

# Poly(amido amine) Dendrimer Adsorption onto 3-Mercaptopropionic Acid Self-assembled Monolayer Formed on Au Surface -Investigation by Surface Enhanced Spectroscopy and Surface Plasmon Sensing-

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Adsorption structure of 3-mercaptopropionic acid (MPA) self-assembled monolayer (SAM) on Au island film and adsorption of amine-terminated poly(amido amine) (PAMAM) dendrimer onto MPA SAM were investigated by the attenuated total reflectance (ATR) surface enhanced infrared absorption (SEIRA) spectroscopy. It was ascertained from the occurrence of absorption bands assigned to carboxylate stretching vibration modes that the only partial proton dissociation of the carboxylic acid groups of MPA SAM and the strong hydrogen-bonding of residual carboxylic acids occurred when water of pH = 3~11 was filled on the SAM. On the other hand, the carboxylic acid species of SAM diminished with adsorption of PAMAM from aqueous solution. This fact supposes that the protonated amine terminal groups of dendrimer bind to carboxylates of SAM by the electrostatic interaction. The surface plasmon resonance (SPR) sensing method was used to confirm the dendrimer adsorption and to determine the thickness of dendrimer adlayer. The depressed molecular structure or the imperfect covering of the adsorbed dendrimers was assumed.

Key words: Dendrimer, Adsorption, Self-Assembled Monolayer, Surface-Enhanced Infrared Absorption Spectroscopy, Surface Plasmon Resonance Sensing

## 1. INTRODUCTION

Technologies for designing interfaces at the molecular level have been developed for the purpose of many applications such as molecular, biomimetic, electronic, and photochromic devices [1,2]. Then, the molecules that produce stable, functional, and select interfaces with facility are required.

In recent years, dendrimers consisting of regular branched structure are of great interest as a building unit of the functional thin film on a substrate [3]. They have a unique surface of plural terminal groups, the number of which can be accurately controlled on a synthesis process. For example, a fourth-generation (G4) poly (amido amine) dendrimer has 64 surface functional groups. Furthermore, it is possible to bind covalently different functional groups at the branches and in the core by divergent synthesis and to conjugate the dendrons of different generations by convergent synthesis [4]. The dendrimers having various structures and properties have been synthesized. Thus, the architectures, such as the thin films, having great functionality and structural stability can be constructed by the dendrimers.

On the other hand, it is not easy to investigate the extremely thin film with thickness less than ~10nm in solution by the infrared (IR) absorption spectroscopy. It has been reported that, for measuring molecules at the microscopic interface, surface-enhanced infrared absorption (SEIRA) spectroscopy is more useful than the conventional IR spectroscopy [5-7]. On the SEIRA spectroscopy, the absorption bands are strongly

enhanced by the surface plasmon effect, which occurs on metal islands by the radiation of infrared ray with longer wavelength than the diameter of islands. Moreover, the adsorption behavior from solution phase to solid surface is investigated by SEIRA spectroscopy with attenuated total reflection (ATR) attachment of Kretschmann arrangement [8].

In this paper, the adsorption structure of carboxylic acid-terminated 3-mercaptopropionic acid (MPA) on Au surface is, first, investigated by ATR-SEIRA spectroscopy. Next, the adsorption of amine-terminated G4 poly (amido amine) (PAMAM) dendrimer onto MPA self-assembled monolayer (SAM) is reported. Moreover, surface plasmon resonance (SPR) sensor [9,10] is used to confirm the adsorption and to determine the thickness of adsorption layer. Figure 1(a) shows chemical structures of MPA and PAMAM.

## 2. EXPERIMENTAL SECTION

MPA and PAMAM purchased from Aldrich Chemical Co. were used without purification. Ethanol was a product of Wako Pure Chemical Industries, Ltd. An ethanol solution of MPA (2mM) and an aqueous solution of PAMAM (10<sup>-2</sup>wt%) were prepared. The pHs of water were adjusted by HCl or NaOH. Deionized distilled-water was used throughout this work.

