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Molecular Recognition Thermodynamics of Pyridine Derivatives by Sulfonatocalixarenes at Different pH Values

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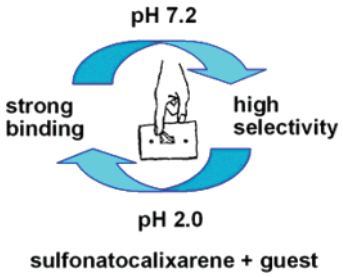

The complex stability constants (K_a) and thermodynamic parameters ($\Delta G°$, $\Delta H°$, and $T\Delta S°$) for 1:1 complexation of water-soluble calix[4]arene, thiacalix[4]arene, and calix[5]arene sulfonates with pyridine and their methylated derivatives have been determined by means of isothermal titration calorimetry at pH 2.0 and 7.2 at 298.15 K, and their binding modes have been investigated by NMR spectroscopy. The results obtained show that sulfonatocalixarenes afford stronger binding ability toward pyridine guests at pH 2.0, attributable to the positive electrostatic interactions and the more extensive desolvation effects, but present higher molecular selectivity at pH 7.2 owing to the strengthened C−H···π interactions. The pH-responsible binding ability and molecular selectivity are discussed from the viewpoint of electrostatic, π-stacking, van der Waals interactions and size-fit relationship between host and guest. A close comparison further demonstrates that the C−H···π interactions and van der Waals interactions play a more important role than π···π interactions in the present inclusion complexation.

Introduction

Calixarenes[1] are a special class of cyclophanes that have been used as such, or after functionalization, for the recognition of neutral molecules,[2] cations,[3] and even anions.[4] Among the various calixarene derivatives, water-soluble calixarenes are becoming increasingly important in supramolecular chemistry because they allow the study of basic forces involved in the host−guest recognition processes in a solvent where most biological processes occur.[5] Moreover, they are also demonstrated to have good potential bio-activities ranging from enzyme inhibition through antithrombotic activity and antiviral activity to antibacterial properties.[6] Sciotto et al. have investigated the recognition behaviors 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. examined the

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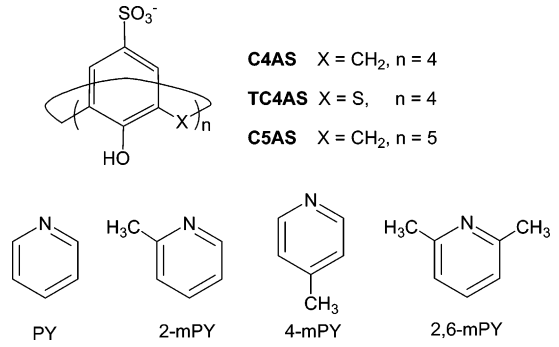
SCHEME 1. Structures of Sulfonatocalixarene Hosts and Guest Molecules



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 synthetic receptors and glycosylaminoglycan (GAG) receptor sequences.[10] Miyano and co-workers studied the complexation behaviors and mechanism of thiacalix- and calixarene tetrasulfonates with monosubstituted benzenes and halomethanes in neutral aqueous solution.[11] However, these investigations are mainly focused on the calix[4]-, calix[6]- or calix[8]arenes; studies on the complexation behaviors of water-soluble calix[5]arenes were rarely reported,[12,13] to the best of our knowledge. Recently, we investigated the molecular binding behavior of sulfonatocalix[4]arenes (C4AS) and sulfonatothiacalix[4]arene (TC4AS) in aqueous solution[14] and found that the position, number, and type of substituent groups introduced onto the guest molecule were the key factors controlling the structural-energetics correlation for the molecular selective binding of these water-soluble calix[4]arenes. Herein, we wish to report a comparative study on the binding behavior of some water-soluble calix[4]arene and calix[5]arene sulfonates toward pyridine and their methylated derivatives (Scheme 1) in both acidic and neutral environment by isothermal titration calorimetry and ^{1}H NMR spectroscopy. These studies will help us to gain a deeper insight into not only the influences of protonation/deprotonation status and electron density of both host and guest on the inclusion complexation behaviors of water-soluble calixarenes but also the potential applications of these calixarenes as drug carriers in different biological environments, such as serum (neutral environment, pH ca. 7.3) or gastric acid (acidic environment, pH ca. 1.5).

