layer is just one continuous bilayer which is stabilized close to the surface but which is not directly coupled to the substrate. Nevertheless this bilayer prooves to be stable with respect to washing and pH changes.The deposition seems to be a two step process. First the vesicles adhere to the surface and in a second step fuse to a continuous bilayer. Details about the interactions which induce the fusion on the surface and stabilize the bilayer are yet unknown.

This work was supported by the Deutsche Forschungsgemeinschaft

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