Bidirectional Inclusion of Free-radical Probe by Water-soluble Calixarene as Detected by Electron Spin Resonance

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The first detection of bidirectional inclusion by water-soluble calixarene is reported. The free-radical probe α-(2,4,6-trimethoxyphenyl)benzyl *tert*-butyl nitroxide is shown to produce two isomeric inclusion complexes with *p*-sulfonatocalix[8]arene in aqueous media, being detected with ESR spectrometry. ESR spectra were assignable to *tert*-butyl-in and phenyl-in complex and the association constants at room temperature were 131 and 45 mol^{-1} dm^3 for *tert*-butyl-in and phenyl-in, respectively.

Calixarenes, bowl-shaped macrocyclic compounds, are the supramolecular host compounds along with crown ethers, cyclodextrins, and paracyclophanes,[1–4] and are widely used in different branches of chemistry, physics, and material science.[5] Water-soluble *p*-sulfonatocalixarenes, synthesized by Shinkai et al.,[6] are good receptors for organic molecules and ions. The study of water-soluble calixarenes' host–guest interactions would help in modeling of recognition and binding process, taking place in enzymatic systems. It is speculated that the important factor regulating the stability of inclusion complex is the direction of inclusion. Thus, previous NMR studies on calixarene inclusion complex assume the presence of bidirectional (bimodal) inclusion complexes, where the guest molecule and calixarene form bidirectional isomeric inclusion complexes.[7–10] This report concerns the first detection of bidirectional inclusion of the guest molecule into a water-soluble calixarene, *p*-sulfonatocalix[8]arene (Calix-S8, Figure 1). The use of unique free-radical probe and ESR spectrometry made it possible to detect bidirectional inclusion in calixarenes.

NMR technique has been a major tool for the detection of calixarene inclusion complexes in solution,[11,12] however, NMR has been unable to separate included/non-included species.[7–12] This is because the time scale of NMR is no more than 10^3 Hz. In contrast, solution ESR has a shorter time scale (ca. 10^6 Hz) than NMR, thus separated ESR spectra were obtained from free and complexed molecules.[13] Also, this is the reason why NMR has been unable to separate bidirectional inclusion complex,[8] where two different inclusion complexes present depending on the direction of the probe inclusion. Thus, by using ESR and free-radical probe, bidirectional-inclusion complex of cyclodextrin has been identified.[14–16] However, ESR detection of bidirectional inclusion in other inclusion systems, such as paracyclophanes has been unsuccessful,[17] and whether or not bidirectional inclusion is a widespread event throughout supramolecular-host compounds remains unclear.

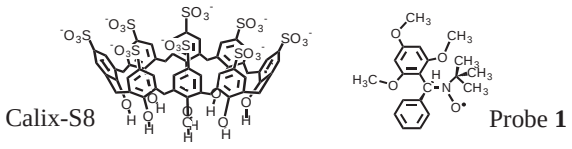

Figure 1. Structures of Calix-S8 and nitroxide probe **1**.

We utilized unique nitroxide free-radical probe as the guest molecule for calixarenes. The nitroxide free-radical probe **1** (1×10^{-4} mol dm^{-3}) (Figure 1) was prepared using the Grignard reaction between phenylmagnesium bromide and 2,4,6-trimethoxyphenyl *tert*-butylnitrone.[14] Calix-S8 was purchased from Dojin Chemicals (Kumamoto, Japan) and used as received. The ESR spectrum shown in Figure 2a was obtained when probe **1** was dissolved in phosphate buffer (pH = 6.9), containing 6.3×10^{-3} mol dm^{-3} Calix-S8. The ESR spectrum can be reproduced with computer spectral simulation by adjusting the relative intensity of ESR spectra of free (Figure 2c) and two complexes (Figures 2d and 2e). Hyperfine splitting constants (hfscs) which were calculated from spectral simulation are listed in Table 1.

R1 and R2 in Table 1 were assigned to *tert*-butyl-in and phenyl-in complex, respectively, based on the changes in hfscs of each complex. In making the assignment, we first postulate that the inclusion should occur only from the *tert*-butyl side or the phenyl side of the probe because the size of the Calix-S8 cavity diameter has been evaluated as 0.75–1.56 nm at the sulfonato side, and 0.64–0.80 nm at the hydroxyl side,[18] assuming that the

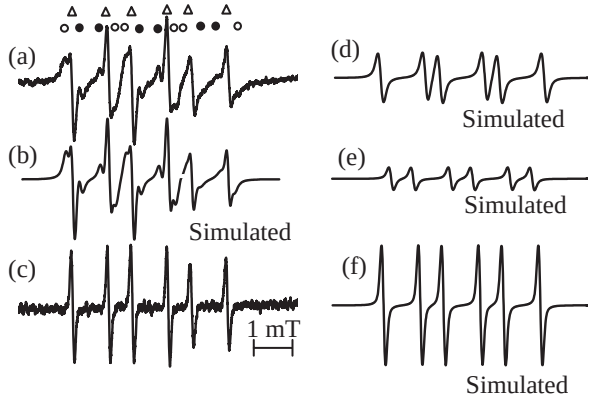

Figure 2. (a) ESR spectrum of probe **1** in aqueous solution containing Calix-S8. The peaks marked with △, ●, and ○ are assigned to free (non-included) and two radical species, respectively (see Table 1). (b) Computer simulated spectrum for (a). (c) ESR spectrum of probe **1** in aqueous solution. Computer simulated spectra for species marked with ○ (d) and ● (e). (f) Computer simulated spectrum for (c).

