stereoelectronic effect of the exo oxygen (exo-anomeric effect) although changes in supramolecular structure could not be eliminated.

As we noted earlier (vide supra), the results from the present study are consistent with a $^4C_1$ conformation for the monomer units. Apparently, the choice of acyl group, concentration, or temperature does little to perturb the basic $^4C_1$ ring conformation. Furthermore, the results from this study suggest that when the acyl group or temperature is varied, change in supramolecular structure rather than in microscopic conformation is primarily responsible for the observed changes in ^1H chemical shifts. For example, at 25 °C the chemical shift order for H1 (moving upfield) is CTA, CTP, and CTB. From the NOESY data (Table VIII) we can see that the H1–H4′ distances do not follow this order as the acyl group is changed. Likewise, we also see that increasing the temperature from 25 to 60 °C shifts H1 upfield by 20 Hz while the H1–H4′ distance remains unchanged. Moreover, as we noted previously, the T_1 values of CTA change abruptly at ca. 53 °C. From Table VIII we can see that neither the basic chair conformation nor the H1–H4′ distance is affected when the interproton distances are measured below and above this transition temperature.

It seems evident that the observed changes in ^1H chemical shifts and the temperature transition in T_1 values are due to changes in supramolecular structure. However, the observed changes due to concentration are more complex. Decreasing the concentration apparently induces an interrelated change in both macroscopic and microscopic conformation. A priori, one expects an increase in T_1 values with decreasing concentration. With CTA (Table IV), a decrease in T_1 values is observed, which we interpret as an increase in chain interaction. Furthermore, the virtual angle between monomer units increases (Table VIII), which suggests that as chain interactions increase, chain helicity increases. Because of this interaction of supramolecular structure and microscopic conformation, the ^1H chemical shifts do not change significantly.

Conclusion

The concept that temperature and concentration can influence solution conformations of macromolecules is well precedented.[17] However, very few studies have been reported that have explored the effect of these variables on high polymers on both a macromolecular and microscopic level. We have shown that carbon-13 NMR relaxation time studies and 2D NOESY studies are suitable tools for probing molecular dynamics and for determining interproton distances in these polymers. Using these tools, we have shown the following: (i) The basic $^4C_1$ conformation of the anhydroglucose monomers is not significantly perturbed by acyl type, temperature, or concentration. (ii) The virtual angle between H1 and H4′ of adjacent anhydroglucose units for cellulose esters is 30–34°, which suggests that these biopolymer derivatives exist as 5/4 helices. (iii) CTA undergoes a unique transition at 53 °C that appears to be due to changes in supramolecular structure. (iv) Decreasing concentration induces a complex, interrelated change in both macroscopic and microscopic conformation.

We recognize that the data we have presented are average values for a set of low-energy conformations interconverting rapidly on the NMR time scale. Nevertheless, our work provides a set of NMR constraints that can give us guidance in constructing a concept of the actions of these polymers in solution. The next step will be to use molecular dynamics simulations to probe for discrete solution structures that will satisfy the experimental constraints.

Acknowledgment. We thank Dr. P. S. Wehner, ECD Research Laboratories, for his aid in implementing the approach of Denk, Baumann, and Wagner for the determination of NOESY peak volumes.

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Effect of pH and Salt Concentration on Bimodal Inclusion of a Nitroxide by Cyclodextrins

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Abstract: The effect of pH and ionic salts on the binding of bimodal inclusion complexes between cyclodextrin and a nitroxide radical probe has been examined by using ESR spectroscopy. A sharp decrease in the amount of inclusion complex relative to the free probe was observed at pH ~ 12, which corresponds to the dissociative pH of protons in the hydroxyl groups on the rim of the cyclodextrin, indicating that the ionized cyclodextrin does not have the ability to include the nitroxide probe selected. The pK_a's of the hydroxyl groups of cyclodextrins are calculated to be 11.3 ± 0.8, 11.7 ± 0.4, and 11.9 ± 0.4 for α-, β-, and γ-cyclodextrin, respectively, on the basis of the pH dependence of the spectra. When an ionic salt was added to the solution of the complex of α-, β-, and γ-cyclodextrin, the association constant of the inclusion complex increases as a function of the salt concentration. The nature of the salt effect in the cyclodextrin system is discussed on the basis of the change of the enthalpy and entropy of association by the addition of salt. In addition, as the concentration of the salt is increased the inclusion of the *tert*-butyl group is enhanced more than that of the phenyl group. This result is discussed on the basis of the difference in the hydrophobicity of the included group.