ATR-SEIRA spectra were recorded on a Bio-Rad FTS 575C FT-IR spectrometer equipped with a cryogenic mercury cadmium telluride detector and with an ATR attachment. Band resolution was 4cm<sup>-1</sup>. All measurements were carried out at room temperature

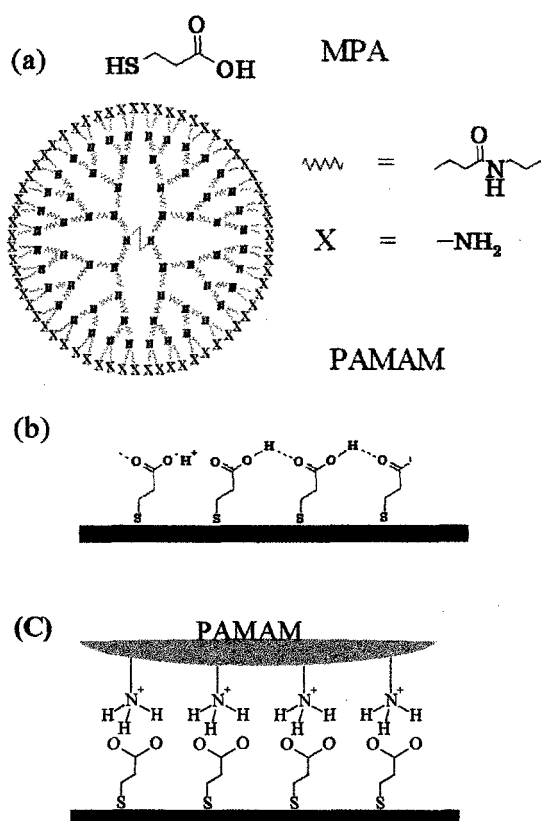


Figure 1. Schemes of (a) samples; 3-mercaptopropionic acid (MPA) and G4 amine-terminated poly (amido amine) (PAMAM) dendrimer, (b) MPA self-assembled monolayer (SAM) on Au surface in water, and (c) PAMAM adsorption on MPA SAM.

( $-25^\circ\text{C}$ ). Au island film on a hemispherical silicon prism was prepared by evaporating Au at a rate of  $0.05\text{\AA s}^{-1}$  under the pressure of  $ca. 3 \times 10^{-4}$  Pa on a Shinku SD-240 vaporizing instrument. The film was controlled to be 15nm in thickness, which was a preferable condition for the surface-enhanced effect [7]. The solution cell with a prism was set on an ATR attachment, and an ethanol solution of MPA was poured into the cell. After the equilibrium of MPA adsorption was reached, the SAM film was rinsed by ethanol and used for ATR-SEIRA spectroscopic measurements in water. An aqueous solution of PAMAM was poured on the MPA SAM film, and a time-resolved ATR-SEIRA spectra were measured. A SEIRA spectrum of an ATR cell filled water was used as a background.

SPR sensing examination was performed on a biosensor analytical system (Nippon Laser & Electronics Lab.). Glass substrate which was evaporated Au (44nm in thickness) was sunk in an ethanol solution of MPA. After the time (30min) sufficient to form SAM passed [8], the SAM-adsorbed substrate was rinsed by ethanol and attached on a hemispherical prism. Aqueous solution of dendrimer and solvent were filled in sensing and reference compartments, respectively. The shift of SPR angle under 670nm wavelength laser light radiation was monitored as a function of adsorption time. A SPR spectrum as a function of incident angle was obtained to

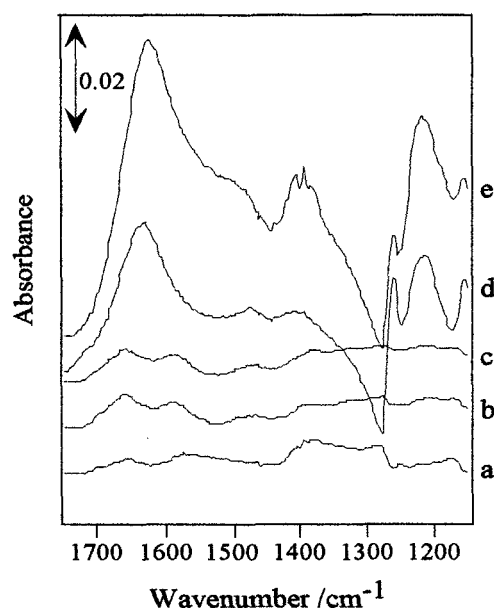


Figure 2. ATR-SEIRA spectra of MPA SAM in water of (a) pH 3.1, (b) p 5.7, (c) pH 9.1, and (d) pH 11.

determine the thickness of the dendrimer layer.