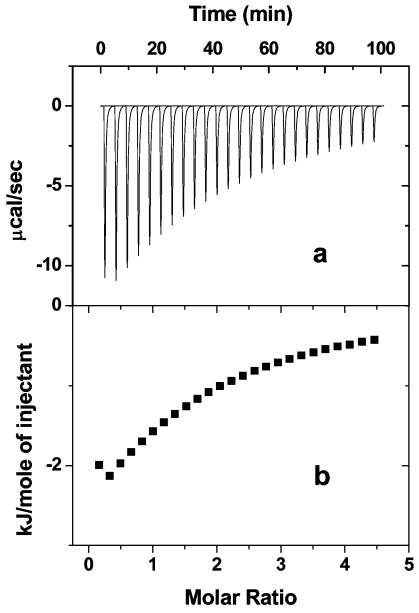


FIGURE 1. Microcalorimetric titration of C5AS with 2-mPY in phosphate buffer solution (pH = 7.2) at 298.15 K: (a) raw data for sequential 25 injections (10 μL per injection) of C5AS solution (19.36 mM) injecting into 2-mPY solution (0.83 mM); (b) apparent reaction heat obtained from the integration of calorimetric traces.

Results and Discussion

Microcalorimetric Titration. A representative titration curve was shown in Figure 1. As can be seen in Figure 1, each titration of C5AS into the sample cell gave an apparent reaction heat, caused by the formation of an inclusion complex between 2-mPY and C5AS. The reaction heat decreases after each injection of C5AS 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 to simultaneously compute the binding stoichiometry (N), complex stability constant (K_a), standard molar reaction enthalpy ($\Delta H°$), and standard deviation from the titration curve. The knowledge of complex stability constant (K_a) and molar reaction enthalpy ($\Delta H°$) enabled the calculation of standard free energy ($\Delta G°$) and entropy changes ($\Delta S°$) according to

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

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

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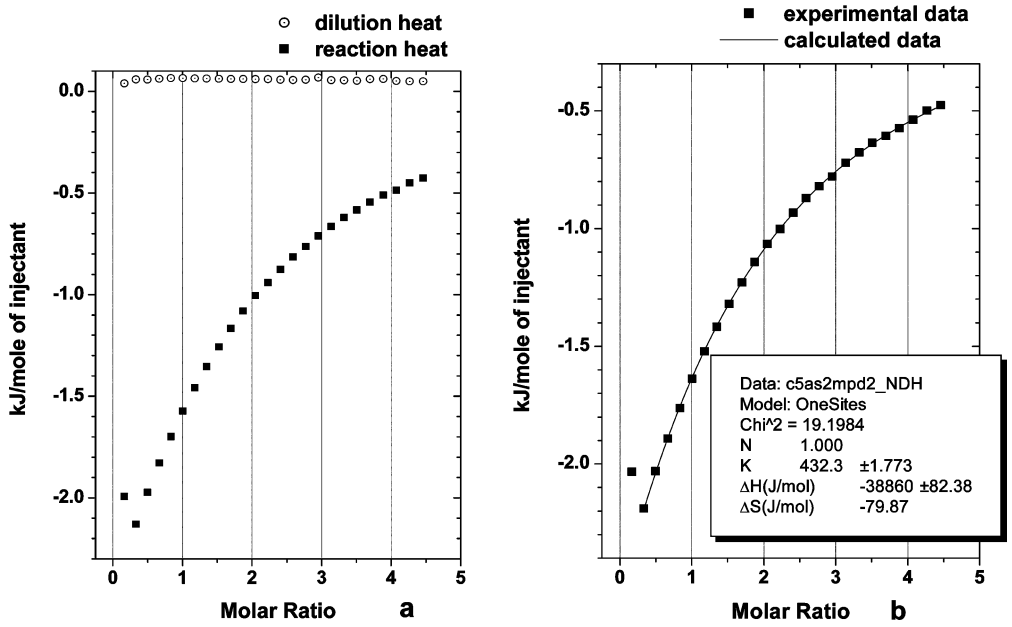

