RESEARCH ARTICLE

Preparation of Dendrimer SAM on Au Substrate and Adsorption/Desorption of Poly-*L*-glutamate on the SAM

Tomoko Yamazaki[1] and Toyoko Imae[1, 2, *]

[1] *Graduate School of Science and* [2] *Research Center for Materials Science,*
Nagoya University, Chikusa, Nagoya 464-8602, Japan

A self-assembled monolayer (SAM) of a dendrimer has been fabricated by covalent bonding of amine-terminated dendrimers to 3-mercaptopropionic acid SAM on an Au substrate, where an amide bond was formed between the amine and the carboxylic acid using amide-coupling reagents. Each reaction step was inspected by transmission surface-enhanced infrared absorption spectroscopy. Reaction was affected by solvents of the coupling reagents and concentrations of the coupling reagents and dendrimers. The pH-dependent adsorption/desorption behavior of sodium poly-*L*-glutamate (PGA) has been examined on the dendrimer SAM prepared. The adsorbed amount was abundant at acid pH below the pK_a of carboxylate, while desorption from PGA-adsorbed dendrimer SAM prepared at acid pH proceeded as the pH was raised. The reaction was reversible and reproducible within 30 min under controlled pH, although the carboxylic acid species adsorbed at pH 4.2 was deprotonated to a carboxylate species after more than 30 min of desorption at pH 9.4.

Keywords: Dendrimer, Poly(amido amine) Dendrimer, Poly(propyleneimine) Dendrimer, Self-Assembled Monolayer, 3-Mercaptopropionic Acid, Poly-*L*-glutamate, Adsorption, Desorption.

1. INTRODUCTION

There has been in increasing importance of designing surfaces at the molecular level. As a block constructing such a surface, the dendrimer is worthy of notice,[1–3] since it has structural characteristics such as a well-defined chemical structure, many functional terminal groups, and an internal void. The novel surface consisting of this functional molecule, dendrimer, is available to various applications including chemical sensors using terminal groups[4, 5] and internal voids[6–8] and biochemical sensors based on the antigen–antibody reaction.[9] In comparison with self-assembly, formation by the acid/base electrostatic bond,[10, 11] covalent bonding avoids the removal of dendrimer from the substrate and increases the chemical stability of the surface against variation of conditions, such as pH change.

In this work, self-assembled monolayers (SAM) of dendrimers were prepared by binding of amine-terminated

dendrimers to 3-mercaptopropionic acid (MPA) SAM prepared on a substrate. Amide bonds were covalently formed between them by use of amide coupling reagents. The dendrimers used are fourth generation (G4) poly(amido amine) (PAMAM) dendrimers and fifth generation (G5) poly(propyleneimine) (PPI) dendrimers, both of which have 64 terminal amine groups. In the process of SAM preparation, effects of solvents and concentrations of coupling reagents and dendrimers were investigated. Each reaction step was verified using surface-enhanced infrared absorption (SEIRA) spectroscopy.[12, 13]

Moreover, we used dendrimer SAM as an interfacial reaction matrix, since close-packed terminal groups of dendrimers would bring high reactivity of the SAM. There are many reports of the complex formation between dendrimers and linear polymers in solutions, which were formed by electrostatic interaction and hydrogen bonding.[14–22] Recently, one of author and her collaborators have reported that sodium poly-*L*-glutamate (NaPGA) makes a complex with PAMAM dendrimer in aqueous solutions by electrostatic interaction at alkaline pH,

---

and phase separation occurs as the pH is lowered.[23–26] In the present work, the adsorption/desorption behavior of NaPGA was examined on PAMAM dendrimer SAM by changing the pH, and the interaction forces of NaPGA with dendrimer on substrate were compared with that of free dendrimer in solution. The clarification of the specific interaction of guest molecules with the host on the substrate is indispensable for the fabrication of sensing systems.