The characteristics of the molecular cavity of cyclodextrin, a cyclic oligomer of glucose, have been well characterized both in the solid and solution phases.[1] In the study of host–guest complexes the effect of pH and salt on the inclusion behavior cannot be studied in the solid phase. From the viewpoint of enzyme-function-directed chemistry the study of the effect of pH and salt is especially important in understanding the phenomena such as the pH dependence of the activity, salting in, salting out, and denaturation.

Recently, Schneider et al.[2,3] studied the effect of solvent and salt on the association constants (binding constants) of organic

(1) For example, see: (a) Bender, M. L.; Komiyama, M. *Cyclodextrin Chemistry*; Springer: New York, 1978. (b) Saenger, W. *Angew. Chem., Int. Ed. Engl.* **1980**, *19*, 344. (c) *Inclusion Compounds*; Atwood, J. L., Davies, J. E. D., MacNicol, D. D., Eds.; Academic Press: London, 1984; Vols. 2 and 3.

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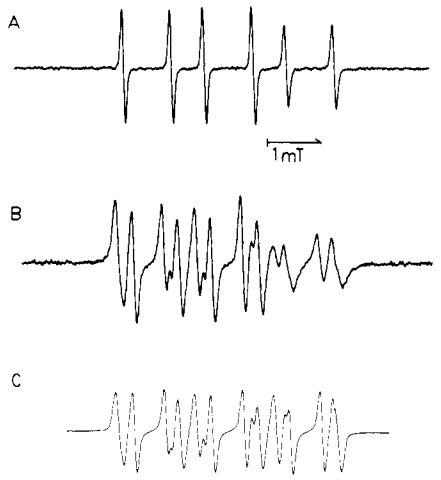


Figure 1. (A) ESR spectrum of probe **1** in aqueous solution. (B) ESR spectrum of probe **1** in aqueous solution in the presence of 0.038 M γ-cyclodextrin. (C) Computer-simulated spectrum for the spectrum B. Weaker intensity in the higher field line in B shows the slowing down of the motion of the probe by the complex formation.

substrates in various macrocyclic compounds including α-cyclodextrin. Unlike other combinations such as between an ionic host and an ionic guest, the salt effect for α-cyclodextrin was reported to cause a slight increase in association constant. Other previous studies have reported the effect of salts on cyclodextrin inclusion complexes of azo dyes and other probes.[4-6] Since it is not clear whether the probes in these studies are ionic or nonionic, association constants of the complexes depend on the type and concentration of the salt in a complicated way.

The study of the pH dependence of β-cyclodextrin complexes by Bergeron et al.[7] concentrated only on the ionic dissociation of the benzoate derivative guest. To our knowledge, the inclusion behavior of cyclodextrins at various pH's or at various salt concentrations has only been checked in relation to the catalytic activity of β-cyclodextrin[1,8,9] but has not otherwise been well investigated as yet. The main reason for this is that cyclodextrin does not show an appreciable change in association constant within a moderate range of pH and salt concentration. In addition, the difficulty in the spectroscopic isolation of the complex from the free species makes previous analyses complicated.

When a paramagnetic substrate and ESR spectroscopy are used, it has been shown that large molecules can undergo bimodal inclusion on the basis of the independent recognition of a functional group by the cyclodextrin cavity.[10-13] The determination of association constants is straightforward in this case because of the good spectroscopic separation of both included species and free species. Thus this probing technique is suitable for the study of the subtle effects caused by the surrounding conditions on the complex.