### 3. RESULTS AND DISCUSSION

#### 3.1 Adsorption structure of MPA SAM in water

Figure 2 shows ATR-SEIRA spectra of the MPA SAM in water (pH 3.1, 5.7, 9.1, and 11). Observed IR absorption band positions and their assignments are listed in Table 1. Vibration bands of the hydrogen-bonded C=O stretching ( $\sim 1655\text{cm}^{-1}$ ), the  $\text{COO}^-$  antisymmetric stretching ( $\sim 1586\text{cm}^{-1}$ ), and the  $\text{COO}^-$  symmetric stretching coupled with C-O stretching and OH in-plane bending ( $\sim 1381\text{cm}^{-1}$ ) appeared. This indicates that some carboxylic acids of the MPA SAM are hydrogen-bonding and the others are deprotonated, as illustrated in Figure 1(b). Even when the pH of the solution was increased up to  $\sim 11$ , the remarkable intensity transition between C=O and  $\text{COO}^-$  stretching bands was not observed, notwithstanding that the dissociation of conventional carboxylic acid derivatives occurs around  $\text{pH} = 5$ . This fact demonstrates the anomalous dissociation behavior of MPA at the interface. This may be due to the remarkably strong hydrogen-bonding of carboxylic acids, which is assumed from a low wavenumber of hydrogen-bonded C=O stretching band. On the other hand, the intensities of bands described above increased above neutral pH with increasing the solution pH. Thus, two possibilities may be realized: (1) It is assumed from a selection rule that the MPA molecules at alkaline pH must be reoriented with perpendicular transition moments of vibration bands. (2) The surface enhancement must be stronger in alkaline pH than in acidic pH.

#### 3.2 PAMAM adsorption onto MPA SAM in water

Time-resolved ATR-SEIRA spectra for PAMAM adsorption onto MPA SAM in water are shown in Figure 3. Band positions and their assignments are included in Table 1. Remarkable changes of the ATR-SEIRA

