A Quartz-Crystal Microbalance Study of Adsorption Behaviors of Artificial Glycoconjugate Polymers onto Chemically Modified Gold Surfaces and Their Interactions with Lectins

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Adsorption behaviors of two different types of lactose-carrying polystyrenes, poly(*p*-vinylbenzamido-β-lactose) (PNLA, **1**) and poly(*N*-*p*-vinylbenzyllactonamide) (PVLA, **2**), onto cast films of polystyrene and self-assembled monolayers (SAMs) of 1-octadecanethiol and cystamine were investigated by applying a quartz-crystal microbalance (QCM). The amphiphilic artificial glycoconjugate polymers were strongly adsorbed on the hydrophobic surfaces, i.e. a polystyrene cast film and a SAM of 1-octadecanethiol, from their aqueous solution according to the Langmuir adsorption isotherm (the apparent association constant: $K_a \approx 10^7$ M^{-1}). On the other hand, they were adsorbed little on a hydrophilic SAM of cystamine. The saturated adsorption amounts of the polymers on the hydrophobic surfaces were 2—5 times larger than that calculated on the basis of an assumption of closed-packing monolayer adsorption, which suggests that the adsorbed polymers may take a loop-train-tail conformation. A SAM of 1-octadecanethiol adsorbed two times more of each polymer than a polystyrene cast film did. β-Galactoside-specific RCA$_{120}$ and PNA lectins bound to the surfaces coated with the galactose-bearing polymers according to the Langmuir adsorption isotherm ($K_a \approx 10^6$ M^{-1}). The binding was stronger than that observed by the inhibition of hemagglutinating activity (about 10^4 M^{-1}).

Carbohydrates are important in cell-cell molecular recognition as well as in structural elements, storage of energy, and metabolism in living systems.[1)] Despite their importance in these specific recognition processes, individual protein–carbohydrate interactions are of low affinity, and hence multiple receptors must be arranged to bind efficiently to multiple saccharide ligands. Compounds called artificial glycoconjugate polymers, substituted with pendant oligosaccharide moieties as polyvalent recognition signals, have been reported to bind strongly to carbohydrate-binding proteins by multivalency or cluster effects.[2)] Consequently, these glycoconjugate polymers have been used as cell-specific culture substrates, artificial antigens, and targeted drug delivery systems.[2a,2b,2c)] We also have developed artificial glycoconjugate polystyrenes,[3a,3b)] polyacrylamides,[3c)] polysaccharides,[3d)] and polypeptides.[3e)] Our glycosidated polystyrenes were designed to construct amphiphilic structures by arranging hydrophobic main chains and hydrophilic pendant oligosaccharides. They were soluble in water and easily adsorbed on polystyrene microtiter plates owing to the hydrophobicity of the polystyrene backbone. The polymer-coated plates have been used as hepatocyte-specific culture substrates,[4a,4b)] erythrocyte attachment substrates,[4c)] immobilization mediators of proteins,[4d)] and acceptor substrates for measuring enzyme activity.[4e)]

In this paper, we investigated the adsorption behaviors of amphiphilic glycoconjugate polystyrenes on hydrophobic and hydrophilic surfaces, and the molecular recognition of the adsorbed polymers by lectins, using a quartz-crystal microbalance (QCM). Chart 1 shows the chemical structures of two different types of lactose-carrying polystyrenes PNLA **1**[3b)] and PVLA **2**.[3a)] QCM is one of the most useful tools to detect molecular adsorptions onto substrates and to measure in situ mass changes both in gas[5)] and solution[6—9)] phases. This tool has been used for in situ sensors for carbohydrate-lectin interaction,[6)] immuno-reaction,[7)] DNA hybridization,[8)] DNA-protein interaction,[9)] and so on. In this study, we used commercially available AT-cut 9 MHz QCMs. The QCMs were modified with a polystyrene cast film and with a self-assembled monolayer (SAM)[10)] of 1-octadecanethiol as models of hydrophobic surfaces, and with a SAM of cystamine as a model of a hydrophilic surface.

