Notes

Multilayer Adsorption and Molecular Organization of Rigid Cylindrical Glycoconjugate Poly(phenylisocyanide) on Hydrophilic Surfaces

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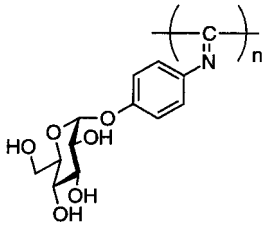
Received June 16, 1999
Revised Manuscript Received November 22, 1999

Introduction

Considerable attention has been given to organized thin films with well-controlled molecular orientations, spacings, and distributions.[1,2] The organization of functional polymers in thin films has been developed by various adsorption techniques including the Langmuir−Blodgett films.[3] Recently, a novel strategy of alternate adsorption using combinations of oppositely charged polyions[4,5] and of recepter proteins−polymeric ligands[6,7] has been developed to form ultrathin film assemblies. Layer-by-layer accumulation of polymer molecules on solid substrates has been attained by the strategy, but two-dimensional orientation of the accumulated polymer molecules is still difficult. On the other hand, the mesoscopic patterning of surfaces of cast films has been widely investigated in view of potential electro- and photochemical applications.[8] Two-dimensionally regulated mesoscopic structures have been attained by self-organization of rigid rodlike molecules. In this respect, we are interested in regulating the molecular orientation in adsorption films by self-organization of rigid cylindrical polymers.

We have been engaged in synthesis and functional analysis of various types of saccharide-carrying polymers.[9−13] These glycopolymers are useful as a tool to investigate molecular recognition because the clustered saccharide chains along the polymer backbones can bind strongly to carbohydrate-binding proteins. We reported that glycosylated polystyrenes were in flexible cylindrical conformations in water owing to the amphiphilic characters and they were adsorbed strongly to hydrophobic surfaces from aqueous solutions.[11,12] Hence, glycosylated polystyrenes were effective as biomedical materials employing the clustered glyco-signals on solid surfaces. However, α-glucosylated poly(phenylisocyanide)[13] (**1**) in Chart 1 exhibited little specific interactions with lectins, despite the crowded saccharide arrays. These glucosylated poly(phenylisocyanide)s have three-dimensionally regulated, rigid cylindrical backbones. Hence, it is assumed that the saccharides are crowded too thickly to be accessible or to be induced-fit to the binding sites. This paper reports that the glycopolymers exhibit unique adsorption and self-organization on hydrophilic surfaces.

Chart 1. Structure of α-D-Glucosylated Poly(phenylisocyanide) (1).



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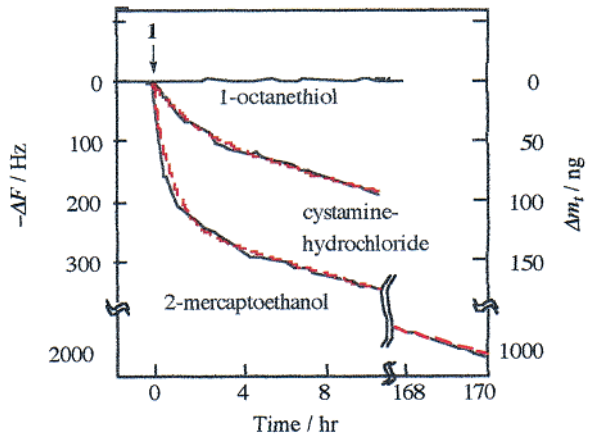

Figure 1. Time courses of frequency change by adsorption of **1** on Au surfaces modified with 1-octadecanethiol, cystamine hydrochloride, and 2-mercaptoethanol. [**1**] = 1.28 mM in deionized water, at 25 °C. Black solid lines: experimental results. Red dotted lines: fitting profiles based on eq 1, using parameters $\Delta m_\infty = 35.9$ ng, $\tau = 1.66$ h, and $k = 8.18$ h^{-1} for cystamine hydrochloride and $\Delta m_\infty = 101.8$ ng, $\tau = 0.62$ h, and $k = 9.44$ h^{-1} for 2-mercaptoethanol, respectively.

