Role of Modest Pressure in Chirally Selective Complexation Interactions

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In contrast with temperature, pressure is often overlooked as an important parameter in condensed phases primarily due to minimal perturbations in bulk properties. Molecular interactions, however, have been demonstrated to be significantly altered by relatively modest pressure (<500 bar) in a number of biologically based studies. In this article, we examine the fundamental role of these pressure perturbations on enantiomeric complexation with β-cyclodextrin. Widely utilized for enantioselective interactions, β-cyclodextrin provides a hard-binding site with a pressure-invariant structure. Using this rigid molecule and enantiomers of warfarin, pressure-induced perturbations in complexation equilibria may be used to determine the change in partial molar volume upon complexation. Complexation measurements by liquid chromatography demonstrate a significant decrease in solute binding for both enantiomers under controlled-pressure conditions (P_{av} < 300 bar). On the basis of these measurements, both enantiomers are shown to exhibit significant changes in molar volume upon complexation ($\Delta V_{comp} = +17 \pm 5.1$ cm^3/mol; $\Delta V_{comp} = +16 \pm 5.0$ cm^3/mol). Moreover, pressure-induced perturbations in chiral selectivity yield a small but significant difference in the change in partial molar volume of the solvated complexes, $\Delta(\Delta V_{comp}) = 1.0 \pm 0.50$ cm^3/mol. For enantiomers, which are expected to have identical molar volumes in solution, this difference in ΔV_{comp} arises primarily from the partial molar volumes of the solvated complexes. These differences in the solvation environment between enantiomers are central to chiral recognition, and further study will be required to elucidate mechanistic distinctions. Generally utilized as a separation technique, liquid chromatography is demonstrated here as a powerful tool for the sensitive determination of pressure-induced perturbations in chiral complexation.

Introduction

The interactions of chiral compounds play a significant role in a wide variety of biological and technological processes. Even though enantiomers have nearly identical physical and chemical properties, small differences in the three-dimensional arrangement of functional groups can result in significant differences in the chemical interactions of enantiomers. In this study, chirally selective interactions are investigated using β-cyclodextrin, a toroidally shaped oligosaccharide capable of selective complexation of enantiomers.[1,2] The pressure-invariant structure of β-cyclodextrin[3] makes it uniquely well suited as a model for hard-site binding interactions in enzymes and proteins.[1−3] Modest pressures (<500 bar) have been demonstrated to affect a number of biological systems,[4−9] even as the fundamental effect of pressure on enantiomeric complexation has not been examined extensively.

In contrast with temperature, pressure can be utilized to elucidate the volumetric components of complexation equilibria without altering the average kinetic energy of the system. Often overlooked due to the limited compressibilities of polar liquids, pressure may have a significant impact on the molecular-level interactions that drive complexation in solution.[5] Moreover, these pressure-induced perturbations may play a role in those enantioselective interactions leading to chiral recognition. Unfortunately, determination of the pressure dependence of these interactions often requires the ability to discern small differences in interaction energies between enantiomers.

Commonly utilized as a separation technique, the application of high-performance liquid chromatography (HPLC) to the fundamental study of pressure effects on chirally selective complexation interactions offers compelling fundamental and practical advantages. The sensitivity of liquid chromatography to small interaction differences makes possible the study of complexation reactions with modest binding constants (K_{comp} < 500 M^{-1}). This sensitivity extends to those very small interaction energy differences between solutes ($\Delta(\Delta G_{comp}) \geq$ 25 J/mol) that are important in enantiomeric complexation but may be difficult or impossible to measure with NMR, fluorescence, or UV−visible techniques. In addition, many of the practical advantages of liquid chromatography as a fundamental technique arise from its separation of analytes. This capability permits simultaneous analysis of several compounds from an impure mixture. In contrast to spectroscopic methods, liquid chromatography requires no measurable shift in solute or host molecule spectra for detection of binding interactions, thus extending the range of complexation interactions that can be assessed fundamentally. In combination, these fundamental and practical advantages make chiral separations in HPLC an excellent system in which to probe the fundamental effect of modest pressures on chirally selective complexation interactions.

