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Influence of Temperature, Pressure, and pH on the Group-Inclusion Equilibrium in Water Soluble Calixarenes

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Previously, we demonstrated, for the first time, that guest probes having more than one bulky group could produce a pair of bidirectional inclusion complexes with water soluble calixarene (*Chem. Lett.* **2006**, *35*, 772). The use of unique free radical probes and electron spin resonance has made this finding possible. In this report, we investigated thermodynamic and pH-dependent properties of the group-inclusion complex formation, i.e., the effects of temperature, pressure and the medium pH on group inclusion by *p*-sulfonatocalixarenes were determined. Temperature-dependence study indicated that enthalpy portions in ΔG play dominant roles in the group-in complexation. Pressure dependence experiments allowed us to calculate the reaction volumes, i.e., intrinsic volume changes related to inclusion into the calixarene cavity, which ranged from -5.1 to $-16.3\,\text{cm}^3\,\text{mol}^{-1}$. Data on pH dependence clarified the role of oxyanions in the entrant group of calixarenes. It should be emphasized that pressure-dependent study provided unique information on the structure of the group-inclusion complex.

Calixarenes are macrocycle oligomers of *p*-substituted phenolic residues bridged by methylene groups and useful host molecules that can be modified with a wide variety of functional groups at the phenolic oxygens (lower rim) and the para positions (upper rim).[1,2] The modification of the macrocycle by sulfonate groups give rise to water-soluble calixarenes, which are useful in the study of the molecular recognition of organic guests in water,[3] that may mimic substrate–enzyme binding. Therefore, we believe it is important to characterize guest–host interactions in *p*-sulfonatocalixarene inclusion complex in aqueous solution. Depending on the kind of guest molecule, several intermolecular interactions for inclusion by calixarenes have been proposed, including (1) ionic, (2) hydrophobic, (3) van der Waals, (4) π–π, (5) cation–π, and (6) hydrogen bonding.[1,2] However, in previous studies, the inclusion data has based on the premise that once the guest–host combination is selected the structure of the complex is unique.

Studies on the structure of inclusion complex of the cyclic oligomer of glucose cyclodextrin indicated that a cyclodextrin cavity can form group-inclusion complex, each bulky group in the guest molecule is included into the cavity. Recently, we have shown that the same happens in calixarene inclusion complex, i.e., calixarene can form more than one inclusion complex from a single guest–host combination. Previously, there have been many reports on inclusion complexation of organic molecules with calixarenes; however, these concern inclusion of a guest molecule as a whole. Few previous NMR studies on calixarene inclusion complex assume the presence of bidirectional (bimodal) inclusion complexes, where the guest molecule and calixarene produce bidirectional isomeric inclusion complexes.[4–7] However, NMR techniques have been unable to separate included/non-included species,[4–7] because NMR has a long timescale ($>10^3$ Hz). In contrast, ESR has a shorter timescale ($\approx 10^6$ Hz) than NMR and could overcome fast exchange difficulties. Obviously, one drawback of ESR is that it requires the use of a free radical probe. By synthesizing unique free radical probes, we have shown that calixarene forms spectroscopically separable group-inclusion complex.[8] Nitroxide free radical probe having more than one bulky functional group was used in that study. For instance, *tert*-butyl group and phenyl group in the probe each forms independent complex (i.e., *tert*-butyl-in and phenyl-in complex (Fig. 1)) with water-soluble *p*-sulfonatocalix[8]arene. These isomeric complexes show distinctive ESR spectrum. In that report, we have determined the equilibrium properties of group-inclusion complex of *p*-sulfonatocalix[8]arene based on pressure dependence of equilibrium constants of the group-inclusion complex which were calculated from the ESR spectra.

In this study, we employed α-substituted 2,4,6-trimethoxybenzyl(*tert*-butyl)-nitroxides as free radical guests and recorded their ESR spectra under varying temperature, pressure and pH. Equilibrium constants (binding constants) of the complex were calculated by using computer spectrum simulation. In order to obtain further information concerning group-recognition of water-soluble calixarenes, we conducted pressure-, temperature-, and pH-dependence studies on the group-recognition properties of *p*-sulfonatocalixarenes. Quantitative information on free energy change, volume change, and ionic contribution was obtained by changing temperature, pressure, and pH, respectively. We concluded that unlike cyclodextrin group-in complex,[9] dominant interaction, which defines the calixarene group-in complex, is the van der Waals interaction.