2. EXPERIMENTAL SECTION

2.1. Materials

MPA, a 10 wt% methanol solution of G4 PAMAM dendrimer, G5 PPI dendrimer, 1-ethyl-3-(3-dimethylamino propyl)carbodiimide hydrochloride (EDC), *N*-hydroxy succinimide (NHS), and ethanolamine were purchased from Aldrich Chemical Co. Ethanol, methanol, 1,4-dioxane, and hydrochloric acid were obtained from Wako Pure Chemical Industries. NaPGA was synthesized by polymerization of glutamic acid. Redistilled and deionized water (MilliQ water) was used throughout the work.

2.2. Measurements

Transmission SEIRA spectroscopic measurement was carried out on a Bio-Rad FTS 575C FT-IR spectrometer equipped with a liquid nitrogen cooled cryogenic mercury cadmium telluride (MCT) detector. Au island film that enhances infrared absorption band intensity was deposited on clean CaF$_2$ substrate, following the method previously reported.[27, 28]

3. RESULTS AND DISCUSSION

3.1. Preparation of Dendrimer SAM

MPA SAM was fabricated by immersing an Au-deposited CaF$_2$ substrate into a 2 mM ethanol solution of MPA for 1 h and rinsing with ethanol. Then the substrate was immersed into a freshly prepared NHS/EDC solution to convert the carboxyl groups of MPA SAM into active ester groups. Solvents used were water or 1,4-dioxane for NHS and water for EDC. These were mixed just before the reaction with MPA SAM. After activation for 30 min, the substrate was rinsed with water and reacted with dendrimer in methanol for 30 min. Chart 1 illustrates a flow chart of the reaction process for the dendrimer SAM preparation. Each reaction step was verified by transmission SEIRA spectroscopy. In this measurement, Au island-deposited CaF$_2$ substrate was used as background.

Figure 1 shows the effect of coupling reagent and its solvent on the dendrimer SAM preparation. An NH$_2$ scissoring band of terminal groups of dendrimer was observed at 1558 cm^{-1} in a SEIRA spectrum after the immersion of MPA SAM into a 0.1 wt% methanol solution of PPI
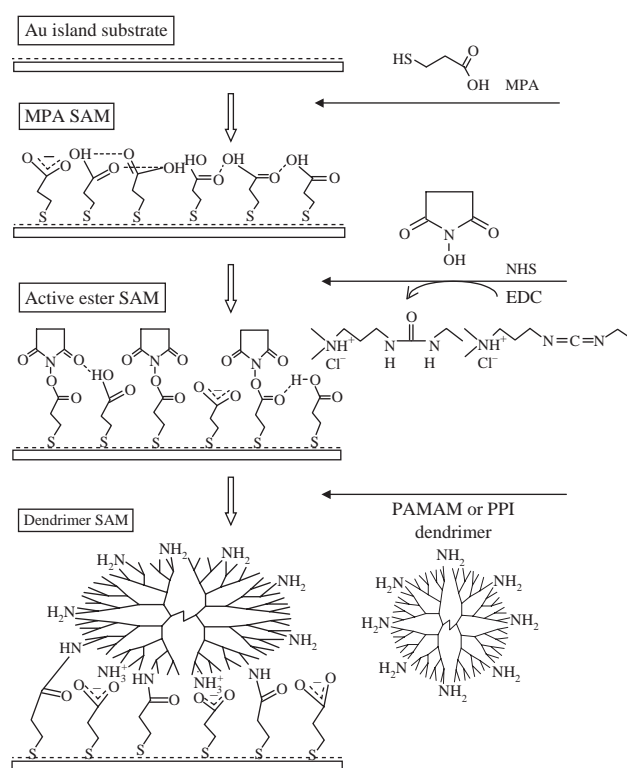

Chart 1. Flow chart of dendrimer SAM preparation.

dendrimer without coupling reagent (see Fig. 1 left). This suggests that PPI dendrimer adsorbs on MPA SAM by the electrostatic interaction. In the process using amide coupling reagents (NHS and EDC) in both water and water/1,4-dioxane, different from the process without coupling reagents, an amide I band at ∼1640 cm^{-1} along with a NH$_2$ scissoring band appeared in the PPI dendrimer SAM spectra (see Fig. 1 right). This indicates that the dendrimer was linked to MPA SAM through a covalent bond after the reaction with amide coupling reagents. Since the amide II band is overlapped on the NH$_2$ scissoring band of free terminals of dendrimer, it is difficult to distinguish those bands.

