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Tethered-type supported lipid bilayer membrane for measurement of membrane potential

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Abstract

A "tethered-type" lipid bilayer membrane is an effective system for the control of the interaction between an artificial bilayer membrane and a solid substrate with spacer molecules on the solid substrate. A method for surface chemical modification retaining the surface roughness below a level of nanometer is demanded because the thickness of lipid bilayer membrane is approximately 5 nm. In this study, we adopted a water-soluble silane coupling agent, carboxyethylsilanetriol, for the termination of solid substrate surface with -COOH group, and immobilized streptavidin. We fabricated sufficiently uniform and flat tethered-type planar lipid bilayer for the investigation of membrane potentials.

1. Introduction

A cell membrane is the outermost organ and acts as a barrier and gates controlling the transportation of materials, information and energy into and out of cells. Membrane potential, which is the difference in electric potential between inside and outside cell across a cell membrane, provides crucial driving force to proteins for membrane transportations. Supported lipid bilayer (SLB) is a cell membrane model system at solid-liquid interface. Previous studies showed that physical and chemical properties of solid substrates affect the structure and properties of SLB [1]. In this study, we constructed a "tethered" type SLB (Fig. 1) to investigate the effect of the surface charges of substrates on membrane potential and its dependence on the distance between SLB and substrates. Tethered-SLB (t-SLB) has spacer molecules between the lipid bilayer and the inorganic substrate, therefore t-SLB has the advantage in the control the interaction between the lipid bilayer and the substrate. It is important to keep the substrate surface flatness on subnanometer scale after the chemical modification of the spacer molecules. The thickness of a lipid bilayer is ~5 nm, thus the surface roughness of a few nanometer significantly affects the structure and physical properties of SLBs. We fabricated fluid and continuous t-SLB keeping the flatness of the substrate surface on subnanometer order using a water-soluble silane coupling agent for covalent conjugation of streptavidin as a tethering molecule.

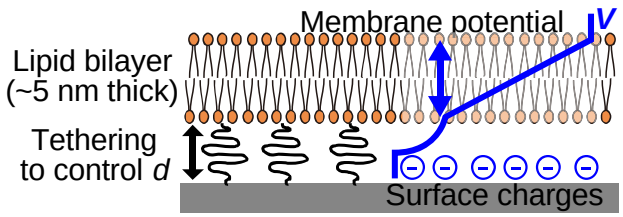

Fig. 1 Schematic of tethered-SLB and membrane potential applied by caused by surface charges of a substrate.

2. Experimental

Thermally oxidized SiO_2/Si substrates were kept in an aqueous solution of carboxyethylsilanetriol (CEST) (25wt%) for 24 h. The CEST-modified surface was treated with 1-ethyl-3-(3-dimethy-laminopropyl) carbodiimide hydro-chloride (EDC), and N-hydroxysuccinimide (NHS), for the conjugation with streptavidin (Fig. 2) [2,3]. Surface morphology at each process was observed with atomic force microscope (AFM) in air using a cantilever of spring constant 2 N/m. SLB and t-SLB were formed by the vesicle fusion method [1,3]. We prepared a vesicle suspension of 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) and biotinylated 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (biotin-DOPE) at molar ratio of 100:1 by suspending a vacuum-dried lipid film in a buffer solution (100 mM KCl, 25 mM HEPES, pH 7.4). SLB and t-SLB were observed with AFM and fluorescence microscope in the buffer solution. SLB and t-SLB doped with dye-labeled lipid (lissamine rhodamine B-DOPE, 0.5mol%) were prepared for fluorescence observation.

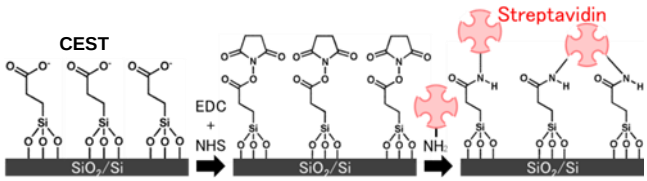

Fig. 2 Schematic of chemical modification of the SiO_2/Si surface with CEST and streptavidin for the fabrication of tethered-SLB.

3. Result and Discussion

Figures 3a and 3b show the AFM topographies of SiO_2/Si substrates before and after the CEST modification. The surface roughness of the substrates was 0.23 nm (rms) and 0.20 nm, respectively. We also detected CH_2 stretching modes of CEST with infrared absorption spectroscopy. These results showed that CEST bound to the surface covalently keeping the surface flatness on atomic level without causing self-polymerization. Figure 3c shows the AFM topography of the CEST-modified SiO_2/Si surface after the modification with streptavidin. Protrusions of 5.7 nm in height in average were observed. This value corresponds to the diameter of a streptavidin molecule (6.0 nm), which was estimated from its molecular mass (60 kDa) [4]. Conjugation efficiency of fluorescence-labeled streptavidin showed that majority of streptavidin were bound to the CEST-modified substrate via covalent bond and that amount of non-specific adsorption was limited.