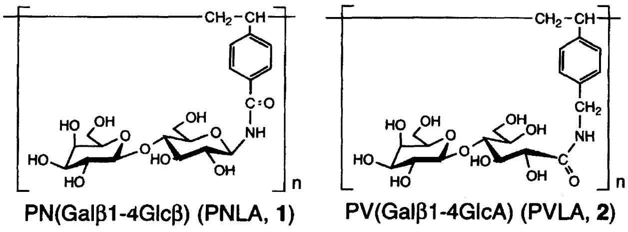


Chart 1. Lactose-carrying glycopolystyrenes.

Experimental

General. The syntheses of PNLA (**1**)[3b] and PVLA (**2**)[3a] were described elsewhere. Number-average molecular weights (M_n) of the polymers were measured with a membrane osmometer Osmomat 090 SA (Gonotec GmbH, Berlin). **1**: $M_n = 5.77 \times 10^4$ and **2**: $M_n = 5.83 \times 10^4$. 1-Octadecanethiol and cystamine were purchased from Tokyo Chemicals. Bovine serum albumin (BSA), RCA_{120} (*Ricinus communis*) lectin, and PNA (peanut, *Arachis hypogaea*) lectin were purchased from Sigma.

QCM Measurement. The QCMs used were commercially available 9 MHz AT-cut quartzes (diameter 9 mm) deposited with Au electrodes on both sides (Sogo Pharmaceutical, Tokyo). A QCM was connected to an oscillation circuit and a frequency counter (Fragrance Sensor SF-105A, Sogo Pharmaceutical) and the frequency changes were 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,[6] according to the calibration using the Sauerbrey equation.[11]

Both sides of the Au electrode of a QCM were washed with a freshly prepared piranha solution of H_2O_2/H_2SO_4 (1/3 v/v) to remove organic adsorbate impurities, and were rinsed with distilled water several times. ***Caution: Piranha solution is very oxidative and dangerous, and direct contact should be avoided.*** The QCM was immediately immersed into an ethanol solution of 1-octadecanethiol or cystamine (1 mM, 1 M = 1 mol dm^{-3}) for 12 h and rinsed with distilled water, giving a SAM-immobilized QCM. A chloroform solution (10 μL) of polystyrene (1 mg mL^{-1}) was cast on the Au electrode, giving a polystyrene-cast QCM. One side of the QCM was covered with a rubber case to avoid contact with water. The modified QCM was immediately used for the following adsorption measurement in water to prevent contamination on the Au electrode.

The QCM was stabilized in water in a 20-mL cell until the drift of the QCM frequency became within ±2 Hz at 25 °C for at least 2 h. An adsorbate was injected into the cell, and the frequency change was recorded until the equilibrium was established. Binding of lectins to the adsorbed artificial glycoconjugate polymers was examined after blocking its remaining hydrophobic area with BSA (0.1 mM) and rinsing with distilled water several times to prevent nonspecific adsorption of the lectins.

Results and Discussion

Adsorption of Artificial Glycoconjugate Polymers onto Chemically Modified Gold Surfaces. Figure 1a shows typical courses of frequency changes (ΔF) of the QCMs that were immobilized with a polystyrene cast film and with SAMs of 1-octadecanethiol and cystamine. The responses were followed in the same concentration (0.8 μM) of PNLA **1** in water at 25 °C. The hydrophobic surfaces, polystyrene cast film and SAM of 1-octadecanethiol, caused significant adsorption of PNLA **1**, which reached equilibrium within 2 h. Contrastingly, the hydrophilic cystamine surface adsorbed little PNLA **1** under the same conditions. When the polymer-adsorbed QCM was immersed in fresh pure water, little frequency change was observed. Thus it has been demonstrated that PNLA **1** was adsorbed strongly on the hydrophobic surfaces and little was desorbed from the surfaces. On the other hand, the corresponding monomer (*p*-vinylbenzamido-β-lactose) of **1** was adsorbed little on both hydrophobic and hydrophilic surfaces even at a higher concentration (0.1 mM) (the data were not shown).