The effect of pH and salt concentration on complex formation of three kinds of cyclodextrins (α-, β-, and γ-cyclodextrin) is reported here. The pH effect is interpreted on the basis of dissociation of hydroxyl groups on the rim of the cyclodextrin. The change of association constants, enthalpy, and entropy of association by the addition of salt is discussed on the basis of hydrophobic–lipophilic interactions between the substrate and the cyclodextrin interior.

Table I. Hyperfine Splitting Constants and Association Constants of the Cyclodextrin Inclusion Complex of **1** in Water at 293 K[a]

complex	A_N,[a] mT	$A_{\beta H}$,[a] mT
free probe **1**	1.642	0.978
α-cyclodextrin[b]		
tert-butyl-in	1.625[c]	0.755[c]
phenyl-in		
β-cyclodextrin		
tert-butyl-in	1.602	1.220
phenyl-in	1.597	0.613
γ-cyclodextrin		
tert-butyl-in	1.594	1.254
phenyl-in	1.574	0.622

[a] Error is ±0.007 mT. [b] In the presence of 4 M KCl. [c] Not assigned.

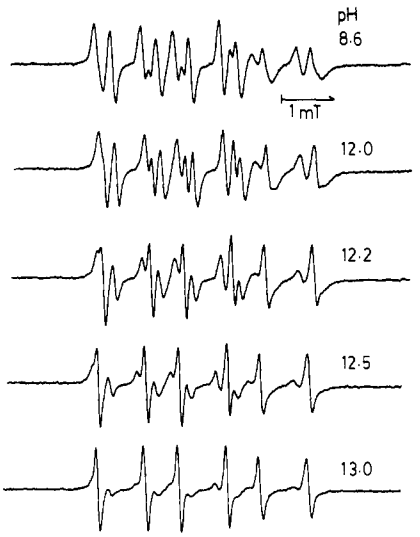
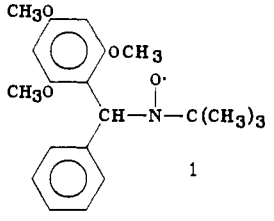


Figure 2. ESR spectra of γ-cyclodextrin inclusion complexes of probe **1** in aqueous solutions at various pH. The concentration of γ-cyclodextrin is 0.038 M.

Results and Discussion

Probes. The ESR spectrum of the inclusion complex of β- and γ-cyclodextrin with 2,4,6-(trimethoxyphenyl)benzyl *tert*-butyl nitroxide (**1**) shows completely separated ESR peaks for two kinds



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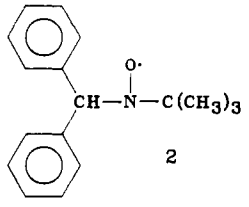

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of complexes.[13,14] Figure 1A shows the ESR spectrum of **1** in water. The addition of γ-cyclodextrin to this solution produces two different spectroscopic species in addition to the free probe (Figure 1B). Since the trimethoxyphenyl group is assumed to have a larger radius than the wider end of γ-cyclodextrin, inclusion probably occurs from either the phenyl side or the *tert*-butyl side of the probe and thus these two inclusion species are assigned to "phenyl-in" and "*tert*-butyl-in" complexes. The complex formation of **1** with α-cyclodextrin was not detected in water except in the presence of high salt concentrations (see later). Thus the ESR spectrum of the complex with probe **2**[11] was monitored in this pH effect study. Hyperfine splitting constants (hfsc) of nitrogen and β-hydrogen nuclei and the assignments of these complexes are shown in Table I. Since the ESR spectrum of the complex of

