Selective Guest Binding onto Nucleic Acid Base Monolayers Immobilized on a Highly Sensitive Quartz-Crystal Microbalance in Gas Phase

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Self-assembled monolayers bearing a thymine or adenine base as a terminal group were immobilized on a highly sensitive (63 MHz) quartz-crystal microbalance. Selective binding processes of guest molecules were obtained as mass increases (frequency decreases) in gas phase.

Molecular recognition by hydrogen bond is one of fundamental processes in biological systems, and many model systems have been proposed in host-guest chemistry and supramolecular chemistry. The host-guest interactions involving hydrogen bonding have been studied mostly in organic solutions[1] and recently at the air-water interface.[2] Molecular recognition in the gas phase would be most simple and fundamental system devoid of solvent effects.[3,4] A quartz-crystal microbalance (QCM) is obviously one of useful techniques to detect directly molecular binding process by measuring mass changes in gas phase.[5-7]

In this paper, we report kinetics of gas phase molecular recognition on a self-assembled monolayer of decanethiol having thymine **1** and adenine **2** at the terminal-position using a highly sensitive (63 MHz) quartz-crystal microbalance (QCM) (see Figure 1).

A 9-MHz, AT-cut QCM was connected to a handmade oscillator designed to drive the quartz at the 7-th overtone (63 MHz).[8] Frequency changes were followed by a universal counter (Hewlett Packard Co., Ltd., Tokyo, model 53131A) attached to the microcomputer system. Calibration of the 7-th overtone 9-MHz QCM showed that 0.11 ng of substrate binding corresponds to 1 Hz of frequency decrease, which was consistent with Sauerbrey equation.[5-8]

The QCM having two Au electrodes on both sides (16 mm^2 x 2) was immersed into 5 mM ethanol solution of the 10-(thymin-1-yl)-1-decanethiol **1** for 12 h. After rinsing with ethanol and Milli-Q water, the QCM was dried and kept under N_2 atmosphere. The frequency was decreased by 870 ± 10 Hz (mass increase, Δm = 100 ± 3 ng) by immobilizing the monolayer **1**. The theoretical mass of the monolayer on two gold electrodes was calculated to be 110 ng, if the surface roughness was assumed to be about 2. These values indicate that the Au electrode was covered as a monolayer of thymine derivative. Similarly, the adenine **2** and decane **3** monolayers were also immobilized onto the QCM.

The monolayer immobilized QCM was set in a flow cell (70 cm^3), in which a mixture of saturated vapor of guest molecules and dry N_2 gas was flowed at a rate of 2 L/min and at various concentrations of guest molecules.

Figure 2 shows typical time courses of frequency changes of a 63 MHz QCM immobilized with the thymine monolayer **1** responding to exposure of the same concentration (1.4×10^{-6} M) of 2-aminopyridine, γ-butyrolactam and aniline in gas phase at 25 °C. We used here 2-aminopyridine and γ-butyrolactam as models of adenine and thymine molecules, respectively, since nucleic acid bases are difficult to be vaporized in air at normal temperature and pressure. An adenine model of 2-aminopyridine

