Formation of Streptavidin-Supported Lipid Bilayers on Porous Anodic Alumina: Electrochemical Monitoring of Triggered Vesicle Fusion

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Received February 9, 2001

A new approach for the self-assembly and quality control of lipid bilayers supported on porous substrates is proposed. Since the pioneering work of the McConnell group,[1] supported lipid membranes, monolayers or bilayers, have been used as models for biological membranes.[2] As reactions in biomembranes depend on the fluid dynamic properties of the bilayer components, special attention has been paid to minimizing the interactions of the bilayer with the support. One increasingly popular strategy is to fix the bilayer on a soft polymer cushion, itself supported or tethered on flat substrates such as gold,[3] glass and quartz,[2c,e,4] or mica.[5] The plane geometry of the support is often essential because it is needed either during bilayer assembly itself (for example, by Langmuir−Blodgett deposition),[2e,4] or for the characterization and quality control of the bilayers, by electrical impedance,[6] surface plasmon resonance (SPR),[3a,7] AFM,[8] or neutron reflectivity.[2c]

Our goal is to produce supported bilayers of large surface area, which are easy to manipulate and allow a high volume concentration of membrane components as in naturally occurring membrane stacking in chloroplasts or mitochondria (Figure 1). Anodically etched porous alumina is a fascinating material for the development of nanometer-sized structures[9] and has been already used as a template for suspended lipid bilayers[10] or hybrid lipid layers.[11] However, the routine formation of polymer-cushioned bilayers inside such microporous structures has to answer two main questions: (i) what kind of driving force can induce vesicle fusion inside the pores, and (ii) how can the quality of the bilayer in this porous material be monitored?

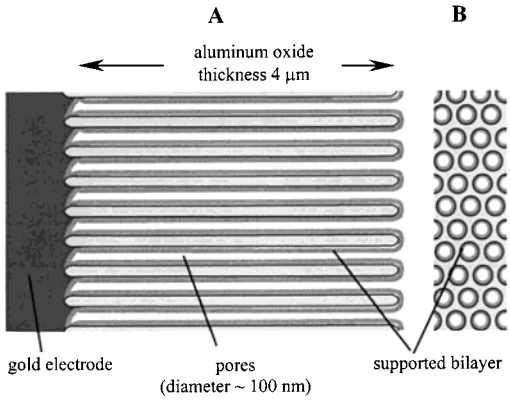

Figure 1. Schematic cross-section **(**A**)** and front view **(**B**)** of the microporous electrode. The honeycomb structure was produced by aluminum anodization and characterized by scanning electron microscopy. The oxide thickness was 4.2 ± 0.3 μm, the average pore diameter 100 ± 10 nm; the oxide surface area was then calculated to be 4.5 ± 0.5 cm^2 for a disk of apparent surface area 0.07 cm^2. The continuous lipid bilayer covering the pores was formed on a cushion of streptavidin molecules according to the procedure described in the text and Figure 2.

The mechanism of vesicle fusion on a solid substrate depends strongly on the nature of the sublayer. For hydrophobic substrates such as alkylated layers, the driving force is clearly the reduction of the free energy of the alkane/water interface during the fusion, and a spreading mechanism was proposed.[12] For hydrophilic substrates, naked or covered with polymers or proteins, the possible driving forces are weaker, and the formation of a supported bilayer involves vesicle adhesion followed by rupture and lateral fusion.[13] In the case of hydrophilic polymers, vesicle fusion was promoted by grafting hydrophobic arms on the sublayer,[3,5,14] these anchoring units ensuring interaction between the substrate and the bilayer.

Formation of the Supported Bilayer. In view of the specific geometry of our structure, the bilayer was loaded on the microporous template in a two-step procedure (Figure 2). In the first step, biotin/streptavidin affinity was used to anchor biotinylated lipid vesicles on a sublayer of streptavidin molecules themselves supported by aluminum oxide. This methodology has already been described for gold interfaces.[15] In the second step, the fusion of the anchored vesicles and the formation of the bilayer were triggered by treatment with a water-soluble fusogen, poly(ethylene)glycol (PEG), which has frequently been used to fuse vesicles in solution.[16]

The Supporting Information gives experimental details for electrode preparation, grafting of the streptavidin layer on aluminum oxide, and preparation of the biotinylated vesicles. The final loading of the streptavidin-cushioned bilayer was achieved by dipping the streptavidin-coated electrodes in the vesicle

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10.1021/ja010361u CCC: $20.00
Published on Web 08/22/2001

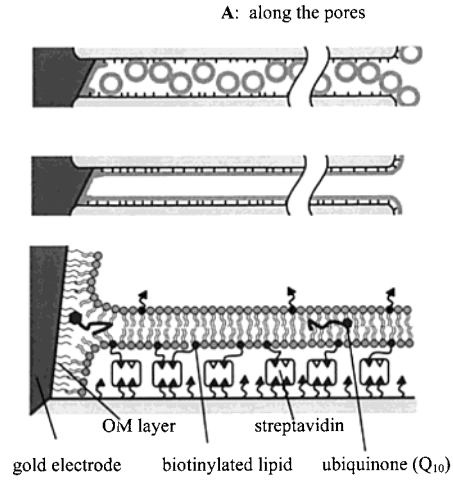

Figure 2. PEG-triggered vesicle fusion inside the microporous electrode. (A) Cross section of a pore before and after PEG treatment. The porous structure, dipped in the vesicle solution, accumulates biotinylated vesicles on the streptavidin sublayer. PEG treatment fuses the lipidic material as a bilayer supported by the inner surface of the pores. (B) At the molecular level, schematic view of the bottom of a pore after fusion. The octadecylthiol treatment (OM layer) of the gold surface is crucial to establish the connection between the bilayer and the electrode interface.

solution for 1 h, then in the PEG solution for 5 min, and rinsing (Figure 3).

