Colorimetric Detection of *Escherichia coli* by Polydiacetylene Vesicles Functionalized with Glycolipid

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Lipids with the structure of diacetylene have many special properties. Some monomers containing diacetylenic moiety can be polymerized under ultraviolet light irradiation into a blue polydiacetylene (PDA) polymer[1−3] when the monomers are appropriately assembled. In single crystals or Langmuir−Blodgett films polydiacetylenes are known to change color from blue to red with environmental perturbations including temperature,[4] mechanical stress,[5] pH,[6] and solvent.[7]

It has been reported by D. H. Charych et al.[8] in 1993 that by chemically connecting a special receptor to the diacetylene molecule and inserting the obtained probe into a diacetylene matrix monolayer, the color of such kind of film will change from blue to red once it adsorbed biomolecules, such as influenza virus. Since then other work[9−12] has been published in this area. A major limitation to this approach is the time-consuming procedures involved in the synthesis of ligands attached to the diacetylene lipids.[13] If these obstacles can be overcome by attaching the ligand to other more conventional lipids this could widely increase the potential applications.

In this work we report a modified approach to improve this situation. The originality of this work lies in inserting the receptor molecules into the PDA matrix by using a glycolipid instead of a diacetylene-based lipid of sialic acid such as dioctadecyl glyceryl ether-β-glucosides (DGG), which is inexpensive and easily obtained (Figure 1).

Two kinds of diacetylenic acid, tricosa-2,4-diynoic acid (TCDA) and 10,12-pentacosadiynoic acid (PCDA) have been used as the matrix lipids for comparison, and DGG served as receptors in them. *E. coli* [ATCC25922] was used as a model bacteria.

Vesicles of a mixture of polymerizable matrix lipid and receptor were formed by a modified probe sonication method.[14] The molar ratio of receptor DGG to PCDA or TCDA was 0.02. A polymerized vesicle functionalized with DGG inserted into the matrix was then obtained by irradiation of the solution with a UV lamp ($\lambda = 254$ nm). The polymerized vesicles (TCDA/DGG) appeared deeply blue and showed an absorption maximum at 650 nm (shown in Figure 2 curve a). After *E. coli*, dispersed in an aqueous solution of sodium chloride (0.85%), was mixed with the polymer vesicles, the system turned from deeply blue to red within several seconds. The absorption maximum shifted from 650 to 540 nm, as shown in Figure 2, curve b. No color change was observed when 0.85% aqueous solution of sodium chloride alone was added to the same system. To directly address the effect of nonspecific adsorption, vesicles were prepared without DGG. Similarly, these vesicles did not change color within 10 min after exposure to *E. coli*. In contrast, although the deeply blue color could also be observed in the PCDA/DGG vesicle after it was irradiated by ultraviolet light at 254 nm for 10 min, there was no color change when the *E. coli* solution was added into that system, showing the obvious difference between these two kinds of PDA.

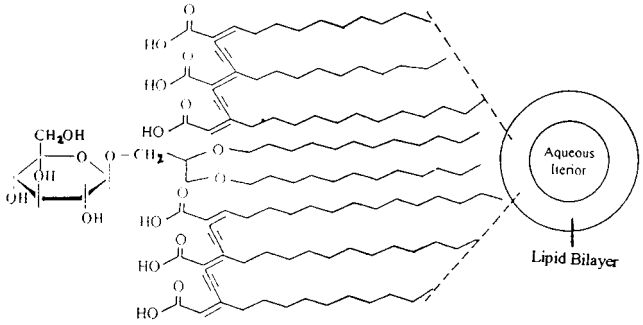
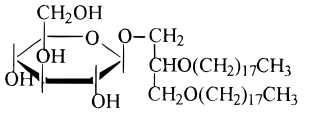


Figure 1. Schematic diagram of a polymerized vesicle composed of TCDA/DGG.

CH_3-$(CH_2)_m$-C≡C-C≡C-$(CH_2)_n$-COOH

n = 0 m = 17 Tricosa-2,4-diynoic acid (TCDA)

n = 8 m = 11 10,12-Pentacosadiynoic acid (PCDA)



Dioctadecyl glycerylether-β-glucosides (DGG)

It is important that when the vesicles were treated with small amino acid molecules, such as aspartic and glutamic acids and alanine, these did not cause the TCDA/DGG vesicle to change its color. Such results showed that the TCDA/DGG system could not "recognize" molecules with relatively low molecular weight like amino acids but was sensitive to larger biomolecules such as bacteria or virus, having great significance in its application in disease diagnose.