Results and Discussion

Adsorption of **1** was investigated with the QCMs, which had been immobilized with hydrophobic and hydrophilic monolayers by treating the gold electrode surfaces of QCMs with mercapto derivatives. Black solid lines in Figure 1 show the time courses of frequency changes of the QCMs in aqueous polymer solutions. A rapid decrease of frequency was observed for hydrophilic 2-mercaptoethanol and cystamine hydrochloride monolayers, but no frequency change was observed for the hydrophobic 1-octadecanethiol monolayer. This is in contrast to the adsorption behavior of flexible glycosyl-

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10.1021/ma990956y CCC: $19.00
Published on Web 03/15/2000

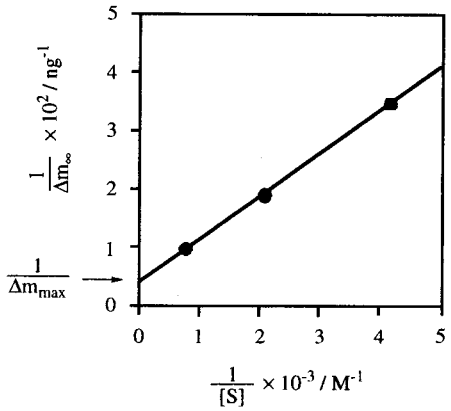


Figure 2. Plot of 1/[S] vs $1/\Delta m_\infty$ obtained by QCM measurements and the subsequent curve-fitting analysis based on eq 1 for 2-mercaptoethanol at several concentrations of **1**.

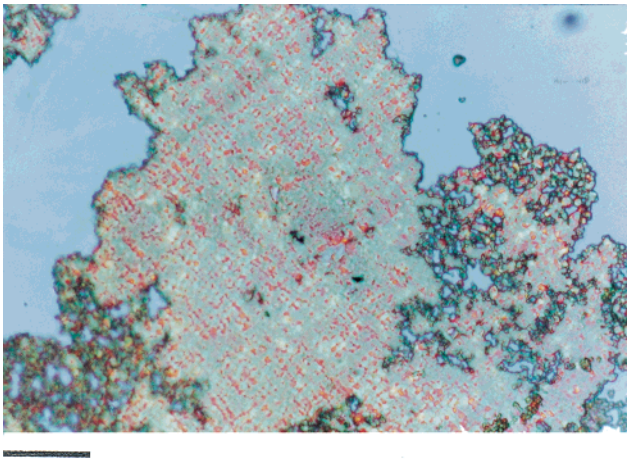


Figure 3. Polarizing microscopic image of **1** adsorbed on a mica substrate from water (through a sensitive color plate). The lower left bar is 50 μm.

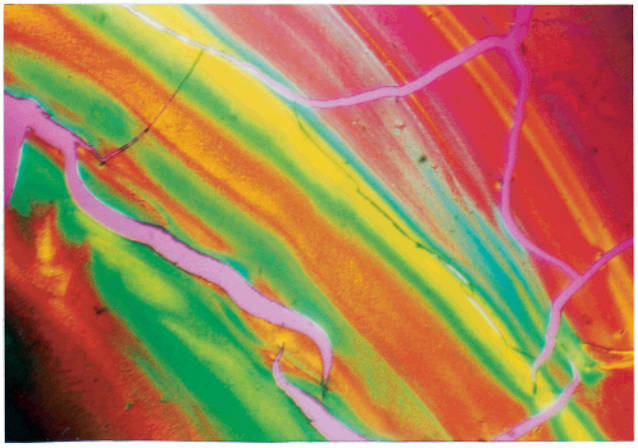


Figure 4. Polarizing microscopic image of the acetylated precursor of **1** cast on a glass substrate from chloroform (through a sensitive color plate). The lower left bar is 100 μm.

ated polystyrenes,[12] which were adsorbed only on hydrophobic surfaces but not on hydrophilic surfaces. We assume that the drastic change in the adsorption behavior was brought about by the steric regulation of the main chain structure.