The effect of solvents of coupling reagents in the active esterification process was also examined. Coupling reagents display characteristic SEIRA bands at 1736 and 1063 cm^{-1}. As shown in Figure 1 right, using a 1,4-dioxane solution of NHS resulted in stronger bands of active ester SAM than using an aqueous solution. This effect may influence greatly formation of amide bonds between dendrimer and MPA SAM. Since dehydration is the driving force in the active esterification, it seems effective to use an organic solvent like 1,4-dioxane.

The surface coverage of dendrimer SAM is controlled by varying the concentration of coupling reagents from 0.013 to 0.065 M, as seen in Figure 2. SEIRA bands of active ester increased with raising the concentration of reagents. This effect was more remarkable when 1,4-dioxane was used as a solvent for NHS than when
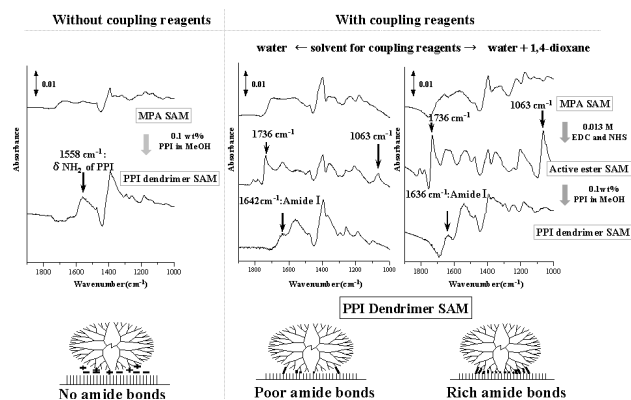
Fig. 1. SEIRA spectra of the preparation process of PPI dendrimer SAM. Effect of coupling reagent and its solvent.

water was. However, it should be noticed that a SEIRA band at 1540 cm^{-1} appeared in dioxane but not in water. This band would be assigned to a urea derivative, which is a byproduct after dehydration of EDC. This charged product is hardly soluble in organic solvent. Attempts to wash the active ester SAM with HCl were not enough to remove this product completely. Even if active esterification in aqueous medium is poor, dendrimer can be linked to MPA SAM with similar content as in dioxane (see Fig. 1), since it has many reactive terminal amine groups. Moreover, using water avoids contamination of byproduct on the SAM. Taking account of these advantages and disadvantages, it can be concluded that water is preferable as solvent. In a water system, SEIRA band intensity of active ester did not increase very much, even if the concentration of reagents was raised from 0.013 to 0.065 M, but it decreased when the concentration was lower than 0.013 M (data are not shown). Then the desirable concentration of coupling reagents is above 0.013 M.