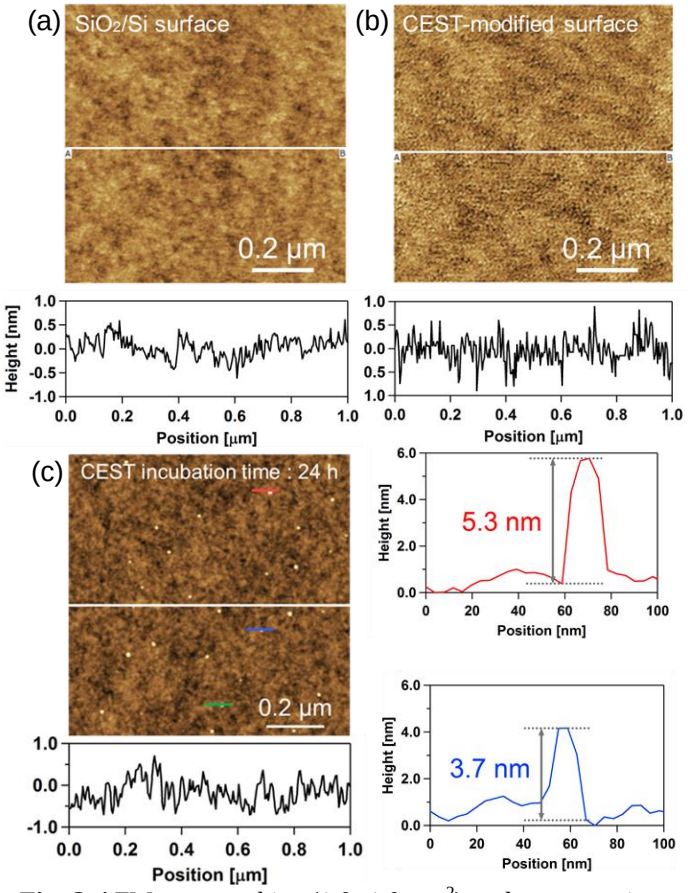

Fig. 3 AFM topographies (1.0×1.0 μm^2) and cross-section profiles of SiO_2/Si substrates: (a) Without chemical modification, (b) after CEST-modification, and (c) after the conjugation of streptavidin molecules.

Figure 4 shows the AFM topography of t-SLB after the preparation with the vesicle fusion method. Protrusions of streptavidin (Fig. 3c) disappeared, and the morphology of the membrane surface was flat and uniform. Fluorescence microscope observation and fluorescence recovery after photobleaching (FRAP) measurement revealed that the t-SLB was uniform on whole the substrate surface and retained the fluidity. The chemical modification method using CEST in this study achieved the preparation of t-SLB keeping the surface flatness on the subnanometer level. We will also describe the results of membrane-voltage sensitive dyes incorporated in SLB and t-SLB on site.

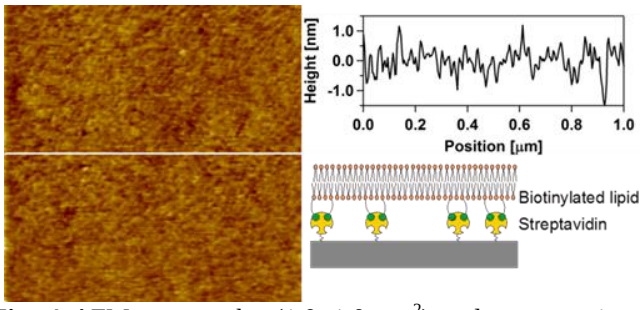

Fig. 4 AFM topography (1.0×1.0 μm^2) and cross-section profiles of t-SLB on the streptavidin-conjugated SiO_2/Si substrates.

4. Conclusions

We demonstrated the protein conjugation on the SiO_2/Si substrate via the COOH-termination with CEST for the fabrication of flat t-SLB system. Single molecule of streptavidin on a flat substrate on subnanometer scale was obtained in the AFM topography. We successfully prepared a flat and fluid tethered-type supported lipid bilayer membrane on the streptavidin-conjugated substrate surface. The chemical modification techniques in this study are useful for the preparation of t-SLB controlling the distance between lipid bilayer and substrate

Acknowledgements

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