Theoretical Predictions

Enantiomeric complexation interactions in liquid solution represent a unique system in which to study the effect of pressure on chiral recognition. While temperature-induced changes to equilibrium thermodynamics are more familiar to most chemists, pressure-induced perturbations to chemical equilibria in solution have been shown to be significant for broad classes of chemical reactions at modest pressures.[5,10,11] Although the bulk properties of polar liquids are not affected significantly at pressures less than 500 bar,[10] the thermodynamic properties of reactions that occur under these conditions may be significantly altered. In contrast to temperature changes, pressure acts upon only the volumetric components of equilibrium. In this way, fundamental studies which examine the effect of pressure on complexation elucidate specific volumetric changes that occur during the chiral recognition process.

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Modest pressures may have a significant impact on equilibrium concentrations of reactants and products during a complexation reaction. The equilibrium complexation constant (K_{comp}) for β-cyclodextrin complexation with a solute is expressed as

$$K_{comp} = \frac{[CD\cdot solute]}{[CD][solute]} \quad (1)$$

where [CD·solute] is the equilibrium molar concentration of cyclodextrin−solute complex and [CD] and [solute] are the equilibrium molar concentrations of unbound cyclodextrin and unbound solute, respectively. The pressure dependence of K_{comp} can be derived from the change in Gibbs free energy for the complexation reaction (ΔG_{comp})

$$\left(\frac{\partial \Delta G_{comp}}{\partial P}\right)_T = -RT\left(\frac{\partial \ln K_{comp}}{\partial P}\right)_T + \Delta nRT\kappa_S \quad (2)$$

where P is pressure and T is temperature. The K_{comp} term in eq 2 measures changes in Gibbs free energy induced by the complexation reaction. This change is mediated by the $\Delta nRT\kappa_S$ term, which corrects for changes in molar concentration with pressure due to compression of solvent. The magnitude of this correction is therefore related to solvent isothermal compressibility (κ_S) as well as the difference in the stoichiometric coefficients of products from reactants (Δn) (approximately −2 cm^3/mol in these studies). The Gibbs equation relates the total change in Gibbs free energy with pressure in eq 2 to a change in partial molar volume upon complexation (ΔV_{comp})

$$\left(\frac{\partial \Delta G_{comp}}{\partial P}\right)_T = \Delta V_{comp} \quad (3)$$

where

$$\Delta V_{comp} = V_{CD\cdot solute} - V_{CD} - V_{solute} \quad (4)$$

The volume (V) terms in eq 4 represent the partial molar volumes of the products and reactants involved in the complexation reaction in their solvated state. Therefore, ΔV_{comp} not only accounts for differences in the partial molar volumes of the products and reactants but also differences in the volumes of the solvent cages associated with each product and reactant. The interrelationship of this change in partial molar volume upon complexation and the change in equilibrium complexation constant with pressure is apparent from the combination of eqs 2 and 3.

$$-RT\left(\frac{\partial \ln K_{comp}}{\partial P}\right)_T + \Delta nRT\kappa_S = \Delta V_{comp} \quad (5)$$

In this way, the change in equilibrium complexation constant with pressure in eq 5 can be used to estimate the change in partial molar volume with solute complexation.

Most complexation reactions exhibit a decrease in partial molar volume with complexation ($\Delta V_{comp} < 0$).[5] As the host molecule and solute associate, their individual solvent cages must often overlap to achieve closest approach. This mutual solvation of the host and solute results in a decrease in solvated partial molar volume upon complexation.[5] Thus, according to eq 5, in most complexation reactions an increase in pressure is predicted to increase the equilibrium complexation constant (K_{comp}).

