A QCM Study on Adsorption of Macrocyclic Sugar-Cluster to Variously-Functionalized Monolayers

Katsuhiko Ariga,# Kouta Isoyama, Osamu Hayashida,† Yasuhiro Aoyama,† and Yoshio Okahata*
Department of Biomolecular Engineering, Tokyo Institute of Technology, 4259 Nagatsuda, Midori-ku, Yokohama 226-8501
†Institute for Fundamental Research of Organic Chemistry, Kyushu University, Hakozaki, Higashi-ku, Fukuoka 812-8581

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Adsorption of the aqueous sugar-cluster molecule **1** to variously-functionalized monolayer was evaluated using a quartz crystal microbalance (QCM). The molecule **1** favorably adsorbed to the anionic (acidic) and OH-condensed lipid monolayers.

Clustered sugar residues are thought to be important as a recognition site on biological membrane surface for protein or lectin bindings,[1-3] and mimic systems have been investigated.[4,5] We have developed macrocyclic sugar-cluster **1**,[6-8] which has eight sugar residues with galactose terminals, four hydrocarbon branches, and a cyclic core of calix[4]resorcarene, as a model of clustered sugar residues (see Figure 1). Investigation on adsorption of **1** to various surfaces will give important implication in understanding of cell adhesion and recognition mechanisms. Parts of authors have been already demonstrated that the cluster can bind to silanol OH[6] and polysaccharides[8] through accumulated hydrogen bonding in water and to rectins[7,8] through the specific recognition. However, further systematic researches must be done to obtain more simple and general information. In this paper, we report adsorption behavior of **1** to variously-functionalized monolayers using a quartz crystal microbalance (QCM).

A QCM is known as a microbalance to give ng-level sensitivity in mass detection. Its frequency is decreased proportionally to mass increase on its electrodes. We have already reported that this device is quite useful to analyze adsorption behavior at the monolayer surface.[4,5] A QCM used in this study (AT-cut, Showa Crystals Co., Chiba) has 27 MHz of parent frequency to give sensitivity of 0.62 ng cm^{-2} Hz^{-1}. Monolayer of amphiphiles[9] listed in Figure 1 was spread on pure water at 20 ± 0.2 °C and was compressed to 30 mN m^{-1}. One side of a QCM plate was covered with rubber to avoid contacting with water and the uncovered side was put horizontally on the compressed monolayer from air.[4] This monolayer-attached QCM plate was transferred into a small vessel in water. This procedure leads to immobilization of the monolayer on the QCM with facing polar head of monolayer to water phase (see Figure 2). After equilibrating the system at 20 ± 0.2 °C, guest molecule (**1** or octyl lactoside) was injected in the water and the QCM frequencies were monitored continuously. Since the frequency fluctuation was only within ± 5 Hz, we can evaluate subnanogram adsorption per cm^2.

Time-course of frequency depended on the kind of monolayer, but the most cases reached adsorption equilibrium within 30–60 min. Therefore, the adsorption was evaluated from frequency change at equilibrium. Typical frequency changes are shown in Figure 2. When 5 μM of **1** was injected to the aqueous phase containing the monolayer of **7** on the QCM, frequencies were decreased and reached equilibrium with changes of 218 Hz

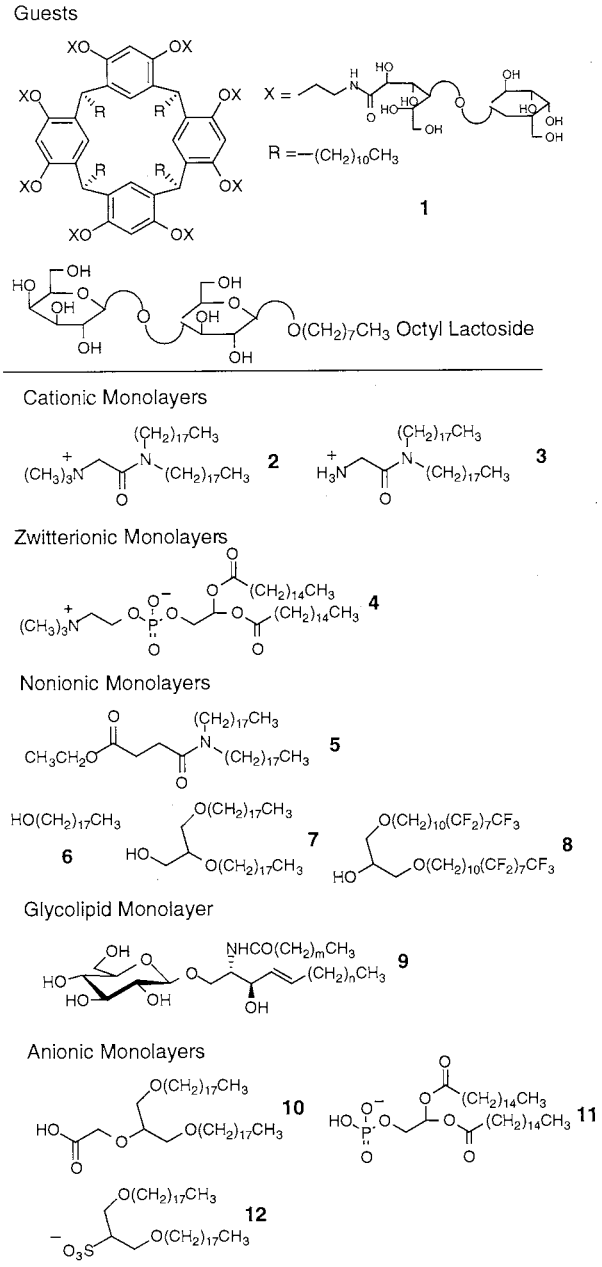

Figure 1. Structures of guests and amphiphiles used in this study.