The effect of dendrimer concentration is shown in Figure 3. SEIRA bands (1736 cm^{-1}, 1063 cm^{-1}) of active ester weakened with increasing concentration of PPI dendrimer in solution in the dendrimer SAM preparation process. This means that the amide coupling reaction depends on the concentration of dendrimer. Then the concentration of PPI dendrimer can be decided as 0.1 wt%. Finally, unreacted active ester was removed by rinsing with an aqueous 1 M solution of ethanolamine. Thus, the free carboxyl group of the SAM reacts with the amine group of ethanolamine and is converted into an ethanolamide group. When the concentration of PPI dendrimer is enough to modify the SAM (≥ 0.1 wt%), the treatment by ethanolamine is needless. Using PAMAM dendrimer is less preferable to PPI dendrimer in order to confirm a link between dendrimer and MPA SAM by IR spectra, since amide bands of PAMAM dendrimer overlapped amide bands of the link between dendrimer and MPA SAM. Nevertheless, it was determined that the preferable concentration of PAMAM dendrimer in the SAM formation was 0.01 wt% from the comparison of
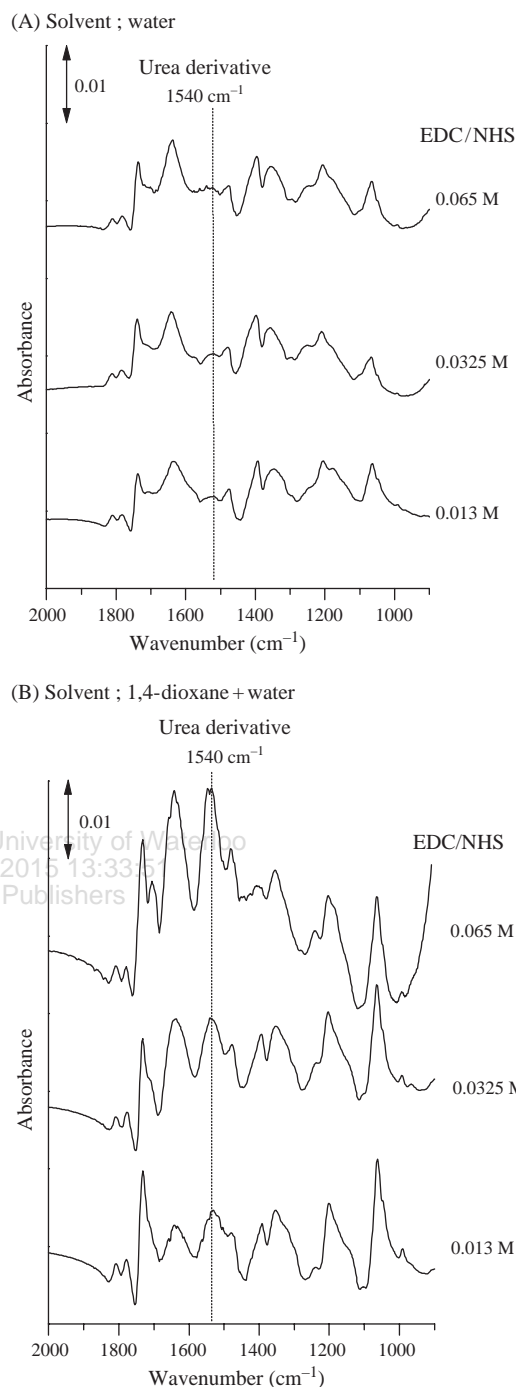

Fig. 2. SEIRA spectra of the active ester SAM in the preparation process of PPI dendrimer SAM. Effect of solvent and the concentration of coupling reagents.

IR spectra between dendrimer SAM and free dendrimer (data are not shown).

