Two-Dimensional Molecular Imprinting:
Binding of Sugars to Boronic Acid Functionalized, Polymerized Langmuir–Blodgett Films

Takashi Miyahara and Kazue Kurihara*
*Institute for Chemical Reaction Science, Tohoku University, 2-1-1 Katahira, Aoba-ku, Sendai 980-8577*

A molecular imprinted surface was prepared by polymerizing the Langmuir film of a mixture of two polymerizable amphiphiles, one of which has a boronic acid group as a binding site for a template molecule, 4-nitrophenyl α-D-mannopyranoside (**3**), dissolved in the aqueous subphase. The imprinted surface exhibited an improved affinity (35%) for the template molecule **3** in 2-butanone compared with the non-imprinted surface.

The molecular imprinting method has been actively used to prepare preorganized binding sites which can specifically recognize a given molecule such as sugars[1] and steroids.[2] Most of them were constructed in three-dimensional polymer matrices or on silica gel particles in order to be used for separation, catalyses, etc. Two-dimensionally organized monolayers are known to have a potential for designing well-defined, unique molecular recognition sites due to their confined geometry.[3,4] Imprinted monolayers should provide novel matrices which could be used for sensors, assay plates, or crystal nucleation.[5] However, only the attempts to imprint cavities on self-assembled monolayers aimed at the size discrimination have been reported.[6] The incorporation of functional groups, which can interact with guest molecules, into monolayers is necessary for designing host surfaces for a wide variety of guest molecules. In this study, we achieved the monolayer imprinting for a sugar derivative using a boronic acid functionalized amphiphile.
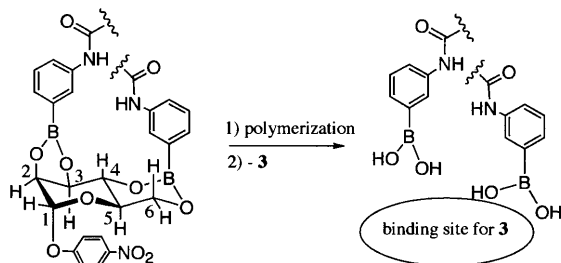
A host monolayer was prepared by polymerization of the 1:2[7] mixture of 3-(10',12'-pentacosadiynamido)phenylboronic acid (**1**)[8] and 10,12-pentacosadiynoic acid (**2**). The amphiphile **1** has a boronic acid group, which can form a cyclic boronic acid ester with a diol at high pH or by dehydration. As a guest molecule, a template, 4-nitrophenyl α-D-mannopyranoside (**3**)[1,9] was chosen because of (i) the two possible binding sites, 2,3-*cis*-diol and *trans*-4-OH·5-CH$_2$OH for the boronic acid group of **1**, and (ii) the 4-nitrophenoxy group which makes it possible to quantify **3** by UV–visible spectroscopy.

First, we examined the behavior of the Langmuir films in the presence of **3** in the aqueous subphase at pH 11 (KOH). A solution of **1**, **2**, or the 1:2 mixture of **1** and **2** in chloroform was spread onto the water surface of a Langmuir trough (USI system,
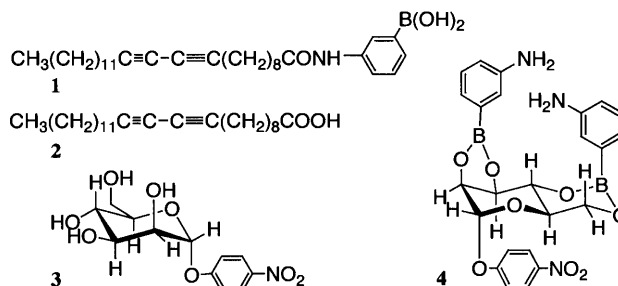
FSD 50). As shown in Figure 1, the surface pressure–area (π–A) isotherm of **1** exhibited an expanded phase with a molecular area of 0.23 nm^2 at π = 20 mN/m (A_{20}). The addition of 1.00×10^{-4} M **3** further expanded the π–A isotherm with an A_{20} of 0.24 nm^2, suggesting that the boronic acid groups interacted with **3** at the air–water interface. On the other hand, the π–A isotherm of **2** at pH 11 was hardly affected by the addition of **3** (the isotherm with **3** not shown), which should be reasonable because no interaction is expected between the caboxyl group of **2** and the hydroxyl group of **3** at pH 11. The π–A isotherm of the 1:2 mixture of **1** and **2** at pH 11 (Figure 1d) showed no effect by **3** on the isotherm and the A_{20} remained at 0.24 nm^2 (the isotherm with **3** is not shown). The density of **1** in the mixed film was one-third of that in its one component film and the boronic acid group may have enough space to form a cyclic ester without expansion.
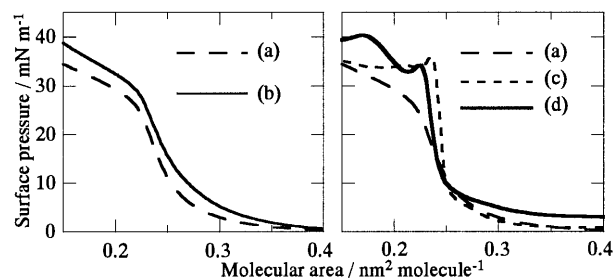