For the QCM immobilized with the 2-mercaptoethanol monolayer, the initial rapid decrease was gradually converted to a slow and monotonic decrease within 5 h, and the slow decrease was continued for more than 170 h to result in 1 μg of total adsorption. Thus, it seems that the glycopolymer was adsorbed as multilayers onto the hydrophilic surface. The adsorption profiles were in good accordance with eq 1 in which the first term is the

$$\Delta m_t = \Delta m_\infty \{1 - \exp(-t/\tau)\} + kt\{1 - \exp(-t/\tau)\} \quad (1)$$

first layer adsorption in pseudo-first-order kinetics and the second term is the subsequent multilayer adsorption, where Δm_∞, τ, and k indicate the equilibrium mass change ($t = \infty$) for the first monolayer adsorption at a certain concentration of **1**, the relaxation time for the first layer adsorption, and the kinetic constant for multilayer adsorptions, respectively.

The QCM measurements and the subsequent curve-fitting analysis for several concentrations of **1** enabled us to estimate the equilibrated mass (Δm_∞) for the first layer at the concentrations. The plots of 1/[S] vs $1/\Delta m_\infty$ gave a linear relationship in accordance with eq 2 as

$$1/\Delta m_\infty = 1/\Delta m_{max} + 1/K_a \Delta m_{max}[S] \quad (2)$$

shown in Figure 2, where [S], Δm_{max}, and K_a indicate the concentration of **1** based on the monomeric unit, the saturated mass change for the first monolayer adsorption, and the association constant of **1** for 2-mercaptoethanol monolayer, respectively. The saturated mass change (Δm_{max}) and the association constant (K_a) were estimated from the slope and intercept of the linearized plot to be 240 ng and 5.8×10^2 M^{-1}, respectively.

The molecular dynamics calculation showed that **1** takes a rigid cylindrical conformation of 2.0 nm diameter in water.[13] If it is assumed that the cylindrical macromolecular chains are adsorbed horizontally in a closely packed fashion, the estimated maximum mass of one monolayer adsorption is 32 ng. Hence, the observed saturated mass for the first monolayer adsorption was 8 times the estimated one, and about 30 layers of the cylinders were adsorbed after 170 h. The observed large monolayer mass probably resulted from the aggregation of the glycopolymer molecules in water and the subsequent adsorption to the surface. The aggregation of the glycopolymer in water was suggested by the molecular weight (M_w) determined by the light scattering. The observed M_w (6.2×10^5) of **1** in water was about 7 times that of the theoretical M_w, which was calculated from that (1.5×10^5) of the acetylated precursor in chloroform.[14] The observed M_w was reduced to 1.9×10^5 in 6.0 M urea aqueous solution, suggesting that hydrogen-bonding network plays an important role to build up the aggregates.

Microscopic observations of the adsorption and self-organization of **1** have been attempted by using mica as a substrate. Since a smaller amount of **1** was adsorbed to mica than to the hydrophilic 2-mercaptoethanol monolayer, the photographs were taken by immersing mica substrates in a rather concentrated aqueous solution (37.5 mM). Mesoscale mosaic domains (5 μm^2 each) were observed on polarizing microscopic photographs (Figure 3), suggesting that the macromolecular chains were regularly oriented in each domain. Similar molecular organization was more easily achieved by casting **1** from an aqueous solution and also casting the peracetylated precursor of **1** from a chloroform solution. The polarization of the latter sample, shown in Figure 4, did not disappear even on heating to 300 °C, which was the upper limit of the apparatus.

