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Effect of Solvation on Induce-Fit Molecular Recognition in Supercritical Fluid to Organic Crystals Immobilized on a Quartz Crystal Microbalance

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Abstract: The inclusion behavior of guest molecules to a solid apohost of an orthogonal anthracene-bis(resorcinol)tetraol (**1**) was investigated in supercritical carbon dioxide ($scCO_2$) by using a 9 MHz quartz-crystal microbalance (QCM). Compound **1** forms crystals composed of molecular-sheet bound together by an extensive hydrogen-bonded network. The selective binding of gaseous ethyl acetate to the apohost-immobilized QCM in $scCO_2$ was observed, and the inclusion amount of ethyl acetate showed a drastic increase above a threshold concentration, $[\text{Guest}]_{\text{th}} = 0.08$ M, and the apparent Gibbs' free energy for the binding was $\Delta G_{\text{app}} = -1.3$ kcal mol^{-1}. Similar selective bindings of ethyl acetate or ethanol had been observed in the gas phase and in water: $[\text{Guest}]_{\text{th}} = 0.002$ M with $\Delta G_{\text{app}} = -3.5$ kcal mol^{-1} and $[\text{Guest}]_{\text{th}} = 0.5$ M with $\Delta G_{\text{app}} = -0.41$ kcal mol^{-1}, respectively. These values obtained in $scCO_2$ were intermediate between those in the gas and water phases. Since various physical properties (viscosity, density, polarity, diffusion constant, and solvation) of supercritical fluid are known to be intermediate between gas and liquid, these values clearly reflect the solvation behavior of guest molecules. Thus, the lower solvation of guest molecules indicates the lower threshold concentration and the larger binding energy in the following order: in air > in $scCO_2$ > in water.

As a model of induced-fit molecular recognition between proteins and their substrates, organic crystals are very interesting in that their crystal structures reversibly changed from apohost to the guest-inclusion host responding to the guest binding.[1−18] The guest binding to organic crystals, however, has been studied mainly by conventional static methods: the host−guest complex has been obtained as precipitated crystals from solutions and analyzed by X-ray diffractions, or the extracted guests from crystals are analyzed by NMR spectra in solution. These static methods have some difficulties for studying kinetics on host−guest chemistry by changing host and guest concentrations. The

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inclusion behavior of guest molecules to the host crystals is favorable for study in the gaseous phase to avoid the solvation effect or the crystal packing force on guest inclusion.[19,20]

We have reported that the inclusion behavior of gaseous guest molecules in a solid apohost of an orthogonal anthracene-bis-(resorcinol)tetraol (**1**) could be kinetically obtained by using a quartz-crystal microbalance (QCM) in the gas phase.[21] A QCM is known to provide a very sensitive mass measuring device because its resonance frequency decreases linearly responding to the mass increase on a QCM plate at the nanogram level both in the gas phase and in aqueous solution.[22−30] Compound **1** forms molecular sheets involving an extensive hydrogen-bonded network with a large (ca. 10 Å) cavity like organic zeolites.[2] The apohost of **1** was cast onto a QCM plate and selective binding of ethyl acetate or methyl ethyl ketone to the apohost could be followed as a function of time by observing frequency decrease (mass increase) on the QCM in the air phase.[21] The inclusion amount was drastically increased above the threshold concentration ($[\text{Guest}]_{\text{th}} = 2.9$ mM) of ethyl acetate in the gas phase. Thus, the structure of the apohost forming hydrogen-bonded networks changed cooperatively in order to bind guest molecules above the threshold concentration. When the guest molecules were removed in a vacuum, the inclusion crystal structure reverted to the apohost. When the inclusion behavior of guest molecules to the apohost **1** was investigated in an aqueous solution, the threshold concentration was very high (0.5 M for ethanol and 4.0 M for methanol) to change the crystal structure.[31] This may be explained by the solvation of guest or host molecules in the water phase.