Electrochemical Monitoring of Bilayer Formation. The electrochemistry of ubiquinone (Q_{10}, a strictly water-insoluble electron carrier) at the gold−bilayer interface was used to monitor the progress of fusion. At sufficiently low Q_{10} concentration (less than 2 mol % of lipid) it is accepted that Q_{10} lies and moves only in the hydrophobic mid-plane of the bilayers.[17,11] It was therefore expected that the amount of Q_{10} able to reach the gold interface by lateral diffusion would reflect the formation and the continuity of the bilayer. The successive voltammograms presented in Figure 3 were recorded at key steps of the PEG-triggered fusion. Peak integration gives the amount of Q_{10} confined in the supported bilayer. After triggered fusion this was 7.9 ± 1.0 μC. From the geometric surface area of the microporous structure (4.5 cm^2), the Q_{10}-to-lipid ratio (2 mol %), and the mean surface area of a lipid in the bilayer (67 $Å^2$ for egg PC),[1,18] the calculated Q_{10} charge is 8.6 ± 1.0 μC. The agreement with the experimental charge shows that PEG treatment has induced fast fusion between vesicles accumulated in the pores, as depicted in Figure 2. Control experiments demonstrated that the final Q_{10} charge was, as expected, proportional to the Q_{10}-to-lipid ratio in the vesicles (from 1 to 3%). The lateral fluidity of the bilayer was ascertained by measurement of the diffusion coefficient of Q_{10}. By a chronocoulometric method[11b] this was $D = (3 \pm 1) \times 10^{-8}$ cm^2 s^{-1} at 30 °C, in good agreement with previous FRAP measurements on vesicles.[17]

Several other control experiments demonstrated that: (i) spontaneous fusion in the pores was possible but very slow without triggering, whatever the vesicle composition; (ii) the presence of phosphoethanolamine heads was crucial for PEG fusion, as was expected from the literature;[16] (iii) the omission of one component like biotin or streptavidin led to low final Q_{10} charges (1−2 μC).

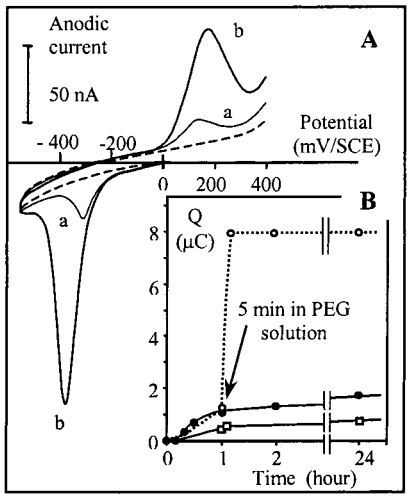

Figure 3. Monitoring of Q_{10}-loading at different steps of supported bilayer formation (loading at 37 °C, electrochemical measurements at 30 °C in 0.1 M phosphate buffer at pH 7). (A) Cyclic voltammograms at low scan rate (2 mV s^{-1}) of Q_{10} solubilized in the mid-plane of the bilayer: (a) the microporous electrode was dipped 1 h in mixed vesicles (64% egg PC; 34% DMPE; 2% Q_{10}; 0.5% biotinylated DMPE); (b) after 5 min in PEG solution (PEG 8000 at 30% w/v). (Dotted line) Background current. (B) Ubiquinone charge from cathodic peaks (2 electrons per Q_{10}): (○) mixed vesicles with DMPE and PEG treatment after 1 h; (●) the same without PEG treatment; (□) mixed vesicles without DMPE but with PEG treatment after 1 h.

Finally, we present in the Supporting Information an independent control experiment performed by fluorescence microscopy on a flat aluminum oxide surface. The efficiency of the PEG-mediated fusion between the immobilized vesicles was demonstrated by the analysis of the fluorescence photobleaching recovery kinetics. Completely immobile before PEG fusion, the lipidic material exhibited a lateral diffusion coefficient in the expected 10^{-8} cm^2 s^{-1} range and a mobile fraction larger than 95% after the triggered connection.

The next step of our strategy is the fusion of proteoliposomes containing transmembrane proteins in the microporous structure, and we are currently working on the incorporation of ubiquinone-dependent enzymes from the mitochondrial electron-transfer chain.

Acknowledgment. The support of this research by the CNRS through PCV Grant 98-091 is gratefully acknowledged. We thank Jacques Pantigny and Nicolas Carreau for help with the confocal microscope experiments and Jacques Moiroux for helpful discussions.

Supporting Information Available: Procedures for preparing microporous electrodes; sequences of scanning confocal microscopy images (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

JA010361U

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