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Detection of Group-in Complexes with Water-Soluble *p*-Sulfonatocalix[8]arene on Addition of Alkali Salts by Using Electron Spin Resonance Spectroscopy

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We found that the addition of alkali salts significantly increased the binding between *p*-sulfonatocalix[8]arene and a guest group. The change in ionic strength of the solution appears to drive the functional group in the probe deep into the calixarene cavity, making it possible to detect the group-in complex by using ESR spectroscopy.

Calixarenes are cyclic oligomers of aryl compounds, and their structures resemble a molecular basket with a hydrophobic cavity. Calixarenes can form a wide variety of host–guest type of inclusion complexes.[1,2] NMR spectroscopy has been a major tool for detecting calixarene inclusion complexes in solution. However, the signals for inclusion complexes cannot be separated from those of a free probe and bidirectional inclusion complexes, which are produced by the inclusion of two functional groups of the guest molecule, because NMR spectroscopy has a timescale of about 10^3 Hz.[3–5] In contrast, electron spin resonance (ESR) spectroscopy has a shorter timescale (ca. 10^6 Hz) than NMR spectroscopy. Thus, using free radical probes, inclusion complexes have been spectroscopically separated from non-included probes in cyclodextrin and calixarene inclusion systems.[6–8]

In a previous paper, using a nitroxide free-radical probe, α-phenyl-2,4,6-trimethoxybenzyl(*t*-butyl)nitroxide[6] (called a phenyl-probe in this report), as a guest molecule, we have detected bidirectional-inclusion complexes of *p*-sulfonatocalix[8]arene (Calix-S8).[8] Both phenyl-in and *t*-butyl-in complexes have been detected with ESR spectroscopy. However, when other α-substituted 2,4,6-trimethoxybenzyl(*t*-butyl)nitroxides were used, the ESR spectra of the guest complex and the non-complexed probe were overlapped. For example, Figure 1a shows the ESR spectrum of *t*-butyl (α-cyclohexyl-2,4,6-trimethoxybenzyl)nitroxide (called a cyclo-probe), and the ESR spectrum of the cyclo-probe in the presence of excess Calix-S8 exhibited ESR peaks for two radical species: a group-in complex and the non-complexed probe (Fig. 1b).

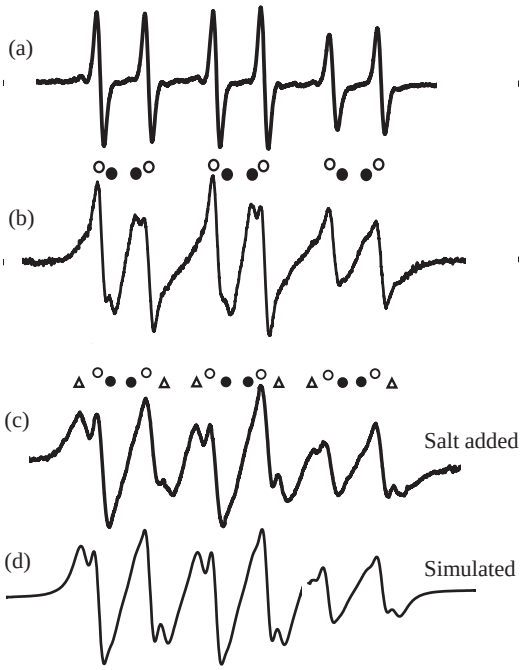

Fig. 1. ESR spectra of cyclo-probe (1.5×10^{-4} mol dm^{-3}) in the presence of various concentrations of Calix-S8 and alkali salts: (**a**) free (non-complexed) cyclo-probe; (**b**) [Calix-S8] = 4.1×10^{-3} mol dm^{-3}, no alkali salt. The peaks marked with ○ and ● are assigned to free (non-included) and cyclohexyl-in complex, respectively; (**c**) [Calix-S8] = 8.7×10^{-3} mol dm^{-3}, [CsCl] = 2.0 mol dm^{-3}. The peaks marked with △ are assigned to *t*-butyl-in complex; and (**d**) Computer-simulated spectra for (**c**). Hfsc's used for the simulation were: free probe ($A_N = 1.684$ mT and $A_H = 0.732$ mT); cyclohexyl-in complex ($A_N = 1.674$ mT and $A_H = 0.448$ mT); and *t*-butyl-in complex ($A_N = 1.671$ mT and $A_H = 1.100$ mT).

