Recognition Ability of Cyclodextrin for Alkyl Groups in Nitroxides As Studied by Electron Spin Resonance

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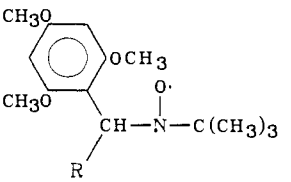
Abstract: The ability of γ-cyclodextrin to recognize various alkyl groups in a substrate to form host–guest inclusion complexes is examined. Electron spin resonance spectra of two kinds of complexes are observed when α-substituted 2,4,6-trimethoxybenzyl *tert*-butyl nitroxides (**1**) are mixed with γ-cyclodextrin. The structures of these complexes are assigned to "*tert*-butyl-in" and "alkyl-in" since it is assumed that the 2,4,6-trimethoxyphenyl group is too large to be included in the γ-cyclodextrin cavity. The association constants as well as the hyperfine splitting constants for the complexes with different alkyl substituents are determined. The ability to recognize various alkyl groups is discussed on the basis of the association constants of each complex. The data suggests that the association constant is dependent only on the characteristics of the included group not on the structure of the whole guest molecule and that the association constant is dependent on the size and hydrophobicity of the included group.

It is widely accepted that the specificity of enzymatic activity originates from the ability of its molecular cleft to recognize specific groups in the substrate molecule. Extensive investigations are now under way to mimic the enzymatic activity or to understand the mechanism of molecular recognition by using synthetic receptors.[1] Cyclodextrins, which are cyclic oligomers of glucose having a cavity structure, also have been studied as a model for enzyme active sites. The structures and the equilibrium properties of their inclusion complexes have been investigated extensively.[2] Also various chemical modifications of the rim of the cyclodextrin cavity have been performed to give catalytic activity similar to an enzyme.[3]

The structural study of host–guest complexes in solution is sometimes hampered by the choice of appropriate spectroscopy. For instance NMR studies give the structure averaged by the fast equilibrium of formation and dissociation of the complex.[4] ESR spectroscopy has a faster time scale than NMR, and spin probes in cyclodextrin solutions show two different ESR spectra corresponding to the included and the free species.[5] In the study of cyclodextrin inclusion complexes using a nitroxide probe, recently it has been clarified that cyclodextrin can recognize a functional group of a guest molecule resulting in the formation of bimodal inclusion complexes.[6-10] For example, when α-phenyl-2,4,6-trimethoxybenzyl *tert*-butyl nitroxide (aminoxyl) (**1** where R =



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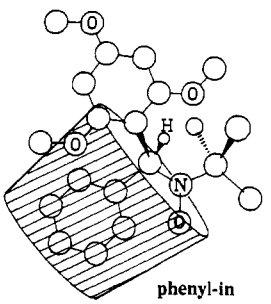
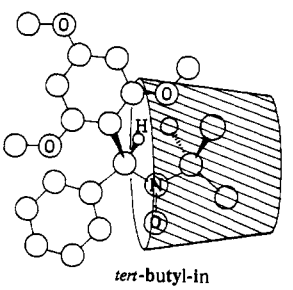
phenyl) is included by γ-cyclodextrin, complete spectroscopic separation of bimodal inclusion complexes is obtained by using ESR spectroscopy.[10] Since the diameter of the trimethoxyphenyl group is expected to be too large to be included in the γ-cyclodextrin cavity, these complexes are attributed to "phenyl-in" and "*tert*-butyl-in" complexes whose structures are schematically shown below. In this report by using various α-substituted 2,4,6-trimethoxybenzyl *tert*-butyl nitroxides the ability to recognize various alkyl groups by γ-cyclodextrin is examined on the basis of the association constant and the thermodynamic parameters of the complex.



phenyl-in



tert-butyl-in

Results and Discussion

ESR Spectra. Various nitroxides having different functional groups in the α-position were synthesized, and ESR spectra of the γ-cyclodextrin complexes were obtained in water. For example, the ESR spectrum of **1** where R = phenyl in water shown in Figure 1A changes into the spectrum in Figure 1B upon addition of γ-cyclodextrin. The spectrum in Figure 1B shows two different sets of six lines as the result of hyperfine splitting (hfs) with one nitrogen and one hydrogen nucleus. When the concentration of