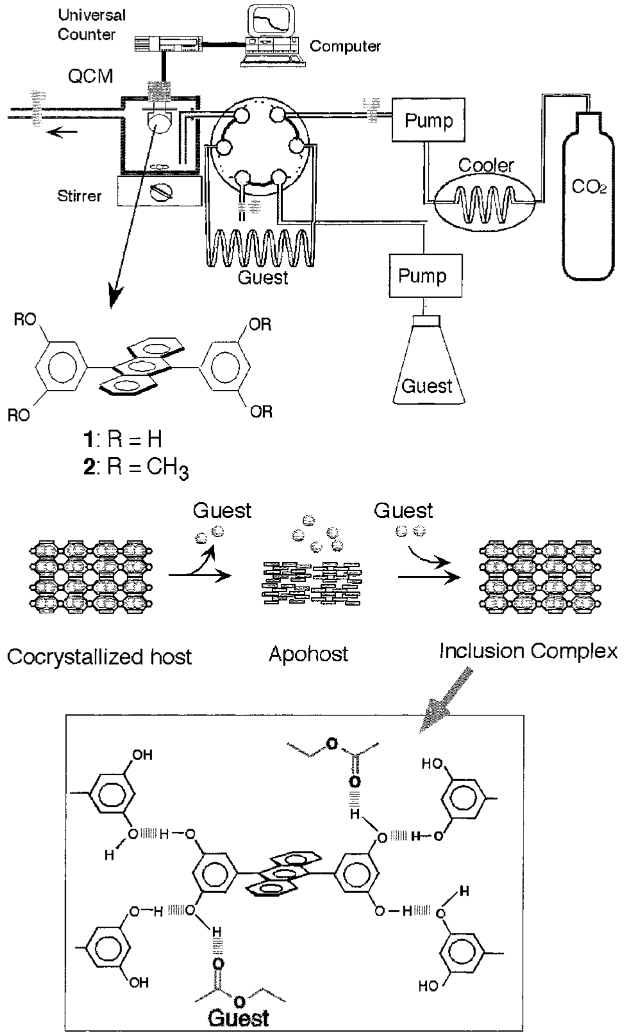

Figure 1. A schematic illustration of in situ detection of gaseous guest binding on the apohost **1** immobilized on a 9 MHz quartz-crystal microbalance (QCM) in supercritical carbon dioxide (scCO_2).

Supercritical fluid will be attracted as the third medium in addition to the gas and liquid phases, because the physical properties (e.g., density, diffusiveness, viscosity, and solvation) are intermediate between those of gas and liquid, and can be manipulated by small changes in pressure or temperature.[32] Several spectral measurements for hydrogen bonding interactions[33] and inclusion phenomena[34] have been carried out in supercritical fluid. It is worth studying and comparing molecular recognition among those three states of matter. The QCM can be used as a mass measuring devise independent of media such as in the gas phase,[21,23−25,29] in aqueous solution,[30] and even in supercritical fluid.[35]

In this paper, we report dynamic studies of gaseous guest binding to the apohost crystals cast on a 9 MHz QCM in

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supercritical carbon dioxide ($scCO_2$) (see Figure 1). The obtained results are compared with those in the gas phase and aqueous solution. The results reflect the difference of solvation of guest or host molecules in three different media.

Experimental Section

QCM Measurements. The QCM employed in this study is a commercially available 9 MHz, AT-cut quartz (diameter 9 mm), purchased from Ukou Electric, Co. Ltd., Saitama. The quartz crystal plate was deposited with Au electrodes on both sides (area 16 mm^2). The 9-MHz QCM was driven by a handmade oscillator, and the frequency changes were followed by a universal frequency counter (Hewlett-Packard Co., Ltd., Tokyo, model 53131A) attached to the microcomputer system.[29,30,35] The following equation has been established for AT-cut shear mode QCM,[22]

