Polydiacetylene Liposomes Functionalized with Sialic Acid Bind and Colorimetrically Detect Influenza Virus

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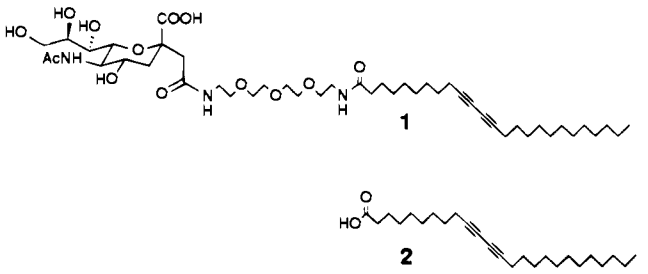
Cell membranes are remarkable structures from a materials science point of view.[1] These highly organized, self-assembled structures[2] provide indispensable functions for cells such as molecular recognition, pumping, gating, energy conversion, and signal transduction.[3] The design of "smart" materials based on membrane structures with specific functional properties is an emerging field of study.[4] We have prepared synthetic, polymerizable liposomes that resemble the organization and functionalization of cell membranes and have employed them as simple colorimetric sensors. The liposomes were designed to specifically bind to influenza virus particles and, in addition, report the binding event by undergoing a visible color change. In effect, these molecular assemblies mimic cell surface molecular recognition as well as signal transduction.

In order to impart both molecular recognition and detection functions to the liposomes, we combined a known ligand–receptor interaction with the unique optical properties of polydiacetylenes. The conjugated backbone of alternating double and triple bonds gives rise to intense absorptions in the visible spectrum. In single crystals or Langmuir–Blodgett films,[5] these materials are known to undergo blue to red color transitions due to a variety of environmental perturbations including heat,[6] mechanical stress,[7] pH,[8] and solvent.[9] In this report, we demonstrate that specific binding of influenza virus to functionalized polydiacetylene liposomes produces an analogous color transition. In earlier work, we showed that similar effects can be obtained with functionalized polydiacetylene Langmuir–Blodgett films.[10]

Influenza virus particles are enveloped by a lipid bilayer to which the hemagglutinin (HA) lectin is anchored. HA binds to terminal α-glycosides of sialic acid on cell-surface glycoproteins and glycolipids,[11,12] initiating cell infection by the virus.[13] Liposomes expressing sialic acid residues have been extensively used as model systems to study the interaction between influenza virus and cell surfaces.[14,15] The polymerized liposomes described here, however, are composed of molecules that allow direct visualization of this specific interaction.

The bifunctional molecule **1** incorporates both the sialic acid ligand for viral binding and the diacetylenic functionality in the hydrocarbon chain for polymerization.[16] The carbon–



glycoside in this compound was designed to prevent hydrolysis by viral neuraminidase.[17] This compound was mixed with 10,12-pentacosadiynoic acid (**2**) and hydrated to form liposomes.[18] Previous studies[16] indicated that optimum viral binding occurs for mixtures of 1–10% compound **1** in the liposome. Therefore, 5 and 10% sialic acid lipid were used in this colorimetric detection study.

Liposomes were prepared using a probe sonication method[19] and subsequently polymerized by irradiation at 254 nm using an ultraviolet pencil lamp. Irradiation of a liposome solution (1 mM in deionized water) for about 5–10 min results in the formation of deeply blue colored liposomes (Figure 1A, solid line). If the polymerization time is longer (between 10 and 30 min), a purple color is observed (Figure 1B, solid line). When influenza virus is added to the liposomes in PBS buffer, the solution immediately changes to a pink or orange color, depending on whether the initial preparation was blue or purple, respectively (Figure 1A and B, dashed curves). These color changes are readily visible with the naked eye and can be quantified by visible absorption spectroscopy. The colorimetric response (CR) is quantified by measuring the percent change in the absorption at 626 nm (which imparts the blue color to the material) relative to the total absorption maxima.[20] As shown in Figure 1, incubation of the blue liposomes (8 min UV) with 60 hemagglutinating units (HAUs)[21] of virus leads to a CR of 47%; incubation of the purple liposomes (24 min