FIGURE 2. (a) Heat effects of the dilution and of the complexation reaction of C5AS with 2-mPY for each injection during the titration microcalorimetric experiment at pH 7.2 and (b) "net" heat effects of complexation of C5AS with 2-mPY for each injection, obtained by subtracting the dilution heat from the reaction heat, which was fitted by computer simulation using the "one set of binding sites" model.

TABLE 1. Complex Stability Constant (K_a), Standard Free Energy Changes ($\Delta G°$), Enthalpy Changes ($\Delta H°$), and Entropy Changes ($T\Delta S°$) for 1:1 Intermolecular Complexation of Guests with Sulfonatocalixarenes in Phosphate Buffer Solution (pH 2.0 and 7.2) at 298.15 K

host	guest	K_a (M^{-1})	$\Delta G°$ (kJ mol^{-1})	$\Delta H°$ (kJ mol^{-1})	$T\Delta S°$ (kJ mol^{-1})
			pH = 2.0		
C4AS	PY[a]	8.31×10^3	−22.4	−29.7	−7.3
	2-mPY[a]	1.33×10^4	−23.5	−34.7	−11.2
	4-mPY[a]	4.45×10^3	−20.8	−28.9	−8.1
	2,6-mPY[a]	2.41×10^4	−25.0	−38.6	−13.6
TC4AS	PY[a]	4.48×10^2	−15.1	−19.9	−4.8
	2-mPY[a]	1.16×10^3	−17.5	−28.2	−10.7
	4-mPY[a]	6.09×10^2	−15.9	−17.6	−1.7
	2,6-mPY[a]	3.05×10^3	−19.9	−28.6	−8.7
C5AS	PY	3.00×10^2	−14.1 ± 0.1	−16.1 ± 0.4	−2.0 ± 0.5
	2-mPY	5.64×10^2	−15.7 ± 0.1	−18.5 ± 0.1	−2.8 ± 0.1
	4-mPY	3.96×10^2	−14.8 ± 0.1	−16.5 ± 0.1	−1.7 ± 0.1
	2,6-mPY	9.88×10^2	−17.1 ± 0.1	−22.2 ± 0.1	−5.1 ± 0.1
			pH = 7.2		
C4AS	PY	3.05×10^2	−14.2 ± 0.1	−41.1 ± 0.7	−26.9 ± 0.7
	2-mPY	1.34×10^3	−17.9 ± 0.1	−45.1 ± 0.1	−27.2 ± 0.1
	4-mPY	5.20×10^2	−15.5 ± 0.1	−41.2 ± 0.1	−25.7 ± 0.1
	2,6-mPY	6.73×10^3	−21.9 ± 0.1	−47.7 ± 0.1	−25.8 ± 0.1
TC4AS	PY	0.55×10^2	−9.9 ± 0.1	−30.5 ± 0.6	−20.6 ± 0.7
	2-mPY	2.09×10^2	−13.2 ± 0.1	−40.5 ± 0.5	−27.3 ± 0.5
	4-mPY	1.39×10^2	−12.2 ± 0.1	−37.6 ± 0.9	−25.4 ± 1.0
	2,6-mPY	1.52×10^3	−18.2 ± 0.1	−43.6 ± 0.1	−25.4 ± 0.6
C5AS	PY	0.73×10^2	−10.6 ± 0.1	−23.3 ± 0.3	−12.7 ± 0.3
	2-mPY	4.32×10^2	−15.0 ± 0.1	−38.9 ± 0.1	−23.9 ± 0.1
	4-mPY	2.67×10^2	−13.9 ± 0.1	−34.2 ± 0.1	−20.3 ± 0.1
	2,6-mPY	2.90×10^3	−19.8 ± 0.1	−38.2 ± 0.1	−18.4 ± 0.2

[a] Reference 14c.