Table 1. Hyperfine splitting constants (±0.005 mT) of probe **1** and association constants

	A_N /mT	A_H /mT	K /mol^{-1} dm^3	assignment
Probe **1** in water	1.683	1.030	—	free
R1	1.691	1.270	131 ± 3	*tert*-butyl-in
R2	1.659	0.619	45 ± 1	phenyl-in

flexible conformation of water-soluble calixarene is fixed to the cone shape upon inclusion of guest molecules.[19] Because the diameter of trimethoxyphenyl group is slightly smaller than the calixarene opening, it may form a shallowly inserted complex, however the binding constants of such complex is expected to be very small. Secondly, CPK modeling tells us that when the inclusion occurs from the *tert*-butyl side, N–O group is located closer to the sulfonato groups than in the case of phenyl-side inclusion. In addition, the snug insertion of the probe's *tert*-butyl or phenyl group into the Calix-S8 cavity slightly rotates H–C–N–O dihedral angle to the opposite direction. This rotation results in the increase (*tert*-butyl-in) or decrease (phenyl-in) of A_H as compared with free probe's A_H (Table 1). Finally, CPK modeling suggests that the inclusion from the *tert*-butyl side forces N–O group to interact with polar sulfonato groups, which could result in the increase in A_N (Table 1) because A_N in nitroxide radical is a useful index of the polarity of the environment,[20] and increases when the surrounding environment is changed from nonpolar to polar. All these inclusion-mediated changes in hfscs are consistent with the assignment made in Table 1 and plausible structure of bidirectional inclusion complex is illustrated in Figure 3. Molecular dynamic calculation could provide additional support for these structures. Such calculation is now in progress.

We then investigated equilibrium properties of group-in complex. Upon the addition of increasing amount of Calix-S8 to the probe solution, ESR spectra changed from Figure 4a to 4d. The peak intensities of phenyl-in and *tert*-butyl-in complexes increase at the expense of the peak of free species as the Calix-S8 concentration increases. Using the concentration of each component which was determined with computer-ESR spectral simulation, binding constants of the bidirectional complex were calculated following the formulation published elsewhere,[16] and we obtained $K_1 = 131 \pm 3\,\mathrm{mol^{-1}\,dm^3}$ for *tert*-butyl-in and $K_2 = 45 \pm 1\,\mathrm{mol^{-1}\,dm^3}$ for phenyl-in at 290 K (Table 1). Calix-S8 favors the inclusion from the *tert*-butyl side than the phenyl side probably because in *tert*-butyl side inclusion N–O group is able to interact with polar Calix-S8 walls. The equilibrium constant K_3 ($= K_1/K_2 =$ [*tert*-butyl-in]/[phenyl-in]) between the bidirectional inclusion complexes can be calculated as 2.9.

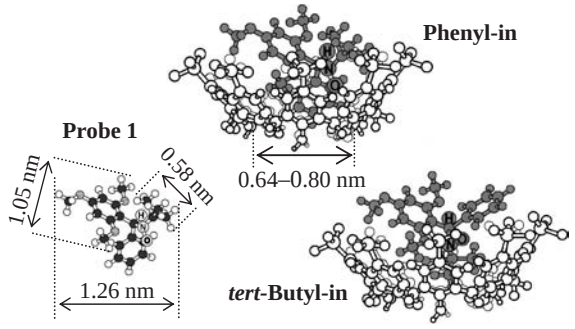

Figure 3. Plausible structure of bidirectional inclusion complex between probe **1** and Calix-S8.

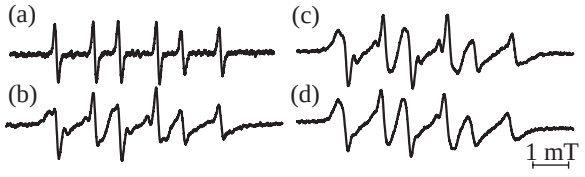

Figure 4. ESR spectra of probe **1** in the presence of various concentrations of Calix-S8: $[\text{Calix-S8}]_0$ = (a) 0, (b) 6.3×10^{-3}, (c) 1.01×10^{-2}, and (d) 3.24×10^{-2} mol dm^{-3}.

The analogs of probe **1** having various alkyl groups (ethyl, propyl, and cyclopentyl groups) instead of phenyl group were synthesized and tested for Calix-S8 complex formation. The ESR spectrum of the group-in complex was not separated from free species (data not shown), probably because the ESR spectrum of the possible group-in complex may have heavily overlapped with free species. We expect that the introduction of a polar group into the phenyl group in **1** would enhance the inclusion and spectral separation. Such experiments as well as more equilibrium studies are in progress. In conclusion, using bulky nitroxide free-radical probe, we were able to obtain an ESR spectroscopic evidence of the bidirectional group-in complexes with Calix-S8. We believe that ESR spectroscopy combined with free-radical probe is a unique means to detect bidirectional inclusion complex of supramolecular host compounds.

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