Since a subtle difference in micro-environment, such as ionic strength around the complex, significantly influences the structure of host–guest inclusion complex,[9] we added alkali salts to the solution of Calix-S8/cyclo-probe system. The ESR peaks for two independent group-in complexes became visible and were assigned to cyclohexyl-in and *t*-butyl-in complexes. The magnitude of separation was dependent on the salt concentration (Fig. 1c). We also showed that the group-in inclusion complexes were stabilized by adding three different alkali salts.

From a visual inspection of a molecular model, we concluded that the size of trimethoxyphenyl group is too big to fit in the Calix-S8 opening.[8] Therefore, we assigned the two species in the ESR spectra to be cyclohexyl-in and *t*-butyl-in complexes of Calix-S8 (Fig. 1). In detail, when the cyclo-probe was dissolved in a Calix-S8 solution ([Calix-S8]/[cyclo-probe] = 27), the ESR spectral peaks separated as the group-in inclusion complexes formed (Fig. 1b). In Fig. 1b, the hyperfine splitting (hfs) corresponds to one nitrogen and one hydro-

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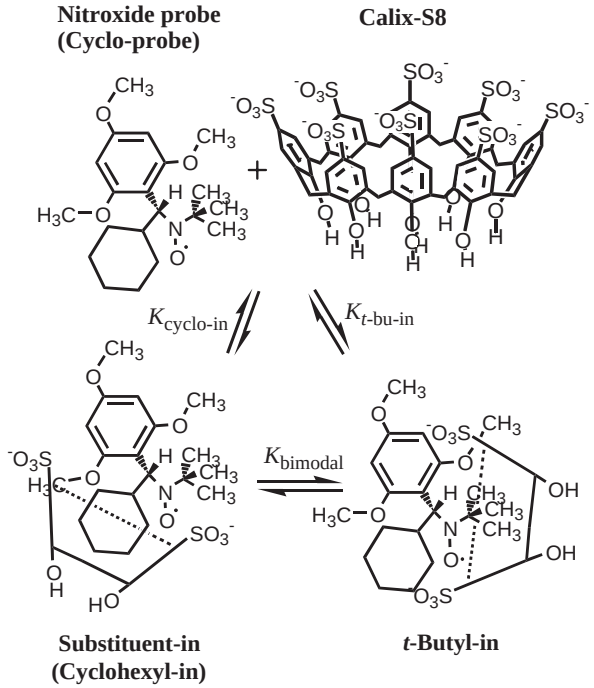

Fig. 2. Reaction scheme for the bidirectional inclusion of cyclo-probe with Calix-S8.

gen nucleus.[8] The lines marked with open circles correspond to the free probe, and the closed circles correspond to the complex. In the presence of excess Calix-S8 ([Calix-S8]/[cyclo-probe] = 58), the ESR spectrum only broadened (spectrum not shown), and only the peaks for one complex were observed. When the alkali salt CsCl was added (2.0 mol dm^{-3}), we were able to detect the other set of ESR peaks (Fig. 1c). Using the sets of hfs constants, R1 ($A_N = 1.684$ mT, $A_H = 0.732$ mT), R2 ($A_N = 1.674$ mT, $A_H = 0.448$ mT), and R3 ($A_N = 1.671$ mT, $A_H = 1.100$ mT), the ESR spectrum could be simulated (Fig. 1d). As suggested by the group inclusion in the Calix-S8/phenyl-probe and cyclodextrin/cyclo-probe system,[7,8] we assigned R1 to be the free cyclo-probe, R2 to be the cyclohexyl-in complex, and R3 to be the *t*-butyl-in complex. The assignment was made based on the fact that A_N is a hydrophobicity indicator of the surrounding media, i.e., a larger A_N means a less hydrophobic environment and vise versa. Therefore, we assigned R1 to be due the molecule exposed to water, i.e., the free probe. Assignment of R2 and R3 to each group-in complex was also made based on analysis of the molecular model, i.e., the direction of torsion at the C–N(O) bond in R2 and R3, because of steric hindrance, is opposite in each complex.[10] Thus, A_H in R2 and R3 shifted in opposite directions from R1. The β-H hfs for group-in complexation of the cyclo-probe is in agreement with that of the phenyl-probe: $A_H = 1.030$, 0.619, and 1.270 mT for free, phenyl-in, and *t*-butyl-in complexes, respectively.[8] We concluded that the addition of salt forced the Calix-S8/cyclo-probe to form a tightly bound inclusion complex.