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

Binding Stoichiometry. It is well-known that calixarenes can form stoichiometric 1:1 complexes with model substrates.[7−11] Herein, the Corey-Pauling-Koltun (CPK) molecular model studies also demonstrate that C4AS, TC4AS, or C5AS can only accommodate one pyridine guest in its hydrophobic cavity. Simultaneously, the 1:1 binding modes for the inclusion complexes of C4AS or TC4AS with pyridine[15] have been also validated by their crystal structures. Therefore, a fixed 1:1 binding stoichiometry is used in the curve-fitting analysis of calorimetric titration.

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TABLE 2. Chemical Shift Changes ($\Delta\delta$, ppm) of Guests in the Presence of C4AS or C5AS (pD 2.0 and pD 7.2)[a]

PY

pH	host	2-H/6-H	3-H/5-H	4-H
2.0	C4AS	−0.25	−0.48	−0.63
	C5AS	−0.27	−0.37	−0.43
7.2	C4AS	−0.63	−0.99	−1.34
	C5AS	−0.13	−0.13	−0.16

2-mPY

pH	host	2-CH_3	3-H	4-H	5-H	6-H
2.0	C4AS[b]	−0.20	−0.51	−0.72	−0.55	−0.30
	C5AS	−0.14	−0.28	−0.35	−0.29	−0.15
7.2	C4AS	−0.24	−0.71	−0.99	−0.89	−0.53
	C5AS	−0.14	−0.26	−0.23	−0.24	−0.26

4-mPY

pH	host	2-H/6-H	3-H/5-H	4-CH_3
2.0	C4AS[b]	−0.15	−0.20	−0.49
	C5AS	−0.424	−0.63	−0.456
7.2	C4AS	−0.45	−0.75	−1.47
	C5AS	−0.08	−0.08	−0.08

2,6-mPY

pH	host	2-CH_3	3-H	4-H
2.0	C4AS[b]	−0.27	−0.71	−0.86
	C5AS	−0.11	−0.25	−0.27
7.2	C4AS	−0.07	−0.25	−0.28
	C5AS	−0.03	−0.07	−0.07

[a] $\Delta\delta = \delta$ (with 1 equiv of host) $- \delta$ (free guest). Negative values indicate upfield shift. [b] Reference 14c.

Binding Mode. To explore the possible binding modes between sulfonatocalixarenes and pyridine guests, ^{1}H NMR spectra were recorded at pD 2.0 and pD 7.2, and some representative chemical shift changes ($\Delta\delta$) of guests in the presence of approximately 1 equiv of sulfonatocalixarenes are listed in Table 2. In all cases, the signals of guest protons are observed as averaged single resonances because of the fast exchange between the free and complexed guest on the NMR time scale.[11a,12] As can be seen in Table 2, the δ values of guest protons appreciably shift to higher fields after complexation with sulfonatocalixarenes as compared with the free one. Therefore, we can deduce that the guest molecule is encapsulated into the calixarene cavity, which thus leads to an efficient shield toward guest protons because of the current ring effect of aromatic nuclei.[11a,12]

