The Structure and Thermodynamics of Calix[*n*]arene Complexes with Dipyridines and Phenanthroline in Aqueous Solution Studied by Microcalorimetry and NMR Spectroscopy

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The complex stability constants (K_S) and thermodynamic parameters ($\Delta H°$ and $T\Delta S°$) for 1:1 intermolecular complexation of three water-soluble calixarenes, that is, *p*-sulfonato calix[4]arene (C4AS), *p*-sulfonato thiacalix[4]arene (TCAS), and *p*-sulfonato calix[5]arene (C5AS), with dipyridines (4-DPD and 2-DPD) and 1,10-phenanthroline (Phen) have been determined by means of titration microcalorimetry in an acidic buffer solution (pH = 2.0) at 298.15 K, and their binding modes have been investigated by ^{1}H NMR and 2D ROESY NMR spectroscopy. The results obtained indicate that 4-DPD, 2-DPD, and Phen are included in the cavity of C5AS with the different patterns, this is, accumbent for 4-DPD, acclivitous for 2-DPD and Phen, while Phen is included upright in the cavity of C4AS. The K_S values decrease with increasing cavity size of host molecules but enhance with extending conjugation degree of guest molecules, and thus C4AS exhibits an exceptionally high Phen/4-DPD selectivity of 22.5. Thermodynamically, the complexation of DPDs/Phen with the water-soluble calixarenes is obviously enthalpy-driven, but the molecular selectivity is mainly governed by the entropy term.

Introduction

Calixarenes are a class of macrocycles, generally made up of phenol units linked via methylene bridges.[1] During the last two decades, the molecular recognition and assembly of calixarenes have attracted a lot of attention because of their potential applications in various fields such as analysis and separation,[2,3] material,[4] enzyme-mimetic systems,[5] self-assembly membrane,[6] an so forth. Among these recognition processes, the molecular recognition of calixarenes in aqueous solution should be more important because most biological processes occur in aqueous solution. In the past few years, Sciotto et al. have extensively investigated the recognition properties of some water-soluble calix[4]arenes toward quaternary ammonium ions,[7] native amino acids,[8] and small neutral organic molecules.[9] Coleman et al.[10] have examined the binding thermodynamics of *p*-sulfonatocalix[*n*]arenes ($n = 4$, 6, and 8) with amino acid and polypeptides to understand the nature and manner of interactions between the synthetic receptors and glycosylaminoglycan (GAG) receptor sequences. In addition, the binding behaviors of some *p*-sulfonatocalix[4]arenes with organic ammonium cations have been also investigated by NMR and microcalorimetry,[11] indicating that the sulfonate groups of hosts appear to serve as anchoring points for positively charged guests to give a more stable inclusion complex. We have demonstrated recently that the different positions of the nitrogen atoms in dipyridiniums play a crucial role in controlling the molecular assemblies and thermodynamic properties of water-soluble calix[4]arenas.[12] We have also reported an investigation of the complexation of some dye molecules with calix[*n*]arenesulfonates and cyclodextrins, which indicates that they lead to different profiles of the fluorescence intensity changes upon complex formation.[13] The fluorescence intensity of the dye guest molecules gradually decreases upon the addition of calix[*n*]arenesulfonates but increases greatly upon the complexation of native cyclodextrins and chemically modified cyclodextrins. However, the alkylation in the lower rim of calix[*n*]arenesulfonates enlarges their hydrophobic cavity, causing the fluorescence intensity of the dye guest molecules to gradually increase.

Though investigation and development of the functions of calix[5]arenes are relatively limited because of the synthetic difficulty of calix[5]arene material and its poor selectivity of modification, calix[5]arene derivitaves are of significant interest as a consequence of the large size of the macrocyclic cavity suggesting the possibility of novel host−guest behavior. Structural studies show that *p*-sulfonato calix[5]arene (C5AS) adopts the cone conformation with all of the phenolic oxygen atoms in an approximate plane, and there is no compromise arising from any preorganization energy required for the binding of hydrophobic moieties within the calixarene cavity the same as *p*-sulfonato calix[4]arene (C4AS).[14] A similar examination of C5AS structures shows that the typical cone conformation and bilayer arrangement were presented by C5AS upon inclusion complexation with metal cations or organic molecules.[15] However, there has been almost no investigation on the recognition mechanism and thermodynamic behavior of calix[5]arene except for a new example that the complexation of quaternary ammonium ions with C5AS was studied by NMR spectra.[16] We wish to report herein our investigation results on the intermolecular complexation of water-soluble calixarenes (Chart 1) with the dipyridines (4-DPD and 2-DPD) and 1,10-phenanthroline in an aqueous phosphate buffer solution (pH 2.0) by titration microcalorimetry and NMR spectroscopy. Comparison of the binding behavior of C5AS with that of C4AS/TCAS, together with the X-ray crystallographic structure of C4AS/Phen complex, will serve our further understanding of the structure-selectivity relationship in the calixarenes complexed.

