INTERACTION OF POLYAMIDOAMINE (PAMAM) DENDRIMERS WITH GLASSY CARBON SUPPORTED BILAYER LIPID MEMBRANES

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ABSTRACT

Electrochemical techniques were used to monitor the formation of a 1,2-dioleoyl-sn-glycero-3-phosphocholine supported bilayer lipid membrane (sBLM) on a glassy carbon electrode and to investigate interactions of polyamidoamine (PAMAM) dendrimers with the sBLM. Dendrimers of generation 2 through generation 4 (G2-G4) did not cause significant damage to the sBLM, whereas G5-G7 dendrimers created large defects in sBLM. In cyclic voltammetry studies, the peak current obtained after dendrimer addition was used to estimate the defect area. Impedance spectroscopy showed an increase in capacitance values after addition of G5-G7 dendrimers, consistent with a decrease in sBLM surface coverage. Nanoparticle mass, diameter, and surface charge density all increase significantly with generation number, suggesting that these variables may influence nanoparticles’ ability to rupture sBLM. The method presented here provides a convenient and sensitive way to detect interactions between nanoparticles and lipid bilayers, and may be useful in evaluating nanoparticles’ safety profile or suitability as drug or gene delivery vehicles.

Keywords: Glassy carbon electrode, supported bilayer lipid membrane, PAMAM, dendrimer, nanoparticles, impedance spectroscopy, cyclic voltammetry, drug delivery.

1 INTRODUCTION

Polyamidoamine (PAMAM) dendrimers are hyperbranched polymeric nanoparticles that are formed in concentric layers. As each subsequent layer (generation) is added, the molecular size, molecular weight, and number of functional groups increases by a known amount. This property makes dendrimers well suited for use as monodisperse nanoparticles having a desired size, shape, and surface charge [1]. Recently, PAMAM dendrimers have also been investigated as non-viral vectors for the gene delivery [2, 3] that offer the potential for low immunogenicity [4, 5] and may protect the gene from DNase activity. The mechanism of gene delivery involves internalization of the charged polymer by host cells, through a mechanism that may involve disruption of the host cell membrane [6]. Dendrimers’ ability to cross cell membranes also makes them an alternative vehicle for drug delivery [7, 8]. Atomic force microscopy studies have shown that PAMAM dendrimers form 15-40 nm holes in 1,2-di-myristoyl-sn-glycero-3-phosphocholine (DMPC) supported bilayer lipid membrane (sBLM) on mica [9-12]. These studies concluded that the high surface charge density of amino groups found in higher generation dendrimers was responsible for disruption of bilayer membranes.

sBLMs are more robust and stable than unsupported planar bilayer membranes [13] and can be deposited on a variety of hydrophilic surfaces, such as oxidized glassy carbon, silica, and mica [13]. Even though a sBLM is only about 5 nm thick, it is an excellent electrical insulator. Thus, disruption of the sBLM by nanoparticles could, in principle, be measured electrochemically. This paper describes a rapid, sensitive, and convenient electrochemical method to detect disruption of sBLM by nanoparticles. The approach involves fabricating a sBLM on a glassy carbon electrode (GCE), and then using electrochemical methods to monitor interactions between nanoparticles and the sBLM. Disruption of a 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) sBLM formed on a GCE was investigated using cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS).

2 EXPERIMENTAL

2.1 Methods

Each GCE (Bioanalytical Systems, West Lafayette, IN) was sequentially polished with 1000, 300 and 50 nm alumina slurry, followed by washing with de-ionized (DI) water and methanol. The electrode was ultrasonicated for 2 min in DI water to remove physically adsorbed alumina, given final rinses with methanol and DI water, and then dried under a nitrogen stream. The GCE was placed in 100 mM NaCl solution and oxidized at a potential of 1500mV for 3 min [14]. It was then washed with DI water and dried under a nitrogen stream. Five μL of dioleoyl-sn-glycero-phosphocholine (DOPC) lipid solution (5 mg/mL in


chloroform) was applied to the electrode surface, and the electrode was immediately immersed in an electrolyte solution (100 mM sodium phosphate buffer, pH 7.4, containing 1 mM potassium ferrocyanide and 1 mM potassium ferricyanide). After 20 min, PAMAM nanoparticles (G2 - G7, provided by Dr. Steve Kaganove, Michigan Molecular Institute, Midland, MI) were added to obtain a final concentration of 20 µM (based on the surface amino groups). CV was then used to monitor changes in the sBLM’s electrochemical properties until a steady-state situation was observed.