Figure 1. Surface pressure-molecular area isotherms at pH 11, 20.0 $\pm$ 0.1 °C: (a,b) **1**, (c) **2**, and (d) the 1:2 mixture of **1** and **2**. Subphase: (a,c,d) in the absence of **3**; (b) in the presence of **3** (1.00×10^{-4} M).

Subsequently, the binding of **3** to the monolayers deposited onto quartz plates was investigated. Quartz plates (12.5 $\times$ 45 $\times$ 1.25 mm) were made hydrophobic by depositing a monolayer of dimethyldioctadecylammonium (DODA) bromide at π = 35.0 mN/m. The imprinted monolayer was prepared with 1.00×10^{-4} M **3** in the subphase, whilst the non-imprinted monolayer (a reference) in the absence of **3**. Ten minutes after the spreading, the monolayer was compressed to π = 20.0 mN/m which was maintained for 10 min. The monolayer was irradiated at 254 nm with a UV lamp (200 μW/cm^2), which was placed about 2 cm above the monolayer, for 3 min to let the amphiphiles polymerize.

During the polymerization the area of the monolayer was decreased by only 5%, which supported that the arrangement of the functional groups was hardly changed by polymerization. The polymerized monolayer was transfered to the quartz plate by horizontal lifting method. The UV–visible absorption spectra of the plate thus obtained showed two peaks at 500 and 540 nm, which is characteristic of polydiacetylene films in the red form[10] and the absorbance of 0.012 at 540 nm was comparable to the results reported previously.[4] The absorbance at 540 nm was decreased by only 2–3% and no significant shifts of the peaks were observed through the procedure described below. These facts warrant that the polydiacetylene monolayer on the plate was stable and did not undergo molecular rearrangement.

It is known that cyclic boronic acid esters are rapidly formed at room temperature by mixing equimolar amounts of a boronic acid and a diol in acetone solution.[11] Similarly, it is reasonable to expect that **3** easily binds to the boronic acid functionalized surface in a form of cyclic boronic acid ester in 2-butanone at room temperature.[12] The fixation and quantification of **3** on the boronic acid modified plate was carried. The plate was immersed in a 2.0×10^{-3} M solution of **3** in 2-butanone (25 mL) for 12 h to let **3** be adsorbed. The plate was washed vigorously in 2-butanone (25 mL) for 10 s and blown dry with a stream of nitrogen. The UV–visible spectrum of the plate was recorded, which was named spectrum I (Figure 2a). Spectrum I showed a shoulder around 300 nm, assigned to the adsorbed **3**. Subsequently, the plate was immersed in pure water (25 mL) for 10 min to remove the adsorbed **3**[13] and blown dry with a stream of nitrogen. The UV–visible spectrum of the plate was recorded again (spectrum II, Figure 2b). In spectrum II, the shoulder around 300 nm observed in spectrum I disappeared, indicating the complete removal of **3**.
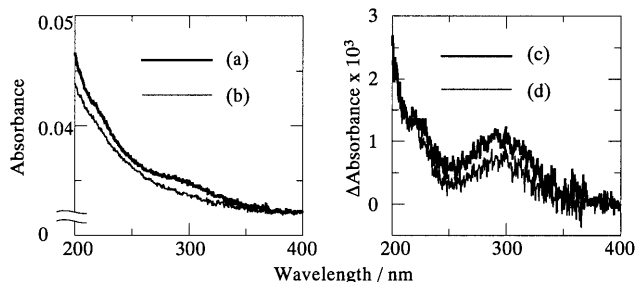

Figure 2. UV-visible spectra of the non-imprinted boronic acid modified plate: (a) spectrum I and (b) spectrum II. Differential UV-visible spectra of spectrum I from II: (c) the imprinted boronic acid modified plate and (d) the non-imprinted boronic acid modified plate.