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(9) Chin, T.-F.; Chung, P.-H.; Lach, J. L. *J. Pharm. Sci.* **1968**, *57*, 44.
(10) Kotake, Y.; Janzen, E. G. *J. Am. Chem. Soc.* **1988**, *110*, 3699.
(11) Kotake, Y.; Janzen, E. G. *Chem. Phys. Lett.* **1988**, *150*, 199.
(12) Janzen, E. G.; Kotake, Y. *J. Am. Chem. Soc.* **1988**, *110*, 7912.
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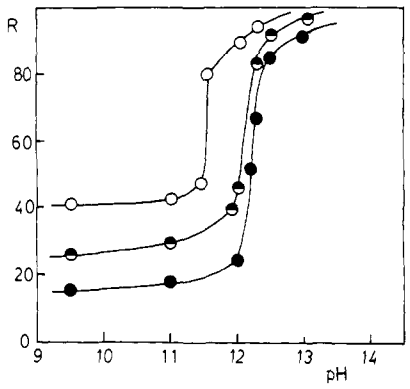

Figure 3. Percent composition of the free probe in aqueous solution of cyclodextrin complex as a function of pH at 293 K: ○, α-cyclodextrin (0.05 M); ⊖, β-cyclodextrin (0.0075 M); ●, γ-cyclodextrin (0.038 M). Probe **1** was used for β- and γ-cyclodextrin and probe **2** was used for α-cyclodextrin.

1 clearly shows isolated peaks for three species, namely the two complexes and the free probe, it is suitable for the study of the effect of environment on the association constant of these complexes.

Effect of pH on the Inclusion Equilibrium. When the pH of the solutions containing the α-cyclodextrin inclusion complex of **2** or β- γ-cyclodextrin inclusion complexes of **1** was changed from neutral to 13 by using an appropriate buffer solution, a striking change in the relative intensity of each species with negligible change in hfsc occurs at around pH 12. Figure 2 shows the ESR spectra of the γ-cyclodextrin inclusion complexes of **1** at various pH's. The relative amounts of free (not included) species are plotted as a function of solution pH in Figure 3. The relative amount of free species starts to increase at about pH 11; however, the ESR intensity ratio of two inclusion complexes stays the same. The complex shows drastic rejection of the substrate at pH 11–12. Cyclodextrin does not decompose even in highly basic media since a reversible change in the spectrum was observed when the solution was neutralized by dilute hydrochloric acid. Also the accompanying change in the salt ion concentration or the ionic strength by changing pH had a negligible effect since the spectrum did not depend on the addition of the same amount of neutral salt to the aqueous solution of the complex.

It has been reported[1a] that hydroxyl groups on the both ends of the β-cyclodextrin cavity have unusually low pK_a's as alcoholic OH, i.e. pK_a = 12.1 based on kinetic data[8] ("normal" alcohols have a pK_a larger than 16[15]). The sharp decrease in the ESR intensity of the complex in this study at around pH 12 can be ascribed to the dissociation of the protons of the hydroxyl groups. The change of this spectral feature is well explained by the assumption that the ionized form of cyclodextrin does not have the ability to include the probe. On the basis of the titration curve in Figure 3 and by the method described in the Experimental Section, the calculated pK_a's for hydroxyl groups in cyclodextrin are pK_a = 11.3 ± 0.8 for α-cyclodextrin, pK_a = 11.7 ± 0.4 for β-cyclodextrin, and pK_a = 11.9 ± 0.4 for γ-cyclodextrin.[8]

It is believed that the hydrogen bond network of the secondary hydroxyl groups on the wider end of the cyclodextrins helps lower the pK_a.[1b] The fact that the dissociated cyclodextrin appears to reject inclusion is in line with the fact that cyclodextrins are poor hosts for ionic guests.[1] The probes used, especially the substituents subject to inclusion, namely *tert*-butyl and phenyl, are very hydrophobic and thus have poor accessibility to the ionic entrance of the cavity. The sensitivity of the association constant to the accessibility of the cavity has already been shown as the difference in the association constant of the complexes between α-, β-, and γ-cyclodextrins and the nitroxide probe.[11] It is known that the reduction of the activity of some enzymes occurs as a result of dissociation of a functional group such as the carboxyl group. This