In most cases, the $\Delta\delta$ values of guest protons after complexation with C5AS are smaller than corresponding values with C4AS, indicating that the shield effect of sulfonatocalixarenes weakens with the enlargement of the cavity. On the other hand, the shapes of NMR signals assigned to the methylene protons in C4AS and C5AS show the obvious differences, although these NMR signals do not shift, after complexation with guest molecules. Without guest molecules, the methylene protons in either C4AS or C5AS give a single NMR peak assigned to a rapid cone-to-cone interconversion of uncomplexed host, indicating that the host sulfonatocalixarenes exists in a flexible conformation. However, after complexation with guests, this single peak of C4AS splits into two sets of double peaks with the same integration intensities, which means that C4AS shows a fixed C_{2v} cone conformation, because the conformation changes of host become slow on the NMR time-scale following the inclusion of guest.[14c] In contrast, the NMR signal assigned to the methylene protons in C5AS still remains in their original shape after complexation with guest molecules, which means that the conformation of C5AS remains flexible during the course of complexation.

A close comparison of the $\Delta\delta$ values of guest protons after complexation with C5AS at pH 2.0 shows that the $\Delta\delta$ values decrease in an order of *para*-H > *meta*-H > *ortho*-H, except for the case of C5AS/4-mPY complex. This order is consistent with those of C4AS and TC4AS.[14c] Therefore, we can deduce that C5AS may adopt a similar binding mode to C4AS or TC4AS upon complexation with these pyridinium guest ions at pH 2.0. That is, the pyridinium ions penetrate into the C5AS cavity from the para position of the N atom, and the protonated N atom is located close to the anionic sulfoante tails of C5AS, giving significant electrostatic interactions between host and guest. Remarkably, the $\Delta\delta$ values for C5AS/4-mPY complex show a unique order of *meta*-H > *para*-H > *ortho*-H. A possible reason may be that 4-mPY is included in the C5AS cavity in an acclivitous orientation, which is different from the perpendicular manner of C4AS.

It is also interesting to compare the $\Delta\delta$ orders at pH 7.2. As can be seen in Table 2, the pyridine guests give the similar $\Delta\delta$ orders after complexation with C4AS at pH 7.2 to those at pH 2.0, which means that C4AS adopts a similar binding mode at pH 7.2 to that at pH 2.0. However, the $\Delta\delta$ orders of guest pyridines after complexation with C5AS become disordered at pH 7.2. A reasonable explanation for this phenomenon is that C5AS adopts a complicated binding mode at pH 7.2 because its wider cavity can adopt relatively small guests (in this cases pyridine derivatives) in several orientations. In addition, because the N atom of guest pyridine is deprotonated at pH 7.2, the geometry restriction arising from the electrostatic interactions between host and guest is thus invalidated, which is also unfavorable to the orientation of guest pyridine in C5AS cavity.

Binding Ability and Thermodynamics. To investigate quantitatively the molecular recognition behavior of water-soluble sulfonatocalixarenes with pyridine guests at different pH values and their thermodynamic origins, the isothermal titration calorimetry (ITC) experiments were performed in aqueous phosphate buffers (pH = 2.0 and 7.2), because the calorimetry measurement is the only method that directly measures the heat changes associated with intermolecular interactions. It is well documented that, among several weak noncovalent interactions working between sulfonatocalixarenes and guest, the hydrogen bond, π-stacking, and van der Waals interactions mainly contribute to the enthalpy changes, while the electrostatic interactions,[16] conformation change, and desolvation effect contribute to the entropy changes. As can be seen in Table 1, all of the intermolecular complexations between sulfonatocalixarenes and guest pyridines are driven by the favorable enthalpy changes ($\Delta H^\circ < 0$), accompanied by the negative entropy changes ($T\Delta S^\circ < 0$), which indicates that the hydrogen bond, π-stacking, and van der Waals interactions are the main driven forces of host−guest complexation.