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(18) Although most natural lipids that form liposomes consist of two alkyl chains, synthetic liposome-forming lipids with only one alkyl chain also exist. See, for example: Hupfer, B.; Ringsdorf, H.; Schupp, H. *Chem. Phys. Lipids* **1983**, *33*, 355–374. Bader, H.; Ringsdorf, H.; Skura, J. *Angew. Chem., Int. Ed. Engl.* **1981**, *20*, 91–92.
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(20) In order to quantify the response of a liposome solution to a given amount of virus, the visible absorption spectrum of the liposome solution without the virus was analyzed as

$$B_0 = I_{626}/(I_{536} + I_{626}) \quad (1)$$

where B_0 is defined as the intensity of absorption at 626 nm divided by the sum of the absorption intensities at 536 and 626 nm. The liposome solution which was exposed to influenza virus was analyzed in the same way as

$$B_v = I_{626}/(I_{536} + I_{626}) \quad (2)$$

where B_v represents the new ratio of absorbance intensities after incubation with the virus. The colorimetric response (CR) of a liposome solution is defined as the percentage change in B upon exposure to virus

$$\mathrm{CR} = [(B_0 - B_v)/B_0] \times 100\% \quad (3)$$

To be consistent with our earlier work, we have arbitrarily chosen the absorption maxima at 626 and 536 nm to calculate the percentage blue absorption for the liposomes solutions. Use of the second absorption maximum at 480 nm for the calculations does not change the relative trend of the results shown.

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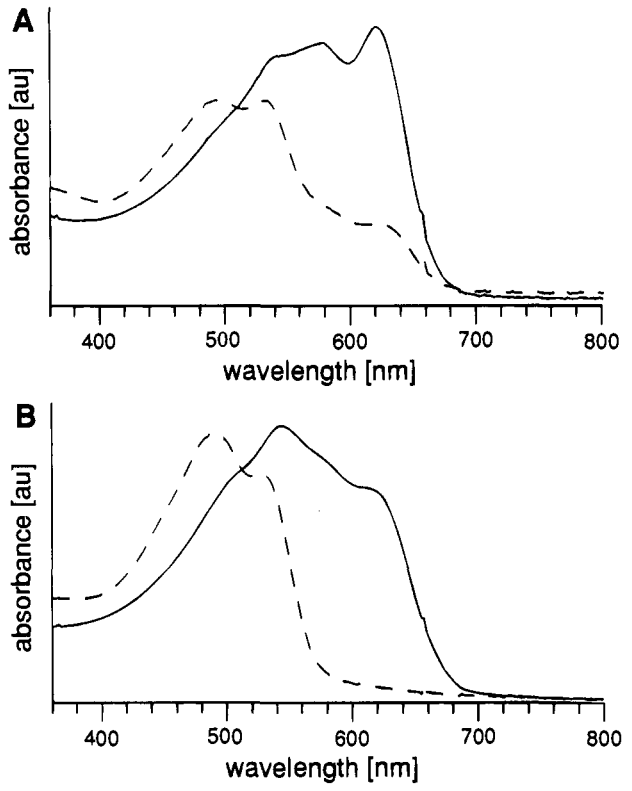

Figure 1. Colorimetric detection of influenza virus by polymerized diacetylene liposomes (5% sialic acid lipid **1**). Visible absorption spectra of (A) blue liposome solution (8 min UV) and (B) purple liposome solution (24 min UV) without virus (solid line) and after incubation with 60 HAUs of influenza virus (dashed line). The concentration of the liposome solutions in PBS buffer was 0.13 mM, and the incubation time with the virus was 1 h.

UV) with the same amount of virus gives a CR of 87%. We speculate that the enhanced sensitivity of the purple liposomes may be due to an increased polymer content, as suggested by their higher optical density (data not shown).