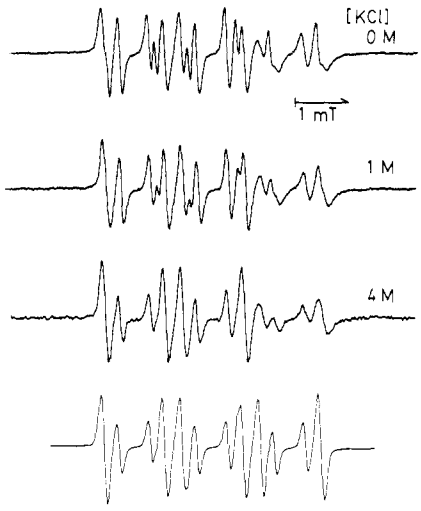

Figure 4. ESR spectrum of γ-cyclodextrin inclusion complexes of probe **1** in aqueous solution as a function of the concentration of potassium chloride at 293 K. The bottom spectrum is a computer spectrum simulation for [KCl] = 3.8 M spectrum. The concentration of γ-cyclodextrin is 0.0095 M.

happens because the dissociated form of the enzyme active site often does not have the ability to form an enzyme–substrate complex.[16]

Salt Effect. When an ionic salt is added to the aqueous solution of the inclusion complex the association constant is altered by a salting-out effect. Whether the association constant is decreased or increased by the addition of salt depends on the nature of the guest as well as the host. Both the cyclodextrin and the nitroxide probes have weak polar characteristics; thus, the association constant increases in all cases upon addition of salt. Debye–Huckel type interactions are not applicable here because they pertain to the decrease of the association constants of ionic complexes. Thus, the effect of salt addition is that the ESR signal for the free probe disappears more readily than that of the complex. However, inclusion in the cyclodextrin cavity provides another way for the probe to stay in the aqueous phase, resulting in an increase in the association constant of the inclusion complex. The addition of salt has only a very small effect on the magnitude of the hfsc's in all cases.

An illustration of a typical effect of salt on the ESR spectral features by the addition of salt is shown in Figure 4 for the γ-cyclodextrin complex of probe **1**. As the concentration of salt is increased, the ESR signal from free species disappears. In the case of the α-cyclodextrin complex of **1**, the presence of complex was detected only in solutions of high salt concentrations. All complexes of probe **1** with α-, β-, and γ-cyclodextrin show an increase in the association constant by adding various concentrations of ionic salt such as potassium chloride, ammonium sulfate, and calcium chloride. The association constants in the presence of salt derived from spectral simulation of the ESR spectra are listed in Table II. In the presence of the same concentration of salt, ammonium sulfate was most effective in increasing the association constant. Ammonium sulfate has the largest ionic strength and it has the greatest influence on the association constants at the same concentration of salt. However, calcium chloride, which has a larger ionic strength than that of potassium chloride, shows a smaller influence on the salting-out effect than potassium chloride.

Salt Effect on Group Inclusion. The temperature dependence of the association constants of β- and γ-cyclodextrin complexes of probe **1** in 2 M potassium chloride solution was observed, and the resulting enthalpies and entropies of association are listed in Table III with those in aqueous solution. These thermodynamic constants for association show that the formation of the complex

(15) For example, see: *Organicum*; Hazzard, B. J., translated; Addison-Wesley: Reading, MA, 1973; p 467.

(16) For example, see: Fersht, A. *Enzyme Structure and Mechanism*; W. H. Freeman: Reading, MA, 1977.

Table II. Association Constants (M^{-1}) of the Cyclodextrin Inclusion Complex with Probe **1** in Aqueous Solution at Various Concentrations of Salt at 293 K[a]

