Signaling of *Escherichia Coli* Enterotoxin on Supramolecular Redox Bilayer Vesicles

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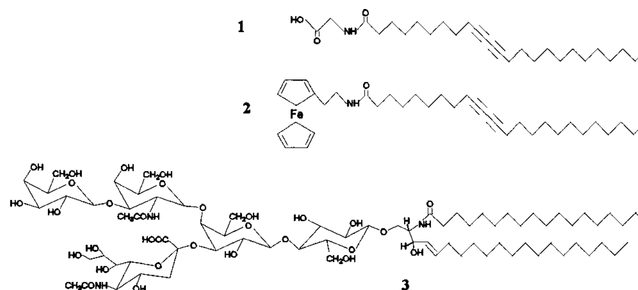
Electron transport in supramolecular assemblies containing redox centers has been a subject of great interest.[1,2] Depending on spatial arrangement of redox moieties in macromolecular structures, transport of electrons may occur via a diffusion mechanism or electron hopping between the neighboring redox sites.[3,4] While research has largely dealt with 3-D redox polymers, some 2-D systems such as self-assembled and Langmuir-Blodgett monolayers have been exploited as well.[5,6] We describe here a new interfacial architecture that combines the high redox concentration in 3-D polymers and controllable structure and functionality of the 2-D monolayer systems. The new interface utilizes structurally defined redox liposomes engineered with biomolecular recognition capability by incorporating cell surface receptor G_{M1} into the bilayer membrane. The design allows for direct inspection of the dependency of electron transport on the state and extent of biomolecular recognition that has taken place on the vesicles and, thus, provides a method for direct measurement of *E. coli* heat-labile enterotoxin binding by electrochemistry.

The redox/receptor liposomes consist of glycine-terminated diacetylene lipid **1**, acetylferrocenic diacetylene lipid **2**, and cell membrane receptor G_{M1}, **3** (Chart 1). G_{M1} is a glycosphingolipid localized to the outer layer of the plasma membrane of vertebrate cells[7] and is known to be the receptor for the 84-kDa *E. coli* enterotoxin.[8] Structurally, G_{M1} contains a saccharide headgroup and a ceramide tail, allowing it to be incorporated into self-assembled monolayers or liposomes for binding assay[9] or biosensors.[10] The enterotoxin binds to G_{M1} through the B_5 subunit of the receptor.[11] Upon binding, the catalytic A subunit inserts part of its structure into the membrane as an anchor to be endocytosed.[12]

Chart 1. Structure of the Lipid Molecules Used for Forming Redox/Receptor Liposomes.



The liposomes formed by sonication (molar ratio of compounds **1**:**2**:**3** is 4:1:0.25) have an average dimension of 150 nm as determined by transmission electron microscopy (TEM). The hydrophilic sol gel film was obtained by hydrolysis of tetraethyl orthosilicate (TEOS) and provides a microporous support matrix on which liposomes are firmly attached. The thin coating, cast on the glassy carbon electrode surface, has an approximate thickness of 500 nm as determined by scanning electron microscopy (SEM).

The binding of enterotoxin to the liposomes and the effect of binding on electron transport were assessed by cyclic voltammetry (CV). The redox/receptor liposomes were first allowed to adsorb on the gel coating of the electrode, and excess liposomes were rinsed off with water before CV measurements were conducted in a buffer solution containing no ferrocene or ferrocene liposomes. With addition of *E. coli* enterotoxin, a sharp drop in the anodic current of ferrocene was observed (Figure 1, insert). The response is time-dependent and the current decays in an exponential manner (Figure 1). After 5 min, the decay of current gradually levels out, indicating that the binding/dissociation has reached equilibrium. With 80 ppm (8.9×10^{-7} M) of toxin added, the change in current can be as large as 24%, corresponding to $\sim$7 μA/cm^2 in current density. The dynamic range for the response was found to extend from 5 to 100 ppm. A detection limit of 3 $\times$ 10^{-8} M *E. coli* enterotoxin was determined using a 3σ signal cutoff.

The control experiment was conducted using a 10-fold molar excess of bovine serum albumin (BSA) which does not provoke any significant current drop within 5 min (Figure 1). Prolonged exposure causes a minor decrease in current (5%), possibly a result of nonspecific binding on the vesicle surface. Similar measurements were pursued using direct adsorption of redox/receptor liposomes on the bare glassy carbon electrode. However, the response became quickly attenuated, and erratic behavior was observed, suggesting that the toxins interacted with both liposome receptors and the electrode and eventually fouled the electrode surface. Use of sol gel thin layer reduces nonspecific binding of protein to the electrode that can cause drastic, electrokinetic retardation.

Interestingly, the 500-nm-thick sol gel thin film is an insulating layer. No noticeable improvement of gel conductivity was observed when various electrolytes were used to replace distilled water for hydrolysis of TEOS. Electrochemical characterization of the thin gel film using $Fe(CN)_6 4^-$ indicates that a great portion (>95%) of current was suppressed as compared with that on the bare electrode. However, a small portion of redox current for $Fe(CN)_6^{4-}$ was readily obtained. As its peak separation is comparable to that on a bare electrode, this suggests the presence