3.2. Adsorption/Desorption of NaPGA on PAMAM Dendrimer SAM

Aqueous NaPGA solutions (0.02 wt%) were prepared and adjusted to pH 3–9 by adding aqueous HCl or NaOH solution. PAMAM dendrimer SAM substrates were
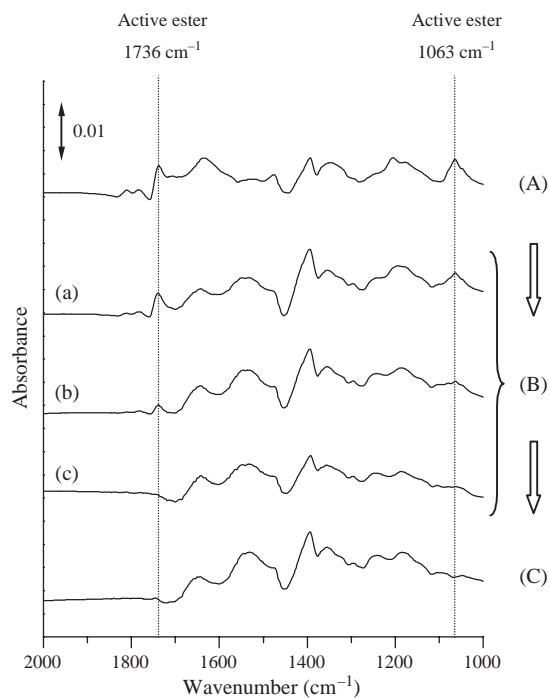
Fig. 3. SEIRA spectra of the preparation process of PPI dendrimer SAM. Effect of dendrimer concentration and ethanolamine treatment. (A) Active ester SAM prepared in an aqueous solution of NHS/EDC (0.013 M). (B) PPI dendrimer SAM prepared from different PPI concentrations: (a) 0.001 wt%; (b) 0.01 wt%; (c) 0.1 wt%. (C) PPI dendrimer SAM after immersed in an aqueous 1 M solution of ethanolamine to remove unreacted active ester (SAM was prepared from an aqueous 0.001 wt% solution of PPI dendrimer).

Chart 2. Flow chart of NaPGA adsorption/desorption.

immersed into the NaPGA solutions. After reaction for 30 min, substrates were rinsed with water. This process is illustrated in Chart 2. Figure 4 shows SEIRA spectra of NaPGA on PAMAM dendrimer SAM. In this measurement, PAMAM dendrimer SAM was used as background. Amide I and II bands of NaPGA appeared at 1658 and 1550 cm^{-1}, respectively. It was indicated from the absorbance increase of these bands that the adsorbed amount of NaPGA increased with lowering the pH. A band at 1715 cm^{-1} assigned to the C=O stretching vibration mode of the hydrogen-bonded COOH group of PGA intensified, as well as amide I and II bands, with lowering the pH. This means that at low pH, PGA adsorbs by hydrogen bonding between the COOH of NaPGA and terminal NH^{3+} and/or amide NH group of the PAMAM dendrimer (see Chart 3).