	α-cyclodextrin[b]		β-cyclodextrin		γ-cyclodextrin	
	*t*Bu-in	Ph-in	*t*Bu-in	Ph-in	*t*Bu-in	Ph-in
in water			4.9×10^2	1.5×10^2	2.0×10^2	1.9×10^2
[KCl], M						
0.3			5.6×10^2	1.6×10^2	3.1×10^2	2.5×10^2
0.6			5.6×10^2	1.6×10^2	3.9×10^2	3.0×10^2
1.0			6.4×10^2	1.7×10^2	5.0×10^2	3.2×10^2
2.0		2.0	8.4×10^2	1.8×10^2	8.8×10^2	5.4×10^2
3.8		4.0			2.6×10^3	1.1×10^3
$[(NH_4)_2SO_4]$, M						
0.3			5.4×10^2	1.5×10^2	5.0×10^2	3.8×10^2
0.6			9.2×10^2	2.4×10^2	5.6×10^2	4.0×10^2
1.0		2.0	1.4×10^3	3.8×10^2	8.8×10^2	5.6×10^2
2.0		5.0	1.7×10^3	4.4×10^2	4.9×10^3	2.5×10^3
$[CaCl_2]$, M						
0.3			5.7×10^2	1.5×10^2	2.8×10^2	2.2×10^2
0.6			5.8×10^2	1.5×10^2	4.2×10^2	3.4×10^2
1.0			6.0×10^2	1.7×10^2	4.7×10^2	3.5×10^2
2.0		2.0	6.8×10^2	1.7×10^2	7.7×10^2	4.5×10^2

[a] Error is ±10%. [b] Complex is not detected except at high salt concentrations. Error in this column is ±25%.

Table III. Association Constants and Thermodynamic Parameters of the Association of the Cyclodextrin Complex of Probe **1**

complex	K_a (293 K)[a]	$\Delta G°$ (293 K), kcal/M	$\Delta H°$,[b] kcal/M	$-T\Delta S°$ (293 K),[c] kcal/M
β-Cyclodextrin				
*t*Bu-in				
in water	5.2×10^2	−3.6	−4.6	0.9
2 M KCl	8.5×10^2	−3.9	−6.0	2.1
Ph-in				
in water	1.5×10^2	−2.9	−7.6	4.7
2 M KCl	1.7×10^2	−3.0	−5.9	2.9
γ-Cyclodextrin				
*t*Bu-in				
in water	2.0×10^2	−3.1	−5.1	2.0
2 M KCl	8.5×10^2	−3.9	−7.0	3.1
Ph-in				
in water	1.8×10^2	−3.0	−1.8	−1.2
2 M KCl	5.2×10^2	−3.6	−4.7	1.1

[a] Error is ±10%. [b] Maximum error is ±18%. [c] Maximum error is ±52%.

of **1** is entirely enthalpy driven except for the phenyl-in complex of γ-cyclodextrin in water. In spite of considerable error in determining these constants, a common interpretation can be made for the effect of salt on the *tert*-butyl-in complex binding. Upon addition of salt to the *tert*-butyl-in complex solution, the increase of enthalpic driving force overrides the unfavorable change of entropy for the increase in the association constant. The solvation structure of the trimethoxyphenyl group and the nonincluded group in both sides of the equilibrium (shown in eq 1 and 2) changes at the same time when the salt is added; thus, there is no contribution to $\Delta H°$ and $\Delta S°$. The effect of salt on the solvation

$$\square + t\text{Bu-Ph} \rightleftharpoons \boxed{t\text{Bu-Ph}} \quad \Delta G°_{t\text{Bu}} \qquad (1)$$

$$\square + \text{Ph-}t\text{Bu} \rightleftharpoons \boxed{\text{Ph-}t\text{Bu}} \quad \Delta G°_{\text{Ph}} \qquad (2)$$

structure of hydrophobic groups in aqueous solution is to cause rearrangement of the solvation shell, resulting in an increase of solvation entropy.[17,18] Thus, the decrease of entropy driven by the addition of salt can be interpreted in terms of the increase of solvation entropy of the *tert*-butyl group on the left-hand side of eq 1. The increase in enthalpic driving force is due to the increase of enthalpy of both water in the cyclodextrin cavity and the solvation of the *tert*-butyl group on the left side of eq 1, while