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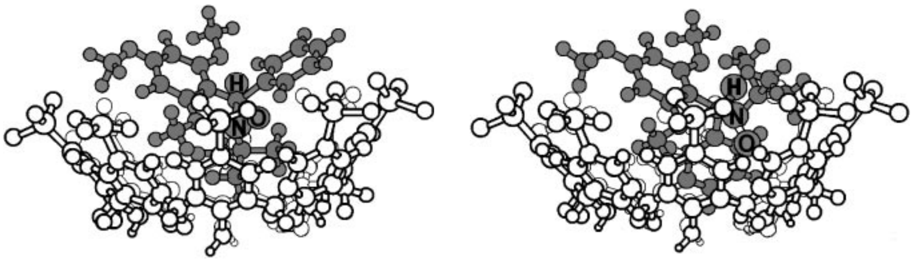
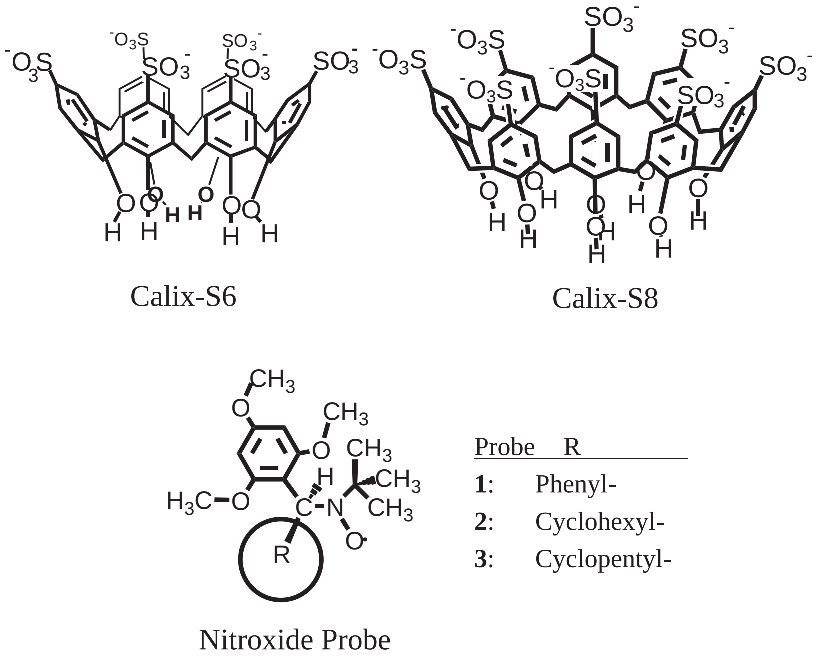


***tert*-Butyl-in** **Phenyl-in**

Fig. 1. Ball and stick structures of *tert*-butyl-in and phenyl-in complex of *p*-sulfonatocalix[8]arene.



Probe	R
1:	Phenyl-
2:	Cyclohexyl-
3:	Cyclopentyl-

Fig. 2. Structures of *p*-sulfonatocalixarenes and nitroxide probes.

Experimental

Materials and Reagents. α-Substituted 2,4,6-trimethoxybenzyl(*tert*-butyl)nitroxides (Fig. 2) were synthesized by the reaction of *N*-(*tert*-butyl)-2,4,6-trimethoxyaniline *N*-oxide, purchased from Aldrich Chemical Co., (Milwaukee, WI, U.S.A.), with Grignard reagents.[10] *p*-Sulfonatocalix[*n*]arenes (Calix-S*n*: $n = 6$, Calix-S6; $n = 8$, Calix-S8 (Fig. 2)) were obtained from Dojin chemicals (Kumamoto, Japan) and used as received. Water was purified by distillation.

ESR Measurements. A phosphate buffer (pH 6.9 and 12.5, ionic strength $I = 0.05$), prepared from phosphate salts, was used as solvent. The concentrations of nitroxide probes were selected small ($<2 \times 10^{-4}$ mol dm^{-3}) to avoid exchange broadening in ESR spectra. pH of the buffer was adjusted by adding diluted solution of either Na_2HPO_4 or NaOH ($I = 0.05$). Sample temperature was adjusted by using JEOL NM-PVT variable temperature unit. Our high-pressure ESR system has been described elsewhere.[9] The probe solution was loaded into a thick wall quartz capillary tubing (i.d. 1 mm, o.d. 6 mm). After applying pressure, the ESR signals were recorded in a JEOL FE3XG X-band spectrometer (Akishima, Japan). The spectrometer settings for ESR measurements were as follow: microwave power, 5 mW; field modulation amplitude, 0.032 mT at 100 kHz; time constant, 0.3 s; field scan rate, 0.31 mT min^{-1}. Computer spectral simulation was carried out using the WIN-RAD program (Radical Research Inc., Hino Japan). For the calculation of equilibrium constants using Eq. 2, the concentration ratio of the inclusion complex and free radical species was determined as follows: 1) recorded first-derivative ESR signal was reproduced by adjusting relative intensity of the two species, 2) simulated ESR signal of each species was independently double-integrated using the integration routine in the software, and 3) the ratio of the two numbers was calculated and adopted as the relative concentration for the calculation of equilibrium constants in Eq. 2.