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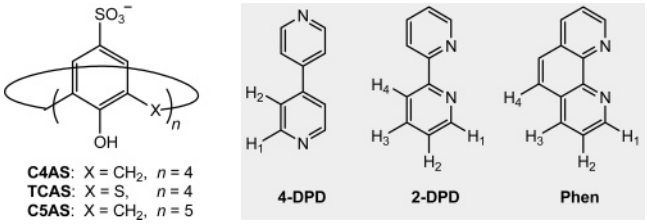
CHART 1: Structures of the Host Calixarenes and Guest Molecules



Experimental Section

Materials. The three *p*-sulfonatocalixarenes, that is, calix[4]arene tetrasulfonate (C4AS),[17] thiacalix[4]arene tetrasulfonate (TCAS),[18] and calix[5]arene tetrasulfonate (C5AS),[19] were synthesized and purified according to the literature reports. Guest molecules, 4,4′-dipyridine (4-DPD), 2,2′-dipyridine (2-DPD), and 1,10-phenanthroline (Phen), were purchased from Acros and were used without further purification. The phosphate buffer solution (pH 2.0) was prepared by dissolving sodium dihydrogen phosphate in distilled, deionized water to make a 0.1 mol·dm^{-3} solution, which was then adjusted to pH 2.0 by phosphoric acid. The pH value of buffer solution was verified on a Sartorius pp-20 pH-meter calibrated with two standard buffer solutions. The phosphate buffer solution (pH 7.2) was prepared by dissolving disodium hydrogen phosphate ($Na_2HPO_4 \cdot 12H_2O$, 25.79 g) and sodium dihydrogen phosphate ($NaH_2PO_4 \cdot 2H_2O$, 4.37 g) in distilled, deionized water (1000 mL) to make a 0.1 mol·dm^{-3} solution. In pH 2.0 solution, every sulfonate group of CAS is in anionic form and all phenolic hydroxyes are protonated according to the reported pK_a values of *p*-sulfonatocalixarenes,[19,20] while the guest 2-DPD and Phen are monoprotonated form and 4-DPD is diprotonated form.[21] Meanwhile, 4-DPD is in a twisted form and 2-DPD adopts the cis-planar conformation.[12,22] In pH 7.2 solution, every sulfonate group of CAS is still in an anionic form, and one of the phenolic hydroxyls is deprotonated.

Measurement. ^{1}H NMR and 2D ROESY (rotating frame Overhauser effect spectroscopy) spectra were recorded at pD 2.0 on a Varian Mercury VX300 spectrometer using 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) as an external reference. The host and guest were mixed in an approximate 1:1 stoichiometry.

A thermostated and fully computer-operated isothermal calorimetry (VP−ITC) instrument, purchased from Microcal Inc., Northampton, MA, was used for all microcalorimetric experiments. The VP−ITC instrument was calibrated chemically by measurement of the complexation reaction of β-cyclodextrin with cyclohexanol, and the obtained thermodynamic data were in good agreement (error <2%) with the literature data. All microcalorimetric titrations between water-soluble calix[*n*]arenes and guests were performed in aqueous phosphate buffer solution (pH 2.0 or pH 7.2) at atmospheric pressure and 298.15 K. Each solution was degassed and thermostated by a ThermoVac accessory before the titration experiment. Twenty-five successive injections were made for each titration experiment. A constant volume (10 μL/injection) of guest (or host) solution (10.0−20.0 mM) in a 0.250-mL syringe was injected into the reaction cell (1.4227 mL) charged with host (or guest) molecule solution (0.5−1.0 mM) in the same buffer solution. A representative titration curve is shown in Figure 1. As can be seen from Figure 1, each titration of TCAS into the sample cell gave an apparent reaction heat caused by the formation of the inclusion complex between 2-DPD and C5AS. The reaction heat

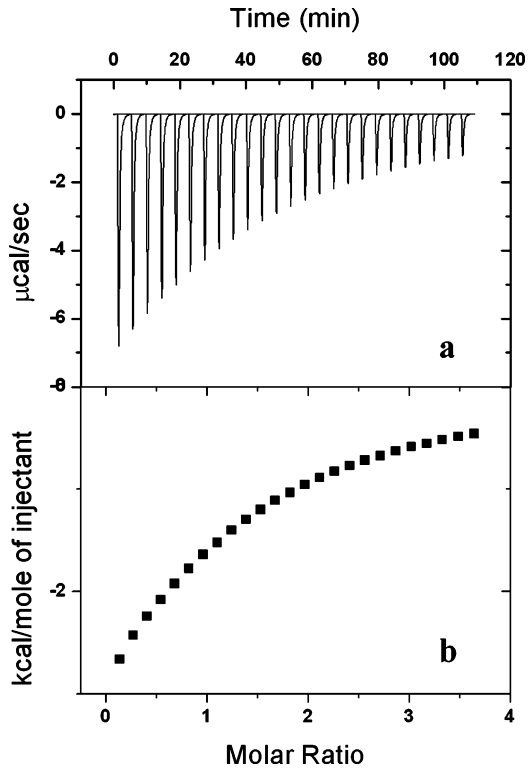


Figure 1. Microcalorimetric titration of C5AS with 2-DPD in phosphate buffer solution (pH = 2.0) at 298.15 K. (a) Raw data for sequential 25 injections (10 μL per injection) of C5AS solution (10.0 mM) injecting into 2-DPD solution (0.53 mM). (b) Apparent reaction heat obtained from the integration of calorimetric traces.

decreases after each injection of TCAS because less and less guest molecules are available to form inclusion complexes. A control experiment was carried out in each run to determine the dilution heat by injecting a guest (or host) buffer solution into a pure buffer solution containing no host (or guest) molecules. The dilution heat determined in these control experiments was subtracted from the apparent reaction heat measured in the titration experiments to give the net reaction heat.

The net reaction heat in each run was analyzed by using "one set of binding sites" model (ORIGIN software, Microcal Inc.) to simultaneously compute the binding stoichiometry (N), complex stability constant (K_S), standard molar reaction enthalpy ($\Delta H°$), and standard deviation from the titration curve. Generally, the first point of the titration curve was removed considering that the concentration of host in the cell far exceeded the concentration of the guest. Knowledge of the complex stability constant (K_S) and molar reaction enthalpy ($\Delta H°$) enabled calculation of the standard free energy ($\Delta G°$) and entropy changes ($\Delta S°$) according to

$$\Delta G° = -RT \ln K_S = \Delta H° - T\Delta S°$$

where R is the gas constant and T is the absolute temperature.