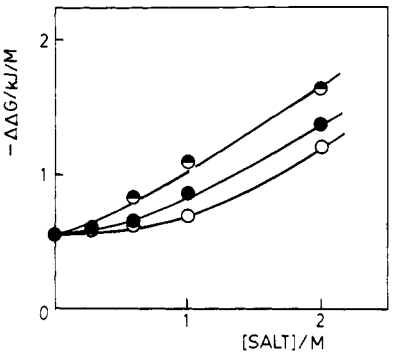

Figure 5. Difference in Gibbs' energy of association (293 K) for *tert*-butyl-in and phenyl-in complex of γ-cyclodextrin and probe **1** as a function of concentration of potassium chloride (●), ammonium sulfate (◓), and calcium chloride (○).

the enthalpy of the complex on the right side increases only by the change of the solvation structure of the phenyl group because the *tert*-butyl group in the cavity experiences only a small influence from the addition of salt to the solution.

In the phenyl-in complex of γ-cyclodextrin in water the association constant was found to be temperature insensitive. The general tendency of the salt effect on the phenyl-in complex is that the role of entropy for the negative influence of the association is more significant than that of the *tert*-butyl-in complex. This could imply that the solvation structure with water is different from that of the *tert*-butyl group although they have very similar hydrophobicity parameters.

It is noted that as the concentration of salt is increased the ratio of the ESR signal intensity of the *tert*-butyl-in complex to that of the phenyl-in complex is increased, indicating that the salt gives more driving Gibbs' energy for inclusion to the *tert*-butyl group than to the phenyl group. For example, the ratio of the association constant between phenyl-in and *tert*-butyl-in complexes changes from 0.81 to 0.43 in 0.3–3.8 M solution of potassium chloride, respectively. In solutions of other salts such as calcium chloride and ammonium sulfate, the relative association constant of the complex also shows similar change. It is noted that the functional groups with the higher hydrophobicity or lipophilicity are subject to more rejection by the ionic solution, thus gaining more driving force to form inclusion complexes.

The difference in the standard Gibbs' energy of these equilibria can be expressed as $\Delta\Delta G°$:

$$\Delta\Delta G° = \Delta G°_{t\text{Bu}} - \Delta G°_{\text{Ph}} \qquad (3)$$

Inspection of eq 1 and 2 shows that $\Delta\Delta G°$ means the difference in the Gibbs' energy of phenyl-in and *tert*-butyl-in complexes in solution; i.e..

(17) Marcus, Y. *Ion Solvation*; Wiley-Interscience: New York, 1980.
(18) Ben-Naim, A. *Hydrophobic Interactions*; Plenum Press: New York, 1980.

$$\boxed{\text{Ph}}\text{-}t\text{Bu} \rightleftharpoons \boxed{t\text{Bu}}\text{-Ph} \quad \Delta\Delta G^\circ \qquad (4)$$

In Figure 5 $\Delta\Delta G$'s calculated by using the equilibrium constants listed in Table II are plotted as a function of the concentration of the salt. The fact that $\Delta\Delta G^\circ$ is enhanced as a function of concentration of the salt indicates that the solvation structure also is accordingly changed. The detailed nature of this change is not quite clear from the numbers in Table III because the change of $\Delta\Delta G^\circ$ by the addition of the salt is the result of complex combination of the change of $\Delta\Delta H^\circ$ and $\Delta\Delta S^\circ$. The hydrophobicity parameter estimated on the basis of the partition of an organic material to water and octanol by Hansch and Leo[19,20] is 1.98 and 1.96 for *tert*-butyl and phenyl, respectively. Although the *tert*-butyl group seems to be slightly more hydrophobic, the difference seems not to be enough to account for the present experimental results. Hydrophobic interactions, which are effective in the salt effect, could be different from the quantitative measure obtained from the extractability by organic solvents especially in the case of the aromatic group. This seems to be in line with the fact that the aromatic group needs a characteristic parameter in molecular mechanics calculations of the structure of inclusion compounds.[21]