Nonetheless, a handful of complexation reactions including those involving inclusion complexes of β-cyclodextrin exhibit an increase in partial molar volume upon complexation ($\Delta V_{comp} > 0$).[3,12−15] This increase in partial molar volume has been posited to arise from the difference in compressibility between β-cyclodextrin and the equivalent volume of solute that fills the binding site when the solute is absent.[3] In contrast with many molecules, β-cyclodextrin is very rigid and incompressible under modest pressure conditions.[3] Of the three components involved in the complexation reaction, the free solute is most able to decrease its solvated molar volume with pressure. The solvated volume of the β-cyclodextrin·solute complex is therefore expected to be greater than the sum of the solvated volumes of the free β-cyclodextrin and free solute, resulting in a positive ΔV_{comp}. In accordance with eq 5, an increase in pressure is predicted to favor a decrease in the equilibrium β-cyclodextrin complexation constant in this case.[3,12−15]

Thermodynamic evaluation of chiral complexation interactions must consider the relationship between equilibrium β-cyclodextrin complexation constants for two enantiomers, which may also be pressure dependent. The equilibrium β-cyclodextrin complexation constants for two enantiomers ($K_{comp,1}$ and $K_{comp,2}$) can be related by a selectivity value (α)

$$\alpha = K_{comp,2}/K_{comp,1} \quad (6)$$

where α is defined as greater than or equal to unity. The magnitude of this selectivity is, in turn, determined by the difference in the change in Gibbs free energy of complexation for two enantiomers, $\Delta(\Delta G_{comp})$.

$$\Delta(\Delta G_{comp}) = -RT \ln \alpha \quad (7)$$

The difference in the change with pressure in the change in Gibbs free energy upon complexation for two enantiomers yields the expression

$$\left[\frac{\Delta(\Delta G_{comp,2})}{\Delta P} - \frac{\Delta(\Delta G_{comp,1})}{\Delta P}\right]_T = -RT\left(\frac{\Delta \ln \alpha}{\Delta P}\right)_T = \Delta(\Delta V_{comp}) \quad (8)$$

where

$$\Delta(\Delta V_{comp}) = |\Delta V_{comp,2} - \Delta V_{comp,1}| \quad (9)$$

In this way, the change in selectivity of two enantiomers with pressure indicated in eq 8 can be used to estimate the difference in the change in partial molar volume of complexation between the enantiomers.

In the unbound state, the partial molar volumes of the enantiomers are expected to be identical. As a result, the difference in the change in partial molar volume of complexation between two enantiomers, $\Delta(\Delta V_{comp})$, simplifies to the difference in volume between the complexes formed with each enantiomer:

$$\Delta(\Delta V_{comp}) = |V_{CD\cdot enantiomer2} - V_{CD\cdot enantiomer1}| \quad (10)$$

Experimental determination of $\Delta(\Delta V_{comp})$ may elucidate complexation interaction differences between enantiomers. A difference in enantiomeric interaction is a necessary condition for chiral selectivity, since the change in Gibbs free energy of complexation must be different between enantiomers in order for a selectivity to be observed. This energetic difference need not give rise to a change in partial molar volume. However, one possible origin for a difference in partial molar volume of β-cyclodextrin·enantiomer complexes may be the fact that the chiral carbons of each enantiomer are, by definition, attached to four different structural groups that may differ considerably

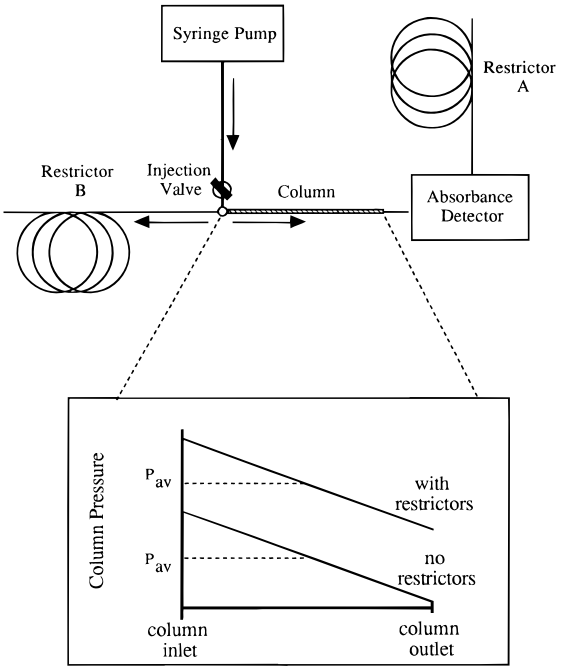

Figure 1. Schematic diagram of pressure-controlled liquid chromatographic system.

in terms of their interaction with the β-cyclodextrin. Some moieties may be included in the β-cyclodextrin cavity, while others may extend into the bulk solution. Differences between enantiomers in the relative placement of these structural groups in the β-cyclodextrin complex may therefore give rise to differences in the solvated volume of their β-cyclodextrin·enantiomer complexes. In this case, pressure-induced perturbations in chiral selectivity can act as a probe of the volumetric components of these enantiomeric interaction differences.