On the other hand, poor adsorption of NaPGA occurred at high pH. At pH 9.2, higher than the pK_a ($\approx$4.8) of PGA,[25] a carboxyl C=O band was replaced by bands of antisymmetric and symmetric COO$^-$ stretching vibration modes at 1568 and 1430 cm^{-1}, respectively. This suggests that although NaPGA can adsorb on dendrimer SAM by the electrostatic interaction between the COO$^-$ of NaPGA and NH^{3+} of dendrimer at a pH lower than the pK_a ($\approx$9.2) of the terminal amine (see Chart 3),[25] a majority
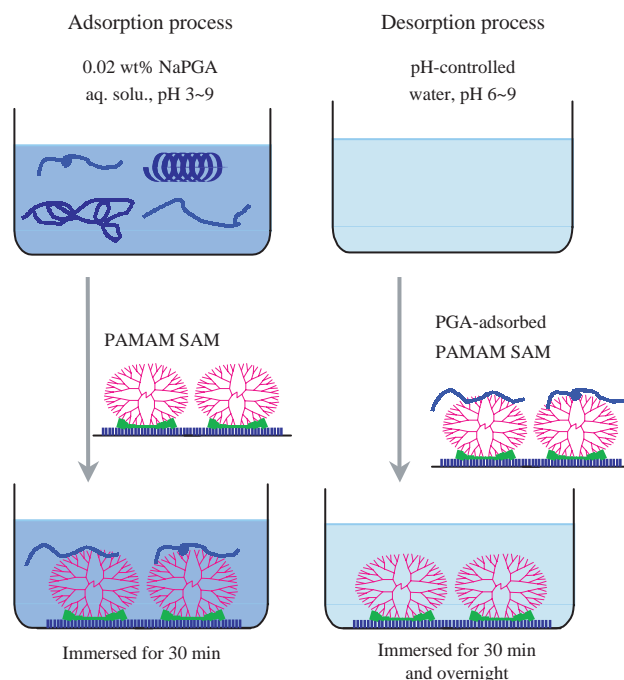
of NaPGA prefers to be free in solution because of hydrophilicity of NaPGA. This is consistent with the behavior of complex formation in solution.[23, 24] Small amounts ($\approx$13%) of dendrimers bound electrostatically to NaPGA
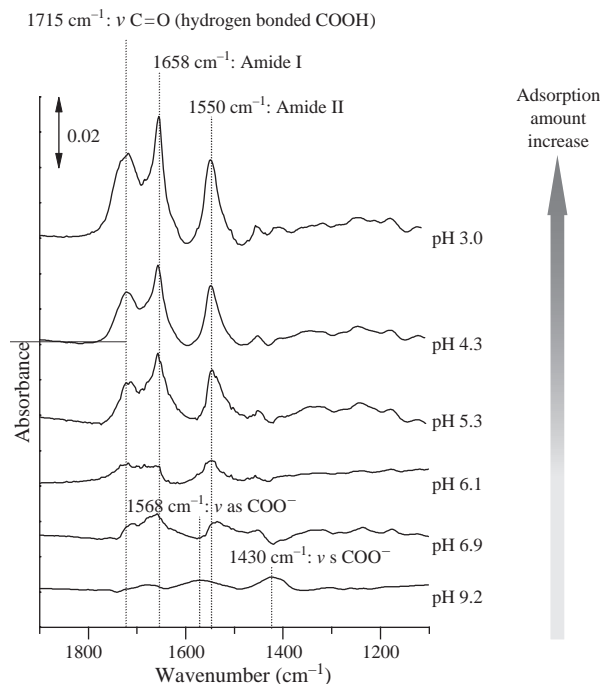

Fig. 4. SEIRA spectra of NaPGA on PAMAM dendrimer SAM after adsorption. Conditions of the adsorption process are given in Chart 2. Dendrimer SAM was prepared through the treatment of MPA SAM by an aqueous solution (0.013 M) of EDC/NHS and a methanol solution (0.01 wt%) of PAMAM dendrimer.
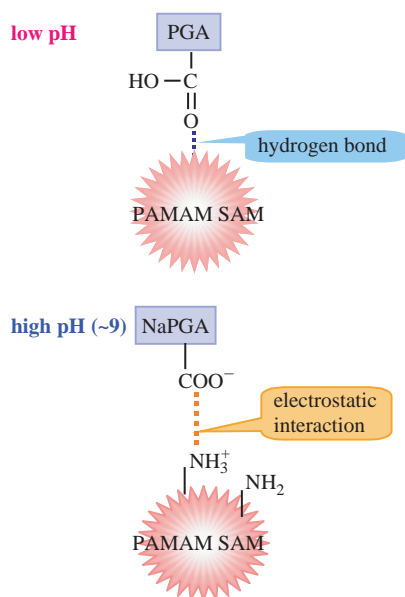
RESEARCH ARTICLE

Chart 3. Illustration of interaction.

Fig. 5. SEIRA spectra of NaPGA on PAMAM dendrimer SAM after desorption. Conditions of the desorption process are given in Chart 2. PGA-adsorbed PAMAM dendrimer SAM was prepared through the treatment of PAMAM dendrimer SAM by an aqueous solution (0.02 wt%) of NaPGA at pH 4.2.

in solution at high pH, but liquid–liquid phase separation due to strong complexation occurred with lowering the pH.