Results and Discussion

Group-in Complex Formation with Calix-S*n*. Figure 3a shows the ESR spectrum of nitroxide probe **1** upon addition of Calix-S8, exhibiting well-separated ESR peaks. The ESR spectrum could be reproduced with computer spectral simulation by adjusting the relative intensity of the ESR spectra of free (Fig. 3e) and two radical species (Figs. 3c and 3d). In the previous paper, based on the characteristic hyperfine splitting constants (hfsc) of group-in complexes, we assigned the two radical species to *tert*-butyl-in and phenyl-in complexes, where either a *tert*-butyl or a phenyl group is included in the calix-

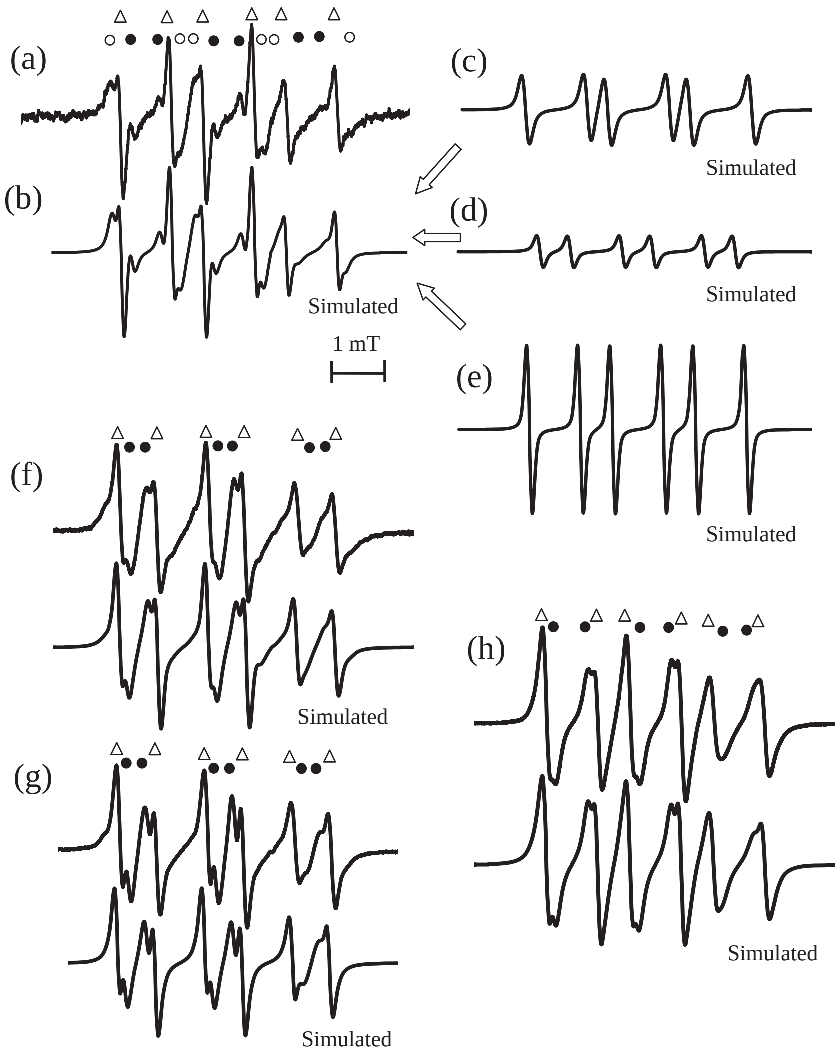


Fig. 3. (a) ESR spectrum of probe **1** ([**1**] = 1.0×10^{-4} mol dm^{-3}) containing Calix-S8 ([Calix-S8] = 9.7×10^{-3} mol dm^{-3}): Δ: free (non-included) radical species, ○: *tert*-butyl-in complex, and ●: phenyl-in complex. Computer-simulated spectra: (b) for ESR spectrum (a) in **1**-Calix-S8 solution; (c) for *tert*-butyl-in; (d) for phenyl-in; and (e) for free radical species. The relative spin concentration ratio of group-in complexes: (c) [*tert*-butyl-in]/[free probe] = 1.27; and (d) [phenyl-in]/[free probe] = 0.433. ESR spectra of probe **2** and **3** containing Calix-S*n* and simulated spectra: (f) [**2**] = 2.0×10^{-4} mol dm^{-3} and [Calix-S8] = 5.6×10^{-3} mol dm^{-3}; (g) [**2**] = 2.0×10^{-4} mol dm^{-3} and [Calix-S6] = 1.5×10^{-2} mol dm^{-3}; and (h) [**3**] = 2.5×10^{-4} mol dm^{-3} and [Calix-S6] = 3.2×10^{-2} mol dm^{-3}.