To understand the reason for the difference between these two kinds of PDA as well as the interaction between matrix and receptor, surface pressure (π)-molecular area (A) isotherms study of TCDA/DGG and PCDA/DGG were conducted. The limiting area per molecule of TCDA on pure water (pH 5.8) was 20.9 Å^2/molecule with collapse pressure at 55 mN/m, and that of PCDA was 27.9 Å^2/molecule with collapse pressure at 15 mN/m, indicating that TCDA could form a more closely packed monolayer than PCTA did. This fact could be reasonably

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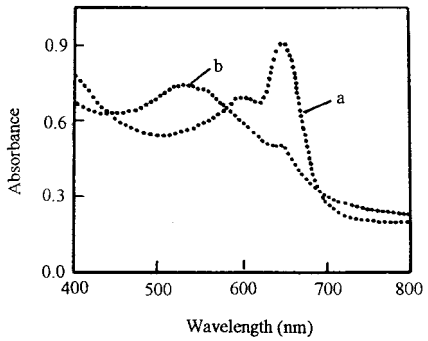

Figure 2. Colorimetric detection of *E. coli* by polymerized diacetylene vesicles (2% DGG). Visible absorption spectra of vesicle solution without *E. coli* (a) and with *E. coli* (b).

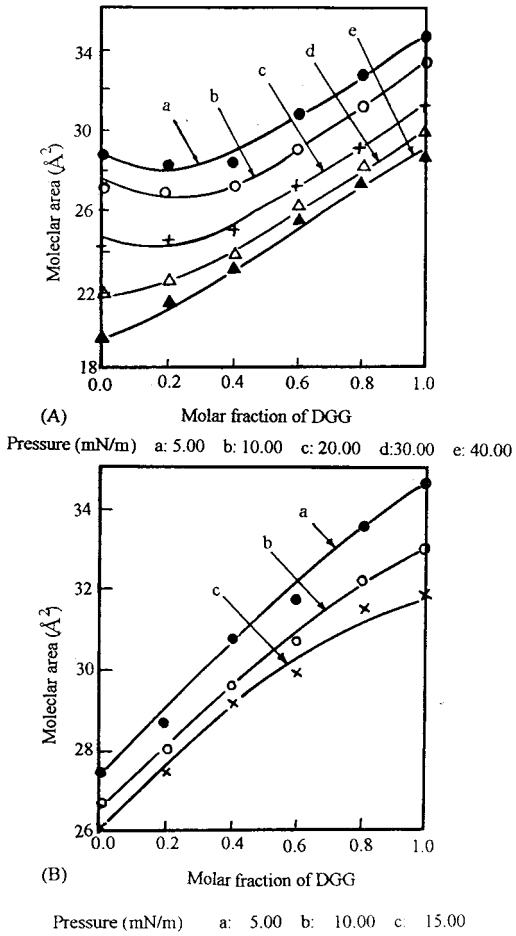

Figure 3. Molecular area of the mixed TCDA/DGG (A) and PCDA/DGG (B) monolayers as a function of the molar fraction of DGG at different surface pressures on pure water, pH 5.8.

explained by the polar group position in these two kinds of PDA. The position of diacetylene in the molecules clearly plays a crucial role in this effect. The molecular areas of TCDA/DGG and PCDA/DGG versus the molar fraction of DGG in the mixed monolayers spread on pure water (pH 5.8) are shown in Figure 3. Negative deviations of the molecular areas from the additivity rule[15] were observed for all molar fractions at all surface pressures studied. This indicated that there exists strong interaction and good miscibility between TCDA/DGG (shown in Figure 3A). On the contrary, for the PCDA/DGG system, the positive deviations of the molecular areas from the additivity rule were observed for all molar fractions at all surface pressures studied (shown in Figure 3B). This demonstrated that there exists a repulsion force between PCDA and DGG and the miscibility of PCDA/DGG is poor.

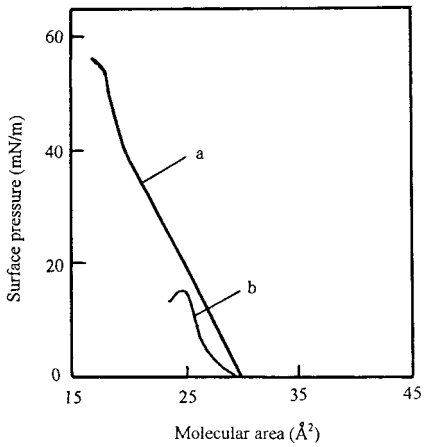

Figure 4. Surface pressure−area isotherms of pure TCDA (a) and PCDA (b) on pure water, pH 5.8.

In summary, we have developed new functionalized diacetylene vesicles where the receptor glycolipids were inserted by physical force rather than directly by the chemical method available in the literature. We have found a useful system, TCDA/DGG, which could be used to detect *E. coli* by a dioctadecyl glyceryl ether-β-glucosides. These receptors rather than sialic acid-diacetylene-lipid derivatives, which are difficult to synthesize and are not generally available, are easily obtained. Also, no color change was observed in the polymer vesicles composed of PCDA/DGG when *E. coli* was added, showing the position of diacetylene in lipids plays a dominant role. The miscibility of TCDA/DGG was also better than that of PCDA/DGG, which may also be related to our observations. This discovery might be of significance in understanding the biomolecular recognition process and designing disease diagnostic biosensors.

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Supporting Information Available: Experimental details (2 pages, print/PDF). See any current masthead page for ordering information and Web access instructions.

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