Adsorption is mainly associated with two types of interactions, electrostatic and hydrogen bonding. In the present system, the latter was superior. It is assumed that at a pH higher than pK_a of PGA, charged COO^- was solvated and most of the NaPGA preferred to exist in the solution bulk phase rather than interact with PAMAM dendrimer SAM. In contrast, at low pH, neutral PGA was hydrogen-bonded with the PAMAM dendrimer. Even after 1 week, NaPGA solutions down to pH 4 were transparent, though a solution at pH 3 was turbid. Thus, adsorption of NaPGA was caused by the interaction with dendrimer SAM, but not precipitation of PGA.

PGA-adsorbed PAMAM dendrimer SAM substrate prepared at pH 4.2 was immersed into aqueous solutions at high pH (5.8, 7.7, 9.4). After reaction for 30 min or overnight, the substrate was rinsed with water (see Chart 2). As seen in Figure 5, the amount of NaPGA after the desorption reaction decreased down to a characteristic adsorption amount in the adsorption process at each pH (see Fig. 4), suggesting the complete progress of the desorption process. Since the absorbance of amide I and II bands after desorption for 30 min scarcely changed after reaction overnight, the desorption process was mostly achieved during the initial 30 min at all pHs examined. On the other hand, at pH 7.7 and 9.4, absorbance of the carboxyl C=O stretching band at 1715 cm^{-1}, which exists at the adsorption for 30 min, decreased after reaction overnight. Simultaneously, bands of antisymmetric and symmetric COO^- stretching vibration modes appeared at 1568 and 1430 cm^{-1}, respectively. Thus, the COOH group, which was maintained at least for 30 min on the desorption process at pHs higher than the pK_a of carboxylate,
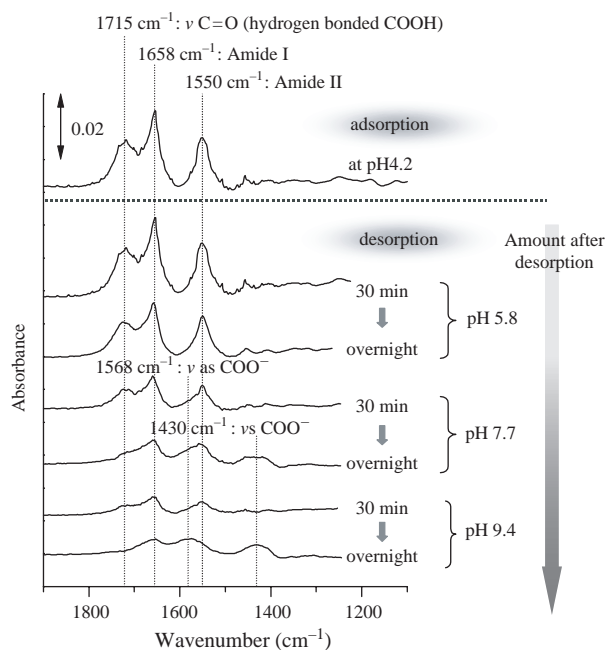
changed to COO^- during the time after the desorption was over. After desorption, the substrate was again immersed into freshly prepared NaPGA solution at pH 4.2. It was found that SEIRA bands produced the same intensity as the first adsorption. This means the adsorption/desorption process is reproducible.

4. CONCLUSIONS

In the present work, we have reported the preparation of dendrimer SAM by covalent bonding on a MPA SAM substrate, which enables the dendrimer to immobilize on the substrate. Interfacial reaction of NaPGA on dendrimer SAM has indicated some attractive behavior beyond the complexation in solution: The adsorption/desorption reaction was reversible under pH control and reproducible for successive reactions. The present report mentions not only basic examination of dendrimer SAM as a reaction matrix but also one of the applications of dendrimer SAM as an available receptor.

References and Notes

1. D. A. Tomalia, A. M. Naylor, and W. A. Goddard, *Angew. Chem. Int. Ed. Engl.* 29, 138 (**1990**).
2. C. J. Hawker and J. M. J. Fréchet, *J. Am. Chem. Soc.* 112, 7632 (**1990**).
3. J. M. J. Fréchet, *Science* 263, 1710 (**1994**).
4. H. Tokuhisa and R. M. Crooks, *Langmuir* 13, 5608 (**1997**).