2.2 Instrumentation

Electrochemical measurements were performed using a CHI660B electrochemical workstation (CH Instruments Inc., Austin, TX). EIS was performed in 100 mM sodium phosphate buffer, pH 7.4, containing 1 mM potassium ferrocyanide and 1 mM potassium ferricyanide. The dc potential was $E^0 = 230$ mV vs a Ag/AgCl reference electrode, and a 5 mV sinusoidal potential was applied across the frequency range of 0.1 to 10,000 Hz. A modified Randle’s equivalent circuit model [15] was fit to the impedance data using Z-view software (Scribner Associates, Southern Pines, NC). CV was performed in same electrolyte solution as used for EIS studies. The potential was cycled in a range of 500 mV to -200 mV at a scan rate of 50 mV/s.

3 RESULTS AND DISCUSSION

sBLM have been deposited on hydrophilic supports such as mica and silica and on the conductive materials such as platinum. sBLM formation on metal surfaces requires cutting the metal while it is immersed in the lipid solution, to ensure that the lipids contact a fresh metal surface [13, 16]. GCE provide a convenient and cost-effective alternative to metal electrodes such as platinum, offering good conductivity, a wide potential window, and the possibility of chemical functionalization [17]. To increase the hydrophilicity of the surface, we oxidized the GCE at 1.5 V for 3 min in a 100 mM NaCl solution. The resulting formation of carboxylate ions imparts a net negative charge to the surface of electrode, which associates with the positively charged choline moiety of the DOPC lipids.

Upon cooling, lipid bilayers undergo a phase transition from a highly fluid, liquid-crystalline phase ($L_o$) to a more viscous, gel phase ($L_p$). Mecke et al [18] reported that polycationic polymers selectively interact with the lipid bilayers in the liquid-crystalline phase (i.e., above the transition temperature). To ensure that the sBLM was fully fluid during the room-temperature studies, the unsaturated phospholipid DOPC (phase transition temperature = -4°C) was chosen. In contrast, saturated phospholipids have a transition temperature of about 28°C.

sBLM formation was monitored using CV and EIS. Figure 1A shows the CV curves for bare GCE and DOPC sBLM. The bare GCE curve exhibited the classic duck shaped profile, with well-defined peaks for ferrocyanide oxidation and ferricyanide reduction. The cathodic and anodic peak currents were 12.3 and 12.5 µA, respectively. The peak splitting of 70 mV was indicative of a reversible redox reaction. The sBLM curve was essentially flat, with no redox peaks, indicating that the sBLM forms a effective dielectric barrier that prevents redox species from reaching the electrode surface.

Figure 1B shows EIS curves of the bare GCE and the DOPC sBLM. The bare GCE showed a very small charge transfer resistance ($6 \Omega$ cm$^2$), and most of the impedance spectrum was representative of Warburg impedance, indicating mass transfer resistance. The bare GCE capacitance ($C_{GCE}$) was 35 µF/cm$^2$. The modified Randle’s equivalent circuit shown in Figure 1B (inset) was fit to the sBLM impedance data, giving a charge transfer resistance ($R_t$) of 85 KΩ cm$^2$. $R_t$ values greater than 1 MΩ cm$^2$ have been reported for tethered lipid bilayer systems, suggesting that the sBLM formed on the GCE has pin-hole defects. Nevertheless, the sBLM still effectively shields the electrode from the ferricyanide.

The Randle’s equivalent circuit assumed for the sBLM involves a parallel arrangement of capacitors: one corresponding to the membrane ($C_m$), and the other corresponding to membrane defects ($C_{defect}$). The overall capacitance is the sum of both capacitances. At the defects, where the electrolyte can contact the electrode surface, the capacitance per area of defect should be similar to $C_{GCE}$. Before dendrimer addition (Figure 1B, squares), the overall capacitance was determined to be 0.75 µF/cm$^2$, a value typical of those reported for sBLM-coated electrodes [19]. Under these conditions, the fraction of the total area occupied by defects, and thus the $C_{defect}$ term, is negligibly small.