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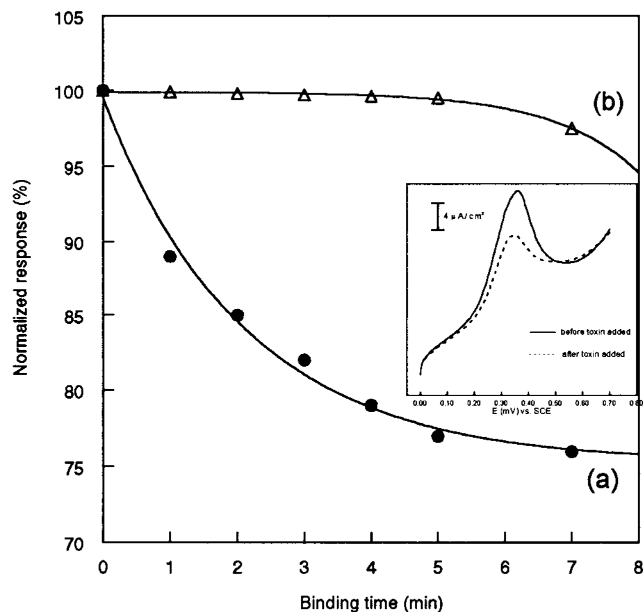

Figure 1. Effect of toxin binding on amperometric response as a function of time. (a) *E. coli* heat labile enterotoxin (80 ppm), (b) BSA (1000 ppm). Insert is the anodic current response for redox liposomes (—) without and (···) with 80 ppm enterotoxin.

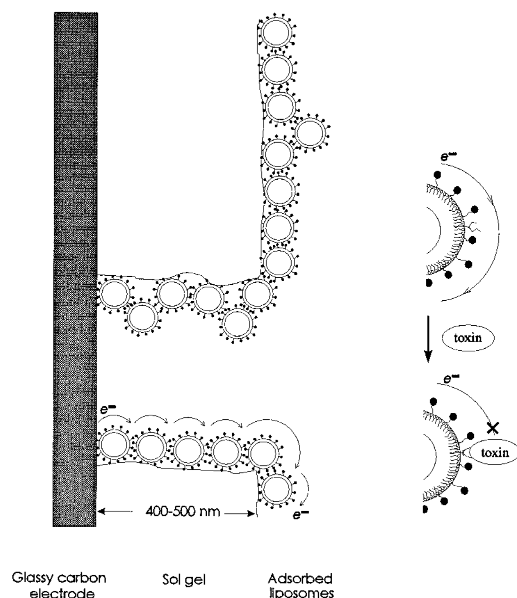

Figure 2. Schematic illustration of gel-crack-assisted electron transport on the sol gel film coated electrode.

of free-electrode surface available for the redox reaction, presumably from gel cracks. SEM experiments revealed the presence of microscale cracks on the gel film, occupying less than 1% of the total area. Given the fact that the current for redox liposomes observed on the gel surface decreases only by 10% as compared with that on the bare electrode, it is obvious that the high current of redox liposomes on the gel-coated electrode is not totally originating from the gel cracks.

A gel-crack-assisted electron transport mechanism is thus suggested to explain the large ferrocene current and its response to enterotoxin binding. The oxidation of ferrocene first occurs between the glassy carbon electrode and the liposomes trapped in the gel cracks, as shown schematically in Figure 2. As the concentration of ferrocene is depleted at the interface, the process requires, for charge neutrality, ionic migration from the bulk into the area. If only the ferrocene lipids in the vicinity of the electrode transfer electrons, the voltammogram would have the feature of a surface-confined reaction. In our experiments, however, the response is a typical diffusion-controlled process (Figure 1, insert). This suggests that ferrocene species near and far from the gel-crack sites participate in the electron transfer. When the enterotoxin is added, its binding to the receptor sites on the liposomes blocks the electron transport path, resulting in a decrease in current magnitude.

The charge transport can be conveniently characterized by the apparent diffusion coefficient D_{app}, which is given by the Dahms-Ruff equation[13]

$$D_{app} = D_{phys} + k_{ex} C_E \delta^2/6 \quad (1)$$

where D_{phys} denotes the diffusion coefficient for physical displacement of the redox species, k_{ex} is the electron self-exchange rate constant, C_E is the concentration of the redox species, and δ is the distance between redox centers at the time of electron transfer. When electron self-exchange makes a significant contribution to the diffusion process, D_{app} should exhibit a linear dependence on the concentration of electroactive sites "frozen" inside the films.[3] We measured the D_{app} at different redox concentrations using the classic chronocoulometric method.[5] The diffusion coefficients were found in the range of 4.73×10^{-8} to 2.30×10^{-8} cm/s^2 for the surface coverage from 5.7×10^{-12} mol/cm^2 to 2.25×10^{-11} mol/cm^2. No linear dependence of D_{app} on redox concentration was found, and the diffusion coefficients in fact show a descending trend with respect to the redox concentration. The rate-determining step of the overall charge-transport process is therefore not the electron-exchange process (hopping). The lack of concentration dependence suggests that the electroreactivity has a mean-field behavior, i.e., charge propagation occurs via physical displacement of the redox element.[3] Considering the unique surface morphology employed in our study, it points to a lateral diffusion mechanism on the liposome's bilayer membrane, similar to the microporous film electrodes studied by Majda and co-workers.[5] The measured diffusion coefficients fall in the same magnitude as reported by Majda[5] and agree well with those measured by other groups using fluorescence recovery after photobleaching (FRAP)[14] and NMR methods.[15] The high D_{app} in liposomes is possibly due to long-range lateral fluidity of the bilayer membranes.[16] However, one cannot totally rule out the contribution from electron hopping, particularly in the boundary between liposomes adjacent to each other. Since lateral diffusion of lipids across the liposome boundary appears to be unlikely, electron transport through percolation should prevail in these regions.

In conclusion, a new method has been developed that couples lateral electron transport with molecular recognition for signaling the binding of pathogenic toxins. This approach takes the advantage of an "open" biosensing platform that allows free access of counterions and thus improves the "diffusion" process. The significance and uniqueness of the work, besides its relevance to charge transport on a newly defined supramolecular structure, extends to the area of designing effective electrochemical sensors for nonelectroactive biological molecules.

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