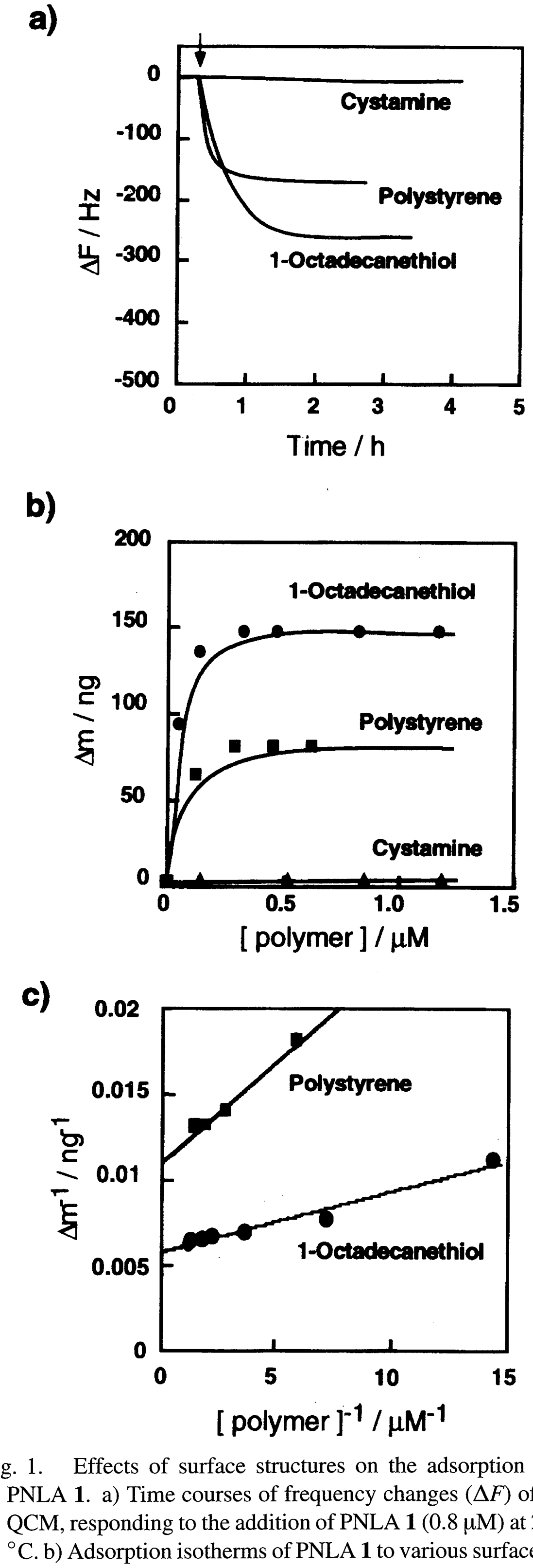


Fig. 1. Effects of surface structures on the adsorption of PNLA **1**. a) Time courses of frequency changes (ΔF) of a QCM, responding to the addition of PNLA **1** (0.8 μM) at 25 °C. b) Adsorption isotherms of PNLA **1** to various surfaces. c) Reciprocal plots.

The adsorption isotherms of PNLA **1** on these surfaces at 25 °C are shown in Figure 1b. Little PNLA **1** was adsorbed on the hydrophilic cystamine surface even in higher concentrations. PNLA **1** was adsorbed on the hydrophobic surfaces according to the Langmuir-type adsorption isotherm, suggesting that PNLA **1** was adsorbed on the hydrophobic surfaces as a monolayer but not as multilayers. The reciprocal plots of the adsorption isotherms provided good linear correlations (Fig. 1c). Apparent association constants (K_a) and maximum adsorption amounts ($\Delta m_{\max}$) of PNLA **1** to the hydrophobic surfaces were calculated from the slopes and intercepts, respectively, according to Eq. 1.