Figure 2 illustrates the equilibrium that is present in Calix-S8/cyclo-probe inclusion system. Equilibrium constants for group inclusion, $K_{t\text{-bu-in}}$ and $K_{cyclo\text{-in}}$ are determined as follows. If [Calix-S8] $\gg$ [cyclo-probe], $K_{t\text{-bu-in}}$ and $K_{cyclo\text{-in}}$ can be

Table 1. Group-in Inclusion Equilibrium Constants

Salts[a)]	$K_{cyclo\text{-in}}$ /mol^{-1} dm^3	$K_{t\text{-bu-in}}$ /mol^{-1} dm^3	$K_{bimodal}$	K_C /mol^{-1} dm^3
CsCl	36.3 ± 1.1	97.1 ± 4.0	2.67	133
KCl	36.0 ± 1.0	94.6 ± 3.0	2.63	131
NaCl	40.2 ± 1.3	122 ± 4	3.03	162
none	58.6 ± 1.8	—	—	58.6

a) The salt concentration is 1.5 mol dm^{-3}.

expressed as follows:

$$K_{t\text{-bu-in}} = \frac{[t\text{-butyl-in}]}{[\text{cyclo-probe}][\text{Calix-S8}]} = \frac{[t\text{-butyl-in}]}{[\text{cyclo-probe}][\text{Calix-S8}]_0}, \quad (1)$$

$$K_{cyclo\text{-in}} = \frac{[\text{cyclohexyl-in}]}{[\text{cyclo-probe}][\text{Calix-S8}]} = \frac{[\text{cyclohexyl-in}]}{[\text{cyclo-probe}][\text{Calix-S8}]_0}. \quad (2)$$

K_C was defined as:

$$K_C = \frac{[t\text{-butyl-in}] + [\text{cyclohexyl-in}]}{[\text{cyclo-probe}][\text{Calix-S8}]_0} = K_{t\text{-bu-in}} + K_{cyclo\text{-in}}. \quad (3)$$

Using these equations, the equilibrium constants could be calculated by using the relative concentration [group-in]/[cyclo-probe], which can be determined from the simulated ESR spectra. The equilibrium constants are listed in Table 1. Inclusion equilibrium constants at various concentrations of three alkali salts, i.e., CsCl, KCl, and NaCl, were determined from ESR spectra, and ln K's were plotted against the salt concentration (Fig. 3). $K_{cyclo\text{-in}}$ decreased with an increase in the salt concentrations regardless of the kind of alkali salt; in contrast, $K_{t\text{-bu-in}}$ increased with an increase in the salt concentrations. Overall, the inclusion constant K_C for cyclo-probe with Calix-S8 increased with an increase in the salt concentration, and the kind of alkali salt had no effect on the group-in inclusion equilibrium constants. This suggests that the effect of the salt is not due to the size of the alkali ions complexed with Calix-S8,[11] but due to a change in the solution properties, such as ionic strength and surface tension.

The large salt effect on the $K_{t\text{-bu-in}}$ values for Calix-S8 complex is similar to that for the cyclodextrin (β-CD) inclusion complex.[9] For the β-CD complex, Örstan and Ross have suggested that surface tension is the major controlling factor, or more explicitly, that a change in the molecular surface area exposed to the solvent is a critical factor in determining the complex stability.[12,13] The surface area change during the inclusion reaction is closely related to the free energy change for the complexation between host and guest molecules. We speculate that the addition of alkali salts to the Calix-S8 solution increases the surface tension of water, resulting in the increase in the association constants. The difference in the stabilities of cyclohexyl-in and *t*-butyl-in complexes at high alkali salt concentrations is probably due to the difference in the surface area exposed to solvent between cyclohexyl- and *t*-butyl-groups in the two group-in complexes, resulting in the shift of the equilibrium between the two group-in complexes.