Table 1. The Association Constants K and Thermodynamic Parameters for Group-in Complexes of **1**–**3** at pH 6.9 in Water

Probe	Calixarenes	Assignment	a_N/mT	a_H/mT	K_1 and K_2/mol^{-1} dm^3		ΔH	ΔS	$T\Delta S$[c)]
					290 K	300 K	/kJ mol^{-1}	/J K^{-1} mol^{-1}	/kJ mol^{-1}
1	Calix-S8	phenyl-in	1.659	0.619	44.6 ± 0.8	33.5 ± 0.6	-18.7 ± 0.7	-33.0 ± 2.4	−9.6
1	Calix-S8	*tert*-butyl-in	1.691	1.270	131 ± 3	105 ± 3	-15.8 ± 0.8	-14.2 ± 2.8	−4.1
2	Calix-S8	cyclohexyl-in	1.674	0.448	58.6 ± 1.8	43.1 ± 1.4[a)]	-15.5 ± 1.1	-19.6 ± 4.1	−5.7
2	Calix-S6	cyclohexyl-in	1.679	0.428	46.1 ± 1.5	31.3 ± 1.0[a)]	-16.6 ± 0.9	-25.6 ± 3.2	−7.4
3	Calix-S6	cyclopentyl-in	1.685	0.786	12.2 ± 0.4	10.3 ± 0.3[b)]	-16.3 ± 0.3	-34.5 ± 1.1	−10.0

a) At 305 K. b) At 298 K. c) At 290 K.

arene cavity (see Fig. 1).[8] The hfsc values of the *tert*-butyl-in and phenyl-in complexes of probe **1** were estimated from computer spectral simulation by combining three sets of triple-doublet spectra (Fig. 3b) and are listed in Table 1. The ESR spectrum of the group-in complex of probe **1** with Calix-S6 did not separate from the free species (data not shown). Possible reasons for this are: (1) Calix-S6 may not form the stable group-in complexes with probe **1**, and (2) the ESR spectra of the possible group-in complexes may have heavily overlapped with free species.

Analogs of nitroxide probes having various alkyl or cycloalkyl groups (ethyl, propyl, butyl, cyclopentyl, and cyclohexyl)

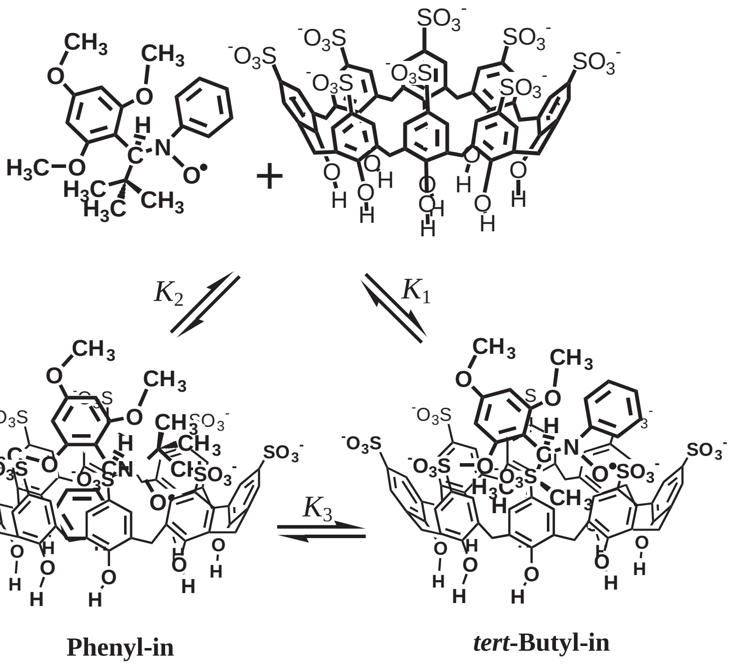


Fig. 4. The reaction scheme for group-in inclusion of nitroxide probe **1** with Calix-S8.

were synthesized and tested for the group-in complex formation with Calix-S8 and -S6. For straight chain alkyl groups, in the ESR spectra group-in complexes were not found. However, probes **2** and **3** that contain cycloalkyl groups formed cycloalkyl-in complexes (Figs. 3f–3h). These ESR spectra were readily reproduced with computer spectrum simulation (Fig. 3). The hfsc values calculated from the spectrum simulation cycloalkyl-in complexes with Calix-S6 and -S8 are listed in Table 1.

