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Effect of phospholipid insertion on arrayed polydiacetylene biosensors

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ABSTRACT

Micro-arrayed polydiacetylene (PDA) vesicles mixed with phospholipids on glass slides were prepared for label-free detection of *Escherichia coli*. When *E. coli* bound to its antibodies chemically attached to polydiacetylene, the fluorescence of the vesicles was dramatically increased. The insertion of dimyristoyl phosphatidylcholine (DMPC) in the vesicles drastically reduced the response time for the fluorescence changes. Vesicles with 20–30% DMPC provided optimal results for bacterial detection. Fourier transform infrared (FTIR) spectra analysis suggested that DMPC insertion decreased the strength of hydrogen bonding among the amide and carboxylic acid groups of the polydiacetylene vesicles. Reduced bonding strength resulted in less rigid structure of the polydiacetylene polymer, allowing more rapid detection upon molecular recognition.

1. Introduction

Conjugated polydiacetylene (PDA) vesicles can be label-free biosensors. Diacetylene lipids, such as 10,12-pentacosadiynoic acid (PCDA), can undergo polymerization via a 1,4-addition reaction upon UV light to form ene–yne alternating polymer chains, producing liposome-like vesicles. The vesicles show a bichromic property from blue to red upon external perturbations, such as heat, pH, and mechanical stresses, providing a cheap and convenient sensor [1–3]. Using the property, PDA-based sensors have been developed to detect biological samples, including influenza virus, cholera toxin, *Escherichia coli*, oligonucleotides, antibodies, antigens, and lipopolysaccharides [4–13]. The colorimetric change causes vesicles to turn fluorescent [3,14,15]. Micro-patterned PDA vesicles can be fluorescence-based sensor chips for external stimulation [16–19], or as a biochip for high-throughput detection of low-volume analytes [20].

PDA vesicles have liposome-like structures, so phospholipids can be incorporated into the structure. Dimyristoyl phosphatidylcholine (DMPC) can be mixed with PDA for the purpose of immobilizing the probes on the vesicles or to increase their flexibility [5,8,10–12]. While there are reports of morphological changes in polydiacetylene polymers containing phospholipids in forms of vesicles or LB films [21–23] the detailed spectroscopic study of the intermolecular interactions has not been reported.

In this study, we demonstrate that mixing phospholipids into the vesicles decreases the response time of PDA vesicles. We studied the effects of the mixing on the molecular interactions by Fourier transform infrared (FTIR) spectroscopy.

2. Experimental

2.1. Materials

10,12-Pentacosadiynoic acid was purchased from GFS chemicals (Powell, OH, USA). PCDA–ABA and PCDA–biotin (Scheme 1) were prepared as described in the literature [24,25]. DMPC was purchased from Sigma–Aldrich (St. Louis, MO, USA). A biotin-labeled polyclonal antibody for detecting an *E. coli* surface protein was purchased from Fitzgerald Industries International, Inc. (Concord, MA, USA). *E. coli* and *Salmonella typimurium* were grown to 1.2×10^7 cfu/ml.

2.2. Methods

2.2.1. Preparation of lipid vesicles and immobilization

DMPC were dissolved in 1 ml chloroform and the solvent was removed by purging with N_2 to generate a thin lipid film on the glass

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