A typical curve fitting result for the complexation of 2-DPD with C5AS at pH 2.0 was shown in Figure 2. To check the accuracy of the observed thermodynamic parameters, two independent titration experiments were carried out to afford self-consistent thermodynamic parameters, and their average values are listed in Table 1 (pH 2.0) and Table 2 (pH 7.2).

Preparation of Crystal of C4AS with Phen. To an aqueous solution of C4AS (0.05 mmol, 20 mL), 4 equiv of Phen was added. Under stirring, 1 M HCl was dropped to adjust the pH

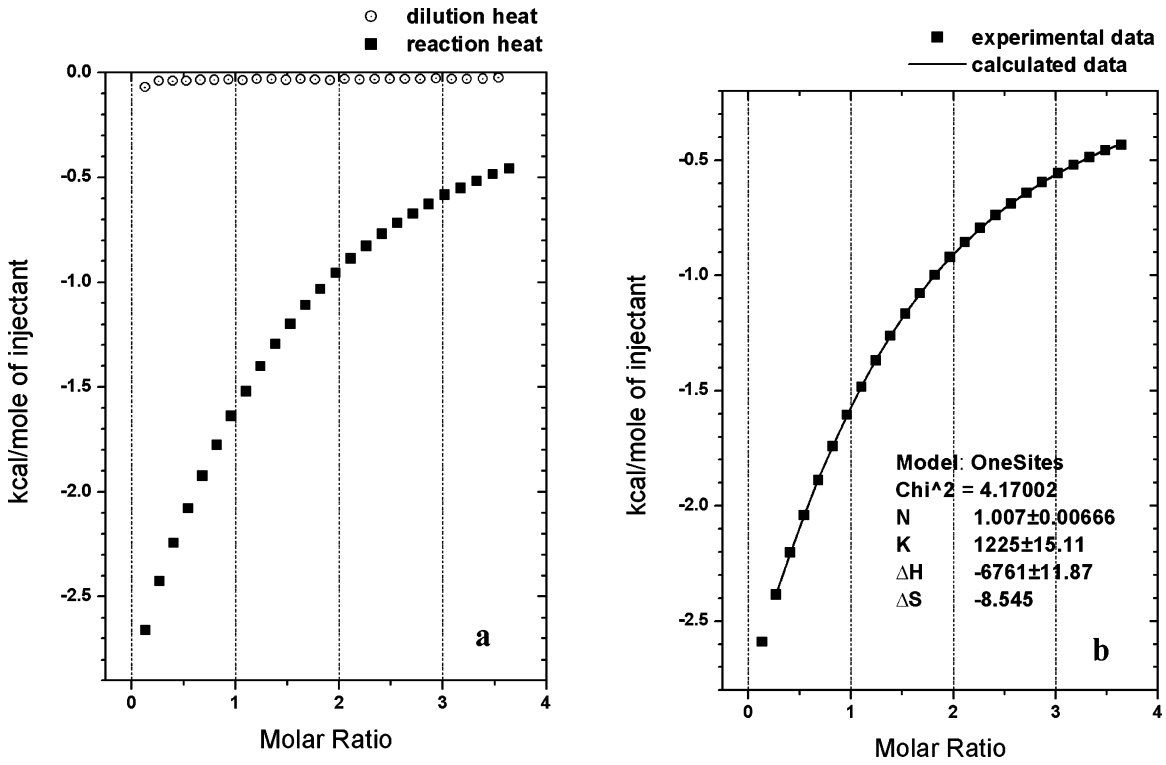


Figure 2. (a) Heat effects of the dilution and of the complexation reaction of C5AS with 2-DPD for each injection during titration microcalorimetric experiment. (b) "Net" heat effects of complexation of C5AS with 2-DPD for each injection, obtained by subtracting the dilution heat from the reaction heat, which was fitted by computer simulation using the "one set of binding sites" model.

TABLE 1: Complex Stability Constants (K_S/M^{-1}), Standard Enthalpy ($\Delta H°$/(kJ·mol^{-1})), and Entropy Changes ($T\Delta S°$/(kJ·mol^{-1})) for 1:1 Intermolecular Complexation of Guests with CAS in Phosphate Buffer Solution (pH 2.0) at 298.15 K

host[a]	guest[b]	N[c]	K_S	$-\Delta G°$	$-\Delta H°$	$T\Delta S°$
C4AS	2-DPD[d]	2	10260 ± 190	22.9 ± 0.0	36.7 ± 0.2	−13.8 ± 0.3
	4-DPD[d]	2	1185 ± 2	17.6 ± 0.1	24.5 ± 0.1	−7.0 ± 0.1
	Phen	2	26665 ± 95	25.3 ± 0.0	44.8 ± 0.1	−19.5 ± 0.1
TCAS	2-DPD[d]	2	1315 ± 20	17.8 ± 0.0	27.5 ± 0.1	−9.7 ± 0.1
	4-DPD[d]	2	523 ± 7	15.6 ± 0.1	18.0 ± 0.1	−2.5 ± 0.2
	Phen	2	4981 ± 127	21.1 ± 0.1	36.6 ± 0.2	−15.5 ± 0.2
C5AS	2-DPD	2	1219 ± 7	17.6 ± 0.0	28.4 ± 0.1	−10.8 ± 0.1
	4-DPD	2	2039 ± 3	18.9 ± 0.0	30.0 ± 0.1	−11.1 ± 0.1
	Phen	2	2281 ± 15	19.2 ± 0.0	38.8 ± 0.1	−19.7 ± 0.1

[a] [Host] = 10.0−20.0 mM. [b] [Guest] = 0.5−1.0 mM. [c] Number of titration runs performed. [d] Reference 12.