$$\frac{1}{\Delta m} = \frac{1}{K_a \Delta m_{\max}[\text{polymer}]} + \frac{1}{\Delta m_{\max}}. \qquad (1)$$

The K_a and $\Delta m_{\max}$ values of PNLA **1** to the various surfaces are summarized in Table 1, together with those of PVLA **2** and bovine serum albumin (BSA). BSA is a hydrophobic protein that is often used as a blocking reagent to avoid nonspecific interaction on hydrophobic plates. The apparent association constants K_a of PNLA **1** and PVLA **2** to both hydrophobic surfaces were about 10^6—10^7 (M of polymer molecule)$^{-1}$, which were comparable with or higher than those of BSA. On the other hand, **1** and **2** were adsorbed little on the cystamine surface, although BSA was strongly ($K_a = 4.5\times10^6$ M^{-1}) adsorbed also on the hydrophilic cystamine surface. The $\Delta m_{\max}$ values of **1** and **2** to the hydrophobic surfaces were within the range of 90—234 ng. If we assume that the monomer units of **1** and **2** were adsorbed as closed-packing monolayers, we can roughly calculate $\Delta m_{\max}$ = 40—50 ng. Therefore the observed $\Delta m_{\max}$ values were 2—5 times the $\Delta m_{\max}$ values predicted on the closed-packing monolayer adsorption. It is reasonable to assume that these polymers take a loop-train-tail conformation on the hydrophobic surfaces in agreement with the conformation of most of flexible polymers adsorbed on surfaces.[12] Since these polymers may be adsorbed as monolayers, but not as multilayers, on the hydrophobic surfaces as mentioned above, we propose a conformation model illustrated in Fig. 2.

The observed $\Delta m_{\max}$ values of these polymers to the SAMs of 1-octadecanethiol are about 2 times that to the polystyrene cast film. A possible explanation of the difference is the irregularity of the surface structures of the polystyrene cast films, although the question still remains.

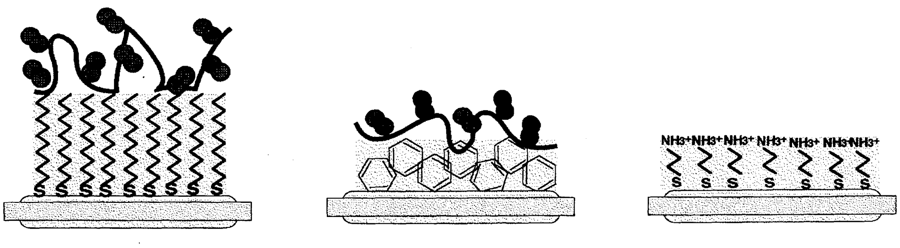
It has been demonstrated by a small-angle X-ray scattering (SAXS) study[13] that these polymers in water take a rod-like conformation which consists of hydrophobic polystyrene moieties as the core and hydrophilic sugar moieties as the outer shell. Thus, the polymeric conformation must change when the polymer chain in an aqueous solution is adsorbed on hydrophobic surfaces. As shown in Table 1, PVLA **2** was adsorbed to the hydrophobic surfaces more strongly and in larger quantity than PNLA **1**. This may be due to some differences in mobility and flexibility between these polymers, since these polymers have the same polystyrene main chain and similar molecular weight (**1**: $M_n = 5.77\times10^4$ and **2**: $M_n = 5.83\times10^4$.

