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Sensitive detection of a pseudo-polyrotaxane ultrathin film by SPR and QCM-D methods

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ABSTRACT

Surface plasmon resonance spectroscopy (SPR) and quartz crystal microbalance with dissipation (QCM-D) are powerful techniques to analyze structure of a thin film in sub-nanometer thickness and 10 ng cm^{-2} mass per area level, respectively. In this study, the pseudo-polyrotaxane in the film state was detected by both methods. The pseudo-polyrotaxane ultrathin film was composed of α-CD molecules and a self-assembled-monolayer (SAM) in which the SAM was fabricated with chains consisting of alkane thiol, a PEG chain, and an azobenzene derivative (MeAzoPEG*n*; PEG chain length *n* = 6-, 10-, and 16-mer). Though polyethylene glycol (PEG) is known to form a polyrotaxane with α-cyclodextrin (α-CD) in aqueous solution and the structure of polyrotaxane was investigated in detail, the pseudo-polyrotaxane in the film state was not studied well. SPR measurement *in situ* indicated resulting increases in layer thickness for a longer PEG chain in a state of equilibrium between the α-CD and the SAM. The results detected by QCM-D, showed that the film mass and rigidity increased with PEG chain length. They also provide evidence for pseudo-polyrotaxane formation. To evaluate it quantitatively, titration of the MeAzoPEG*n* SAMs with α-CD was measured by SPR. The increase in film thickness was found to depend on the *m*th order of the α-CD concentration in equilibrium state. SPR and QCM-D were found to be useful to detect the pseudo-polyrotaxane in the film state.

1. Introduction

Surface plasmon resonance spectroscopy (SPR) and quartz crystal microbalance with dissipation (QCM-D) are widely used for bio-sensing [1–5]. Each resolution for detection can be sub-nanometer in film thickness and 10 ng cm^{-2} in mass per area, respectively, and therefore there are advantages in applying them to a bio-sensor. The resolution or sensitivity for detecting depends on the instrumental condition of SPR and QCM-D, individually. For SPR instrument, the wavelength of a light source and the dielectric constants of substrate and metal coating used are, and for QCM-D the fundamental frequency and the stability of temperature are principal. In this study, nano-structure of a highly dense pseudo-polyrotaxane which was formed between α-cyclodextrin and self-assembled-monolayer (SAM) into water was detected by SPR and QCM-D.

Rotaxanes and polyrotaxanes have been studied as one of the most interesting and useful phenomena of supramolecular architecture and these materials have been applied in various new fields such as nano-machinery, molecular wire, shuttles [6,7], and motors [8–10]. To date, however, there have been few studies of the formation of high density polyrotaxane film on SAM [11,12]. The creation of novel polyrotaxane film or colloid where the rotaxane-chain axis arrangement at the surface is normal could lead to its application in areas such as drug delivery systems (DDS) and related release control systems. At the same time, it would be interesting from a purely scientific point of view relating to interface structure, dynamics, and functions. Polyrotaxane film formed in a non-homogeneous system would be expected to have different kinetics and stability properties from polyrotaxane formed in a homogeneous solution system.

Of the various kinds of polyrotaxane, one which has interested many researchers is that formed by PEG and α-CD. PEG is one of the most promising biomaterials because of its water solubility and its property of suppressing non-specific intermolecular interaction with proteins and bio-molecules. Cyclodextrins, meanwhile, a class of sugars, form attractive compounds allowing insertion of various molecules. Polyrotaxane formation between polyethylene glycol (PEG) and α-cyclodextrins (α-CD) in solution has been

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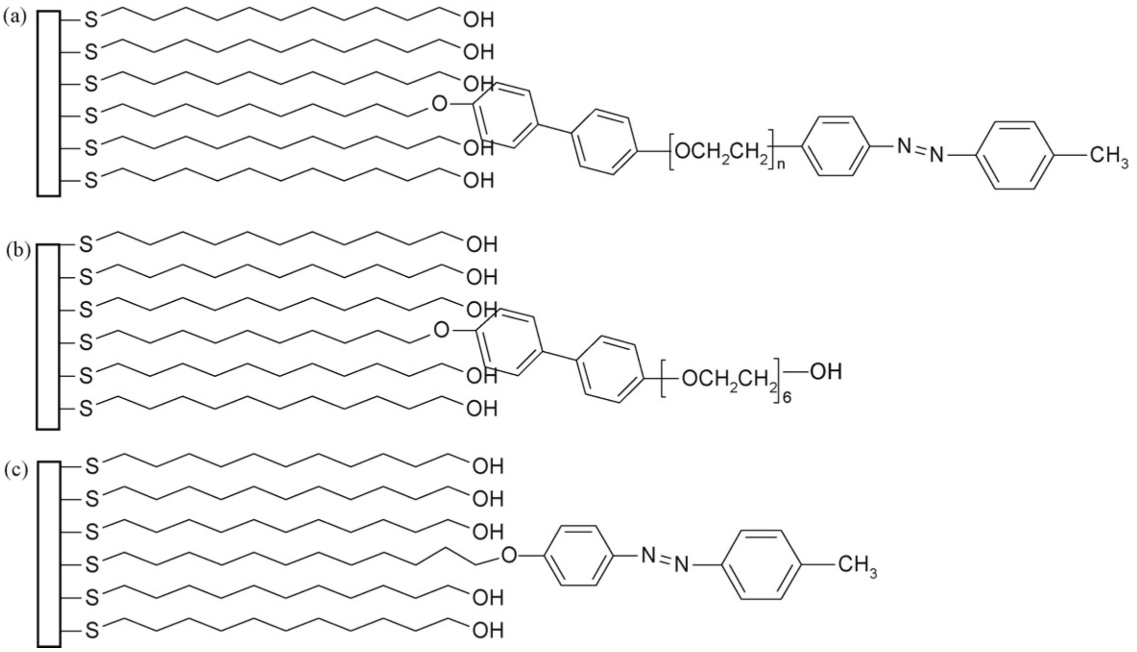