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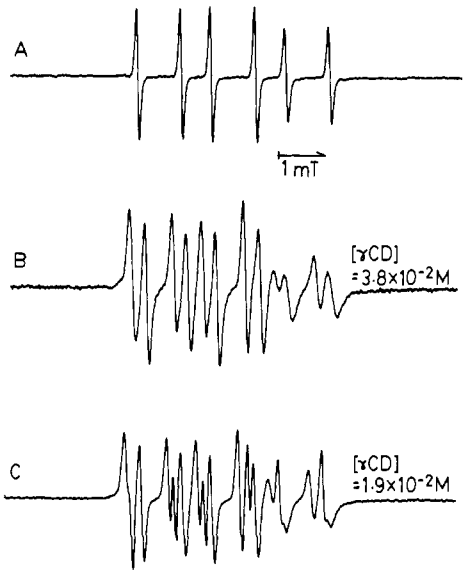

Figure 1. ESR spectra of α-phenyl-2,4,6-trimethoxybenzyl *tert*-butyl nitroxide (**1**): A, in water; B, in the presence of 3.8×10^{-2} M γ-cyclodextrin; C, in the presence of 1.9×10^{-2} M γ-cyclodextrin.

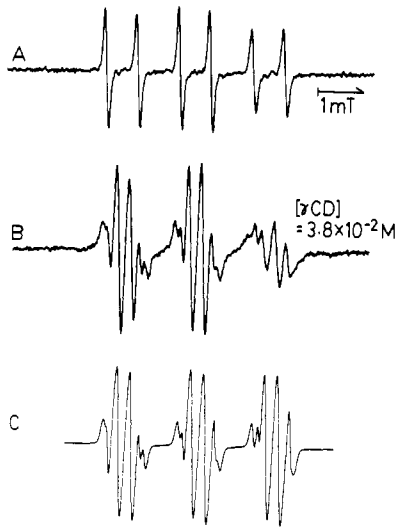

Figure 2. ESR spectra of α-cyclohexyl-2,4,6-trimethoxybenzyl *tert*-butyl nitroxide: A, in water, B, in the presence of 3.8×10^{-2} M γ-cyclodextrin; C, computer simulated spectrum for B. The concentration ratio of three species is 0.11 free, 0.13 *tert*-butyl-in, and 0.76 cyclohexyl-in.

γ-cyclodextrin is decreased, ESR peaks from the free (noninncluded) species also appear in the spectrum (Figure 1C). Figure 2 shows the spectrum from the cyclohexyl-substituted nitroxide as another example. The species with the larger proton-hfs is similar to that of the hfs in the *tert*-butyl-in complex of **1** where R = phenyl, while the other species has a smaller proton-hfs and stronger intensity than in the case of the phenyl-in complex of **1** where R = phenyl. Also the relative ESR intensity of the bimodal inclusion complex is different. In most cases we have found that the proton-hfs of one of the species becomes larger by complexation while the other decreases. Also the higher field lines which belong to the $M_I = 1$ manifold of nitrogen nuclear Zeeman level increases in line width upon formation of an inclusion complex because of the restriction in tumbling motions.

Assignment of the Spectra. In general all the inclusion complexes show smaller nitrogen-hfs than those of the corresponding free species (Table I). It is well-known that the nitrogen-hfs in nitroxides decreases when the solvent is changed from polar to nonpolar. The decrease in nitrogen-hfs in these complexes shows that the NO group is surrounded by a less polar environment than in water. This surrounding is assumed to be interior of the cyclodextrin. However, the change is very small in some cases

Table I. Hyperfine Splitting Constants and Association Constants of γ-Cyclodextrin Inclusion Complexes of Nitroxides at 293 K