TABLE 2: Complex Stability Constants (K_S/M^{-1}), Standard Enthalpy ($\Delta H°$/(kJ·mol^{-1})), and Entropy Changes ($T\Delta S°$/(kJ·mol^{-1})) for 1:1 Intermolecular Complexation of Guests with C4AS and TCAS in Phosphate Buffer Solution (pH 7.2) at 298.15 K

host[a]	guest[b]	N[c]	K_S	$-\Delta G°$	$-\Delta H°$	$T\Delta S°$
C4AS	2-DPD	2	84 ± 1	11.0 ± 0.0	37.8 ± 0.5	−26.8 ± 0.5
	4-DPD	2	44 ± 3	9.4 ± 0.2	8.3 ± 0.6	1.1 ± 0.7
	Phen	2	278 ± 3	14.0 ± 0.0	46.7 ± 0.1	−32.8 ± 0.4
TCAS	2-DPD	2	57 ± 1	10.0 ± 0.1	26.5 ± 0.3	−16.5 ± 0.3
	4-DPD	2	49 ± 3	9.7 ± 0.2	16.7 ± 0.8	−7.1 ± 0.9
	Phen	2	285 ± 1	14.0 ± 0.0	41.9 ± 0.1	−27.9 ± 0.1

[a] [Host] = 10.0−20.0 mM. [b] [Guest] = 0.5−1.0 mM. [c] Number of titration runs performed.

to ≤1. Followed by filtration, the filtrate was placed to evaporation for about 3 days. Then, the colorless crystal formed was collected along with its mother liquor for the X-ray crystallographic analyses. Data: yield 43.4 mg, 53%. Anal. Calcd for $C_{64}H_{67}N_6O_{25.5}S_4$ (M = 1636.7): C, 52.78; H, 4.64; N, 5.77; S, 8.80%. Found: C, 53.21; H, 4.13; N, 6.02; S, 9.16%.

The X-ray intensity data for the complex C4AS/Phen was collected on a standard Siemens SMART CCD Area Detector System equipped with a normal-focus molybdenum-target X-ray tube (λ = 0.71073 Å) operated at 2.0 kW (50 kV, 40 mA) and a graphite monochromator at T = 293(2) K. The structures were solved by using direct method and were refined employing full-matrix least squares on F^2 (Siemens, SHELXTL-97). X-ray structural data: $C_{64}H_{67}N_6O_{25.5}S_4$, M = 1456.48, monoclinic, a = 14.257(4) Å, b = 38.099(10) Å, c = 16.146(4) Å, β = 105.981(5)°, space group $P2(1)/n$, Z = 4, calculated density 1.147, crystal dimensions (mm^3): 0.38 × 0.34 × 0.32. μ = 0.183 mm^{-1}, $2\theta_{max}$ = 38.6°, 42 792 measured reflections of which 14 700 were unique ($R_{(int)}$ = 0.0918), final R indices [$I/\sigma(I) > 2$]: R_1 = 0.1654, wR_2 = 0.3793, R indices (all data): R_1 = 0.2965, wR_2 = 0.4583, GOF on F^2 1.236. CCDC-278919 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB21EZ, United Kingdom; fax: (+44) 1223-336-033 or deposit@ccdc.cam.uk).

Results and Discussion

Binding Stoichiometry. It is well-known that CAS can form typical 1:1 complexes with model substrates.[7−11] The microcalorimetric experiments of C4AS, TCAS, and C5AS with DPDs and Phen showed typical titration curves of 1:1 complex formation. The stoichiometric ratios (N value) that we observed from curve-fitting results of the binding isotherm fell within the range of 0.86−1.13:1. This clearly indicates that the majority of the inclusion complexes had a 1:1 stoichiometry of guests and CAS. Simultaneously, the 1:1 binding modes for the inclusion complexations of C4AS or TCAS with 2-DPD[12] as well as C4AS with Phen have been also validated by their crystal structures.

TABLE 3: The Chemical Shift Change $\Delta\delta$ Values (ppm) of DPDs and Phen in the Presence of C4AS or C5AS[a]

	4-DPD		2-DPD				Phen			
host	H1	H2	H1	H2	H3	H4	H1	H2	H3	H4
C4AS	[b]	[b]	[b]	[b]	[b]	[b]	−0.79	−1.36	−1.79	−1.58
C5AS	−0.82	−1.15	−0.66	−1.11	−0.99	−1.63	−0.57	−0.76	−1.75	−2.1

[a] $\Delta\delta = \delta$ (presence of 1 equiv of host) $- \delta$ (free guest). Negative values indicate upfield shift. [b] The ^{1}H NMR spectra were not measured because of the complexes of C4AS with DPDs being nonsoluble in D_2O.[12]