$$\Delta F = \frac{-2F_o^{\;2}}{A\sqrt{\rho_q \mu_q}\,\Delta m} \tag{1}$$

where ΔF is the measured frequency shift (Hz), F_o the fundamental frequency of the QCM (9×10^6 Hz), Δm mass change (g), A the electrode area (16 mm^2), ρ_q the density of the quartz (2.65 g cm^{-3}), and μ_q the shear modulus of the quartz (2.95×10^{11} dyn cm^{-2}). Calibration of the 9 MHz QCM in $scCO_2$ medium gave the same constant value (eq 2) as in the gas (0.94 ± 0.01)[21,29] and the aqueous solution (0.95 ± 0.05).[30]

$$\Delta m = [-(0.95 \pm 0.03) \times 10^{-9}]\Delta F \tag{2}$$

The fundamental frequency (F_o) of the 9 MHz QCM decreased ca. 200 Hz in $scCO_2$ at 45 °C with 7.6 MPa and ca. 3000 Hz in water at 25 °C, compared with the gas phase at 25 °C. This reflects the difference in viscosity or density of three different media: physical properties of $scCO_2$ are intermediate between gas and liquid. Since the fundamental frequency was affected by temperature, it was calibrated at each temperature in different media.[21,29,30]

Apohost Casting. Syntheses of the host compound **1** and host analogue compound **2** [an orthogonal anthracene-bis(resorcinol)-tetramethoxyl] were described elsewhere.[2a] A solution of host **1** in ethyl acetate (1 mg mL^{-1}) was cast on both sides of the gold electrode of the QCM at room temperature. It has been confirmed from powder X-ray analyses that the host **1** was cocrystallized together with guest in the cavity with a molecular ratio of 2:1.[2] When 2 μL of solution was cast on the electrode and dried in air, the frequency decreased 3030 ± 10 Hz (Δm = 2880 ± 10 ng) in the air, which is assumed to be the total mass of 2000 ng (5.08 mol) of the cast host **1** and 950 ± 30 ng (11.0 ± 0.5 nmol) of cocrystallized ethyl acetate with a molar ratio of guest:host = 2:1. When the host−guest complex on the QCM was dried under vacuum at 140 °C for 4 h, the frequency increased 1100 ± 50 Hz (mass decrease of Δm = 960 ± 30 ng), which agrees with the expected mass of included ethyl acetate. Thus, the as-cast crystal of **1** contained ethyl acetate as a guest with a molar ratio of 2:1 (guest to host), and ethyl acetate was completely evaporated after drying in vacuo to the apohost **1** on the QCM plate (see Figure 1). Immobilization of the host analogue **2** was also carried out in the similar procedure descried above.

Guest Binding in $scCO_2$. The QCM immobilized with the organic solid apohost was set in a pressure-resist vessel (7 cm^3) in the $scCO_2$ flow cell system as shown in Figure 1. Liquid CO_2 was pumped into a pressure-resist vessel with use of a LC pump (Jasco PU-980 HPLC pump) connected to a CO_2 cylinder. Carbon dioxide exists as the supercritical state above 31.0 °C and 7.29 MPa.[32] Carbon dioxide did not bind to the apohost on the QCM in $scCO_2$ at 40−60 °C with 7.5−10 MPa. CO_2 molecules, however, incorporated into the apohost at the lower temperature (32−40 °C) and at the higher pressure (12−15 MPa) due to the cluster formation of $scCO_2$.[32−34] Therefore, the guest binding experiments were carried out mainly at 45 °C with 7.6 MPa to avoid CO_2 adsorption into the apohost. The concentration of guest molecules in the high-pressure-resistant QCM vessel was controlled by changing the volume of the sample loop of the injection valve. To avoid the influence of water molecules, guest molecules were distilled at atmospheric pressure and CO_2 was dyed up with anhydrous sodium sulfate.

