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RESEARCH ARTICLE

Fluorescence Signal Enhancement of Polydiacetylene Vesicle Stacks

Hyun Choi[1], Insung S. Choi[2], Gil Sun Lee[1,3,*], and Dong June Ahn[1,*]

[1] Department of Chemical and Biological Engineering, Korea University, Seoul 136-701, Korea
[2] Department of Chemistry, Korea Advanced Institute of Science and Technology, Daejeon 305-701, Korea
[3] Research Institute of Clean Chemical Engineering Systems, Korea University, Seoul 136-701, Korea

Polydiacetylenes provide a good biological/chemical label-free detection system owing to their blue to red color change and fluorescence change from non-fluorescence to red fluorescence. One of the important factors to consider before applying polydiacetylenes to various sensors is to enhance the sensitivity against specific molecules. This paper reports two methods for stacking polydiacetylene vesicles for fluorescence signal enhancement of polydiacetylene on solid substrates. This focuses on how to achieve a highly sensitive sensor chip by introducing two strategies to immobilize the vesicles effectively on substrates. One method is layer-by-layer deposition through reactions of vesicles and avidins alternately on an avidin treated substrate. The other is to prepare a clustered vesicles solution by mixing the appropriate amount of a biotinylated vesicle solution and avidin solution, and then immobilize the clustered vesicles on the substrates. The former allows easy control of the layer thickness, whereas the latter can shorten the process time. These strategies can be applied to a range of biological/chemical sensors for fluorescence signal enhancement.

Keywords: Polydiacetylenes, Signal Enhancement, Layer-by-Layer, Clustered Vesicle.

1. INTRODUCTION

Polydiacetylene supramolecules have attracted considerable interest for sensing because of their blue to red color change and self-emitting fluorescence characteristics from non-fluorescence to red fluorescence upon exposure to outer stimuli, such as mechanical stress, ligand–receptor interaction, temperature, pH, electrochromic stress, and solvent stress.[1–7] Well organized diacetylene monomers under 254 nm UV light go into polymerization. The change in the delocalized π-electrons conjugation length is related directly to the color transition and red fluorescence emission.[8]

Thus far, many studies have examined LB (Langmuir-Blodgett) and LS (Langmuir-Schaefer) films on substrates or vesicles in the liquid phase using polydiacetylene supramolecules. LB and LS films have relatively higher stability and lower intensity than the vesicle form. One strategy to use polydiacetylene supramolecules for more useful materials in terms of the sensor is to immobilize the vesicles on a solid substrate. Ahn et al. proposed a chip based sensor system for detection with a small amount of vesicles, simple handling of a chip, high stability, and high sensitivity.[9–12]

Sensitivity is one of prime considerations in biosensors. Every ligand–receptor or antibody–antigen interaction is not always strong enough to induce a color change of polydiacetylene to verify the analytes. To enhance the sensitivity using polydiacetylene, many studies focusing on controlling the flexibility of polydiacetylenes upon outer stresses have been reported by altering the molecular structure of the alkyl chain length of the hydrophobic group,[13–14] introduction of a fluorescence dye, insertion of phosphoric lipids to fabricate heterogeneous vesicles,[15] pH control in a vesicle solution,[16] control of the polymerization time,[17] and control of the vesicle size.[18] Strategies to fabricate multilayered polydiacetylene vesicles have been studied recently.[19–20]

This study examined how to achieve a highly sensitive sensor chip by introducing two strategies to immobilize the vesicles effectively on substrates. As shown in Figure 1, the first method was layer-by-layer deposition by a reaction of a heterogeneous polydiacetylene vesicle solution and avidin solution alternately on avidin treated substrates. In this way, the layer thickness can be controlled by adjusting the number of alternate dipping steps into the vesicle solution and avidin solution. The second method is the

*Authors to whom correspondence should be addressed.

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