Interaction between Artificial Glycoconjugate Polymers and Lectins. The interaction of galactose-specific lectins was investigated with glycopolymer-coated surfaces on SAMs of 1-octadecanethiol on QCMs. When the glycopolymer-coated surface was treated with BSA, the adsorption of the lectins was observed with $\Delta m_{\max}\approx30$ ng. This indicates that there still remained a hydrophobic area of the glycopolymer-coated surface and the area could be blocked with BSA as illustrated in Fig. 3d. In the absence of the polymer, BSA was adsorbed on the SAM surface with $\Delta m_{\max} = 58$ ng. This also suggests that the glycopolymers on the SAM did not form a closely-packed adsorption but a loop-train-tail

Table 1. K_a[a] and $\Delta m_{\max}$ in Adsorption of Glycopolymers on Various Surfaces

	1-Octadecanethiol		Polystyrene		Cystamine	
Polymer	K_a/M^{-1}	$\Delta m_{\max}$/ng	K_a/M^{-1}	$\Delta m_{\max}$/ng	K_a/M^{-1}	$\Delta m_{\max}$/ng
PNLA (**1**)	1.7×10^7 (1.4×10^5)	169	9.5×10^6 (7.9×10^4)	90	< 5000	0
PVLA (**2**)	3.1×10^7 (2.6×10^5)	234	4.1×10^7 (3.4×10^5)	115	< 5000	0
BSA	3.3×10^6	58	9.9×10^5	97	4.5×10^6	90

a) K_a was calculated using molarity (mol L^{-1}) of the polymer molecule and the value in the blacket using molarity (mol L^{-1}) of the monomeric unit.



a) 1-Octadecanethiol   b) Polystyrene   c) Cystamine

Fig. 2. Illustrations of the adsorptions of the glycopolystyrenes onto various surfaces.

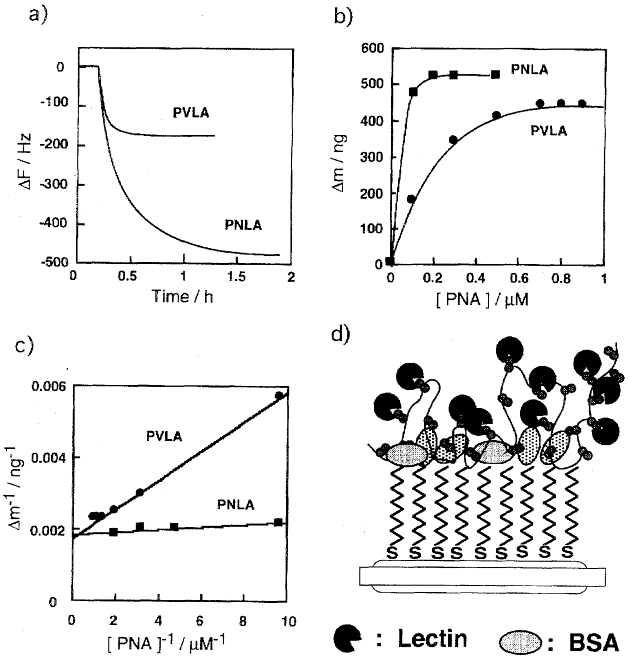


Fig. 3. Interaction of PNA with lactose-carrying polystyrenes **1** and **2** on the 1-octadecanethiol surface. a) Time courses of frequency changes (ΔF) of a QCM, responding to the addition of PNA (0.1 μM) from an aqueous solution (25 °C). b) Saturation binding behavior of PNA to polymer. c) Reciprocal plots. d) Illustration of the interaction between lectin and glycopolymer on the hydrophobic surface.

type adsorption.

PNA lectin was added to these QCMs coated with both glycopolymers and BSA. The courses of frequency changes (ΔF) of the QCM were traced in Fig. 3a. A larger amount of PNA lectin was found to bind to PNLA-coated QCM than to PVLA-coated QCM. The binding isotherms and their reciprocal plots are shown in Figs. 3b and 3c, respectively. The apparent binding constants (K_a) and maximum binding amounts ($\Delta m_{\max}$) of PNA lectin to the polymers were calculated from the slopes and intercepts, respectively. These binding data of galactose-specific lectins, PNA and RCA_{120}, with PNLA **1** and PVLA **2** are summarized in Table 2, which also the results of inhibition of hemagglutionation. Since the immobilized amount of PVLA **2** on the SAM was about twice of that of PNLA, the maximum binding amounts of lectins were normalized as $\Delta m^n_{\max}$ (g mol^{-1}) by dividing $\Delta m_{\max}$ by the mole amount of the sugar moiety of adsorbed polymer. These lectins were little bound to the BSA-coated surface, and hence we could detect specific binding of the lectins to the sugars tethered to the polymers.