Miyano et al. have reported that electron-deficient aromatic guests displayed a stronger binding than their electron-rich counterparts with calixarenes and provided an indication that $\pi\cdots\pi$ electronic interactions, while weak, may be more important in the inclusion of substituted benzenes than unspecific hydrophobic interactions.[11a] Herein, similar results are also found by comparing the binding ability of sulfonatocalixarenes toward

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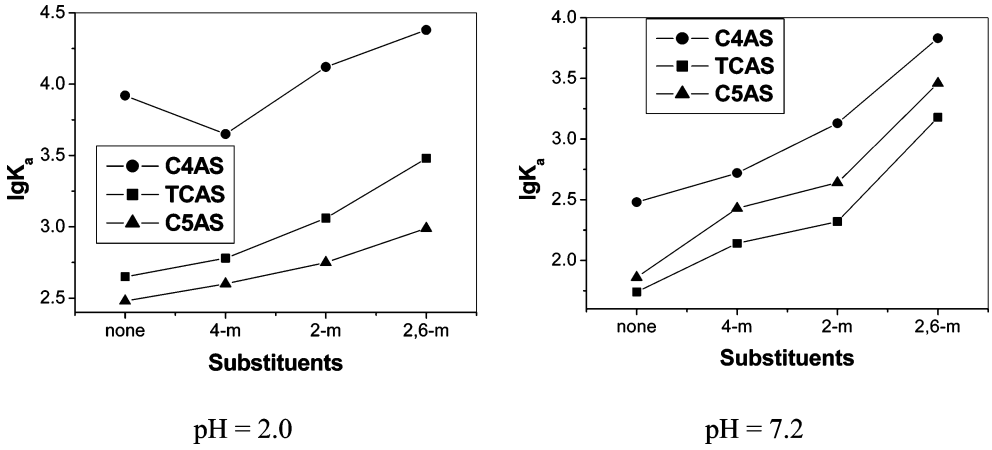

FIGURE 3. Plots of binding constants (K_a) versus the substitutes of guest pyridines upon complexation with sulfonatocalixarenes.

pyridine guests with that toward benzene and toluene molecules at pH 7.2. As can be seen in Table 1, the K_a values for the complexation of sulfonatocalixarenes with pyridine guests vary in the range of 0.55×10^2 to 6.73×10^3 M^{-1}, which are higher than the corresponding values with benzene and toluene (K_a = 16−24 M^{-1}).[11a] A possible reason for the stronger binding ability of sulfonatocalixarenes toward pyridine guests may be that the lower π electron density of pyridines enhances the efficiency of $\pi\cdots\pi$ interactions between the pyridine ring and the phenolic groups of sulfonatocalixarenes. However, a close comparison among the binding ability of four selected pyridine guests with sulfonatocalixarenes shows that the K_a values increase in an order of PY < 4-mPY < 2-mPY < 2,6-mPY, except for the C4AS/PY system at pH 2.0 (Figure 3), which means that the introduction of methyl groups (electron-donating groups) on the pyridine ring actually leads to the higher binding abilities. We have previously demonstrated that 4-mPY gives the stronger binding with sulfonatocalixarenes than PY because of the additional C−H$\cdots\pi$ interactions, while 2-mPY shows the stronger binding than 4-mPY because of the closer contact of both the pyridine ring and the methyl substituent of 2-mPY with the aromatic rings of sulfonatocalixarenes, which leads to stronger $\pi\cdots\pi$ and C−H$\cdots\pi$ interactions, and the better size-fit between 2-mPY and sulfonatocalixarenes, which leads to stronger van der Waals interactions.[14c] Possessing two methyl groups, 2,6-mPY gives the strongest C−H$\cdots\pi$ interactions with sulfonatocalixarenes. Moreover, the better geometrical fit of the larger 2,6-mPY into host sulfonatocalixarene cavities than other selected pyridine guests also contribute to the higher stability of 2,6-mPY complexes, leading to the stronger van der Waals interactions between host and guest. As a joint result of these two factors, 2,6-mPY shows the strongest binding with sulfonatocalixarenes. These results indicate that, with an increasing number of the methyl groups of pyridine guests, the strengthened C−H$\cdots\pi$ interactions and van der Waals interactions not only compensate the unfavorable $\pi\cdots\pi$ interactions arising from the increased π electron density on the pyridine ring, but also lead to the enhanced binding abilities. Therefore, we can deduce that the C−H$\cdots\pi$ and van der Waals interactions are more important than $\pi\cdots\pi$ interactions in the present inclusion complexation of calixarenes.