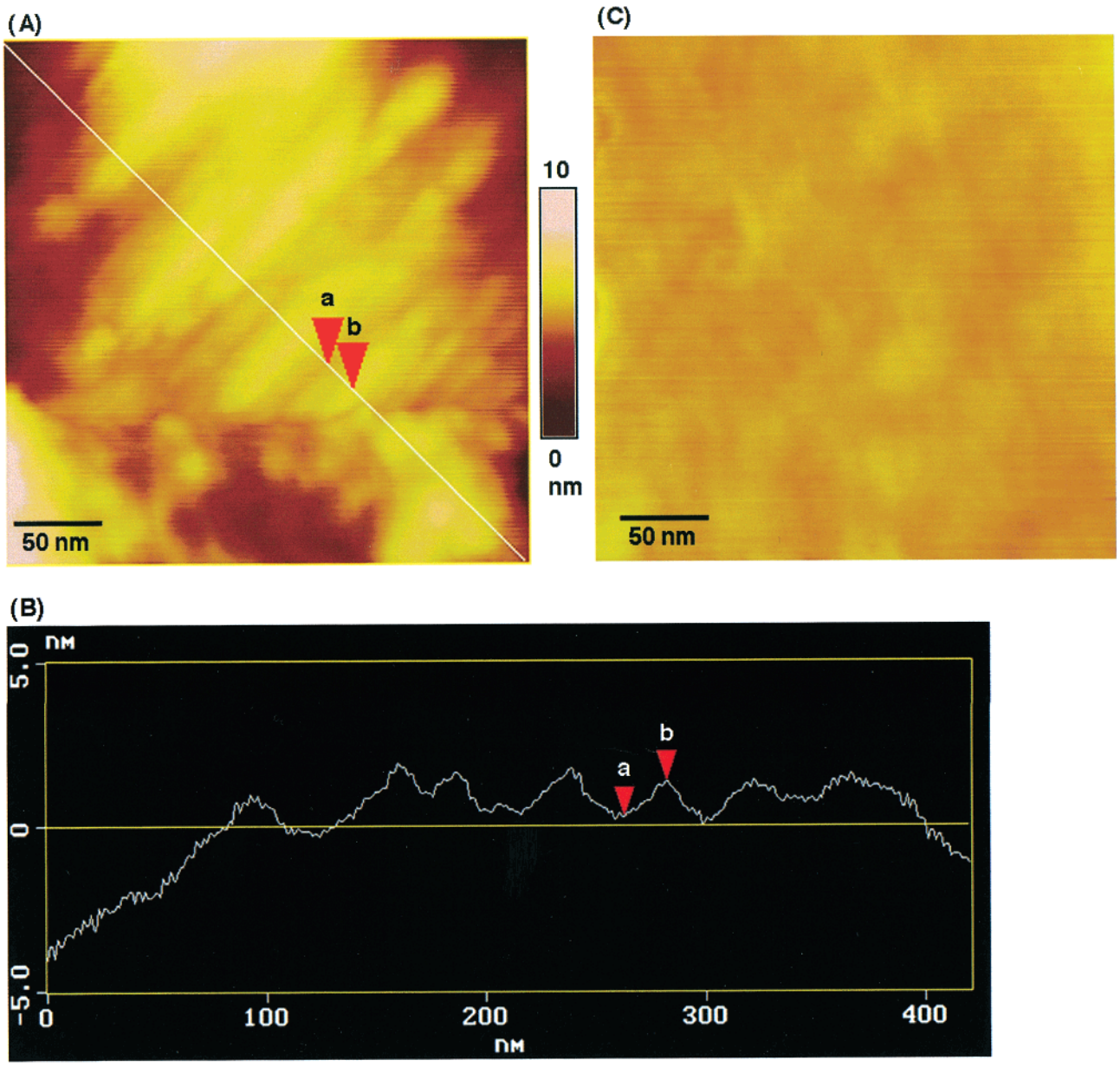
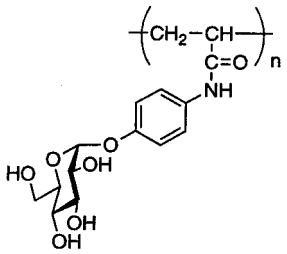


Figure 5. AFM images of cast films on a mica substrate from water: (A) top view of a cast film of **1**; (B) cross-section profile of A; (C) top view of a cast film of flexible polyacrylamide-type polymer.