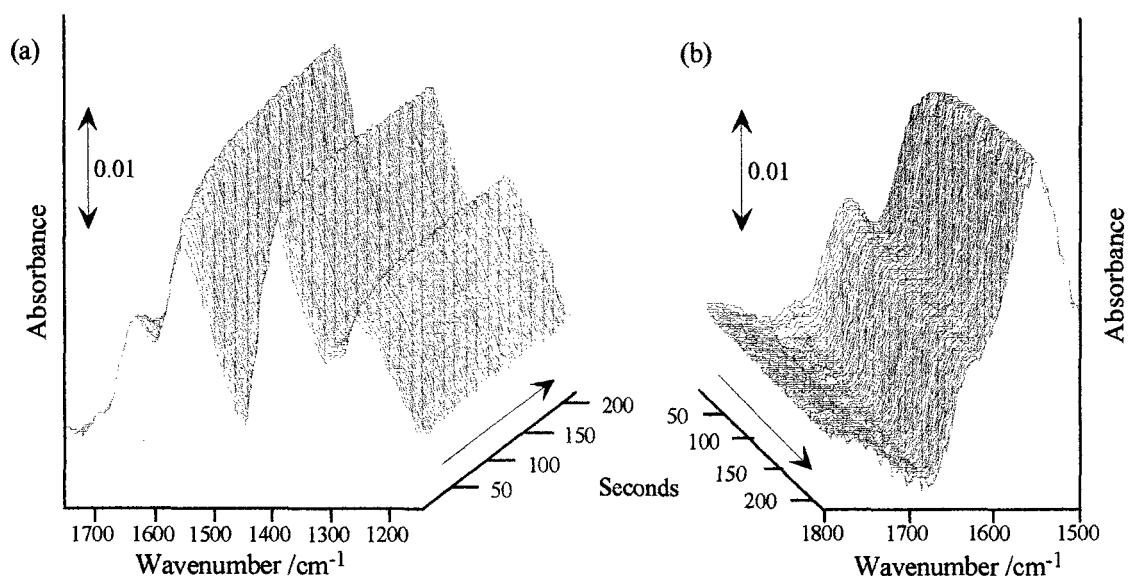


Figure 3. Time-resolved ATR-SEIRA spectra of PAMAM adsorption onto MPA SAM from aqueous solution of PAMAM (0.01wt%). (a) Time passes from near side to far side at 1150-1750 $\text{cm}^{-1}$  region; (b) time passes from far side to near side at 1500-1800 $\text{cm}^{-1}$  region.

spectra during the time course are a disappearance of C=O stretching vibration band (1698 $\text{cm}^{-1}$ ) and a decrease of hydrogen-bonded C=O stretching band overlapped with amide I band (1644-1632 $\text{cm}^{-1}$ ). On the other hands, the COO<sup>-</sup> antisymmetric stretching band overlapped with the amide II of PAMAM (1552-1550 $\text{cm}^{-1}$ ) and the COO<sup>-</sup> symmetric stretching band overlapped with C-O stretching and OH in-plane bending bands (1397-1390 $\text{cm}^{-1}$ ) are increased with the adsorption time. Thus, it is noted that carboxylic acid species of the MPA SAM change to ionized species and PAMAMs are adsorbed on the ionized MPA SAM.

It is reported that the pK value, which is the negative logarithm of the acidic dissociation constant, is 9.75 for the primary amine end groups (-NH<sub>2</sub>) on the periphery of G4 and G5 poly(propyleneimine) dendrimers in salt-free water [11]. Incidentally, the pK of the ternary amine groups (>N-), which are situated at the branching points, is around 6.1. Therefore, the former amines are partly dissociated but the latter amines are not ionized in aqueous solution of PAMAM, since the pH of the

aqueous solution of PAMAM (0.01wt%) was 8.7. Some carboxylic acids of the MPA SAM are not dissociated in the basic solution of this pH region, as apparent from Figure 2. These facts demonstrate that the dissociation of carboxylic acids of the MPA SAM occurred with the adsorption of the terminal amines of the PAMAM, but not originally in the basic solution of the PAMAM. Moreover, the carboxylates bind with the protonated primary amines of the PAMAM in water by the electrostatic interaction.

### 3.3 Thickness of PAMAM adlayer on MPA SAM in water

A time-depending SPR angle shift on the adsorption of PAMAM onto Au surface from aqueous solution was measured, and the adsorption thickness is shown as a function of adsorption time in Figure 4. The vertical axis is converted from the SPR angle shift to the adlayer thickness by using the standard Fresnel theory for the four-layer model [12,13].

Table I IR Band Positions( $\text{cm}^{-1}$ )<sup>a</sup> and Their Assignments<sup>b,c</sup>.

assignment	ATR-SEIRA				ATR-SEIRA (time-resolved)	
	MPA SAM in water				PAMAM on MPA SAM in water	
	pH3.1	pH5.7	pH9.1	pH11	immediate	in equilibrium
C=O str	-	-	-	-	1698sh	-
hydrogen-bonded C=O str (amide I)	1655w	1659w	1631m	1622s	1644m	1632sh
COO <sup>-</sup> antisym str (amideII)	1586w	1587w	sh	sh	1552m	1550s
CH <sub>2</sub> scissor	1473w	1468w	1471w	1474sh	sh	1416sh
COO <sup>-</sup> sym str + C-O str + OH ip bend	1381vw	1382vw	1407w	1406m	1397m	1390s
CH <sub>2</sub> wag (amide III)	1277vw	1275vw	1261w	1261w	1258m	1258m
CH <sub>2</sub> wag	1216w	1217w	1216m	1221m	-	-
C-C str	1172vw	1174vw	1156m	1157m	sh	1189m

<sup>a</sup> s, strong; m, medium; w, weak; vw, very weak; sh, shoulder. <sup>b</sup> str, stretching; antisym, antisymmetric; scissor, scissoring; sym, symmetric; ip, in-plane; bend, bending; wag, wagging. <sup>c</sup> The assignments in parenthesis are originated only from PAMAM.