After sBLM formation, PAMAM nanoparticles were added to the electrolyte solution, and their interaction with the sBLM was monitored electrochemically. The system typically reached steady state 15 min after the addition of dendrimer solution, but the electrochemical parameters were monitored for 30 min to ensure a constant value. The dendrimer concentration was calculated based on surface amino groups to ensure that the same number of charged groups was used in all experiments.
Figure 1: (A) Cyclic voltammogram for bare GCE (curve 1) and sBLM on GCE (curve 2) and, (B) impedance spectrum of sBLM (squares) compared with bare GCE (triangles). The assumed equivalent circuit is shown in the inset.

The CV curves obtained after addition of G2 and G3 dendrimers are shown in Figure 2A. No redox peaks were obtained for G2, and only a small peak current of 0.12 μA was obtained for G3. These results indicate that lower generation dendrimers cause minimal defects in the sBLM, and are consistent with the AFM study by Mecke et al [9], which found that G2 and G3 primarily adsorbed on the sBLM, without creating obvious holes. The CV curves obtained after addition of G4-G7 dendrimers are shown in Figure 2B. The peak current increased roughly linearly with generation number for G5 - G7, suggesting that larger dendrimers more effectively create defects in the bilayer, through which the redox species can reach the electrode surface. The corresponding EIS curves (data not shown) indicated that the overall capacitance increased significantly with generation number, presumably due to a larger fraction of electrode area being exposed to the electrolyte solution, and hence a significant $C_{\text{defect}}$ value.

The peak current values for G2-G7 dendrimers are summarized in Table 1. These values were used with the following equation to calculate the approximate sBLM defect area value for each generation [15]:

$$i_p = (2.69 \times 10^5) n^{3/2} A C_{\text{bulk}} (Dv)^{1/2}$$

where $i_p$ is the peak current (μA), $n$ is the number of electrons transferred (1), $A$ is the area of electrode (0.0706 cm$^2$), $C_{\text{bulk}}$ is the bulk concentration of redox species (1 $\times$ 10$^{-6}$ mol/cm$^3$), $D$ is the diffusion coefficient (cm$^2$/s) and $v$ is the scan rate (0.05 V/s). The diffusion coefficient of the redox species ($8.4 \times 10^{-6}$ cm$^2$/s) was calculated from the slope of the plot of peak current vs scan rate for a bare GCE surface.

Figure 2: Cyclic voltammograms showing the effect of (A) lower generation PAMAM dendrimers: G2 (curve 2) and G3 (curve 3) and, (B) higher generation PAMAM dendrimers: G4 (curve 4), G5 (curve 5), G6 (curve 6) and G7 (curve 7). Curve 1 represents the sBLM before dendrimer addition.
<table>
<thead>
<tr>
<th>Generation</th>
<th>Mol. weight</th>
<th>Surface amino groups/molecule</th>
<th>Diameter (nm)</th>
<th>Peak current (μA)</th>
<th>Area of electrode exposed (mm²)</th>
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<td>512</td>
<td>7.6</td>
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<td>Bare GCE</td>
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<td>N/A</td>
<td>N/A</td>
<td>12.3</td>
<td>7.0639</td>
</tr>
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</table>

Table 1: Characteristics of PAMAM dendrimers, along with the parameters showing their effect on SBLM

Based on the data provided in Table 1, G7 dendrimers have a 36 times greater mass, 2.6 times greater diameter, and a 4.6 times greater surface charge density than G2 dendrimers. The relative contributions of these properties on the efficacy of SBLM disruption by PAMAM dendrimers is not yet understood and requires further study.

4 CONCLUSION

A biomimetic interface consisting of a sBLM deposited on a GCE was used to study the interaction of G2-G7 PAMAM dendrimers with lipid bilayers. CV and EIS studies showed that dendrimer addition dramatically decreased impedance and increased ferricyanide peak currents, presumably by creating defects in the sBLM. Higher generation dendrimers had a much stronger effect on the sBLM than did lower generation dendrimers. CV data were used to estimate the defect area as a function of generation. EIS results showed an increase in overall capacitance values following dendrimer addition, consistent with a decrease in sBLM surface coverage. The molecular mass, diameter, and surface charge density of PAMAM dendrimers all increase with generation number, although the relative roles of these nanoparticle properties in efficacy of lipid bilayer disruption is not understood. The method described in this paper provides a rapid and convenient way to screen nanoparticles for strong interactions with lipid bilayers and may have utility in assessing nanoparticles' toxicity or suitability for intracellular genes or drug delivery.

Acknowledgement

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REFERENCES