The differential spectrum I minus II[14] was similar as the UV–visible spectrum of **3** dissolved in water although the peak at 306 nm observed in water was shifted to 295 nm (the spectrum of **3** in water not shown). The amount of the adsorbed **3** was estimated from the difference. In order to confirm the reproducibility, five pairs of the imprinted and the non-imprinted surfaces were prepared and separately tested; the average values were used for comparison. The absorbance of the differential spectrum obtained from the imprinted plate was $(8.9 \pm 0.7) \times 10^{-4}$ (Figure 2c). Using the molar extinction coefficient of **3** in water, 1.03×10^4, at 306 nm, the molecular density of **3** on the imprinted surface was estimated to be 0.52 ± 0.04 molecule/nm^2.[15] Assuming that all of the boronic acid groups captured the **3** mole-

cules in a 2:1 ratio, the molecular density of the captured **3** is 0.69 $(= 1/(2 \times 0.72))$ molecule/nm^2. Therefore, it can be presumed that 75 percent of the binding sites of the imprinted surface were occupied by **3** during the immersion in the 2-butanone solution. On the other hand, the absorbance of the differential spectrum from the non-imprinted plate was $(6.6 \pm 0.7) \times 10^{-4}$, which meant that the molecular density of **3** was 0.39 ± 0.04 molecule/nm^2 and the coverage of the binding sites on the non-imprinted surface was only 57 percent. The comparison of the amount of adsorbed **3** showed that the imprinted surface possessed an improved affinity for the template molecule **3** in 2-butanone by 35 percent. This increase can be attributed to the appropriate arrangement of the amphiphiles for **3** in the imprinted Langmuir film. The rotation of the head groups of **1** can allow the boronic acid groups to adjust finely to the optimum orientation for **3**.

In conclusion, the molecular imprinted surface has been prepared which has an improved affinity for the template molecule. More versatile molecular imprinted surfaces can be designed by combining more than two kinds of functional groups.

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7　We performed similar experiments for the 1:1, 1:3, and 1:4 mixture of **1:2**. In the former case, both of the absorbances of the adsorbed **3** on the imprinted and the non-imprinted monolayer were 1.1×10^{-3} and no imprinting effect was obserbed. For the two latters, the absorbances were too small ($< 5 \times 10^{-4}$) to examine.
8　The condensation of 10,12-pentacosadiynoyl chloride and 3-aminophenylboronic acid produced **1** in 80% yield. **1**: ^{1}H NMR (400MHz, DMSO-d_6) δ 9.77 (1H, s), 7.99 (2H, s), 7.80 (1H, s), 7.70 (1H, d, $J = 8.3$ Hz), 7.43 (1H, d, $J = 7.3$ Hz), 7.22 (1H, t, $J = 7.7$ Hz), 2.26 (6H, m), 1.56 (2H, quintet, $J = 6.4$ Hz,), 1.42 (4H, m), 1.22 (26H, apparent two peaks), 0.83 (3H, t, $J = 6.8$ Hz).
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12　It was confirmed that 4'-nitrophenyl α-D-mannopyranoside-2,3;4,6-di-O-(3"-aminophenylboronate) (**4**) was quantitatively prepared by the dehydration (azeotropic distillation) of the 1:2 mixture of **3** and 3-aminophenylboronic acid in dioxane.
13　The cyclic boronic acid ester **4** was rapidly decomposed to the starting materials in the presence of water.
14　The UV–visible spectrum recorded just before being immersed in the solution of **3** in 2-butanone was not the same as spectrum II because the DODA monolayer deposited on the back side was dissolved in 2-butanone. Therefore, the spectrum II was used as the blank.
15　The molar extinction coefficient of **3** adsorbed on the boronic acid modified plate was assumed to be the same as that of **3** in water. Abs. $= \epsilon\,cd = 10^{17}\epsilon\,D/N_A$, where ϵ, c, d and D are molar extinction coefficient, concentration [mol/dm^3], optical path length [cm], and molecular density [molecule/nm^2], respectively.