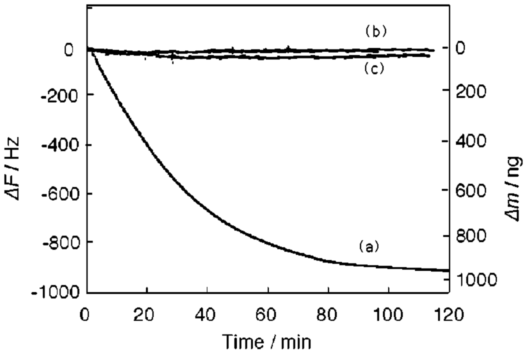

Figure 2. Time courses of frequency changes of the QCM immobilized with the apohost **1** (2000 ng, 5.08 nmol) responding to the exposure of gaseous guest molecules (150 mM, 520 μmol in 7 cm^3) at 45 °C with 7.6 MPa: (a) ethyl acetate, (b) benzene, and (c) cyclohexane.

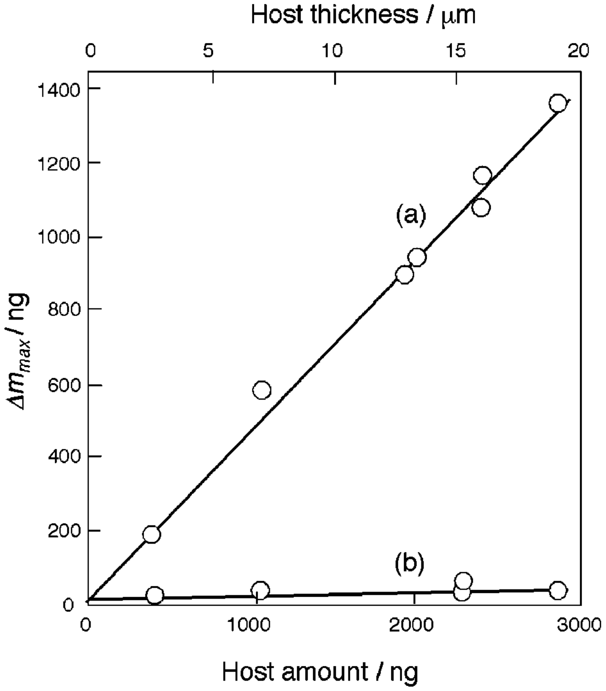

Figure 3. Effects of the amount (thickness) of apohost **1** on the equilibrium inclusion amount (Δm_{max}) in $scCO_2$ at 45 °C with 7.6 MPa at [Guest] = 150 mM: (a) ethyl acetate and (b) cyclohexane.

FT-IR Spectroscopy. The apohost **1** and the ethyl acetate-included host were cast on a gold substrate and FTIR-RAS spectra were taken in $scCO_2$ (at 45 °C with 7.6 MPa) in a pressure-resistant stainless steel vessel with ZnSe windows by using a FTS-6000 spectrometer (BIO-rad) with HgCdTe (MCT) detector.[35] FT-IR spectra of ethyl acetate were measured with an A-100 IR spectrometer (JEOL, Japan) in the transmission mode.

Results and Discussion

Guest Binding in $scCO_2$. Figure 2 shows typical time-resolved changes of frequency of the QCM immobilized with the apohost **1** (2000 ng, 5.08 mol) responding to exposure of the same concentration (150 mM in 7 cm^3) of ethyl acetate, benzene, and cyclohexane in $scCO_2$ at 45 °C with 7.6 MPa. Ethyl acetate was significantly included by the apohost **1** with time and reached equilibrium within 2 h. These changes can be