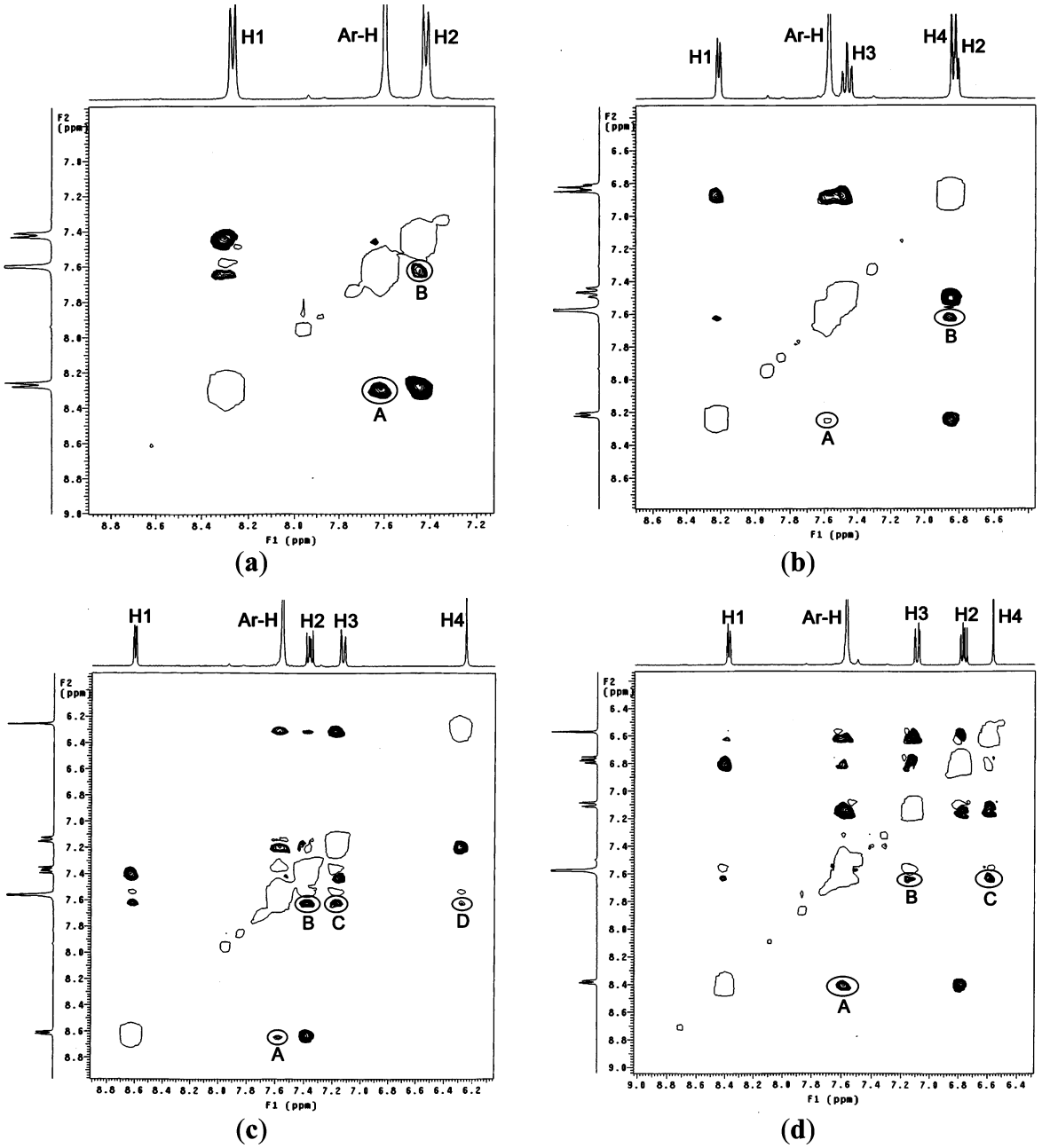


Figure 3. The 2D ROESY NMR spectra of (a) C5AS + 4-DPD, (b) C5AS + 2-DPD, (c) C5AS + Phen, and (d) C4AS + Phen in D_2O with a mixing time of 300 ms at 25 °C. The concentrations of both hosts and guests are about 10 mM.

Binding Mode. To obtain detailed information about the solution structure of the resulting complexes of CAS with DPDs/Phen, ^{1}H NMR spectra of DPDs/Phen-D_2O solutions in the presence and absence of CAS and the 2D NMR experiments of their complexes were measured at pD 2.0. The corresponding chemical shift changes ($\Delta\delta$) of guests in the presence of approximately 1 equiv C5AS or C4AS are listed in Table 3, and the ROESY spectra are shown in Figure 3. The guest protons are observed as a single resonance because of fast exchange between a free guest and a complexed one on the NMR time scale. Usually, a complexation causes a deprotonation of the guest or host molecule to stabilize the complex, because the guest tends to lay at hydrophobic environment. In the present event, the resonance values of guest protons obviously shift toward high field after complexation with CASs (Table 3), but the crystal structures of the complexes C4AS/2-DPD,[12] C4AS/4-DPD,[23] TCAS/2-DPD,[12] TCAS/4-DPD,[12] and C4AS/Phen have validated that the guest molecule in the

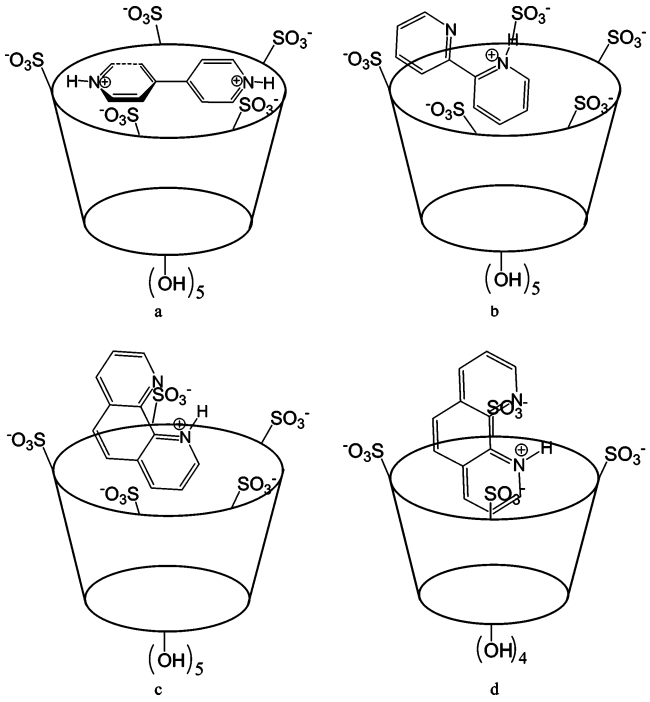


Figure 4. The deduced binding modes of guests with C5AS/C4AS hosts according to 2D NMR spectra.