Experimental Section

Chemicals. A racemic mixture of warfarin ((*R*,*S*)-3-(α-acetonylbenzyl)-4-hydroxycoumarin) was obtained from Sigma (St. Louis, MO) and used without further purification. Mobile-phase solutions were prepared using high-purity acetonitrile and methanol (Baxter Healthcare, Muskegon, MI) with high-purity acetic acid (Aldrich, Milwaukee, WI) and HPLC-grade triethylamine (Fisher, Fair Lawn, NJ).

Chromatography. As illustrated in Figure 1, mobile-phase delivery was accomplished using a high-pressure syringe pump (Model 260D, Isco, Lincoln, NE) equipped with a high-precision pressure transducer (±1 psi). Probe compounds were dissolved in mobile phase at 1.15 mM concentration and introduced onto a packed-capillary column from a 1-μL internal volume injection valve (Valco Instruments, Houston, TX) using the split injection technique (split ratio = 74 ± 9; V_{inj} = 13 nL). Analyte detection at 305 nm utilized an absorbance detector (Model UVIS-205; Linear, Chicago, IL) fitted with a high-pressure capillary flow cell (76 μm i.d. and 357 μm o.d.; Polymicro Technologies, Inc., Phoenix, AZ).

A single, packed capillary column containing β-cyclodextrin bonded stationary phase (Cyclobond I 2000, Advanced Separations Technology, Whippany, NJ) was utilized throughout this study. Cyclobond I 2000 features β-cyclodextrin moieties chemically bonded with a 3-glycidoxylsilane spacer arm to a spherical silica gel support (d_p = 5 μm). Preparation and characterization of these phases have been described in detail elsewhere in the literature.[16,17] In this study, a fused-silica capillary (251 μm i.d. and 360 μm o.d.; Polymicro Technologies, Phoenix, AZ) was packed with a slurry of stationary phase and 80:20 v/v acetone−20 mM NH_4NO_3 under moderate pressure (380 bar). The resulting chromatographic column was terminated using a quartz wool frit at a final column length of 37.5 cm. A mobile phase consisting of 90:10:0.004:0.004 v/v/v/v acetonitrile−methanol−acetic acid−triethylamine was employed throughout this study.[18]

The chromatographic system illustrated schematically in Figure 1 has been designed to allow the average pressure (P_{av}) on the column to be controlled while maintaining a constant column flow rate. With no added restrictors, the average column pressure arises solely on the basis of the pressure necessary to create mobile-phase flow through the column. In this case, the column outlet is at atmospheric pressure and therefore the average column pressure may be approximated as simply one-half of the column inlet pressure (inset, Figure 1). If restrictors producing equivalent back-pressures are added at the inlet and outlet, the column inlet and outlet pressure are increased to the same extent. In this manner, the column pressure gradient remains constant, while the average pressure on the column is increased by the magnitude of the pressure change generated by the restrictors. Throughout this study, the column flow rate was maintained at 1.54 ± 0.04 μL/min resulting in a column pressure gradient of 24.9 bar. In this manner, the heat of friction generated from mobile-phase flow is effectively constant.[19,20] Moreover, the use of a packed-capillary column ensures effective heat dissipation and rapid column temperature equilibration with ambient conditions (T = 23 ± 0.5 °C). Constant flow rate conditions were confirmed on the basis of the reproducibility of the void time throughout these studies (SD = 0.16 min; RSD = 1.6%), which was determined from replicate injections of methanol. For consistency with conventional column studies, this volumetric flow rate was chosen to correspond with the mobile-phase linear velocity (3.0 cm/min) present at 0.50 mL/min in 4.6 mm internal diameter columns. Solute retention reversibility after high pressure was confirmed by direct comparison of chromatographic figures of merit at low pressure after high-pressure perturbation.