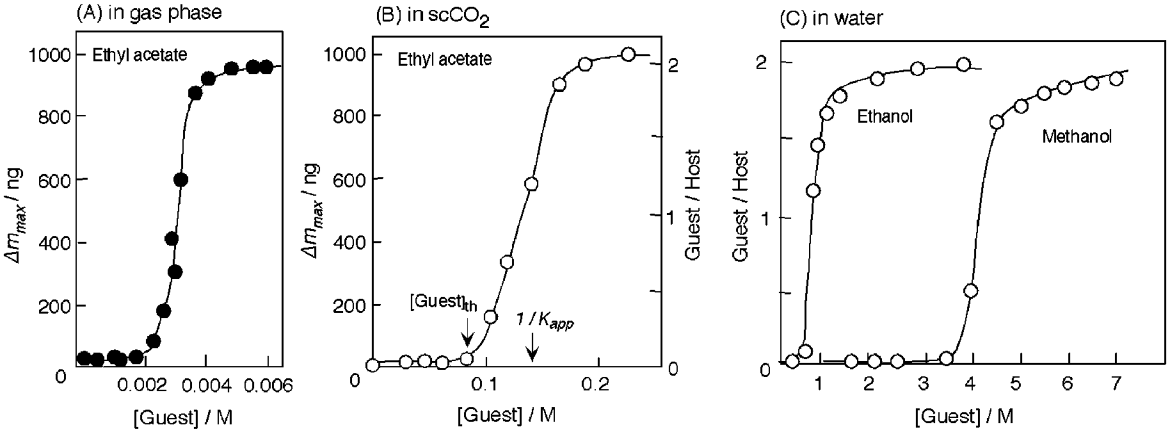

Figure 4. Effects of concentrations of guests on the equilibrium inclusion amount (Δm_{max}) or the guest/host ratio to the apohost **1** (2000 ng, 5.08 nmol): (a) ethyl acetate binding obtained by a QCM in the gas phase at 25 °C from ref 21a, (b) ethyl acetate binding obtained by a QCM in $scCO_2$ at 45 °C with 7.6 MPa, and (c) ethanol and methanol bindings obtained by X-ray diffraction in water at room temperature (ca. 25 °C) from ref 31.

approximated with the first-order kinetics and the obtained curve was fitted by the following equation,

$$\Delta m_t = \Delta m_{\mathrm{max}}[1 - \exp(-t/\tau)] \qquad (3)$$

where Δm_t and Δm_{max} are binding amount of guests at t and infinite time. Parameter τ is relaxation time on the binding. Curve fitting was always satisfactory in all the cases (correlation coefficient, $r > 0.98$). The Δm_{max} values obtained from Figure 2 were 950 ± 20 ng (10.8 nmol). Since 5.08 nmol of apohost was cast on the QCM, ethyl acetate was included by the apohost **1** in 2:1 (guest to host) stoichiometry at saturation. This was in good agreement with the observation of the host−guest complex (1:2) by X-ray analyses,[2b] and with the evaporated mass when the cocrystallized host−guest complex was dried in a vacuum (see Experimental Section). On the contrary, benzene and cyclohexane were hardly included by the apohost **1**. These selective binding behaviors showed good agreement with the fact that cocrystals were not obtained from these solvents.[2a] Hydrocarbons such as benzene and cyclohexane having no hydrogen-bonding ability adsorbed very weakly and/or adsorbed near the surface only.

To know hydrogen bonding between host and guest molecules, IR spectra were measured of the apohost **1**, the host **1** with included ethyl acetate, and ethyl acetate only in $scCO_2$ at 45 °C and 7.6 MPa. The peak position of $\nu_{C=O}$ of ethyl acetate in the inclusion complex shifted to 1700 cm^{-1} compared to 1740 cm^{-1} for ethyl acetate only. This indicates that ethyl acetate is included into the apohost **1** by hydrogen bonding. This IR result agrees with those observed for the inclusion complex of **1** with ethyl acetate form the gas phase.[21]

When a host analogue **2**, four hydroxyl groups of which were substituted by methoxy groups, was immobilized on a QCM and is kept in $scCO_2$ at 45 °C with 7.6 MPa, it was easily pealed off from the QCM plate within 30 min due to the solvation of $scCO_2$. The methoxy derivative **2** has been confirmed not to form a hydrogen-bonded network and the cavity.[2b] Thus, the formation of the cavity-forming hydrogen-bonded network is important in forming a stable host crystal.