complexes maintains the original protonated form. In controlling experiment, the ^{1}H NMR spectrum of 4-DPD with 4-phenolsulfonic sodium displays that the peak shifts in 4-DPD are negligible (<0.06 ppm). Hence, the observation of the high-field shift suggests that the guest molecule should be encapsulated into the cavity of CAS.[16,24]

On the other hand, though the chemical shift values of methylene bridges in both C4AS and C5AS do not change upon complexation with guests, the proton signals in C4AS become obtuse to be steamed bread shape, while those in C5AS still present the spiculate peak. These observations provide the evidence for a rigidification of the cone conformation of C4AS[12,25] upon complexation with the large planar aromatic guest Phen, and no compromise of conformation shift is needed to accommodate DPDs and Phen for C5AS because of its larger cavity. That is to say, the conformation of C5AS is invariable before and after complexation with guests.

Unlike complexation-induced shifts, ROESY cross-peaks are indicative of specific proximity relationships between host and guest protons (generally 4 Å or less).[26] It should be first emphasized that no correlation is observed between any of the protons in DPD/Phen and methylene protons in C4AS/C5AS, suggesting that the guest molecules cannot be completely included in the cavity of the calixarenes. As can be seen from Figure 3a, the ROESY spectrum of the complex of C5AS with 4-DPD exhibits clear cross-peaks (peaks A and B) between H1 and H2 of 4-DPD and aromatic protons (Ar−H) of C5AS, and the cross-peak of H2 with Ar−H (peak B) is weaker than that of H1 (peak A), which indicates that H2 in 4-DPD must be further away from the aromatic protons in C5AS than H1. These results show that 4-DPD should be accumbently included into the cavity of C5AS from the upper rim. In the ROESY spectrum of the complex of C5AS with 2-DPD (Figure 3b), the correlation between H2 and H4 in 2-DPD and Ar−H (peaks B) are stronger than that between H1 and Ar−H (peak A), suggesting that 2-DPD penetrates into the cavity of C5AS in acclivitous orientation, and its two N atoms are away from the cavity, as illustrated in Figure 4. However, the ROESY spectrum of the

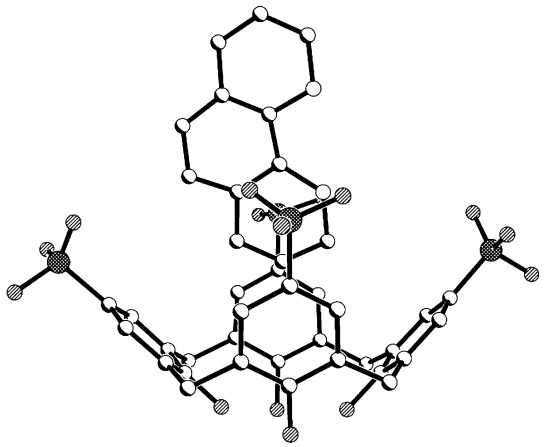


Figure 5. The structural view of C4AS/Phen complex arising from the solid-state single crystal.

complex of C5AS with Phen is different from that of C4AS. For the former (Figure 3c), there are obvious cross-peaks originating from Ar−H of C5AS with all of the four protons of Phen (peaks A−D), in which the correlation between H2 and H3 (peaks B and C) are much stronger than those of H1 and H4 (peaks A and D). For the latter (Figure 3d), only three cross-peaks between H1, H3, H4, and Ar−H of C4AS (almost same intensity) are observed. The absence of the correlation of H2 with Ar−H implies that H2 is far away from the aromatic protons of C4AS in the upper rim. Therefore, one may reasonably deduce that Phen should be slantways encapsulated into the cavity of C5AS resembling the 2-DPD case, and C4AS accommodates Phen in an almost vertical manner, as illustrated in Figure 4. The complexation mode of C4AS with Phen is otherwise validated by the crystal structure obtained. As can be seen from Figure 5, the Phen molecule penetrates into the cavity of C4AS with a depth of 4.268 Å,[27] in which C4AS adopts the C_{2v} symmetry conformation with the SS (trans sulfonate) approaches of 8.414 Å and 11.722 Å. The difference of binding modes between C5AS and C4AS is in hindsight comprehensive that the larger cavity of C5AS can include Phen in another manner besides upright orientation as in comparison with C4AS.

The order of $\Delta\delta$ values of guests in ^{1}H NMR spectra is thought to provide the valuable information for the structures of complexes.[16,24] Commonly, the larger the $\Delta\delta$ values, the more prior the portion of guest is suggested to immerse the cavity of CAS. However, Nau et al.[28] pointed out that larger chemically induced shifts cannot be necessarily interpreted in terms of their deeper immersion into the CAS cavity. In the present system, the $\Delta\delta$ value of H2 in 4-DPD is larger than that of H1, but H2 does not penetrate deeper into the C5AS cavity than H1 according to 2D ROESY NMR experiment; the $\Delta\delta$ values of H3 and H4 in Phen are markedly larger than those of H1 and H2, while H2 immerses deeper into the C5AS cavity than H4. That is to say, the experiential rule about chemically induced shifts is not always suitable to these larger guest species.