		hfs,[a] mT		association constant,[b] M^{-1}	
R	complex	proton	nitrogen	K_{Bu}	K_R
methyl	free	1.361	1.677	150	
	t-Bu-in	1.672	1.672		
	R-in				
ethyl	free	1.122	1.658	150	
	t-Bu-in	1.334	1.610		
	R-in				
n-propyl	free	1.050	1.661	130	50
	t-Bu-in	1.274	1.608		
	R-in	1.198	1.660		
isopropyl	free	0.718	1.632	120	20
	t-Bu-in	1.126	1.606		
	R-in	0.570	1.615		
n-butyl	free	1.087	1.660	120	60
	t-Bu-in	1.275	1.602		
	R-in	0.883	1.601		
sec-butyl	free	0.703	1.631	120	60
	t-Bu-in	1.125	1.601		
	R-in	0.368	1.615		
n-pentyl	free	1.080	1.663	140	70
	t-Bu-in	1.186	1.633		
	R-in	0.920	1.615		
cyclopentyl	free	1.014	1.637	130	130
	t-Bu-in	1.244	1.631		
	R-in	0.604	1.662		
n-hexyl	free	1.089	1.660	170	150
	t-Bu-in	1.121	1.633		
	R-in	0.910	1.623		
cyclohexyl	free	0.683	1.632	130	750
	t-Bu-in	0.850	1.615		
	R-in	0.281	1.604		
phenyl	free	0.978	1.642	160	130
	t-Bu-in	1.254	1.594		
	R-in	0.612	1.574		

[a] Error is ±0.007 mT. [b] Error is ±10%.

compared to the solvent effect observed by changing the solvent from water to benzene[11] indicating that the nitroxide may be located on the rim of the cyclodextrin cavity. Since the change in nitrogen-hfs as well as inspection of molecular models indicates that the γ-cyclodextrin cavity can include the probe as far as the nitroxyl function from either *tert*-butyl or R side, it is not possible to assign the direction of inclusion on the basis of the nitrogen-hfs alone as in the case of β-cyclodextrin.[6]

The assignments of the inclusion complexes listed in Table I were made on the basis of the inspection of the magnitudes of the β-hydrogen-hfs as well as association constants. Firstly the β-hydrogen-hfs of the spectrum assigned to the *tert*-butyl-in complexes is relatively insensitive to complex formation as compared to those assigned to R-in complex (see Table I). This fact shows that inclusion occurs at the remote site from where the β-hydrogen is located. Also the magnitude of the β-hydrogen-hfs assigned to *tert*-butyl-in always increases upon inclusion. Conformational studies of nitroxide radicals similar to **1** have clarified that the introduction of a group more bulky than *tert*-butyl makes the β-hydrogen-hfs larger.[12-14] The fact that inclusion is identical with making the included group more bulky is consistent with this assignment. Also in the spin trapping chemistry of α-2,4,6-(trimethoxyphenyl)-*N*-butylnitrone it is found that the spin adduct with the more bulky addend within a series with the same functional group shows a smaller β-hydrogen-hfs.[15] Therefore the spectra with the smaller β-hydrogen-hfs is assigned to the R-in

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Table II. $\Delta G°$ for Inclusion Complex Formation of Alkyl Group and Transfer from *n*-Octanol to Water

alkyl group	$\Delta G°$, kJ/mol	
	inclusion (293 K)	transfer[18]
n-propyl	9.5	8.6
isopropyl	7.3	7.4
n-butyl	9.9	11.4
sec-butyl	9.9	10.2
n-pentyl	10.3	14.3
cyclopentyl	11.8	
cyclohexyl	16.1	
phenyl	11.8	

complex since these show smaller β-hydrogen-hfs values than in the free species (the only exception is the *n*-propyl adduct).

Association Constants. The association constant for each complex was calculated based on the ESR intensity of the complex and the free probe present in the aqueous phase by using computer spectrum simulation. These are listed in Table I with the hfs constants used for the simulation.

The association constants for the *tert*-butyl-in complexes for all the alkyl- and phenyl-substituted nitroxides do not vary substantially (about ±12%). On the other hand, the association constant for the R-in complexes changes dramatically, namely from zero for methyl or ethyl to 750 M^{-1} for cyclohexyl. Also there is a steady increase in inclusion tendency with increase in chain length of hydrocarbon; thus for methyl, ethyl, *n*-propyl, *n*-butyl, *n*-pentyl, and *n*-hexyl the association constant is zero, very small, 50, 60, 70, and 150 M^{-1}, respectively. The effect of branching seems to depend on the size of the group used; for example the association constant for isopropyl is less than for *n*-propyl the same for *n*-butyl and *sec*-butyl but the association constant for cyclopentyl is greater than for *n*-pentyl. The very large association constant for cyclohexyl is noteworthy.

In summary the ability to recognize a functional group by γ-cyclodextrin is well-defined by the magnitude of the association constant (binding constant) of the complex in which the functional group is included in the cavity. Moreover the magnitude of the association constants of *tert*-butyl-in complexes is not dependent on the functional group attached to the other side of the nitroxide group, and the association constant of R-in complexes is a function of the bulkiness as well as the number of carbon atoms in this group. This strongly suggests that inclusion does occur on the basis of functional group recognition not by whole molecule recognition. Thus the important conclusion drawn here is that the association constants for multimodal inclusion complexes is determined by the properties of the functional group to be included.