Previous studies have suggested that color transitions in polydiacetylenes arise from changes in the effective conjugation length of the polydiacetylene backbone[22,23] and that the electronic structure of the polymer backbone is strongly coupled to side chain conformation.[24,25] We can only speculate at this point that specific virus–liposome interactions may serve to alter side chain conformation, reducing the effective conjugation length of the ene-yne backbone. Indeed, theoretical calculations suggest that very slight rotations around the C–C bond of the polymer backbone decrease the π electron delocalization.[26,27]

No color change could be detected if pure PBS buffer or a solution of BSA in PBS buffer (1 mg/mL) was added to the liposome solution (CR $\leq$ 5% within 2 h). In order to directly address the effects of nonspecific adsorption, liposomes were prepared without sialic acid lipid **1**. Similarly, these liposomes did not change color after exposure to virus. Additionally, the specific nature of the interaction between the influenza virus and the sialic acid liposomes was confirmed by a competitive inhibition experiment. Incubation of a liposome solution (10% sialic acid lipid **1**) with 54 HAUs of influenza virus yields a

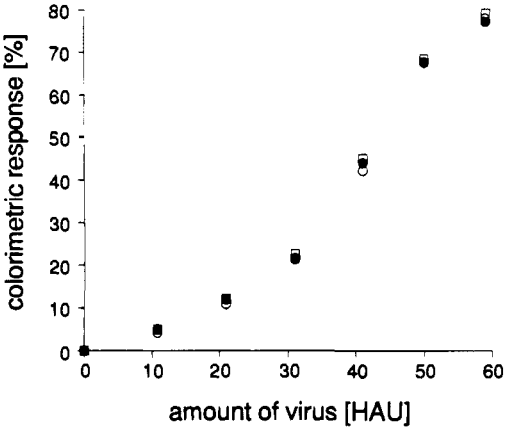

Figure 2. Plot of the colorimetric response of a purple liposome solution (5% sialic acid lipid **1**, 24 min UV) versus successive additions of influenza virus. The liposomes were incubated for 30 min following each addition of virus, and the visible absorption spectrum was recorded. The CR for each virus concentration was obtained in three independent experiments.

CR of 31% for blue and 70% for purple liposomes. Performing the same experiment with a slight excess of α-*O*-methyl-neuraminic acid, a known inhibitor for influenza virus hemagglutination, results in no color change.

Kinetic experiments show that the color change induced by the addition of an aliquot of virus reaches a plateau after 30 min, although the change becomes apparent within 5 min. For a given polymerization time, the CR depends on the amount of added virus, as shown in Figure 2. Given that the color change of the liposomes in buffer without virus is less than 4% within 2 h, a CR of 5% or more in a few minutes is considered significant. Therefore, the amount of virus required to produce a CR just above this value defines the detection limit of the method. The titration curve in Figure 2 shows that as little as 11 HAUs can be detected. This corresponds to approximately 11×10^7 virus particles by electron microscopy count.[28]

In conclusion, we have demonstrated that polymerized liposomes are biomolecular materials that provide a molecular recognition function (sialic acid) and a detection element (polydiacetylene backbone), all within a single supramolecular assembly. The binding event is transduced to a visible color change, readily seen with the naked eye and quantified by absorption spectroscopy. Specificity of the color change was demonstrated by competitive inhibition studies. In addition, nonspecific adsorption, if it occurs, does not appear to affect the color of the liposome solutions. Current studies focus on understanding the structural changes responsible for the color change, as well as the structural differences that arise from different polymerization times and their described effects on sensitivity. These will be the subject of a future, detailed report.

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Supplementary Material Available: Experimental details of the formation of the liposomes, the colorimetric detection of the virus, and the virus titration (3 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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(21) A hemagglutinating unit is a measure of the highest dilution of the virus solution that still completely agglutinates a 1% solution of red blood cells. See also supplementary material.

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