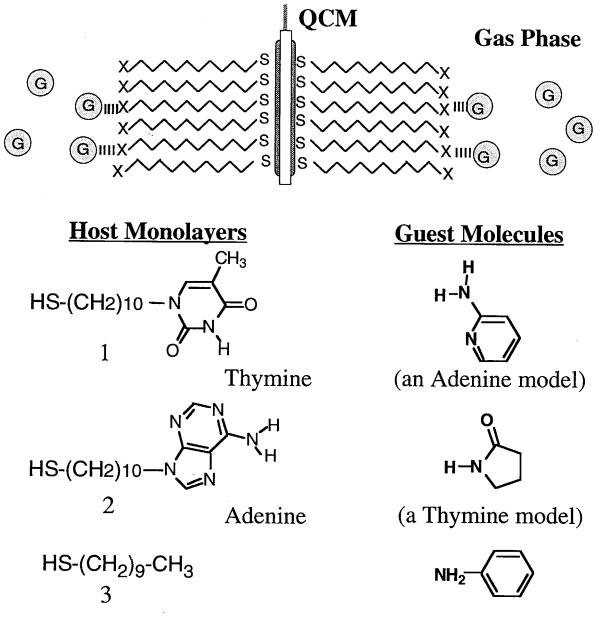


Figure 1. Schematic illustration of binding of guest molecules from gas phase onto a self-assembled monolayer immobilized on a highly sensitive 63 MHz quartz-crystal microbalance (QCM).

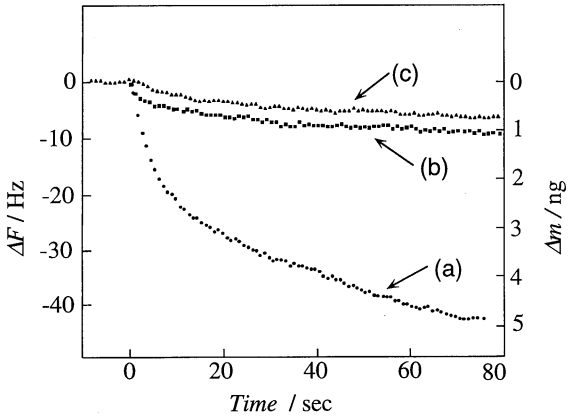


Figure 2. Time courses of frequency changes of the QCM immobilized with the thymine monolayer **1**. (a): 2-Aminopyridine, (b) γ-Butyrolactam, and (c) Aniline. Gas phase concentration of guests was 1.4×10^{-6} M at 25 °C.

fairly bound onto the thymine monolayer **1** probably due to complementary two-point hydrogen bonding ability with the host membrane. In spite of bearing two-point hydrogen bonding ability with thymine, γ-butyrolactam (a thymine model) hardly adsorbed onto the thymine monolayer **1**, as well as aniline that would bind by one-point hydrogen bond with the thymine membrane.

The time course of binding behavior of guest molecules onto a host membrane is expressed as following equations.

$$[\text{Host}] + [\text{Guest}] \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} [\text{Host/Guest}] \quad (1)$$

$$\Delta m_t = [\text{Host/Guest}]_t = \Delta m_\infty \{1 - \exp(-t/\tau)\} \quad (2)$$

$$\text{where } \tau^{-1} = k_1[\text{Guest}] + k_{-1} \quad (3)$$

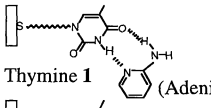
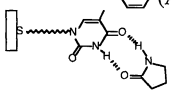
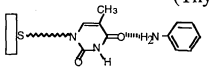
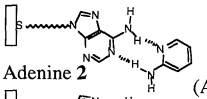
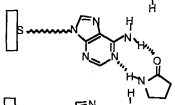
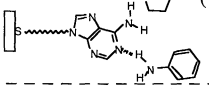
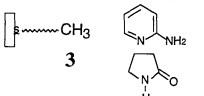
The binding and dissociation rate constants (k_1 and k_{-1}, respectively) were obtained from eq. 3 at several different concentrations of guest molecules. Association constants (K_a) were obtained from k_1/k_{-1}, and kinetic parameters are summarized in Table 1.

Selective binding between the thymine monolayer **1** and 2-aminopyridine (an adenine model) showed about 100 times larger K_a value than other host-guest combinations. This large selectivity of K_a value is due to the larger binding rate constant (k_1) and the smaller dissociation rate constant (k_{-1}) than those of non-selective bindings. The similar selective binding kinetics were observed in the combination between the adenine monolayer **2** and a thymine model of γ-butyrolactam, although the difference of K_a value is not so large (5-20 times). When the self-assembled monolayer of 1-decanethiol **3** was used, all of these guest molecules were hardly bound to the alkane membrane. These results clearly indicate that guest molecules selectively bind to the nucleic acid base membrane by using selective hydrogen bonding even in gas phase.