Figure 3 shows the effect of a cast amount (thickness) of the apohost on the QCM plate on the equilibrium inclusion amount (Δm_{max}) of guests. In the case of ethyl acetate as a guest, the Δm_{max} increased linearly as the amount (thickness) of the cast apohost increased with a slope of 2.0 ± 0.1 molar ratio (900 ng/2000 ng = 5.1 nmol/2.5 nmol). This indicates that ethyl acetate adsorbs and penetrates deeply and completely into the solid apohost even through 20 μm thickness (3000 ng on a 16 mm^2 electrode), to form a complex in which two guest molecules fit into one host site. On the other hand, for cyclohexane as the guest, the equilibrium inclusion amount was independent of the apohost thickness, which shows that there is simple surface adsorption.

Threshold Concentration and Cooperativities. The effect of the concentration of ethyl acetate in $scCO_2$ (45 °C and 7.6 MPa) on the Δm_{max} to the apohost **1** (2000 ng, 5.08 nmol) is shown in Figure 4B. The inclusion amount of ethyl acetate increased sigmoidally with an increase in the guest concentration with the threshold concentrations of $[\mathrm{Guest}]_{\mathrm{th}} = 0.08$ M. Similar sigmoid-type binding of ethyl acetate has been observed when the same apohost-immobilized QCM experiment was carried out in the gas phase under an N_2 stream with the very low threshold concentration of 0.002 M (Figure 4A).[21a] With the inclusion experiments of ethanol or methanol into the solid apohost in water, the inclusion amount was calculated from X-ray diffraction pattern, similar sigmoidal curves were obtained with the very high threshold concentration of 0.5 or 3.5 M, respectively.[31] The Δm_{max} and guest/host ratio were always 2.0 ± 0.1 independent of media. Those values obtained in three different media are summarized in Table 1.

The cooperativities on binding ethyl acetate, ethanol, or methanol suggest that below the threshold concentration the guest molecules just adsorb near the solid surface of the collapsed crystal structure of the apohost. The cooperativities also suggest that above the threshold guest concentration, an extensive hydrogen-bonded network is formed to give an expanded, porous crystal structure, as shown schematically in Figure 1.

In the aqueous solution, the apohost **1** had been reported to be stabilized by the incorporated water (up to 12−16 molecules per a host) and guest molecules have to remove solvating water molecules to be included in the cavity.[31] On the contrary, in the gas phase and in $scCO_2$, the apohost was observed from the QCM experiments not to be solvated by the medium (N_2 and CO_2). Thus, the binding behavior in the gas and $scCO_2$ media reflects simply the inclusion of guest to the empty apohost. The cooperative binding behavior in the gas and $scCO_2$ could be analyzed more precisely, according to the Hill equation (eq 4) in which n is the cooperativity factor, K is binding constant, and y and y_∞ are amount of bound guest per host (guest/host) at [Guest] and infinite guest concentration, respec-

Table 1. Binding Parameters of Guest Molecules into the Apohost **1** in Three Different Media of Gas Phase, $scCO_2$, and Water

medium	guest	$[Guest]_{th}$/M	guest/host ratio	n^d	K^d/M^{-n}	$K_{app}{}^e/M^{-1}$	$\Delta G_{app}{}^f$/kcal mol^{-1}
gas[a]	ethyl acetate	0.002	2.02 ± 0.07	13.8 ± 6.7	1.4×10^{36}	340	−3.5
$scCO_2$[b]	ethyl acetate	0.08	2.3 ± 0.1	10.1 ± 5.1	3.5×10^{18}	8.3	−1.3
water[c]	ethanol	0.5	2.0 ± 0.1			2.0	−0.41
	methanol	3.5	2.0 ± 0.1			0.25	−0.05