Gibb's Energy of Inclusion. $\Delta G°$ derived from the association constant at 293 K is the special constant peculiar to the given functional group. Also $\Delta G°$ for the groups included should be additive so that one can estimate the association constant for a given molecule if this molecule has two functional groups which can be included at the same time.

The components of $\Delta G°$ for complex formation consist of the removal of solvated water from the group to be included and from the cyclodextrin cavity in addition to the Gibb's energy gained by the formation of the hydrophobic bond between the group and the cyclodextrin interior. This situation is very similar to the extraction of an organic compound in water by an organic solvent such as *n*-octanol. The partition of organic compounds between *n*-octanol and water has been used to determine the hydrophobicity of the functional group.[16-19] Gibb's energy of transfer of alkyl groups from *n*-octanol to water is listed in Table II with $\Delta G°$ for the complex. There seems to be considerable coincidence between the two numbers which supports the notion that complexation is driven mainly by hydrophobic interactions.

It should be noted that the α-position of the probes is sterically crowded. If the alkyl substituent is small it is hidden by the bulky trimethoxyphenyl group resulting in no formation of a complex from this side as exemplified in the case of the methyl- and ethyl-substituted nitroxides. The distortion generated by complex formation also makes a contribution to $\Delta G°$, which is different from the hydrophobic interaction.

Experimental Section

Preparation of Spin Probes. All nitroxides were prepared by the reaction of appropriate Grignard reagent or organolithium with 2,4,6-trimethoxyphenyl-*tert*-butylnitrone (**2**). To a benzene solution of 3 mg of **2** was added 0.2 mL of ether solution (2 M) of Grignard reagent. The solution was gently bubbled with oxygen gas for 5 min and then was washed by 1 mL of 0.1 M aqueous solution of sodium bicarbonate twice. The organic portion was separated, and the solvent was purged by nitrogen gas flow. The dry residue obtained was added to 0.5 mL of ethanol. The radical concentration of this solution was ca. 1×10^{-4} M. Ethanol was purged from 10 μL of the probe solution, and the γ-cyclodextrin solution of appropriate concentration was added to make up the sample solution.

Materials. γ-Cyclodextrin was obtained from Sigma Chemical Company and was used without further purification. Grignard reagents and organolithium were purchased from Aldrich Chemical Company and used as received. Water was distilled and Millipore treated. Spin trap, α-2,4,6-trimethoxyphenyl-*N*-*tert*-butylnitrone was synthesized and purified by sublimation in these laboratories.

ESR Measurements. The sample solution was loaded in a Pyrex tube of 1 mm i.d. and 2 mm o.d. ESR spectra were measured by a Bruker ER 200D-SRC spectrometer equipped with 100 kHz field modulation. The field modulation amplitude setting was 0.2 G, and the incident microwave power was 10 mW. Temperature of the sample was controlled at 293 K by using a Bruker ER4111VT variable temperature unit.

Determination of the Association Constants. The association constant of the complex is calculated by assuming the following equilibrium

$$\text{R} + \text{CD} \leftrightarrow \text{R-CD} \quad (1)$$

where R, CD, and R-CD denote the probe, cyclodextrin, and the complex, respectively. The one-to-one association constant is defined by

$$K_a = [\text{R-CD}]/[\text{R}][\text{CD}] \quad (2)$$

where [ ] means the concentration. Equation 2 can be rewritten by using the relative concentration of the free probe and the complex

$$K_a = (r_1/r_0)(C - Pr_1/(r_1 + r_0))^{-1} \quad (3)$$

where r_0 and r_1 denote the relative concentrations of the free probe and the complex, respectively, and C and P give the initial concentration of cyclodextrin and the probe. The concentration of the probe was much lower than that of cyclodextrin thus eq 3 simplifies to

$$K_a = (r_1/r_0)/C \quad (4)$$

The relative concentrations of the complex and the free probe were determined by computer spectrum simulation. The relative area intensity of the simulated spectrum was used as r_0 and r_1. In order to test the fit, simulations were performed at two different concentrations of cyclodextrin.

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