Although atomic force microscope (AFM) images of adsorbed films of **1** on mica substrates could not be obtained owing to the low coverage, an AFM image of a cast film was observed as shown in parts A and B of Figure 5. Rigid rod or cylindrical structures lie horizontally on the surface with the same orientation. The diameter of the structures was 20−30 nm, suggestive of the aggregation of **1**. On the other hand, none of the regulated structures were detected on the cast films of the flexible polyacrylamide-type glycopolymers (Chart 2, Figure 5C). We propose a mechanism of self-organization of **1** on the substrate as illustrated schematically in Figure 6. Self-organization of **1** has been attained via aggregation of several chains of rigid rodlike molecules in water followed by adsorption to the substrates.

It is reasonable to assume that the two-dimensionally oriented multilayer adsorption is caused by hydrogen bondings. Hydrogen bondings in aqueous solutions are usually too weak to induce such spontaneous adsorption. However, amplification of hydrogen bondings could be

Chart 2. Structure of Flexible Polyacrylamide-Type Glycopolymer Bearing α-D-Glucoside Moieties.



attained through the multivalency and structural rigidity of α-glucosylated poly(phenylisocyanide) which has highly regulated cylindrical conformation, as proposed for glyco-calixarenes.[15]

In conclusion, multilayer thin films, oriented horizontally and two-dimensionally on hydrophilic surfaces in an aqueous solution, could be constructed from α-glucosylated poly(phenylisocyanide) (**1**). A rigid rodlike structure and spatially regulated saccharide chains

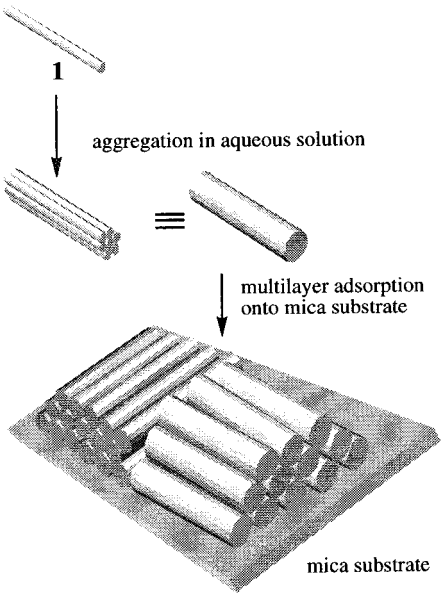


Figure 6. Schematic illustration of the proposed mechanism of regulated molecular orientation of **1** on a mica substrate.

were essential to induce the adsorption and self-organization using amplified multivalent hydrogen-bonding as the driving force. This has been achieved by the stereoregulation of the glycopolymer backbone structure.

Experimental Section

Materials. α-Glucosylated poly(phenylisocyanide) was prepared by the polymerization of peracetylated α-glucosyl phenylisocyanide with $NiCl_2(H_2O)_6$ in methanol/chloroform (1:1) at room temperature and subsequent deacetylation as reported elsewhere.[13]

Measurements. The surfaces of gold electrodes of QCMs (9-MHz, AT-cut, Sogo Pharmaceutical Co., Ltd.) were treated with 2-mercaptoethanol, cystamine hydrochloride, and 1-octadecanethiol. The QCM was immersed in 20 mL of water, the frequency was monitored, and then, an aqueous solution of **1** was injected into the water layer. A QCM was connected to an oscillation circuit (USI System, Fukuoka). The frequency changes were counted by a frequency counter (Hewlet Packard 53131A universal counter) and recorded with a microcomputer system. A frequency decrease of 1 Hz corresponded to a mass increase of 0.5 ng on the QCM electrode in an aqueous solution, according to the calibration using the Sauerbrey equation.[16] Polarizing microscopic images were taken by an Olympus BH-2 polarizing microscope. AFM images were taken by a Nanoscope IIIa (Digital Instruments, Santa Barbara, CA) with tapping mode in air. The normal spring constant was 20−100 Nm^{-1}. Drive frequency was 300 kHz. Scanning speed was at a line frequency of 1 Hz with 256 pixels per line.

