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Chiral Ice Chromatography

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Abstract: Water-ice particles simultaneously doped with β-cyclodextrin and a salt enabled chromatographic separation of enantiomers without synthetic processes, and enhanced chiral recognition occurring in the liquid-water phase coexistent with the solid-ice phase.

Despite the fact that it is a common material, water ice has attracted a number of researchers in a wide variety of scientific fields because ice is related to various natural phenomena and plays important roles in the global environment.[1−4] Some atmospheric reactions proceed in so-called cryogenic reactors, including the quasi-liquid layer on the surface of ice and micropockets developed at the triple junction of ice grain boundaries.[4] It is also known that some reactions are accelerated when confined in ice.[5] These examples suggest that a liquid-water phase in ice (WPI) acts as a new class of reaction medium.

In contrast to extensive studies to reveal the characteristic features of water ice, there have been very few attempts to utilize its unique functionalities for productive developments in chemistry and related disciplines. Ice chromatography, in which ice particles are used as a chromatographic stationary phase, was first attempted by Dasgupta and Mo.[6] We have devised an effective way to prepare ice particles suitable for this purpose and have revealed the interfacial chemistry of water ice.[7−9] In this method, the presence of the WPI allows the mechanistic shift from adsorption mode to partition mode. This shift is enhanced by doping a salt into the ice stationary phase.[9]

This communication discusses the chiral recognition reaction in the WPI studied using chiral ice chromatography measurements. This approach not only reveals the suitability of the WPI as a platform for the chiral recognition reaction but also will lead to the preparation of a new class of chiral stationary phase. Chiral chromatography constitutes a dominant sector of chiral separations conducted in various branches of chemical, biological, pharmaceutical, and medical science.[10,11] In most of the contemporary stationary phases, chiral selectors are chemically bonded on a solid matrix such as silica gel or polymer beads. Chiral ice chromatography may allow us to skip the synthetic processes required for the preparation of the current chiral solid phases.

In order to realize chiral ice chromatographic separation, β-cyclodextrin (β-CD) was incorporated as a chiral selector into an ice stationary phase throughout this work. The mobile phase was hexane containing a small amount of tetrahydrofuran (THF); the concentration of THF was adjusted for the retentivity of a solute. In general, native β-CD is not suitable for chiral separation in a nonpolar medium such as hexane because solvent molecules are accommodated in the cavity of β-CD and interfere with its chiral recognition.[10] Various structures have been introduced onto the CD to reduce this effect in commercialized CD-bonded normal-phase chiral stationary phases. In the present system, a salt as well as β-CD is doped in ice to facilitate the development of the WPI, in which the chiral recognition occurs; native CD can thus be used. We previously studied chromatography with salt-doped ice and found that the growth of the WPI can be probed chromatographically.[9] KCl or NaCl was selected as a dopant in this work because the phase diagrams of the water−KCl and water−NaCl systems are well-known. The literature data for the phase boundaries in these systems were fitted using an appropriate polynomial, as shown in Figures S1 and S2 in the Supporting Information. The temperature−molar ratio diagrams were used for the calculation of the volume of the WPI at a given temperature and salt concentration in the entire system (i.e., its concentration before freezing), whereas the temperature−molar concentration diagrams were utilized for the estimation of the salt concentration in the WPI equilibrated with the ice phase at a given temperature.

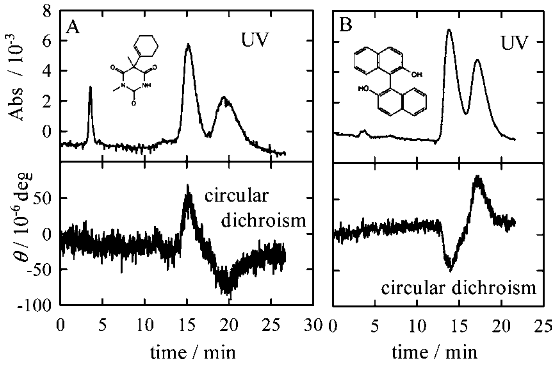

Figure 1. Chiral ice chromatographic separation of (A) hexobarbital and (B) 1,1′-bis-2-naphthol with using (top) UV and (bottom) circular dichroism detection. (A) Stationary phase, ice doped with 0.5 mM β-CD and 75 mM KCl; mobile phase, 0.5% (v/v) THF in hexane; temperature, −8 °C; detection at 230 nm. (B) Stationary phase, ice doped with 10 mM β-CD and 100 mM KCl; mobile phase, 0.1% (v/v) THF in hexane; temperature, −8 °C; detection at 280 nm.