Inclusion equilibrium constants were calculated based on the relative concentration of the complex and free species, which were also determined with spectral simulation. The equilibrium based on the reaction scheme for the group-in complex formation of probe **1**–**3** with calixarenes shown in Fig. 4 is expressed as follows:

$$K = \frac{[\text{P-Calix-S}n]}{[\text{Probe}][\text{Calix-S}n]}, \tag{1}$$

where P-Calix-S*n* denotes the group-in complex. Under the condition of [Calix-S*n*] $\gg$ [probe], Eq. 1 can be rewritten as follows:

$$\frac{[\text{P-Calix-S}n]}{[\text{Probe}]} = K[\text{Calix-S}n]_0, \tag{2}$$

where $[\text{Calix-S}n]_0$ denotes the initial concentration of calixarenes. Thus, the inclusion equilibrium constants (K) can be calculated from plots of the relative abundance of free probe and group-in complex against the initial concentration of calixarenes. In Fig. 5, a plot of [P-Calix-S8]/[Probe] vs. $[\text{Calix-S8}]_0$ for probe **1** gave a straight line with the slope K, which passed through the origin. Equilibrium constants (K_1 and K_2) obtained for the group-in complexes of probe **1**–**3** with Calix-S*n* are listed in Table 1.

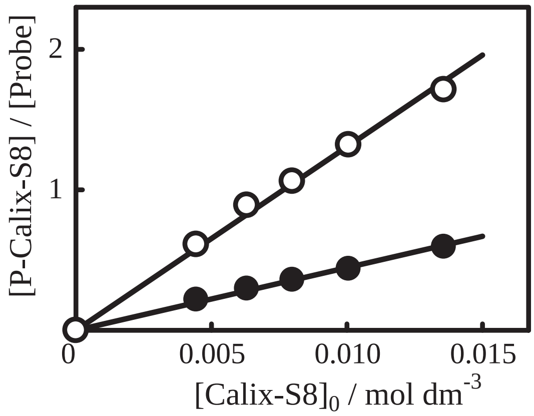


Fig. 5. Relative abundance of group-in complex and free radical probe **1**, plotted as a function of the initial concentration of Calix-S8: *tert*-butyl-in (○) and phenyl-in (●) complex formations.

Temperature Dependence of Group-in Inclusion. We have examined the temperature dependences of the inclusion equilibrium (K) for group-in complexation. Figure 6 shows the ESR spectra of probe **1** and **2** in the presence of excess Calix-S8 at various temperatures. With an increase in the temperature, the ESR signal intensities of group-in complexes (*tert*-butyl-in and α-substituent-in) decreased, and those of free (non-complexed) probes increased. Figure 7 shows the plots of $\ln K$ (inclusion equilibrium constant) against T^{-1} (a van't Hoff plot). From the slope and intercept of the plots, thermodynamic parameters (ΔH and ΔS) were estimated and are given in the right-hand side of Table 1. All of the values of ΔH are similar, and the values of ΔS were negative in the range of -14–$-35\,\mathrm{J\,K^{-1}\,mol^{-1}}$.

The comparison of ΔH with $T\Delta S$ is informative for the inclusion mechanism. The relatively large negative values of ΔH for the group-in complexation as compared to $T\Delta S$ are indicative that group-in complex formation is favored by

