Nucleobase molecular recognition in supercritical carbon dioxide by using a highly sensitive 27 MHz quartz-crystal microbalance

Toshiaki Mori, Masanobu Naito, Yoshinobu Irimoto and Yoshio Okahata*

Department of Biomolecular Engineering, Tokyo Institute of Technology, 4259 Nagatsuda, Midori-ku, Yokohama 226-8501, Japan. E-mail: yokahata@bio.titech.ac.jp

Received (in Columbia, MO, USA) 14th September 1999, Accepted 8th November 1999

Nucleobase molecular recognition on a self-assembled monolayer was studied in supercritical carbon dioxide and obtained kinetics were compared with those in air and liquid phases.

Molecular recognition is an essential phenomenon in living systems, as observed in complementary hydrogen bond formation in DNA. In recent years, a number of artificial receptors containing base pairing models of DNA have been reported.[1] They confirm selective binding of small guest molecules *via* complementary hydrogen bonds in hydrophobic organic solvents and at the air-water interface, although it hardly occurs in bulk water.[2] Molecular recognition in the gas phase has also been investigated, as it would form a most simple system avoiding any solvation effects on both host and guest molecules.[3] Supercritical fluid is attractive as a third medium, in addition to the liquid and gas phases, because its physical properties (*e.g.* solvation, density, diffusiveness and viscosity) are intermediate between those of a gas and a liquid, and can be manipulated by small changes in pressure or temperature.[4] Several spectral studies of hydrogen bonding interactions[5a–c] and inclusion phenomena[5a,e] have been carried out in supercritical fluid. It is useful to study and compare molecular recognition in these three different states of matter. A quartz-crystal microbalance (QCM) is a useful instrument for detecting directly guest molecular binding processes by measuring mass changes at a host monolayer, as it is independent of media and can be used in an air phase,[3a–c] in aqueous solution,[6] and even in supercritical fluid.

Here we report the binding behavior and kinetics of small guest molecules in supercritical carbon dioxide ($scCO_2$) at a self-assembled monolayer of decanethiol having thymine at the terminus on a highly sensitive 27 MHz QCM (see Fig. 1); these data are then compared with those obtained in the gas phase[3a–c] and in aqueous solution.[6]

A 27 MHz, AT-cut QCM was connected to a handmade oscillator.[6,7] Frequency changes were followed by a universal counter (Hewlett Packard Co., Ltd., model 53131A) attached to a microcomputer system. Calibration of the 27 MHz QCM showed that 0.62 ng cm^{-2} of substrate binding corresponds to 1 Hz of frequency decrease, which was consistent with the Sauerbrey equation[8] and our previous papers.[6,7] The thymine monolayer was immobilized on Au electrodes on both sides (4.9 $mm^2 \times 2$) of the QCM according to the previous papers.[3a–c] The frequency decreased by 500–600 Hz (mass increase, Δm = 310–370 ng cm^{-2}) due to the monolayer immobilization. The theoretical mass of the monolayer on the two gold electrodes was calculated to be 350 ng cm^{-2}, if the surface roughness was assumed to be about 2. These values indicate that the Au electrode was covered with a monolayer of the thymine derivative.

Liquid CO_2 was pumped into a reservoir vessel containing guest molecules at 10–15 MPa using a LC pump (Jasco PU-980 HPLC pump) connected to a CO_2 cylinder. The vessel was warmed up to 40 °C to create the supercritical state. A mixture of guest saturated $scCO_2$ and pure $scCO_2$ was passed at a rate of 3–5 ml min^{-1} into a reaction vessel at 40 °C and 10 MPa, in which the monolayer immobilized QCM was set with a magnetic stirrer. Guest concentrations were controlled by changing the mixture ratio of the two fluids.

