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Rapid Report

Self assembly of covalently anchored phospholipid supported membranes by use of DODA-Suc-NHS-lipids

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Abstract

We present a novel preparation method for the self assembly of covalently anchored phospholipid supported membranes. The surface is gold covered by cysteamine. Vesicles containing DMPC and activated DODA-Suc-NHS-lipids assembled on this surface. The whole self-assembly process is monitored conveniently by Near Infrared Surface Plasmon Resonance (NIR-SPR). Comparing the data to those obtained by Ca^{2+}-mediated vesicle fusion, confirmed this interpretation.

Keywords: Near infrared surface plasmon spectroscopy; Supported membrane; Vesicle fusion

Many methods for the preparation of supported bio lipid membranes have been proposed: Monolayer and multilayer systems deposited by Langmuir–Blodgett technique [1], self assembled phospholipid bilayers attached to planar surfaces [2] or beads [3]. Polymer cushions to support bilayers are investigated [4]. Lipids with thiol groups coupled to their headgroups have been synthesized, which allow binding to gold surfaces [5]. Others have synthesized lipid anchors on polymer backbones to mediate organization of lipid bilayer formation on solid surfaces [6]. The reasons for these various attempts are obvious: Biofunctionalization of solid surfaces is of major interest in biomedical applications to ensure biocompatibility and reduce unspecific adsorption processes. Molecular recognition processes play the essential role in many biological mechanisms, e.g., cell–cell interaction or immunological response. Those mechanisms can not only be studied, employing supported membranes, the formation of the bilayer system presented in our contribution can even be regarded as an elementary model for the recognition process itself.

Most of the common techniques to prepare supported membranes are relatively complicated: they require either a film balance or sophisticated molecules which are difficult to synthesize and sensitive to improper handling. Inspired by the synthesis of F_{ab}-fragment-lipids [7], we found a different way of preparing lipid bilayers on solid supports which we describe in the present contribution. The process is monitored and demonstrated by Near Infrared Surface Plasmon Resonance [8].

In [7] activated phospholipid molecules were used. We chose the DODA-Suc-NHS system (see Fig. 1) for reasons of easier handling. The synthesis of *N*-[(hydroxysuccinimidyl)-*N*-succinyl]dioctadecylamine (DODA-Suc-NHS) is described elsewhere [9].

1,2-Dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) and 1,2-dimyristoyl-*sn*-glycero-3-phospho-(1-*rac*-glycerol) (DMPG) were purchased from Sigma (Deisenhofen, Germany). Cysteamine (2-aminoethanethiol) was purchased from Fluka (Neu-Ulm, Germany). Bis(1,1'-carboxypentadecane-15,15'-disulfide) was a kind gift from Consortium der Electrochemischen Industrie of Wacker Chemie (Munich, Germany).

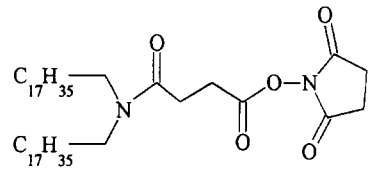

Fig. 1. The molecule *N*-[(hydroxysuccinimidyl)-*N*-succinyl]dioctadecylamine (DODA-Suc-NHS) which serves as a anchor and covalently binds the phospholipid membrane to the surface.

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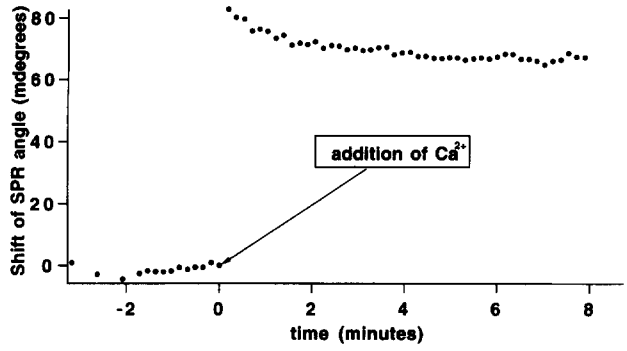

Fig. 2. Ca^{2+}-mediated vesicle fusion: A gold surface had been coated with bis(1,1'-carboxypentadecane-15,15'-disulfide) which self-assembled from an 1 mg/ml ethanol solution. Small tense vesicles of DMPG-lipids which are negatively charged at pH 6.0 (as well as the surface described) were added within 10 mM Hepes (pH 6.0). At time 0 1 mM $CaCl_2$ was added. A shift of the surface plasmon resonance angle shows the rise in thickness of the surface-coating layer and demonstrates how vesicles fuse to a supported bilayer.

The vesicles were prepared as follows:

DMPG was dissolved in $CHCl_3$/MeOH (3:1, v/v). The solution was dried under vacuum overnight. The dried film was resuspended in 10 mM Hepes (pH 7.0). The vesicles formed by incubating the lipids in this buffer for 2 h at 50°C.