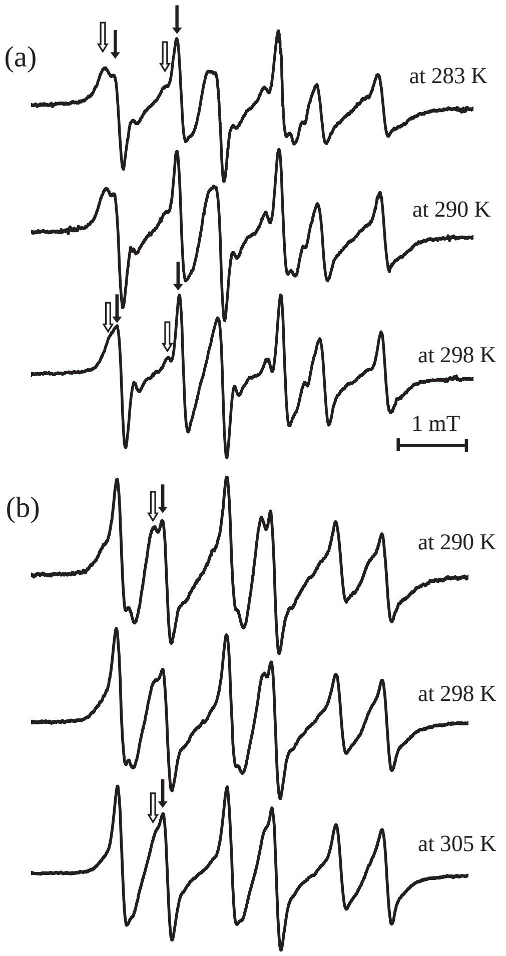


Fig. 6. ESR spectra of probes **1** and **2** in the presence of Calix-S8 at various temperatures: (a) $[\mathbf{1}] = 1.0 \times 10^{-4}$ $\mathrm{mol\,dm^{-3}}$ and $[\text{Calix-S8}] = 9.7 \times 10^{-3}\ \mathrm{mol\,dm^{-3}}$; and (b) $[\mathbf{2}] = 2.5 \times 10^{-4}\ \mathrm{mol\,dm^{-3}}$ and $[\text{Calix-S8}] = 5.6 \times 10^{-3}$ $\mathrm{mol\,dm^{-3}}$. Open arrows and closed arrows are assigned to the group-in complexes and free species, respectively.

enthalpic contributions and disfavored by entropic contributions. The major contribution to enthalpy of inclusion most likely comes from the van der Waals interactions, because probes **1**–**3** are neutral and electrostatic interaction can be ignored. In the study of the complexation mechanism of *p*-sulfonatocalixarenes with dipeptides or tripeptides, Douteau-Guevel et al. have suggested that the binding process for the alkyl chain is controlled by the favorable enthalpy resulting from the inclusion of the apolar part of the guest into the hydrophobic cavity of host through van der Waals interactions.[11] This is in agreement with our observation for the apolar group inclusion by *p*-sulfonatocalixarenes.

Effects of Pressure on Group-in Inclusion. Figure 8 shows ESR spectra of probes **1**–**3** in the presence of Calix-S8 and -S6 at high pressures. When a static pressure of 490 bar (1 bar $= 1 \times 10^5$ Pa) was applied to the solution, the ESR spectral intensities of group-in complexes increased. These ESR spectra were reproduced using computer simulation by adjusting relative abundance of the two or three radical species, but keeping the hfs constants the same. Inclusion equilibrium constants (K) for the group-in complex of probes **1**–**3** at various pressures are listed in Table 2. The K values increased as a function of increasing external pressure.

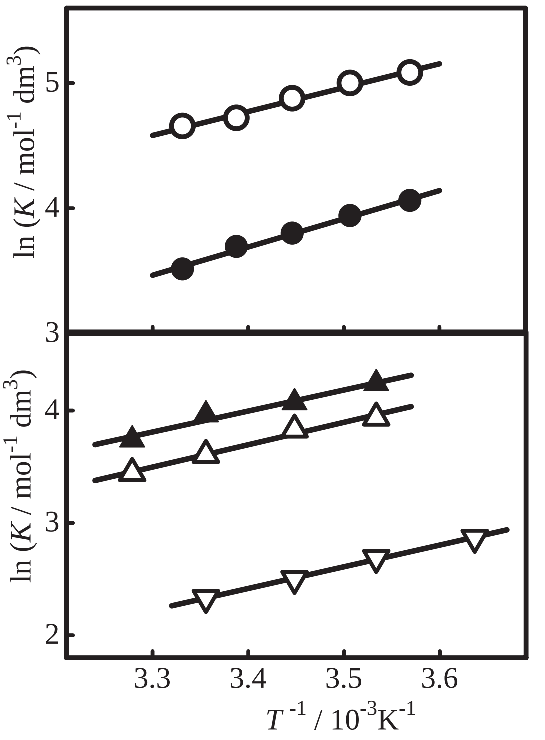


Fig. 7. Plots of $\ln K$ against T^{-1} for group-in equilibrium of probes **1**–**3**. *tert*-Butyl-in (○) and phenyl-in (●) complex formation of probe **1** with Calix-S8. Cyclohexyl-in complex formation of probe **2** with Calix-S8 (▲) and Calix-S6 (△). (▽) cyclopentyl-in complex formation of probe **3** with Calix-S6.

Pressure dependence data can be analyzed with following formulations. The change in the volume accompanied by the group-in complex formation (reaction volume ΔV) was calculated according to the following equations:

$$\ln K = aP + b, \tag{3}$$

$$\Delta V = -RT\left(\frac{\partial \ln K}{\partial P}\right)_T - \kappa_T RT, \tag{4}$$

where κ_T is the thermal compressibility of the solvent[12] and $\kappa_T = 4.43 \times 10^{-5}\ \mathrm{bar^{-1}}$. In the group-in complex formation of calixarenes, all probes showed negative ΔV values (Table 2). ΔV that is accompanied by chemical reaction can be divided into two terms: 1) an intrinsic volume change, and 2) a volume change arising from reorganization of the solvent molecules. Similarly, the volumetric contributions during formation of an inclusion complex with calixarenes can be expressed by two discrete volume changes:

$$\Delta V = \Delta V_{\mathrm{inclu}} + \Delta V_{\mathrm{desolv}}, \tag{5}$$

where $\Delta V_{\mathrm{inclu}}$ denotes volume changes related to inclusion of guest molecules in the calixarene cavity ($\Delta V_{\mathrm{inclu}} < 0$), and $\Delta V_{\mathrm{desolv}}$ denotes accompanying desolvation around the guest and host molecules ($\Delta V_{\mathrm{desolv}} > 0$). $\Delta V_{\mathrm{desolv}}$ for inclusion was assumed to be small, because probe **1**–**3** are neutral. Therefore, the reaction volume observed for group-in complexation corresponds to the change in the intrinsic volume. Unlike in the case