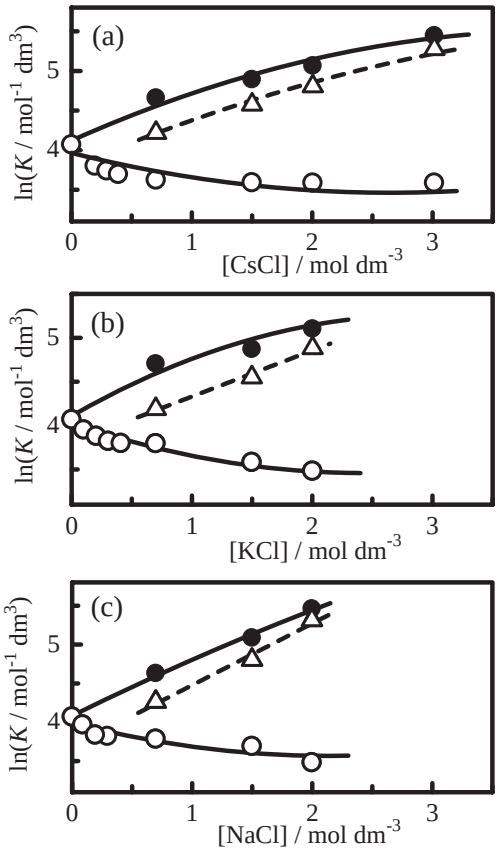

Fig. 3. Dependence of alkali salts ((a) CsCl, (b) KCl, and (c) NaCl) on the group-in inclusion equilibrium constants: (○) $K_{\text{cyclo-in}}$, (△) $K_{t\text{-bu-in}}$, and (●) K_{C}.

In the presence of excess salt, inclusion from the *t*-butyl side was favored over the cyclohexyl side (Fig. 3). The equilibrium constants K_{bimodal} (=[*t*-butyl-in]/[cyclohexyl-in]) between the bidirectional inclusion complexes reflect the difference in stability between the two group-in complexes in salt solution. The K_{bimodal} values, estimated from the $K_{t\text{-bu-in}}$ and $K_{\text{cyclo-in}}$ values, indicate that the equilibrium between the two group-in complexes shifts to the *t*-butyl-in side when the salt concentration is increased: for example, $K_{\text{bimodal}} = 1.81$, 2.67, and 5.4 at [CsCl] = 0.70, 1.5, and 3.0 mol dm^{-3}, respectively. In the presence of the other salts, K_{bimodal} also shows similar changes, which are in agreement with the observations for β-CD inclusion.[9]

In conclusion, the addition of ionic salts enhanced the inclusion ability or binding constant of Calix-S8, and bidirectional group-in complexes could be observed using ESR spectroscopy, which is due to effects of the salt on the functional groups and not on the whole molecule.

Experimental

Materials and Reagents. *t*-Butyl(α-cyclohexyl-2,4,6-trimethoxybenzyl)nitroxide was synthesized according to the method reported by Kotake and Janzen.[6] The Grignard reagent and *N*-*t*-butyl-α-(2,4,6-trimethoxyphenyl)nitrone were purchased from Aldrich Chemical Co. (Milwaukee WI, USA). Calix-S8 was obtained from Dojin Chemicals (Kumamoto, Japan) and used as received. Water was purified by distillation.

ESR Measurements. A phosphate buffer (pH 6.9 and μ(ionic strength) = 0.05), prepared from phosphate salts, was used as solvent. The nitroxide probe concentration was small ($<3 \times 10^{-4}$ mol dm^{-3}) to avoid line broadening from intermolecular spin exchange. The alkali salts (CsCl, KCl, and NaCl) were added to aliquots of the solution, and the ESR signals were recorded at 290 K with a JEOL FE3XG X-band spectrometer (Akishima, Japan). The spectrometer settings for ESR measurements was as follows: microwave power 5 mW; field modulation amplitude 0.032 mT at 100 kHz; time constants 0.3 s; field scan rate 0.31 mT min^{-1}. Computer simulation of the spectra was conducted using WIN-RAD program (Radical Research Inc., Hino, Japan).

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