A mixture of 10 weight percent of DODA-Suc-NHS in DMPC was dissolved in $CHCl_3$/MeOH (3:1, v/v). The solution was dried under vacuum overnight. The dried lipids were resuspended in 10 mM NaH_2PO_4 (pH 5.0), 150 mM NaCl and incubated for 2 h at 50°C. Shortly before the self assembly process the buffer was carefully adjusted to pH 8.0 with 0.1 M NaOH.

From both suspensions small vesicles under tension were obtained by ultra sonicating them for 10 min.

The supporting surfaces consisted of silicon wafers on which 50 nm gold had been evaporated.

Some of the gold-coated wafers were prepared by incubating in 1 mg/ml bis(1,1'-carboxypentadecane-15,15'-disulfide) in ethanol. Others were left in 2 mM ascorbic acid, 1 mM cysteamine, PBS buffer. After 3 h the gold surfaces were thoroughly rinsed with Millipore water.

As analytical tool for label free real time monitoring of the coupling processes we used Near Infrared Surface Plasmon Resonance. Surface plasmon spectroscopy has been very successfully applied to study the binding of biomolecules on glass/gold substrates ([10], [11] and references cited therein). The method allows quantitative measurements of average surface coverage [12]. SPS in the visible or the UV based on the convenient Kretschmann configuration [13], is restricted to devices using glass substrates. Surface plasmon spectroscopy in the Mid to Far Infrared with water as ambient is inhibited by strong absorption of water in the IR [14]. We recently introduced near infrared plasmon resonance (NIR-SPR) [8] as an improvement of conventional SPR: Working at a wavelength of 1300 nm, where both silicon and water are light transmissive, we use convenient smooth silicon wafers as substrate. 1300 nm light is widely used in fiber optics, thus mature, specific and inexpensive components (as light sources and detectors) are at hand. Visible light in the reaction chamber does not interfere with the measurements, one can easily observe the experimental setup by eye. Due to the wavelength dependence of noble metals, surface plasmons with sharper resonance are applied in the detection of surface alterations. More details on NIR-SPR can be found in [8].

The results shown in Fig. 2 enabled us to attribute the shift in resonance angle in our second experiment (Fig. 3) to bilayer fusion. To obtain a standard for the shift in NIR-SPR-resonance angle caused by bilayer fusion to a surface we used the well studied bilayer fusion of DMPG vesicles to bis(1,1'-carboxypentadecane-15,15'-disulfide).

Both DMPG vesicles and the surface of bis(1,1'-carboxypentadecane-15,15'-disulfide), bound to gold by their thiol groups, are negatively charged. The carbon-acid surface and the vesicles repel each other. Adding 1 mM $CaCl_2$ to the suspension results in bilayer fusion [15], as demonstrated in Fig. 2.

Fig. 3 shows an experiment, in which a solution of vesicles containing 10% DODA-Suc-NHS was added to cysteamine-covered gold. Fig. 4 serves to illustrate the fusion process which takes place in the experiment. Rins-

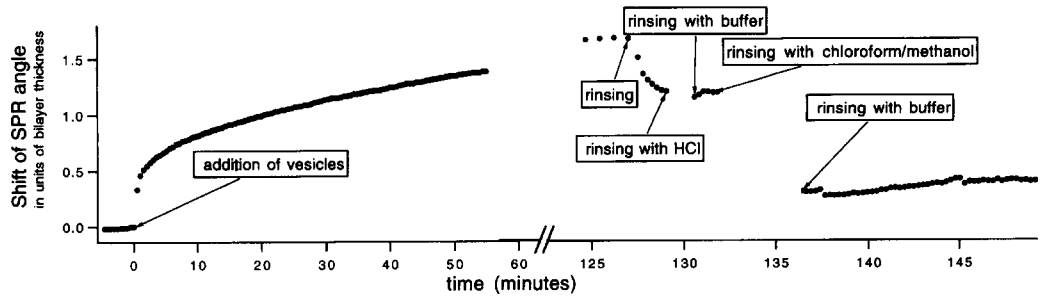

Fig. 3. Fusion of lipid vesicles to a surface through activated anchor lipids on an aminated surface. In this case the surface had been coated with cysteamine from an aqueous solution. At time 0 small tense DMPC/DODA-Suc-NHS 9:1 lipid vesicles were added. The rise in resonance angle indicates self assembly of the bilayer. Washing with buffer removes excess lipids. The remnant could not be removed even with pure HCl. Washing with organic solvent removed most of the bilayer's molecules. The time gap and the two different time scales indicated in the time axis should be recognized.