Fig. 8. ESR spectra of probes **1**–**3** in the presence of excess Calix-S*n* under 1 and 490 bar at 290 K. (a) [**1**] = 2.5×10^{-4} mol dm^{-3} and [Calix-S8] = 8.0×10^{-3} mol dm^{-3}. (b) [**2**] = 2.5×10^{-4} mol dm^{-3} and [Calix-S8] = 7.0×10^{-3} mol dm^{-3}. (c) [**2**] = 2.0×10^{-4} mol dm^{-3} and [Calix-S6] = 1.5×10^{-2} mol dm^{-3}. (d) [**3**] = 2.5×10^{-4} mol dm^{-3} and [Calix-S6] = 3.2×10^{-2} mol dm^{-3}. Open arrows and closed arrows are assigned to the group-in complexes and free radical species, respectively.

Table 2. The Association Constants K under External Pressures and Reaction Volumes for Group-in Complexes of **1**–**3** at 290 K

Probe	Calixarenes	Assignment	pH	K_1 and K_2/mol^{-1} dm^3				ΔV /cm^3 mol^{-1}
				1 bar	98 bar	294 bar	490 bar	
1	Calix-S8	phenyl-in	6.9	44.6 ± 0.8	48.7 ± 0.8	55.6 ± 1.0	60.9 ± 1.1	-16.3 ± 2.0
1	Calix-S8	*tert*-butyl-in	6.9	131 ± 3	135 ± 3	148 ± 5	156 ± 4	-10.0 ± 1.5
2	Calix-S8	cyclohexyl-in	6.9	58.6 ± 1.8	59.7 ± 1.5	61.5 ± 1.6	63.6 ± 1.9	-5.1 ± 1.1
2	Calix-S8	cyclohexyl-in	12.5	34.8 ± 1.5	36.5 ± 1.2	39.4 ± 1.5	44.6 ± 1.7	-13.0 ± 1.7
2	Calix-S6	cyclohexyl-in	6.9	46.1 ± 1.5	47.0 ± 1.5	48.6 ± 1.1	50.3 ± 1.5	-5.4 ± 0.9
2	Calix-S6	cyclohexyl-in	12.5	31.4 ± 1.3	33.4 ± 1.3	35.9 ± 1.8	40.0 ± 1.5	-12.5 ± 1.6
3	Calix-S6	cyclopentyl-in	6.9	12.2 ± 0.4	12.5 ± 0.5	13.6 ± 0.5	14.2 ± 0.6	-9.1 ± 1.5

of cyclodextrin inclusion complex, ΔV ($\approx \Delta V_{\mathrm{inclu}}$) for probes **2** and **3** with Calix-S6 and -S8 (-5–-9 cm^3 mol^{-1}, at pH 6.9) are insensitive to the size of guests.[9] The large negative ΔV for the phenyl-in complex formation of probe **1** by Calix-S8 indicates deep penetration from the phenyl group side into the Calix-S8 cavity, which may also be responsible for the large negative value of ΔS. This explains the small a_{N} value of the phenyl-in complex, because a_{N} in nitroxide radicals becomes larger in a polar environment.[13] In the group-in complex of probe **1**, Calix-S8 favors the inclusion from the *tert*-butyl side than the phenyl side, probably because the inclusion from the *tert*-butyl side forces N–O group to interact with polar sulfonato groups, resulting in the increase in a_{N} (Table 1). The small negative ΔV for the *tert*-butyl-in complex formation as compared with that of the phenyl-in complex indicates shallow inclusion from the *tert*-butyl side, which may be also responsible for the small negative value of ΔS.