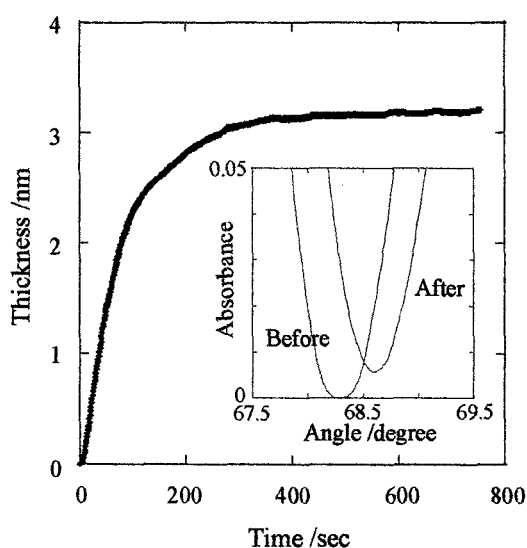


Figure 4. Adlayer thickness of PAMAM on MPA SAM, as a function of adsorption time, evaluated from SPR angle shift. Insert displays a SPR angle shift at equilibrium state.

The adlayer thickness of PAMAM was gradually increased with the adsorption time and converged to 3.2nm. This value is slightly smaller than diameter of PAMAM (3.6nm) calculated from Corey-Pauling-Koltun (CPK) model. However, it is a reasonable value, when two possible reasons are considered. First, the depressed molecular structure of PAMAM is assumed on the solid surface. Some investigators have already reported this kind of dendrimer structure on the surfaces [10,14]. The other possibility is that the PAMAM binds to the MPA SAM at the condition so as to cover imperfectly the binding areas of SAM.

#### 4. CONCLUSIONS

The formation of MPA SAM and the PAMAM adsorption onto the MPA SAM film were investigated by the ATR-SEIRA spectroscopy. It was verified that IR spectroscopy enhanced by the surface-plasmon effects is powerful for the detection of tiny amount of adsorbed molecules on solid substrate. The SPR sensing examination informed us the adsorption kinetics on the monolayer formation and the adsorption thickness.

It was demonstrated by the ATR-SEIRA spectroscopy that the most carboxylic acids of MPA SAM were immediately dissociated with the beginning of the PAMAM adsorption, and the protonated terminal amines were bound to the carboxylates of MPA SAM by the electrostatic interaction. Similar adsorption behavior was observed for the adsorption of cationic surfactant, hexadecyltrimethylammonium chloride ( $C_{16}TAC$ ), on MPA SAM [8].  $C_{16}TAC$  adsorbed on MPA SAM by the electrostatic interaction but the additional physisorption occurred on the first  $C_{16}TAC$  adlayer, different from the present case. In this connection, the adsorption of  $C_{16}TAC$  proceeded abundant on 1-dodecanethiol SAM owing to the hydrophobic interaction but less and slow on 3-mercapto-1-propanol SAM on which the specific interaction sites with  $C_{16}TAC$  are poor [15].

The thickness of the PAMAM adlayer on the MPA SAM was reasonable for the monolayer adsorption. This result is comparable with the adsorption of ammonium chloride-terminated PAMAM dendrimer on Au or glass surface without SAM [10,14]. The adsorption of PAMAM dendrimer was extremely less than monolayer or took much time up to monolayer adsorption, although an amine group/n-hexyl group terminated amphiphilic surface-block dendrimer adsorbed on Au surface more than PAMAM [16].

The molecular adsorption to the surface attracts the attention from the point of view of molecular recognition. The absolute amount of the adsorbed molecules, molecular structure on the surface, and the specialization of the intermolecular interaction are indispensable for the investigation of molecular recognition on the solid surface. The present work performs such investigations by the simultaneous utilizations of multiple surface nanotechnologies.

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