Fig. 1. SAM mixed with HSC11OH and (a) MeAzoPEG*n*, (b) PEG6, and (c) MeAzoPEG0.

extensively studied by Harada et al. [13,14], who report that polyrotaxane is formed when a row of α-CD rings is tied together at the ratio of two ethylene glycol units to one α-CD. The pseudo-polyrotaxane ultrathin film with α-CD studied here was arranged on the substrate using a SAM synthesized as a MeAzoPEG*n* composed of an alkyl chain modified with a mercapto group, a PEG moiety, and an azobenzene moiety. The complex of α-CD molecules with the MeAzoPEG*n* interface may take one of the following forms: (pseudo-)polyrotaxane film formation; inclusion complex capped with an azobenzene moiety; or adsorption to the interface. In order to clarify which, the interaction process was observed *in situ* by SPR and QCM-D. The scale of the polyrotaxane film studied corresponds to ∼nm in film thickness and ∼100 ng in mass. SPR and QCM-D are suitably sensitive measurement methods. The pseudo-polyrotaxane ultrathin film was successfully constructed by penetrating the α-CD from one chain end of the azobenzene group side.

In order to clarify the stoichiometry of pseudo-polyrotaxane formation, titration with CD was also measured by SPR. Although a binding constant for both solution and solid surface was established from kinetic analysis of 1:1-molecular recognition, no binding constant was established for 1:multi-pseudo-polyrotaxane film as a non-homogeneous system. In the present study, the binding constant for 1:multi-pseudo-polyrotaxane film was analyzed by treatment of the multi-step complex formation under stepwise equilibria such as evaluation of 1:3-rotaxane formation in solution [15–17]. The evaluation method applied to pseudo-polyrotaxane film was corroborated by that observed in multi-ion adsorption to the interface [18–20].

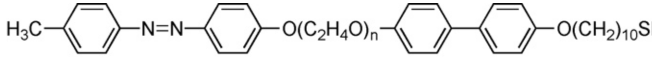
2. Experimental

2.1. Materials

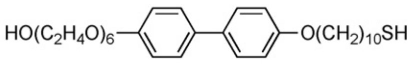
Dodecane thiol (HSC12) and 11-mercapto-1-undecanol (HSC11OH) were purchased from Aldrich and used as the main diluent thiols to prepare a mixed SAM of MeAzoPEG*n* or PEG6 [Fig. 1(a)–(c)]. Thiol-PEG with molecular weight of 5000, HSPEG110 (HS-CH_2CH_2-$(OCH_2CH_2)_n$-OCH_3), was purchased from NOF (SUNBRIGHT ME-050SH). The other thiols shown in Fig. 1(a)–(c) were synthesized as described below. α-cyclodextrin (α-CD) was purchased from Wako. All of the thiols and CDs were used without further purification. α-CD was prepared as a saturated aqueous solution at 145 mg/ml. As glass substrates for SPR measurement, LaSFN9 (Hellma Optik, Jena, Germany) with a high dielectric constant ($\varepsilon' = 3.41$ at wavelength of 632.8 nm) was used. The gold (Au) targets (purity 99.99%) for sputtering were obtained from Furuuchi Chemical Corporation (Tokyo, Japan).

2.2. Synthesis

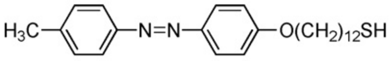
All chemicals for synthesis were of commercially available purity and used without further purification. Three compounds consisting of azobenzene, PEG (6-, 10-, and 16-mer), and alkane thiol moieties (MeAzoPEG*n*; *n* = 6, 10, and 16) were synthesized according to the outline shown in the figure insets. In order to evaluate the influence of the azobenzene and PEG moieties on rotaxane formation, two analogues without the azobenzene or PEG moiety, PEG6 (Fig. 1(b)) and MeAzoPEG0 (Fig. 1(c)), were also synthesized in the same way. In general synthetic procedures, the first step was the incorporation of PEG and hydroxyalkane and the second the introduction of the azobenzene moiety. The final step, conversion of the hydroxy group to a mercapto group, was carried out using potassium thioacetate. Details are given in SI.



1. MeAzoPEG6 (n=6)
2. MeAzoPEG10 (n=10)
3. MeAzoPEG16 (n=16)



4. PEG6



5. MeAzoPEG0