Figure 1 shows chiral separations of hexobarbital and 1,1′-bis-2-naphthol using the ice stationary phase doped with both β-CD and KCl. The circular dichroism spectrometric detection clearly indicated that the enantiomers were successfully separated by the doped ice stationary phase. The effects of the concentrations of β-CD and the salt as well as the effect of temperature on the retention of the enantiomers of hexobarbital are shown in Figures S3−S5. We can summarize some characteristics of chiral ice chromatographic separation from these figures: (1) the effect of temperature is smaller than those of other factors; (2) doping with a salt is essential for separation of the enantiomers (no separation occurred with β-CD-doped ice that was not doped with salt); (3) increasing the β-CD concentration enhances the chiral separation, as expected.

The effects of the experimental variables on the chiral separation can be comprehensively interpreted on the basis of the following

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model. The retention factor (k) of a solute in the present system is represented by

$$k = k_{\mathrm{ads}} + k_{\mathrm{par}} \tag{1}$$

where the contributions to k from adsorption on the ice stationary surface and from partitioning into the WPI are given by k_{ads} and k_{par}, respectively. When β-CD is incorporated into the ice stationary phase, complexation of β-CD with a solute (S) occurs in the WPI; this enhances k_{par}. Substitution of the volume of the WPI (V_{WPI}), the volume of the mobile phase in the column (V_{mob}), and the concentrations of complexed and uncomplexed solute in the two phases into eq 1 yields

$$\begin{aligned} k &= k_{\mathrm{ads}} + \frac{V_{\mathrm{WPI}}}{V_{\mathrm{mob}}} \frac{[\mathrm{S}]_{\mathrm{WPI}} + [\mathrm{S{-}CD}]_{\mathrm{WPI}} + [\mathrm{S{-}(CD)_2}]_{\mathrm{WPI}}}{[\mathrm{S}]_{\mathrm{mob}}} \\ &= k_{\mathrm{ads}} + \frac{V_{\mathrm{WPI}}}{K_{\mathrm{h/w}} V_{\mathrm{mob}}} (1 + K_1[\mathrm{CD}]_{\mathrm{WPI}} + \beta_2[\mathrm{CD}]_{\mathrm{WPI}}^2) \end{aligned} \tag{2}$$

where K_1 (M^{-1}) and β_2 (M^{-2}) are the association constants for 1:1 (S−CD) and 1:2 [S−(CD)$_2$] complexes between the solute and β-CD, respectively, and $K_{\mathrm{h/w}} = [\mathrm{S}]_{\mathrm{mob}}/[\mathrm{S}]_{\mathrm{WPI}}$ is the partition coefficient of uncomplexed solute between the mobile phase and the WPI phase.

The adsorption of a solute on the doped-ice stationary phase is the result of several processes, namely, adsorption on the solid-ice surface, adsorption on the surface of the WPI, and the interaction with β-CD at these interfaces. It should be noted that these mechanisms are not enantioselective; the first two have no enantioselective origins, and the last one is also non-enantioselective because the formation of inclusion complexes of β-CD is very weak in the presence of a nonpolar solvent such as hexane, as discussed above.[10] The contribution from these processes to k_{ads} should vary with experimental conditions such as the temperature and the concentrations of the salt and β-CD. The quantitative discussion of k_{ads} is not straightforward. However, since the enantioselectivity in chiral ice chromatography appears only in the k_{par} term, we can discuss the enantioselectivity of the ice stationary phase on the basis of the difference between the enantiomer k values, which allows the cancellation of the contribution from the non-enantioselective k_{ads} term:

$$\Delta k = k_2 - k_1 = \frac{V_{\mathrm{WPI}}}{K_{\mathrm{h/w}} V_{\mathrm{mob}}} (\Delta K_1[\mathrm{CD}]_{\mathrm{WPI}} + \Delta\beta_2[\mathrm{CD}]_{\mathrm{WPI}}^2) \tag{3}$$

where ΔK_1 and $\Delta\beta_2$ are the differences in the complexation constants for the two enantiomers. Although the temperature dependence of $K_{\mathrm{h/w}}$ is negligibly small, its dependence on the salt concentration is so large that this effect must be taken into account to interpret the separation data (Figure S6). Thus, we can correct Δk in terms of V_{WPI}, V_{mob}, and $K_{\mathrm{h/w}}$. V_{WPI} can be calculated from the phase diagram, and V_{mob} can be determined from the void volume of the column; $K_{\mathrm{h/w}}$ can be obtained from Figure S6 using the salt concentration in the WPI at the working temperature. Obviously, the corrected Δk values are described by a quadratic equation in $[\mathrm{CD}]_{\mathrm{WPI}}$.