Results and Discussion

In investigating the fundamental role of pressure on enantiomeric complexation interactions, the experimental design must effectively isolate pressure from all other parameters. This study of complexation interactions is accomplished using high-performance liquid chromatography, in which the solute is introduced into a flowing solution or mobile phase. In order to isolate the effects of mobile-phase flow rate on thermodynamic parameters, the column flow rate is kept constant in all experiments while the absolute pressure on the chromatographic column is varied, as discussed previously.

The host molecule β-cyclodextrin is selected for these studies of enantiomeric complexation due to its rigidity and incompressibility in the pressure range of interest (< 500 bar).[3] These properties make this host uniquely well suited as a model for hard-binding site interactions in proteins and other macromolecules.[1−3] In addition, β-cyclodextrin has found extensive practical use as a stationary phase for liquid chromatography due, in large part, to its ability to form complexes that are chirally selective.[21−23] The enantiomers of warfarin (Figure 2) are chosen as model chiral solutes because their separation using β-cyclodextrin stationary phase and 90:10:0.004:0.004 v/v/v/v acetonitrile−methanol−acetic acid−triethylamine mobile phase exhibits low capacity factors (<1) but high chiral selectivity.[18] Thus, a large proportion of the interactions of warfarin with β-cyclodextrin are chirally selective, and any mediating effect

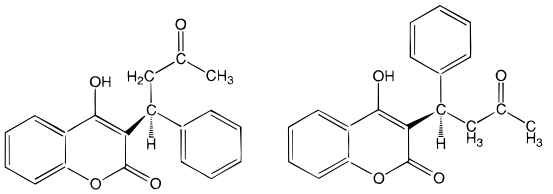

Figure 2. Structure of warfarin enantiomers.

of nonselective interactions need not be considered. In addition, warfarin possesses no ionizable protons in the pH range of the mobile phase. For this reason, the effects of pressure-induced changes in solute ionization state on β-cyclodextrin complexation[14,24−26] are minimal for this model system.

Chromatographic Measurements. Liquid chromatographic retention is well modeled as an equilibrium process even as the flow of mobile phase creates an inherently nonequilibrium condition.[27] As the solute zone passes through the packed bed, solute molecules interact with the stationary phase on the solid support. The continual movement of this solute zone into regions of differing solute concentrations creates a dynamic nonequilibrium condition. The majority of the impact of this nonequilibrium occurs at the boundary regions of the solute zone, thus minimizing the effect on the movement of the center of the zone profile. As a result, the movement of the centroid of the solute zone may be well modeled as an equilibrium process, and chromatographic retention directly correlated to equilibrium thermodynamics.[27]

In liquid chromatographic separations using β-cyclodextrin bound to a stationary support, the predominant solute retention mechanism is β-cyclodextrin·solute complexation.[16,28−30] For this reason, the equilibrium distribution coefficient of the solute between the stationary phase and mobile phase (K_{dist}) is assumed to be equal to the fundamental equilibrium complexation constant (K_{comp}) for β-cyclodextrin complexation with the solute. This equilibrium complexation constant can therefore be determined by measurement of the chromatographic capacity factor, k, as well as ϕ, which is the ratio of volumes of the stationary and mobile phases.

$$K_{comp} \cong K_{dist} = k/\phi \tag{11}$$

The solute capacity factor is defined as the ratio of moles of solute residing in the stationary phase and mobile phase and can be readily calculated as

$$k = (t_R - t_0)/t_0 \tag{12}$$

where t_0 is the time required for a narrow plug of mobile phase to traverse the column length and t_R is the time required for the solute of interest to travel the same distance. Capacity factor is a sensitive measure of the comparative strength of solute interactions with the mobile phase and stationary phase. An increase in capacity factor indicates an increase in solute interactions with the stationary phase relative to solute interactions with the mobile phase. Therefore, for this model system, an increase in warfarin capacity factor indicates an increase in the strength of warfarin interaction with β-cyclodextrin relative to the solution-phase environment.