Fig. 2 shows typical time courses of frequency changes of the 27 MHz QCM immobilized with the thymine monolayer responding to exposure to the same concentration (1.0×10^{-4} M) of various guest molecules in $scCO_2$ at 40 °C and 10 MPa. Both 9-ethyladenine and 2-aminopyridine (an adenine analogue) were bound reasonably well onto the thymine monolayer, probably due to complementary two-point hydrogen bonding with the host thymine membrane. In spite of having a two-point hydrogen bonding ability with thymine, 1-cyclohexyluracyl and 2-pyrrolidone (a uracil analogue) were hardly adsorbed onto the thymine monolayer. Similar selective binding of adenine derivatives to the thymine monolayer was observed when the binding experiments were carried out in the air phase[3a–c] and on the thymine monolayer at the air-water interface.[6]

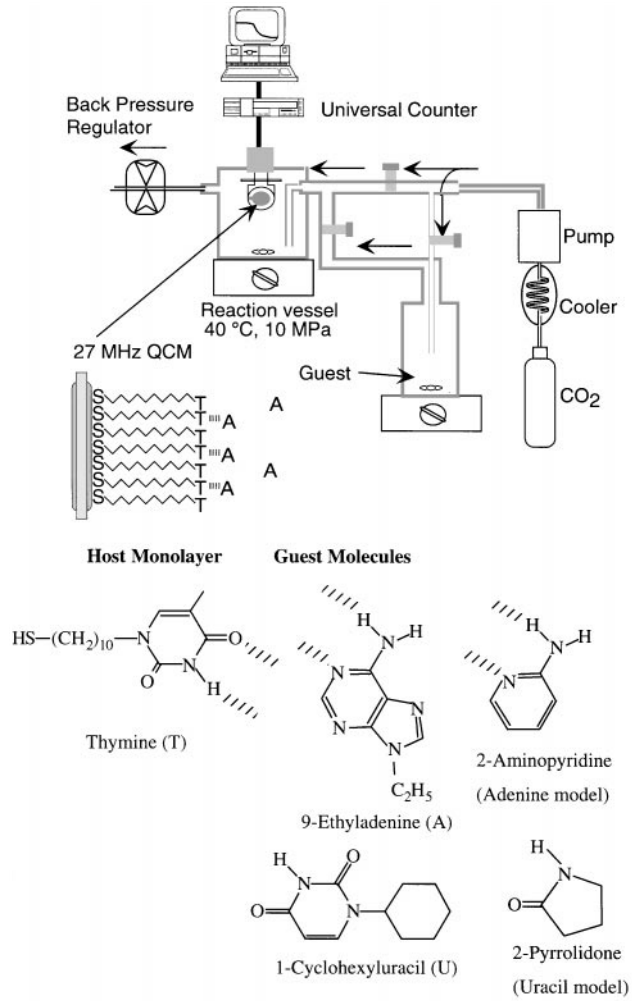

Fig. 1 Schematic illustration of nucleobase binding to a thymine monolayer on a 27 MHz QCM in a supercritical CO_2 flow system.

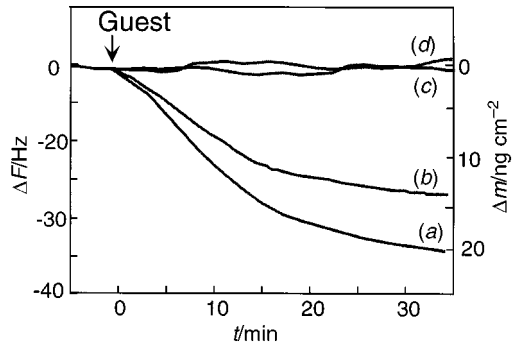

Fig. 2 Time courses of frequency decreases (mass increases) of the 27 MHz QCM immobilized with the thymine monolayer, responding to exposure of guest molecules (1.0×10^{-4} M) in supercritical CO_2 (40 °C and 10 MPa); (*a*) 9-ethyladenine, (*b*) 2-aminopyridine (an adenine model), (*c*) 1-cyclohexyluracil and (*d*) 2-pyrrolidone.

Table 1 Association constants (K_a) and initial binding rate constants (k_1) for guest bindings to the thymine monolayer in various media

Media	Guests	$K_a/10^3$ M^{-1}	k_1/M^{-1} s^{-1}
$scCO_2$[a]	9-ethyladenine	1.2	2.0
	2-aminopyridine (an adenine model)	2.6	8.0
	1-cyclohexyuracil	0.02	0.1
	2-pyrrolidone (a uracil model)	0.02	0.2
Air[b]	2-aminopyridine (an adenine model)	73 000	190 000
	2-pyrrolidone (a uracil model)	780	21 000
Water[c]	adenosine	0.23	0.5
	uridine	< 0.01	< 0.01

[a] 40 °C, 10 MPa. [b] 25 °C, see refs. 3(*a*) and 9. [c] 25 °C, pH 7.5, 0.01 M phosphate buffer, see refs. 6 and 10.