corresponding to adsorption of 135 ng cm^{-2}. In contrast, adsorption of **1** to the cationic monolayer of **2** was hardly detected. Adsorption amounts (Δm) of 5 μM (40 μM of sugar

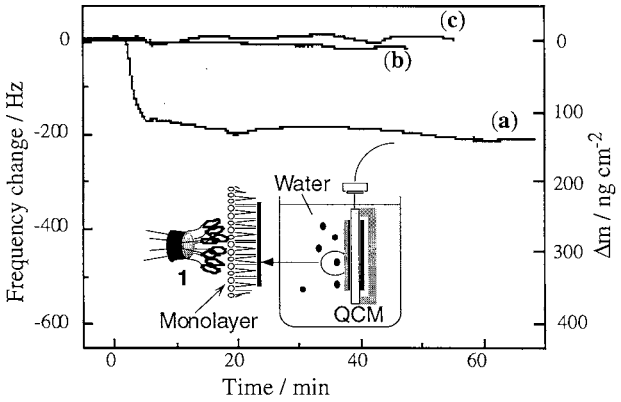

Figure 2. Frequency changes upon adsorption. (a) 5 mM of **1** to monolayer of **7**, (b) 40 mM of octyl lactoside to monolayer of **7**, and (c) 5 mM of **1** to monolayer of **2**. Inserted figure represents schematic image of the measurement.

Table 1. Adsorption amount (Δm) of **1**[a] to variously-functionalized monolayers

Monolayer		Δm[b] (ng cm^{-2})
Cationic	**2**	0
	3	0
Zwitterionic	**4**	0
Nonionic	**5**	90
	6	180
	7	135
	8	30
Glyco	**9**	270
Anionic	**10**	270
	11	210
	12	210

[a] Concentration of **1** was 5 mM corresponding to 40 mM of sugar residues.
[b] Adsorption amount at equilibrium.

residues) of **1** to variously-functionalized monolayers are summarized in Table 1. The adsorption behavior of **1** largely depended on the surface of the monolayers. Monomeric sugar molecule (octyl lactoside) did not adsorb onto any monolayers tested as seen in a curve (b) of Figure 2. This result strongly suggests that clustering of sugar residues are necessary in effective adsorption.

Adsorption of **1** to these monolayers showed interesting tendency. Adsorption to cationic and zwitterionic surfaces (**2, 3,** and **4**) were hardly detected, while all the anionic (acidic) surfaces (**10**, **11**, and **12**) can accommodate **1** with almost monolayer-coverage: maximum packing density of **1** (0.28 molecule nm^{-2}, see reference 6) gives monolayer coverage of 225 ng cm^{-2}. It suggests that the cluster **1** forms slightly cationic surface. Hydrogen bond formation between neighboring two OH groups might produce δ^+ on remaining hydrogen through -O-H···O-H interaction as seen in resorcinol crystal systems.[10] Our previous report[6] showed strong adsorption of **1** to acidic silanol glass surface and the adsorption was broken only by the addition of other bases. Molecule **1** also showed high affinity to anionic dyes. These former results also suggest that clustered sugar residues induce the positively-charged surface, resulting in the high affinity to the anionic surface.

Adsorption of **1** to the nonionic surface significantly depends on hydrophilic head groups of monolayer surface. Ethyl ester (**5**) did not show any affinity to **1**, implying that the observed adsorption is not mainly driven by hydrophobic interactions. Monolayers of alcohol derivatives **6**, **7**, and **8** showed weaker affinity to **1**, and the adsorption amount increased in order of **8** (30 ng cm^{-2}) < **7** (130 ng cm^{-2}) < **6** (180 ng cm^{-2}). This tendency can be explained by density of OH groups, *i.e.*, the most slender **6** can produce highest density of OH leading to the greatest adsorption.[11] Glycolipids **9** showed higher affinity to **1** than these alcoholic monolayers probably due to higher density of OH groups. However, further experiments with various glycolipids showed more complicated dependency on sugar derivatives (data not show). Selectivity of **1** to glycolipids cannot be simply explained by structure of single sugar residues and it may be influenced by nature, orientation, and density of exposed OH groups at membrane surfaces.

In conclusion, adsorption behavior of macrocyclic sugar-cluster **1** to variously-functionalized surface was investigated. The cluster prefers to adsorb to anionic (acidic) surfaces and OH-condensed surfaces rather than cationic or zwitterionic surfaces. The obtained results might lead to new understanding of molecular recognition on sugar-clustered surface.

References and Notes

Present address: Graduate School of Materials Science, Nara Institute of Science and Technology (NAIST), 8916-5 Takayama, Ikoma 630-0101.

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9 Lipids **5** (Nichiyu Liposome Co.) and **9** (Snow Brand Milk Products Co.) were used as purchased. Details of syntheses of the other amphiphiles will be reported in future publication.

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11 Molecular area of **6** at 30 mN m^{-1} is 0.2 nm^2 molecule^{-1} (5 OH group nm^{-2}). Adsorption amount of **1** (180 ng cm^{-2}) gives sugar density of 1.8 residue nm^{-2}. Therefore, several OH groups probably interact with one sugar residue of **1**.