5. H. Tokuhisa, M. Zhao, A. A. Baker, V. T. Phan, D. L. Dermody, M. E. Garcia, P. F. Peez, R. M. Crooks, and T. M. Mayer, *J. Am. Chem. Soc*. 120, 4492 (**1998**).

6. M. Wells and R. M. Crooks, *J. Am. Chem. Soc.* 118, 3988 (**1996**).

7. M. Schlupp, T. Weil, A. J. Berresheim, U. M. Wiesler, J. Bargon, and K. Müllen, *Angew. Chem. Int. Ed.* 40, 4011 (**2001**).

8. S. Chen, Q. Yu, L. Li, C. L. Boozer, J. Homola, S. S. Yee, and S. Jiang, *J. Am. Chem. Soc.* 124, 3395 (**2002**).

9. H. C. Yoon, M.-Y. Hong, and H.-S. Kim, *Langmuir* 17, 1234 (**2001**).

10. H. Nagaoka and T. Imae, *Trans. Mater. Res. Soc. Jpn.* 26, 945 (**2001**).

11. H. Nagaoka and T. Imae, *Int. J. Nonlinear Sci. Numerical Simulation* 3, 223 (**2002**).

12. M. Osawa, *Bull. Chem. Soc. Jpn.* 70, 2861 (**1997**).

13. T. Imae and M. Dekker, *Encyclopedia Surf. Colloid Sci.* 3547, (**2002**).

14. Y. Li, P. L. Dubin, R. Spindler, and D. A. Tomalia, *Macromolecules* 28, 8426 (**1995**).

15. H. Zhang, P. L. Dubin, R. Spindler, and D. A. Tomalia, *Ber. Bunsenges. Phys. Chem.* 100, 923 (**1996**).

16. H. Zhang, P. L. Dubin, J. Ray, G. S. Manning, C. N. Moorefield, and G. R. Newkome, *J. Phys. Chem. B.* 103, 2347 (**1999**).

17. N. Miura, P. L. Dubin, C. N. Moorefield, and G. R. Newkome, *Langmuir* 15, 4245 (**1999**).

18. V. A. Kabanov, A. B. Zezin, V. B. Rogacheva, Zh. G. Gulyaeva, M. F. Zansochova, J. G. H. Joosten, and J. Brackman, *Macromolecules* 32, 1904 (**1999**).

19. V. A. Kabanov, V. G. Sergeyev, O. A. Pyshkina, A. A. Zinchenko, A. B. Zezin, J. G. H. Joosten, J. Brackman, and K. Yoshikawa, *Macromolecules* 33, 9587 (**2000**).

20. C. L. Gebhart and V. A. Kabanov, *J. Contr. Rel.* 73, 401 (**2001**).

21. T. Imae, T. Hirota, K. Funayama, K. Aoi, and M. Okada, *J. Colloid Interface Sci*. 263, 306 (**2003**).

22. A. Mitra and T. Imae, *Biomacromolecules* 5, 69 (**2004**).

23. D. Leisner and T. Imae, *J. Phys. Chem. B* 107, 8078 (**2003**).

24. T. Imae and A. Miura, *J. Phys. Chem. B* 107, 8088 (**2003**).

25. D. Leisner and T. Imae, *J. Phys. Chem. B* 107, 13158 (**2003**).

26. D. Leisner and T. Imae, *J. Phys. Chem. B* 108, 1798 (**2004**).

27. Z. Zhang and T. Imae, *J. Colloid Interface Sci.* 233, 99 (**2001**).

28. Z. Zhang and T. Imae, *J. Colloid Interface Sci.* 233, 107 (**2001**).

RESEARCH ARTICLE