Acknowledgment. We are grateful to Professor Y. Okahata of Tokyo Institute of Technology for the AFM measurements and to Professor T. Yamane and Associate Professor A. Suzuki of Nagoya University for the polarizing microscopic measurements.

References and Notes

(1) Fuhrhop, J.-H.; Koning, J. *Membrane and Molecular Assemblies, The Synthetic Approach*; Royal Society of Chemistry: London, 1994.
(2) Vogtle, F. *Supramolecular Chemistry*; Wiley: Chichester, England, 1993.
(3) Ulman, A. In *An Introduction to Ultrathin Organic Films from Langmuir-Brodgett to Self-Assembly*; Ulman, A., Ed.; Academic Press: New York, 1991.
(4) Lvov, Y.; Decher, G.; Mohwald, H. *Langmuir* **1993**, *9*, 481.
(5) Ariga, K.; Lvov, Y.; Kunitake, T. *J. Am. Chem. Soc.* **1997**, *119*, 2224.
(6) Lvov, Y.; Ariga, K.; Ichinose, I.; Kunitake, T. *J. Chem. Soc., Chem. Commun.* **1995**, 2313.
(7) Muller, W.; Rump, E.; Wildburg, G.; Zhang, X.; Angermaier, L.; Knoll, W.; Liley, M.; Spinkle, J. *Science* **1993**, *262*, 1706.
(8) Okahata, Y.; Kobayashi, T.; Tanaka, K.; Shimomura, M. *J. Am. Chem. Soc.* **1998**, *120*, 6165.
(9) Kobayashi, K.; Kobayashi, A.; Tobe, S.; Akaike, T. In *Neoglycoconjugates*; Lee, Y. C., Ed.; Academic Press: San Diego, CA, 1994; p 261.
(10) Kobayashi, K.; Tsuchida, A.; Usui, T.; Akaike, T. *Macromolecules* **1997**, *30*, 2016.
(11) Wataoka, I.; Urakawa, H.; Kobayashi, A.; Schmidt, M.; Kajiwara, K. *Macromolecules* **1998**, *31*, 1816.
(12) Matsuura, K.; Tsuchida, A.; Okahata, Y.; Akaike, T.; Kobayashi, K. *Bull. Chem. Soc. Jpn.* **1998**, *71*, 2973.
(13) Hasegawa, T.; Kondoh, S.; Matsuura, K.; Kobayashi, K. *Macromolecules* **1999**, *32*, 6595.
(14) The molecular sizes of the hydroxylated and acetylated polymers were also compared by SEC, which was carried out with use of water and chloroform as eluents and pollulans and polystyrenes as standards, respectively. The estimated M_n (2.0×10^5) of the hydroxylated polymer was larger than that (2.5×10^4) of its acetylated precursor by a factor of 8, whereas their molecular weight distributions ($M_w/M_n = 1.63$ and 1.60 for the hydroxylated and acetylated polymers, respectively) were similar to each other. This is also evidence in support of aggregation of hydroxylated polymer in aqueous solution.
(15) Fujimoto, T.; Shimizu, C.; Hayashida, O.; Aoyama, Y. *J. Am. Chem. Soc.* **1997**, *119*, 6676.
(16) Okahata, Y.; Ebara, Y.; *J. Am. Chem. Soc.* **1994**, *116*, 11209.

MA990956Y