Molecular Binding Ability and Molecular Selectivity. In this context, the cavity size of the three calixarene hosts increases in the order C4AS < TCAS < C5AS. To visualize the intermolecular complexation behavior of the water-soluble calixarenes with the guests, the changing profiles of K_S values for host−guest complexation are illustrated in Figure 6.

As shown in Table 1 and Figure 6, when the calixarenes interact with 2-DPD or Phen guests, the K_S values obtained decrease with increasing cavity size. Thus, the host C4AS shows

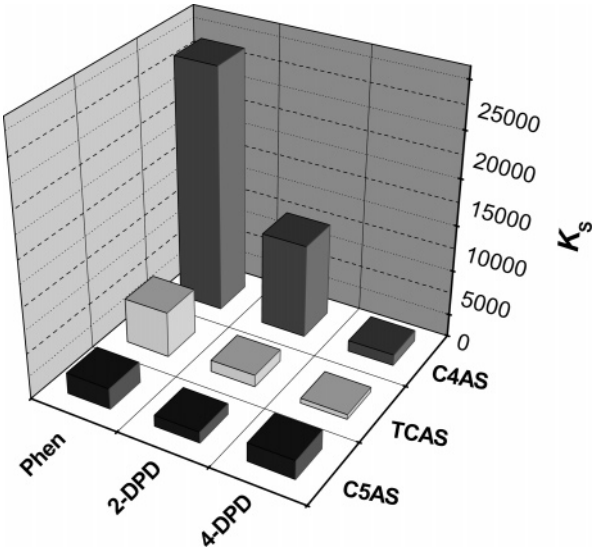


Figure 6. Complex stability constants (K_S) for the complexation of guests 4-DPD, 2-DPD, and Phen with CAS in phosphate buffer solution (pH 2.0) at 298.15 K.

the strongest binding abilities for 2-DPD (10260 M^{-1}) and Phen (26665 M^{-1}). One reasonable explanation for the declining profile of K_S is that C4AS adapts itself to C_{2v} conformation upon complexation with 2-DPD or Phen to some different extent, which is particularly suited to the inclusion of planar aromatic guest species.[29] On the other hand, C4AS can include the guests tighter than TCAS and C5AS because of its smaller cavity and relatively higher π electron density, leading to a closer distance of π-stacking interaction. However, an "abnormal" binding ability profile is observed for complexation of the three hosts with 4-DPD, that is, the K_S value is the biggest for C5AS, the second for C4AS, and the smallest for TCAS. The crystal structures of the complexes C4AS/4-DPD[23] and TCAS/4-DPD[12] together with the above solution study of complex C5AS/4-DPD would be useful for our understanding of these observations. Compared with three binding modes obtained, just 4-DPD in complex C5AS/4-DPD can protrude into the cavity of calixarenes, so the host−guest interaction between C5AS and 4-DPD is stronger than those of C4AS/TCAS.

It is significantly noted that there are two pyridine units in every guest molecule. Two pyridine rings in 4-DPD array in an approximately vertical manner, those in 2-DPD are almost located in a same plane as mentioned above, and two pyridine rings in Phen are completely coplanar with a big π-conjugated system. As shown in Figure 6, the K_S values obtained for the complexation of the guests with C4AS and TCAS show a simple profile, declining with decreasing conjugation degree of guest molecules. The regular changes endow C4AS an exceptionally high molecular selectivity for 2-DPD of 8.7 over 4-DPD or even the Phen/4-DPD selectivity of 22.5. In the case of C5AS, the K_S for Phen is still the largest among the three guests, but that for 4-DPD goes beyond 2-DPD. As a consequence of such a unique changing profile of the K_S for C4AS, TCAS, and C5AS, the relative molecular selectivity between 2-DPD and 4-DPD is dramatically inverted just by extending the cavity size, switching from the 2-DPD/4-DPD selectivity of 8.7 for C4AS and 2.5 for TCAS to the 4-DPD/2-DPD selectivity of 1.7 for C5AS. One possible reason for the observations is that the larger the conjugated π system of guest, the stronger the π-stacking interaction between aromatic rings and the phenolic units of the host cavity, which leads to the largest K_S for Phen because of its broad conjugated π system. In addition, possessing the wider cavity that can accommodate 4-DPD in unique mode, C5AS presents the most efficient binding stability among the three calixarenes.

Thermodynamic Parameters. It is well documented that, among several weak noncovalent interactions working between host and guest, the electrostatic, hydrogen bond, $\pi-\pi$, C−H···π, and van der Waals interactions mainly contribute to the enthalpic changes, while the conformation change and the desolvation effect contribute to the entropic changes.[10b] As can be seen from Table 1, all intermolecular complexations between CAS and DPDs and Phen guests are mainly driven by the favorable enthalpic changes ($-\Delta H^\circ$ = 18.0−44.8 kJ/mol), accompanied by negative entropic changes ($-T\Delta S^\circ$ = 2.5−19.7 kJ/mol), which indicates that the electrostatic, hydrogen bond, π-stacking, and van der Waals interactions may play a crucial role in the host−guest complexation.