Experimental Section

ESR Spectroscopy. Probes **1** and **2** were prepared from phenyl lithium and α-2,4,6-(trimethoxyphenyl)-*N*-*tert*-butyl nitrone (**3**) and phenyl *N*-*tert*-butylnitrone (**4**) by the method described previously.[10] All solutions were prepared by using distilled water treated with Millipore Milli Q system. The pH of the solution was adjusted by using an appropriate buffer: pH 7–9, NaH_2PO_4, NaOH; pH 11–12, Na_2HPO_4, NaOH; pH 12–13, KCl, NaOH. All chemicals except **3**, **4**, α-, and γ-cyclodextrin were obtained from Aldrich Chemical Co. α- and γ-cyclodextrin were purchased from Sigma Chemical Co. Spin traps **3** and **4** are available in these laboratories. Sample solutions whose probe concentrations were less than 10^{-4} M were loaded in Pyrex tubes, 1 mm i.d. and 2 mm o.d. ESR spectra were obtained with a Bruker ER 200D-SRC spectrometer. The field modulation width was set less than 0.016 mT, and the incident microwave power was set less than 6.3 mW. The temperature was controlled by using a Bruker ER 4111T variable-temperature unit. Relative concentrations of each spectral species are determined by using computer spectrum simulation. ESR lines with nitrogen nuclear spin 0 and 1 (center- and high-field wing of nitrogen hfs) were used for the fitting.

Calculation of pK_a. In order to simplify the calculation, all hydroxyl groups of cyclodextrin are assumed to dissociate at a single pH; then the dissociation equilibrium present in this system is simplified as

$$CD \rightleftharpoons CD^- + H^+ \qquad K_a = [CD^-][H^+]/[CD] \qquad (5)$$

where CD, CD^-, and H^+ show cyclodextrin, cyclodextrin anion, and hydronium ion, respectively. Thus, the concentration of cyclodextrin in this equilibrium is[16]

$$C_1 = C_0/(1 + K_a/[H^+]) \qquad (6)$$

where C_0 denotes the initial concentration of cyclodextrin. From the inclusion equilibrium of the complex, the association constant of the complex is expressed as[10]

$$K_{CD} = (r_1/r_0)[C_1 - [R]r_1/(r_1 + r_0)]^{-1} \qquad (7)$$

where r_0 and r_1 denote the relative concentration of the free and induced species and [R] shows the initial probe concentration. Since $C_1 \gg [R]$, the term including [R] can be neglected. Combining eq 6 and eq 7, one can obtain the following equation:

$$pK_a = pH - \log (r_0 C_0 K_{CD}/r_1 - 1) \qquad (8)$$

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NMR Template Analysis of Biphenomycin: The Prediction of Conformational Domains Defined by Clustered Distance Constraints

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Abstract: The stereochemistry of the cyclic tripeptide antibiotic biphenomycin has been assigned by using conformational information derived from NMR studies. Kannan and Williams[1] argue from a smaller and less quantitative set of NMR observations in the assignment of stereochemistry than do Brown and co-workers.[2] Energy-based modeling studies[2] suggest that both data sets are sufficient to assign the chirality of residue 3 and demonstrate that the smaller data set cannot be used to assign chirality at residue 1. Template analyses[3,4] of the two data sets are reported in this study. This analysis predicts conformational domains defined by sets of clustered NMR observations and identifies regions of the molecule where conformational variability associated with unconstrained rotatable bonds or "links" joining conformational domains can be expected in modeling studies. NMR template analysis is an ab initio analysis of the distance constraints and is not derived from the results of modeling studies. The results of the energy-based modeling analysis of the two data sets reported by Brown et al.[2] are consistent with the predictions of the template analysis.

Biphenomycin A (Figure 1) is a cyclic tripeptide antibiotic that shows potent antibacterial activity against gram-positive organisms.[5] The stereochemistries of residues 1 (atom 14) and 3 (atoms 8 and 7) have been assigned by using a combination of NMR and computational techniques.[1,2] The chirality of residue 2 (atom 11) was assigned independently.[5] All three residues are characterized by *S* chirality at the α carbon, while the β carbon of residue 3 is *R* (atom 7). Two groups of workers undertook the assignment of the chiral centers of residues 1 and 3 using con-

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