Based on the reaction scheme in Fig. 4, the inclusion equilibrium between *tert*-butyl-in and phenyl-in complexes of probe **1** can be expressed as:

$$K_3 = \frac{[\textit{tert}\text{-butyl-in}]}{[\text{phenyl-in}]}, \tag{6}$$

where K_3 denotes the equilibrium constant between group-in complexes. The value of K_3 was calculated to be 2.9 at 290 K using the data in Table 2. The volume change ΔV_3 of the group-in complexes (*tert*-butyl-in and phenyl-in) of probe **1** with Calix-S8 was evaluated as follows:

$$\Delta V_3 = \Delta V_{\textit{tert}\text{-butyl-in}} - \Delta V_{\text{phenyl-in}} = 6.3\,\mathrm{cm^3\,mol^{-1}}. \tag{7}$$

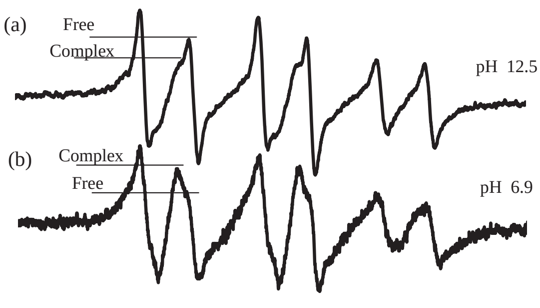


Fig. 9. ESR spectra of probes **2** in the presence of excess Calix-S8 at pH (a) 12.5 and (b) 6.9: [**2**] $= 1.5 \times 10^{-4}$ mol dm^{-3} and [Calix-S8] $= 7.45 \times 10^{-3}$ mol dm^{-3}.

This ΔV_3 value shows that with an increasing external pressure, the group inclusion equilibrium shifts to the phenyl-in complex side.

pH Dependence of Group-in Inclusion. Shinkai et al. have reported that the pK_a values of the OH groups in Calix-S6 and -S8 are p$K_1 < 1$, p$K_2 = 3.0$, p$K_3 = 4.0$, p$K_4 > 11$ for Calix-S6 and p$K_1 = 3.69$, p$K_2 = 4.34$, p$K_3 = 8.04$ for Calix-S8.[14,15] Therefore, in Calix-S6 and -S8, three and two of the OH groups are dissociated at the neutral pH region (pH 6.9), respectively. We determined the effects of the H-dissociated oxyanions (lower rim depicted in Fig. 2) on the group-inclusion of *p*-sulfonatocalixarenes by varying the pH of the solvent. In very acidic medium, such as pH 2.0, the instability of probes **1**–**3** hampered the experiment. In very basic medium (pH 12.5), a group-in complex of probe **1** was not observed, but those of probes **2** and **3** were observed. Therefore, we performed the ESR measurements of group-in complexes of probe **2** with Calix-S6 and -S8 at pH 12.5.

Figure 9 show the ESR spectrum of probes **2** in the presence of excess Calix-S8 at pH 12.5 and 6.9. The ESR spectral intensity of cyclohexyl-in complex decreased with an increase in the pH of the medium. From the spectral simulation, the hfsc of cyclohexyl-in complex (probe **2**) with Calix-S6 and -S8 at pH 12.5 were: $a_N = 1.679$ mT and $a_H = 0.444$ mT for Calix-S6, and $a_N = 1.674$ mT and $a_H = 0.485$ mT for Calix-S8. The a_N values at pH 12.5 are the same as in pH 6.9. The a_H values at pH 12.5 are slightly larger than at pH 6.9. The equilibrium constants for cyclohexyl-in complex formation at pH 12.5 were evaluated according to Eq. 2 and listed in Table 2. When the pH was changed from 6.9 to 12.5, the inclusion equilibrium constants for cyclohexyl-in complex became smaller, whereas $|\Delta V\ (\approx -13\,\text{cm}^3\,\text{mol}^{-1})|$ at pH 12.5 was large as compared with that ($-5\,\text{cm}^3\,\text{mol}^{-1}$) at pH 6.9. In the basic pH region (pH 12.5), four H-dissociated oxyanions are situated on the small lower rim of Calix-S6. The repulsion between the oxyanions may result in the conformational change of calixarene. The pH dependence in hydrogen hfs (a_H) occurs probably because the group inclusion of the group causes a slightly different magnitude of torsion at the N–α-C bond.[16] In the group inclusion of probe **2** with β-cyclodextrin (CD), the a_H value (=0.48 mT) for the cyclohexyl-in complex was larger (=0.28 mT) than γ-CD with a larger cavity.[9] The increase in the a_H values for group-in complexes of calixarene with an increase in the pH suggests that the size of the Calix-S*n* cavity diameter (upper rim of sulfonato group side) decreases in highly basic media, resulting in deeper encapsulation into the Calix-S*n* cavity. We speculate that the decrease in the portal size of Calix-S*n* is responsible for the smaller K values. Deep inclusion causes the larger negative intrinsic volume change, which is indicated by large negative ΔV's.

In conclusion, we determined basic thermodynamic and pH dependent properties of newly found group-inclusion complex of calixarene. In particular, the high-pressure studies provided useful insights for the functional group-recognition of calixarenes.

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