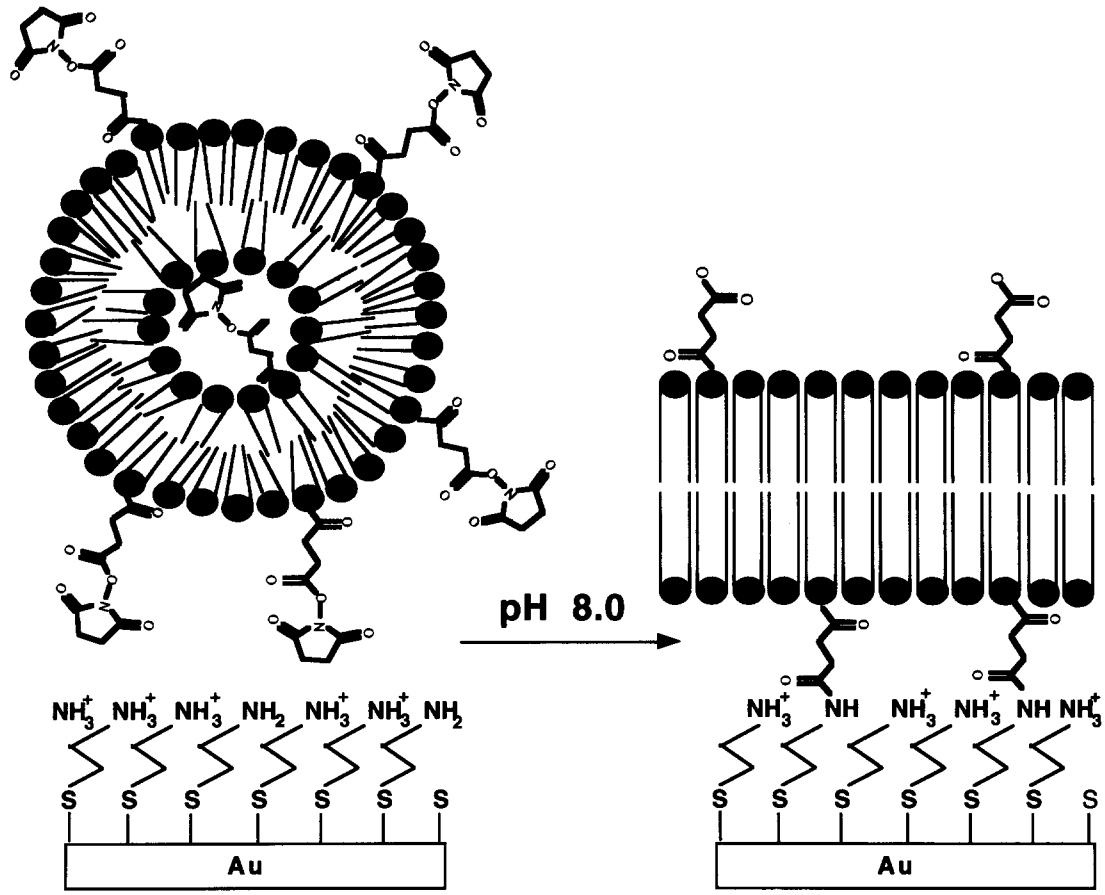

Fig. 4. Concept of bilayer attachment through our activated anchor lipids. The left picture shows a vesicle approaching the surface, the right one the planar bilayer formed as a result.

ing with phosphate buffer removed surplus lipid and left a reproducible amount of lipids, which can be identified with a bound bilayer. Washing with pH 3 buffer did not alter the lipid thickness, however, organic solvent was able to remove the bilayer.

As control experiment vesicles of pure DMPC were added to the cysteamine-functionalized gold surface. In this case a deposition occurred as well, but was removable with phosphate buffer.

The most prominent advantage of the self assembled supported lipid bilayer formation presented in this report is its simplicity. There are some similarities to the application of lipid molecules with thiol headgroups [5]. Our method, however, requires not only less synthetic effort. It is to be expected that the application of vesicle fusion will help to produce homogeneously mixed lipid supported membrane systems and to insert membrane proteins.

Other model systems similar to the one presented here, will have to be considered. First experiments of adsorbing streptavidin on gold surfaces and assembling phospholipid bilayers with biotinylated lipid anchors showed promising results (Weissmueller, G., Feder, T., Brink, G. and Sackmann, E., in preparation).

Proteins like serum albumin and fibrinogen adsorb very well on gold. Adsorbed protein could supply the amino group in a modification of our binding experiment instead of cysteamine as used in the present contribution.

Experiments to attach the bilayer on a gel or porous carrier are in preparation.

Nature often uses compartmentalization in recognition processes or for separation of two chemical processes under different conditions. Supported lipid membranes on porous carriers could be applied in biotechnology in like manner.

Label free monitoring techniques have recently become commercially available [11]. They now form a basis to promote studies as presented in this contribution: They allow monitoring of the assembly process as well as the application of the assembled model systems in further studies, especially since ready-to-use chemically modified (e.g., aminated) surfaces or protein (e.g., streptavidin) coated substrates are supplied by the manufacturers of such tools. NIR-SPR as used in the present contribution is a convenient tool and the employment of the silicon wafers as a substrate allows easy and simple preparation of label free monitoring experiments.

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