The experimentally determined pressure dependence of capacity factor can be directly related to the pressure-dependence of the equilibrium complexation constant by eq 11. Therefore, substitution of equation 11 into the pressure dependence of K_{comp} described in eq 5 permits estimation of the change in molar volume of complexation directly from the measured change in capacity factor (k) with pressure, assuming that ϕ does not change significantly with pressure.

$$-RT\left(\frac{\Delta \ln k}{\Delta P}\right)_T + \Delta nRT\kappa_S = \Delta V_{comp} \tag{13}$$

For the modest pressure conditions encountered in liquid chromatography, the partial differential in eq 5 can be accurately represented as a simple difference.

In addition to measuring the change in molar volume of complexation, the chromatographic behavior of solutes can be used to assess the relationship between two equilibrium complexation constants. The selectivity of β-cyclodextrin for two solutes (α) can be readily assessed from the measured capacity factors of each solute

$$\alpha = k_2/k_1 \tag{14}$$

where k_2 is the capacity factor of the more retained solute. The pressure dependence of chromatographically determined chiral selectivity can be related to the enantiomeric difference in the change with pressure in the change in Gibbs free energy as well as the difference in the change in molar volume of complexation between two enantiomers, $\Delta(\Delta V_{comp})$.

$$\left[\frac{\Delta(\Delta G_{comp,2})}{\Delta P} - \frac{\Delta(\Delta G_{comp,1})}{\Delta P}\right]_T = -RT\left(\frac{\Delta \ln \alpha}{\Delta P}\right)_T = \Delta(\Delta V_{comp}) \tag{15}$$

In this way, chromatographic measurements are used to directly measure changes in fundamental thermodynamic parameters with pressure.

TABLE 1: Pressure-Induced Changes in Solute Retention (k) and Selectivity (α) for the Separation of Warfarin Using β-Cyclodextrin Stationary Phase[a]

avg. column pressure	capacity factor 1st enantiomer (k)	capacity factor 2nd enantiomer (k)	chiral selectivity (α)
12.4 bar	0.330 ± 0.0097	0.399 ± 0.0098	1.21 ± 0.006
298 bar	0.263 ± 0.0050	0.322 ± 0.0066	1.22 ± 0.004
percent change	−20%	−19%	+1%

[a] Mobile phase: 90:10:0.004:0.004 v/v/v/v acetonitrile−methanol−acetic acid−triethylamine; $T = 23 \pm 0.5$ °C; $F = 1.5$ μL/min; methanol used as the void marker. Uncertainty values are the standard deviation of replicate measurements.

Results. As shown in Table 1, modest pressure has a significant impact on capacity factor and chiral selectivity for a separation of warfarin enantiomers using β-cyclodextrin stationary phase. Using eq 13, shifts in capacity factor with pressure are used to estimate changes in partial molar volume of complexation (ΔV_{comp}) for warfarin 1 and warfarin 2 of +17 ± 5.1 and +16 ± 5.0 cm^3/mol, respectively. Reported errors, in this case, are determined on the basis of propagation of error from replicate measurements of solute capacity factor under controlled-pressure conditions. Errors in all parameters are included in this determination, and repetition of the experiment yielded statistically identical determinations. The observed decrease in equilibrium complexation constant (K_{comp}) for both enantiomers with pressure is fully consistent with previous studies of pressure-dependent β-cyclodextrin complexation. However, the magnitude of the equilibrium complexation constant shift with pressure is greater for β-cyclodextrin·warfarin than for any previous studies.[3,12−15]

This enhancement in the change in partial molar volume of complexation (ΔV_{comp}) for warfarin enantiomers may arise because all previous investigations of the effect of pressure on β-cyclodextrin complexation have been conducted in water-based solvents. It is important to note that the difference in bulk isothermal compressibilities for these two solvent systems is considerable (κ_S of water $= 4.57 \times 10^{-5}$ bar^{-1}, κ_S of acetonitrile $= 1.11 \times 10^{-4}$ bar^{-1}, both at 25 °C and atmospheric pressure[31,32]). However, concentration effects from solvent compression do not account for this difference since correction has already been made for this perturbation through the $\Delta nRT\kappa_S$ term in eq 13. By contrast, pressure-dependent solvation differences are expected to be evident in the overall observed ΔV_{comp} and may result in a different ΔV_{comp} for complexation reactions measured in an organic solvent environment.