[a] Obtained by a QCM method at 25 °C under N_2 stream, see ref 21a. [b] Obtained by a QCM method at 45 °C and 7.6 Mpa. [c] Obtained by X-ray diffraction at room temperature (ca. 25 °C), see ref 31. [d] Obtained from eq 4. [e] Obtained from the inverse of the concentration of the half Δm_{max}. [f] obtained from $\Delta G_{app} = -RT \ln K_{app}$.

tively.[36] The results are summarized in Table 1.

$$y = y_\infty \frac{K[\mathrm{Guest}]^n}{1 + [\mathrm{Guest}]^n} \qquad (4)$$

The binding stoichiometry value (y_∞) was obtained to be 2.0 ± 0.1 in the gas phase and $scCO_2$ medium, which showed good agreement with the guest/host ratio obtained in Figure 4A,B. In both cases, n values are larger than unity, indicating that the system has a positive cooperativity on binding. Unexpectedly large n values (13.8 and 10.1) were observed in the gas and $scCO_2$ phases, respectively. The cooperative factor does not usually exceed the binding stoichiometry as seen in oxygen binding to hemoglobin: the binding site stoichiometry is 4 and the cooperative factor is 2.8.[37] The large cooperative factor for ethyl acetate to the apohost **1** is probably attributed to the continuity of host structures. A cooperative factor larger than its stoichiometry was also reported for adenine binding to a host-site array assembled on a monolayer of orotate-type lipid.[38] Guest binding to the apohost **1** would be driven by reconstruction of the collapsed host structure. If the collapsed apohost maintains a partial hydrogen-bonding network and sheet structure within the network intercalates with each other, only a small number of bond guests would by required to recreate a large number of active binding sites by opening intercalated sheet structures.[2b] This tendency in the gas phase may be larger than that in $scCO_2$ due to the large n value in the gas phase.

Since it is difficult to compare directly multiordered K values (1.4×10^{36} $M^{-13.8}$ in the gas phase and 3.5×10^{18} $M^{-10.1}$ in $scCO_2$) in three different media, apparent binding constants, K_{app} (M^{-1}), were obtained from the inverse of the concentrations of the half Δm_{max} values. Then, Gibbs's free energy, ΔG_{app}, was calculated, since the inclusion experiments were carried out at different temperatures in three media. Data are summarized in Table 1. The $[Guest]_{th}$ and ΔG_{app} values in $scCO_2$ were intermediate between those in the gas and water phases. The supercritical fluid has been reported to have intermediate physical properties such as viscosity, density, polarity, diffusion constant, and solvation between the gas and liquid phases.[32] Different inclusion behaviors in three media may be explained by solvation of host and guest molecules. In the gas phase, both apohost and guest are not solvated by medium, and the smallest $[Guest]_{th} = 0.002$ M and $\Delta G_{app} = -3.5$ kcal mol^{-1} are given. On the contrary, in the water phase, both apohost and guest molecules are largely solvated and the largest $[Guest]_{th} = 0.5-3.5$ M and $\Delta G_{app} = -0.41$ to 0.05 kcal mol^{-1} are given. In supercritical fluid, only the guest molecules are slightly solvated by $scCO_2$ and the intermediate $[Guest]_{th} = 0.08$ M and $\Delta G_{app} = -1.3$ kcal mol^{-1} are given.

Conclusion

We have observed the selective induced-fit inclusion of ethyl acetate to a solid organic host on the QCM even in supercritical fluid. In comparison with the same experiments in the gas and water phases, the inclusion behavior reflects the solvation of guest or host molecules. Supercritical fluids are interesting for investigation of the solvation effect on molecular recognition as the third medium in addition to the gas and liquid phases. The QCM technique is also useful to detect directly molecular recognition on the solid host as a mass change, independent of media.

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