As shown in Table 1, the global profiles of K_S for the complexation of any of the calixarenes with three guests are completely consistent with those of enthalpy changes, namely, the larger the exothermic enthalpy changes the higher the complex stability and vice versa. These observations are readily understood by previous explanation about K_S. However, except for 4-DPD, the consistency is not observed in the complexation of 2-DPD or Phen with three calixarene hosts. The K_S values for TCAS with 2-DPD and Phen (1315 M^{-1} for 2-DPD/TCAS and 4981 M^{-1} for Phen/TCAS) are larger than those for C5AS (1219 M^{-1} for 2-DPD/C5AS and 2281 M^{-1} for Phen/C5AS), but the corresponding exothermic enthalpy changes for TCAS (27.5 kJ/mol for 2-DPD/TCAS and 36.6 kJ/mol for Phen/TCAS) are smaller than those for C5AS (28.4 kJ/mol for 2-DPD/C5AS and 38.8 kJ/mol for Phen/C5AS). These results indicate that the large enthalpic changes do not always mean high K_S values.

In contrast to enthalpy, the complexation of CAS with DPDs or Phen exhibits unfavorable entropy changes ($T\Delta S^\circ$ = −2.5 kJ/mol to −19.7 kJ/mol), and the global profiles of entropy ($T\Delta S^\circ$) is reversed as compared with the K_S values, that is to say, the smaller the entropy changes the higher the complex stability and vice versa. It is reasonable to suppose that the negative entropy changes mainly result from the loss of conformational degrees of freedom for the hosts and structure freezing upon complexation, and the structurally slight difference among C4AS, TCAS, and C5AS as well as 4-DPD, 2-DPDP, and Phen would lead to the distinct entropy values. The entropy loss upon complexation with the same guest enhances to a different extent (1.1∼8.6 kJ/mol) on going from TCAS to C4AS (C5AS). Replacing methylene bridges with sulfur atoms, thiacalixarenes possess more flexibility than classic calixarenes, so its loss of conformational degrees of freedom is certainly less, thus making the entropy losses upon complexation with DPDs or Phen smaller than that of C4AS and C5AS. Upon complexation with 2-DPD, C4AS presents a more unfavorable entropy ($T\Delta\Delta S^\circ$ = −3.0 kJ/mol) than C5AS; however, C4AS gives a more favorable entropy ($T\Delta\Delta S^\circ$ = 4.1 kJ/mol) than C5AS upon complexation with 4-DPD, resulting in the inverted molecular selectivity between 2-DPD and 4-DPD. One reasonable explanation for the thermodynamic behaviors is that, despite the similar inclusion modes between 2-DPD/C4AS[12] and 2-DPD/C5AS, the conformational loss of C5AS should be smaller than C4AS because of its wider cavity. Therefore, the molecular selectivity is mainly governed by the entropy term.

Effect of pH Values. It is well-known that the protonation of guest molecules should significantly affect the K_S values, molecular selectivity, and thermodynamic parameters upon

complexation of host with guest. The guest 2-DPD and Phen are monoprotonated form, and 4-DPD is diprotonated form in pH 2.0 solution, but these guests are not protonated at all in pH 7.2 solution. To compare pH value effects, we also performed ITC experiments of C4AS and TCAS with three guests in pH 7.2 buffer solution. As can be seen from Table 2, the K_S values in this pH value are 1−2 orders of magnitude less than the corresponding K_S values in pH 2.0. It indicates that the protonation of guests exerts predominant influence over the host−guest binding ability and selectivity. A close examination on the thermodynamic parameters in the different pH values shows that, except for those of complexation of C4AS with 4-DPD, the corresponding enthalpy changes in the two pH values do not obviously change, while the entropy changes reduce in pH 7.2. That is, the decrease of K_S values in pH 7.2 does not result from the enthalpy term but from the entropy term. The differential entropy changes in different pH values may be attributed to the desolvation effect of guests. In acidic solution, the guest molecules protonated give heavily solvated pyridinium ions through ion−dipole interactions. Then, the complexation with calixarenes requires fairly extensive desolvation of both DPD and Phen upon complexation, affording the relatively high entropy changes, as observed (Table 1). In neutral solution, the solvation to the guest molecules nonprotonated is not so heavy as those protonated, which probably reduces the entropic contributions to the complex stabilities. These observations exclude the electrostatic interaction as a crucial role in the host−guest complexation. On the other hand, the entropy losses of TCAS in neutral medium are smaller than that of C4AS, which is because TCAS possesses more flexibility than C4AS, and then its loss of conformational degrees of freedom is certainly less than C4AS. 4-DPD is in diprotonated form, and therefore the electrostatic interaction with C4AS is not neglectable, resulting in the larger difference of enthalpy change.

Conclusion

In summary, the binding modes, binding ability, molecular selectivity, and thermodynamic origins of three water-soluble calixarenes upon complexation with DPDs and Phen have been investigated by ITC and NMR spectra. The 2D NMR studies show that 4-DPD, 2-DPD, and Phen are included in the cavity of C5AS with the different patterns, that is, accumbent for 4-DPD and acclivitous for 2-DPD and Phen, and the cone conformation of C5AS is invariable before and after complexation with guests. On the contrary, Phen is included upright in the cavity of C4AS with the conformation rigidified, while 4-DPD is located outside C4AS, and its cone shape is disrupted by 4-DPD to assume the so-called 1,3-alternate conformation.[23] The thermodynamic investigation indicates that the complexation of DPDs/Phen with water-soluble calixarenes is obviously enthalpy-driven, but the molecular selectivity is mainly governed by the entropy term. The conjugation degree of guest molecules and the cavity size of host molecules are two key factors to the formation of inclusion complexes, while the electrostatic interaction does not play a crucial role in the host−guest complexation.

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Supporting Information Available: The cif file of the crystal C4AS/Phen. This material is available free of charge via the Internet at http://pubs.acs.org.

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