The observed $\Delta m_{\max}$ values of the lectins to the adsorbed polymers were much larger than the predicted and observed values (below 100 ng)[6] on the adsorbed glycolipid (ganglioside, GM3 and GM3 lactone) monolayer on a QCM. This also suggests that the adsorbed polymers on the SAM of 1-octadecanethiol take a loop-train-tail conformation. The adsorbed glycoconjugate polymers strongly bound each lectin with $K_a = 10^6$—10^7 M^{-1}, which was much larger than the reciprocal of the minimum inhibitory concentration (about 10^{-4} M) of the polymers for hemagglultination.[3c] This suggests that the galactose-specific lectins bind the polymers adsorbed on the hydrophobic surface more strongly than the polymer in an aqueous solution. We assumed that the loop-train-tail conformation of the adsorbed polymers brought about more efficient multipoint binding with lectins.

Both the apparent binding constant (K_a) and normalized maximum binding amount ($\Delta m^n_{\max}$) of PNA lectin to PNLA **1** were larger than those to PVLA **2**, while these values of RCA_{120} lectin were similar in the two polymers. It is known that PNA lectin recognizes Galβ1-3GalNAc disaccharide[14] and RCA_{120} lectin recognizes Galβ monosaccharide.[15] Therefore we assumed that PNA lectin recognized the closed glucopyranose ring structure in PNLA **1** more strongly than the open chain gluconamide structure in PVLA **2**, but RCA_{120} lectin did not recognize the difference of these glycoconjugate polymers.

In conclusion, it was found that amphiphilic artificial glycoconjugate polymers, PNLA **1** and PVLA **2**, were adsorbed preferentially on hydrophobic polystyrene cast films and SAMs of 1-octadecanethiol surfaces with $K_a \approx 10^7$ M^{-1}, but not on hydrophilic SAMs of cystamine surfaces. The adsorbed glycoconjugate polymers recognized galactose-specific lectins strongly according to the Langmuir adsorption isotherm with $K_a \approx 10^6$ M^{-1}. We assumed that the adsorbed

Table 2. K_a[a] and $\Delta m_{\max}$ in Interaction between Lectins and Glycopolymers on the 1-Octadecanethiol Surfaces

Lectin	Coating polymer	K_a/M^{-1}	$\Delta m_{\max}$/ng	$\Delta m^n_{\max}$[a]/g mol^{-1}	MIC[b]/M
	PNLA (**1**)	9.6×10^7	267	758	5×10^{-4}
RCA_{120}	PVLA (**2**)	2.5×10^7	528	745	4×10^{-4}
	BSA	$< 10^5$	< 10	—	—
	PNLA (**1**)	6.8×10^7	270	648	3×10^{-4}
PNA	PVLA (**2**)	5.2×10^6	275	453	7×10^{-4}
	BSA	$< 10^5$	< 10	—	—

a) Normalized maximum binding amount was estimated by dividing $\Delta m_{\max}$ by the mole amount of sugar moiety of adsorbed polymer. b) Minimum concentration of polymer for inhibition of hemagglutination.

glycoconjugate polymers on the hydrophobic surfaces take a loop-train-tail conformation for the following reasons. The $\Delta m_{\max}$ values of the polymers on the surface were larger than the $\Delta m_{\max}$ values predicted on the closed-packing monolayer adsorption. The amount of galactose-specific lectins bound to the adsorbed polymer were much larger than the predicted and observed amounts of the lectins bound to the adsorbed glycolipid monolayer. The quartz-crystal microbalance (QCM) is useful as a tool to investigate adsorption behaviors and conformations of glycopolymers. The information is essential for development of composite materials.

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