The selectivity (about 10^2 times) for K_a values of the thymine monolayer **1** with 2-aminopyridine in gas phase is comparable to the selectivity reported for association of 1-cyclohexyluracil (U) and 9-ethyladenosine (A) in $CHCl_3$ obtained by IR spectroscopy (K_{AU} = 140 M^{-1} and K_{UU} = 6.1 M^{-1} at 25 °C).[9] However, binding constants obtained in gas phase (10^7 M^{-1}) is very large compared with those obtained in organic solvents (10^2 M^{-1}). This is probably due to devoid of solvent effects in gas phase.

We are currently studying the similar molecular recognition comparing in gas phase, in aqueous solution, in organic media, and at the air-water interface by using the QCM system.

Table 1. Kinetic parameters for binding of guest molecules from gas phase onto the host monolayers at 25 °C

Host membranes	Guests	k_1 / 10^3 $M^{-1}s^{-1}$	k_{-1} / 10^{-2} s^{-1}	K_a / 10^5 M^{-1}
 Thymine **1**	(Adenine model)	**190**	**0.26**	**730**
	(Thymine model)	21	2.7	7.8
		18	5.4	3.3
 Adenine **2**	(Adenine model)	230	25	9.2
	(Thymine model)	**45**	**0.79**	**57**
		19	9.2	2.1
 **3**		7.9	1.2	6.6
		22	6.7	3.3
		6.8	4.7	1.4

References and Notes

1 a) J. Rebek, Jr., *Angew. Chem. Int. Ed. Eng.*, **29**, 245 (1990). b) A. D. Hamilton, *J. Chem. Educ.*, **67**, 821 (1990).

2 a) K. Kurihara, K. Ohta, Y. Honda, and T. Kunitake, *J. Am. Chem. Soc.*, **113**, 5077 (1991). b) R. Ahuja, P.-L. Caruso, D. Möbius, W. Paulus, H. Ringsdorf, and G. Wildburg, *Angew. Chem. Int. Ed. Engl.*, **32**, 1033 (1993).

3 a) I-H. Chu, D. V. Dearden, J. S. Bradshaw, P. Huszthy, and R. M. Izatt, *J. Am. Chem. Soc.*, **115**, 4318 (1993). b) K. D. Schierbaum, T. Weiss, E. U. Thoden van Velzen, J. F. J. Engbersen, D. N. Reinhoudt, and W. Göpel, *Science*, **265**, 1413 (1994).

4 M. Dey, F. Moritz, J. Grotemeyer, and E. W. Schlag, *J. Am. Chem. Soc.*, **116**, 9211 (1994).

5 G. Sauerbrey, *Z. Phys.*, **155**, 206 (1959).

6 M. D. Ward and D. A. Buttry, *Science*, **249**, 1000 (1990).

7 a) Y. Okahata and H. Ebato, *J. Chem. Soc., Perkin Trans. 2*, 475 (1991). b) Y. Okahata, Y. Matsunobu, K. Ijiro, M. Mukae, A. Murakami, and K. Makino, *J. Am. Chem. Soc.*, **114**, 8299 (1992). c) Y. Okahata, K. Yasunaga, and K. Ogura, *J. Chem. Soc., Chem. Commun.*, **1994**, 469. d) Y. Ebara and Y. Okahata, *J. Am. Chem. Soc.*, **116**, 11209 (1994).

8 a) K. Matsuura, Y. Ebara, and Y. Okahata, *Thin Solid Films*, in press. b) Y. Okahata, K. Matsuura, K. Ito, and Y. Ebara, *Langmuir*, in press.

9 Y. Kyogoku, R. C. Lord, and A. Rich, *J. Am. Chem. Soc.*, **89**, 496 (1967).