In addition, the significant increase in partial molar volume of complexation (ΔV_{comp}) for warfarin enantiomers may also be the result of fundamental differences between the types of complex formed in this study and classic inclusion complexation. In water-based solvents, the β-cyclodextrin complexation mechanism is predominantly the formation of an inclusion complex, whereby the hydrophobic moieties of the solute are included within the hydrophobic interior of the β-cyclodextrin cavity.[1,2,33] However, the chiral complexation mechanism for acetonitrile-based solvent systems and β-cyclodextrin stationary phase has been posited to be quite different. In this mode of interaction, the enantiomeric solutes interact primarily with the hydrophilic secondary hydroxyl groups on the outer rims of the cyclodextrin and may form a "cover" over the β-cyclodextrin cavity.[18,34−36] No significant inclusion of the solute inside the cavity implies that the partial molar volume of the solvated β-cyclodextrin·enantiomer complex may be greater in this case. Inspection of eq 4 reveals that the enhancement in the solvated volume of the complex ($V_{CD\cdot solute}$) caused by lack of inclusion may result in an enhancement of the observed ΔV_{comp} in acetonitrile-based mobile phases compared to aqueous solutions.

When $\Delta(\Delta V_{comp})$ is estimated from pressure-induced changes in chiral selectivity using eq 15, the difference in the changes in partial molar volume of complexation between the enantiomers is only 1.0 ± 0.50 cm^3/mol. As previously discussed for ΔV_{comp}, the reported error in $\Delta(\Delta V_{comp})$ is determined on the basis of propagation of error. It is interesting to note that although ΔV_{comp} determinations for the individual enantiomers are statistically indistinguishable, the $\Delta(\Delta V_{comp})$ value is statistically significant. This result is due in large part to the cancellation of uncertainty in the compressibility term for the $\Delta(\Delta V_{comp})$ determination. Since $\Delta(\Delta V_{comp})$ is effectively the difference in volume between the complexes formed with each warfarin enantiomer, this significant but small $\Delta(\Delta V_{comp})$ suggests that the warfarin enantiomers exhibit complex structures of slightly differing volume. Further studies employing a greater number of pressure values will be needed to more accurately determine the precise enantiomeric complexation volume differences for β-cyclodextrin·warfarin.

These changes in volume during β-cyclodextrin·solute complexation are the result of several processes that are predicted to change the partial molar volume of various components of the reaction. These processes include the partial solvation of solute by β-cyclodextrin, inclusion and release of solvent from the β-cyclodextrin cavity, and possible conformation changes of the solute upon complexation.[12,13,37] Further investigations using high-pressure NMR are in progress, which will provide the mechanistic information necessary to clarify the origins of the change in volume implied by ΔV_{comp}. It is important to note that this study has centered on β-cyclodextrin that is surface bound. The impact of this reduction in degrees of freedom on pressure-dependent complexation behavior is currently under investigation. Finally, the chromatographic technique employed in these studies is limited in the choice of solution-phase environment. The mobile-phase choice is constrained by the conditions necessary to achieve chiral selectivity. Studies are presently underway to extend these studies to a wider range of solution environments using capillary electrophoresis in the high-pressure domain.[38]

Conclusions

Modest pressures are demonstrated to have a significant effect on complexation interactions in condensed phases. Previously considered chiefly in the highly compressible regimes of gases and supercritical fluids, pressures less than 500 bar are shown to have a direct impact on the complexation of enantiomers in condensed phases. This pressure-induced perturbation in complexation is observed even under conditions where one of the species is surface attached. The role of limited degrees of freedom on solute complexation may be considerable and is presently under investigation. Finally, the feasibility of measuring the differential partial molar volume between enantiomeric complexes is successfully demonstrated. Often considered as only a separation system, pressure-controlled liquid chromatography is shown to be a valuable measurement tool for the study of fundamental complexation processes.

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