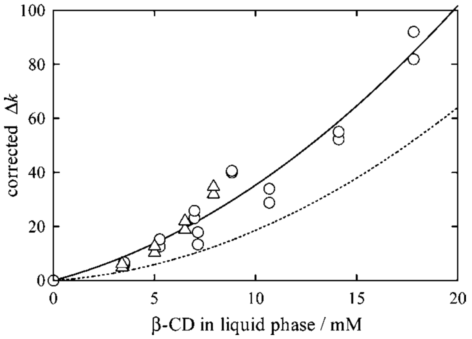

Figure 2. Relation between the corrected Δk for the enantiomers of hexobarbital and the concentration of β-CD in the WPI in KCl-doped (○) and NaCl-doped (△) ice stationary phases. The solid curve was obtained by curve fitting and is given by $y = (1.97 \times 10^3)[\mathrm{CD}]_{\mathrm{WPI}} + (1.56 \times 10^5)[\mathrm{CD}]_{\mathrm{WPI}}^2$. The dashed curve was drawn using the values determined for bulk complexation.

Figure 2 shows the relation between the corrected Δk values and $[\mathrm{CD}]_{\mathrm{WPI}}$, which was calculated by assuming that β-CD added in the stationary phase is completely dissolved in the WPI. Although there is some scatter in the data, all of the corrected Δk values obtained with NaCl- and KCl-incorporated ice stationary phases fall on the solid quadratic curve. From the coefficients of the fitting curve, the chiral ice chromatographic retention data imply that $\Delta K_1 = 1.97 \times 10^3\ \mathrm{M}^{-1}$ and $\Delta\beta_2 = 1.56 \times 10^5\ \mathrm{M}^{-2}$ for the enantiomers of hexobarbital in the WPI. In contrast, as shown in Figure S7, solvent extraction experiments at −5 °C gave $\Delta K_1 \approx 500\ \mathrm{M}^{-1}$ and $\Delta\beta_2 = 1.26 \times 10^5\ \mathrm{M}^{-2}$. These values give the broken curve depicted in Figure 2. An enhancement of β-CD chiral recognition in the WPI relative to that in bulk solutions is suggested. Another point that should be noted here is the solubility of β-CD in the WPI. The solubility of β-CD in water is reported to be ~16 mM but is enhanced up to ~70 mM in the presence of an electrolyte.[12] We found that its solubility in the hexane-saturated aqueous KCl solution (1.1−2.8 M) was almost constant at 2−2.5 mM over the temperature range −10 to 10 °C. If the concentration of β-CD in the WPI were constant irrespective of the extent of its growth, $K_{\mathrm{h/w}}\Delta k$ should be proportional to V_{WPI} according to eq 3. However, the $\Delta k - V_{\mathrm{WPI}}$ plot was entirely scattered and did not suggest any trends. This strongly implies that the solubility of β-CD in the WPI is enhanced in comparison with that in bulk solutions. Although we attempted chiral separation using silica gel impregnated with an aqueous β-CD solution, no separation was confirmed. This again indicates the specific physicochemical properties of the WPI, which plays an essential role in successful chiral separation in the present scheme. The present results indicate that the WPI well dissolves β-CD or excludes hexane and facilitates formation of the inclusion complex of β-CD. If the WPI has properties similar to those of high-density water, which is under debate in relation to the polyamorphism of water, the present findings may be interpreted.[13]

Hence, the present method has several advantages. First, the separation performance can be adjusted by (1) changing V_{WPI} through changing temperature and/or the salt dopant concentration and (2) varying $K_{\mathrm{h/w}}$ through changing the organic solvent or modifier. Second, the specific medium property of the WPI can result in enhanced chiral separation; the function of the WPI and its molecular origin should be revealed in more detail. Although separation using β-CD has been discussed here, any water-soluble chiral selector can be incorporated into the ice stationary phase in a similar fashion. We believe that ice chromatography opens a new era for both ice chemistry and chiral separation.

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Supporting Information Available: Experimental details and results of chromatographic experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

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