When the concentration of guest molecules increased (2×10^{-5} to 5×10^{-4} M), the typical saturation binding curves were obtained. From the reciprocal plots, association constants (K_a) could be obtained from linear correlations. From the initial slopes of the frequency decreases (mass increases) of Fig. 2, the apparent binding rate constants (k_1) were calculated. These data are summarized in Table 1, as are those values obtained using the same QCM system in the air[3a,9] or on the monolayer at the air-water interface.[6,10]

Complementary binding between the thymine monolayer and 9-ethyladenine or 2-aminopyridine (an adenine model) showed $K_a = (1.2–2.6) \times 10^3$ M^{-1}, which is about 100 times larger than K_a = 10–20 M^{-1} for noncomplementary binding of 1-cyclohecyluracil or 2-pyrrolidone. Similar selectivity was observed between the thymine monolayer and 2-aminopyridine (an adenine model) or 2-pyrrolidone (a uracil model) in the air phase at 25 °C, and between the thymine monolayer and adenosine or uridine in the aqueous solution (25 °C, pH 7.5, 0.01 M phosphate buffer). In the air phase, 2-aminopyridine and 2-pyrrolidone were employed as adenine and thymine models, respectively, since nucleobases are difficult to vaporize under the usual conditions. In aqueous solution, adenosine and uridine were employed due to their high solubility to aqueous solution.

Complementary hydrogen bonding between nucleobases has been confirmed by FT-IR spectral measurements in $CDCl_3$ solution.[11] We measured FT-IR spectra in the bulk $scCO_2$ using a pressure-resistant stainless steel vessel with ZnSe windows.[12] The $\delta_{N–H}$ at 1604 cm^{-1} for 2-aminopyridine (an adenine model) was shifted to 1616 cm^{-1} in the presence of an equal amount of 2-pyrrolidone (a uracil model) in $scCO_2$ (40 °C, 10 MPa), indicating that both molecules interact through hydrogen bonding as efficiently as in $CDCl_3$ solution.[11]

The selectivity (about 100 times) of the thymine monolayer for complementary guest molecules was similar among the three phases (air, water and super critical fluid). However, association constants (K_a) and initial binding rate constants (k_1) largely depended on the medium. K_a and k_1 values obtained in the air phase were very large due to the lack of solvent effects in the gas state, and those values in supercritical fluid were closer to, but still 10 times larger than, those in the aqueous phase. In supercritical fluid, the medium itself is thought to form fluid clusters, by which substrates are weakly solvated like a monolayer.[4a] This means that the very thin, weak solvation of the host and/or guest molecules in the supercritical fluid significantly reduced the interaction compared with the bare interaction in gas phase, and became close to the fully solvated interaction in the solution. Although the reaction conditions and guest molecules are slightly different for the three media, the K_a and k_1 values reflect the physical properties such as solvation and diffusiveness in each medium.

In conclusion, we could observe selective interactions between nucleobases in a supercritical fluid, and the QCM system is useful to monitor this binding behavior even in supercritical fluid, as well as in the liquid and air phases. One of advantages of supercritical fluid as a medium is that its physical properties, such as density, diffusion rate and solvation, can be reversibly changed by varying the temperature or pressure of the $scCO_2$ state.[13] We are currently studying the effect of solvation on molecular recognition by changing the physical state of the supercritical fluid.

Notes and references

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10 In the aqueous phase experiments, the monolayer of the amphiphile having thymine as a hydrophilic head group was spread on the water phase, the bare QCM plate was attached horizontally from the air phase on the amphiphile monolayer, and then guest molecules were injected into the water phase (ref. 6).

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12 Although we attempted direct detection of the hydrogen bond formation on the monolayer using an RAS-IR at the interface, we could not obtain clear results due to a lack of the sensitivity.

13 For example, density and relative permittivity can be changed over the ranges 0.1–0.8 and 0.1–0.5, respectively, by changing the pressure from 7 to 20 MPa at 40 °C.

Communication a907478c