We can also find in Table 1 that, in most cases, sulfonatocalixarenes give the stronger binding toward pyridine guests at pH 2.0 than at pH 7.2. Typically, C4AS shows a K_a value up to 8.31×10^3 M^{-1} toward PY at pH 2.0, which is 27 times higher than the corresponding value at pH 7.2. However, a close examination on the thermodynamic parameters at different pH values shows that the enthalpy changes at pH 7.2 are more negative than those at pH 2.0, but the entropy changes at pH 7.2 are much more negative than those at pH 2.0. This means that the higher K_a values at pH 2.0 are not attributed to the large enthalpy changes, but to the less unfavorable entropy changes, although the complexation of sulfonatocalixarenes with pyridine guests are driven by the enthalpy term. A possible reason may be that, at pH 2.0, the protonation of guest pyridine reduces the electron density on the methyl groups. Moreover, because all of the phenolic hydroxyl groups in sulfonatocalixarenes are protonated, the π-electron density of sulfonatocalixarene at pH 2.0 should be lower than the case at pH 7.2, where one of the phenilic hydroxyl groups in sulfonatocalixarene is deprotonated. These two factors jointly lead to the weakened C−H$\cdots\pi$ interactions between host and guest, and subsequently result in the relatively low enthalpy gain at pH 2.0. Possessing a higher π electron density, sulfonatocalixarenes give the stronger $\pi\cdots\pi$ and C−H$\cdots\pi$ interactions with pyridine guests at pH 7.2, and thus show the more negative enthalpy changes. On the other hand, the protonated pyridinium ions should be heavily solvated at pH 2.0, and the solvent molecules around the guest are highly ordered. During the complexation, before the pyridine guests enter the calixarene cavity, it has to lose its solvation shell. This process causes the disorder of system to increase and thus leads to a favorable entropy gain, which compensates the entropy loss arising from the loss of conformational freedom upon complexation in a great degree. Moreover, the electrostatic interactions between the protonated guest and the anionic sulfonate groups of sulfonatocalixarenes also contribute to the entropy gain to some extent.[16] Therefore, the complexation of sulfonatocalixarenes with pyridine guests at pH 2.0 gives the less unfavorable entropy loss. With a less extensive desolvation effect and without the electrostatic interactions, sulfonatocalixarenes show the more unfavorable entropy changes and weaker binding ability at pH 7.2, although they display the more negative enthalpy changes.

Interestingly, sulfonatocalixarenes display the higher molecular selectivity at pH 7.2 than at pH 2.0. As can be seen in Table 1, the highest molecular selectivity among the four guests employed are as low as 5.4 by C4AS to 2,6-mPY/4-mPY pair, 6.8 by TC4AS to 2,6-mPY/PY pair, and 3.3 by C5AS to 2,6-mPY/PY pair at pH 2.0, but much enhanced to 22 by C4AS, 28 by TC4AS, and 40 by C5AS to 2,6-mPY/PY pair at pH 7.2.

This means, sulfonatocalixarenes give higher or lower K_a values that critically depend on the number of methyl groups of guests, and this situation is more obvious at pH 7.2. Therefore, we can deduce that the molecular selectivity of sulfonatocalixarenes toward pyridine guests is mainly dominated by the C−H···π interactions, which are remarkably affected by the number of methyl groups of pyridine guests and the pH of the system. Possessing the stronger C−H···π interactions, sulfonatocalixarenes show the higher molecular selectivity at pH 7.2. However, the C−H···π interactions are inevitably weakened owing to the decreased electron densities on both the methyl groups of pyridine guests and the aromatic rings of sulfonatocalixarenes at pH 2.0, which consequently results in the lower molecular selectivity.

As can be seen in Table 1, sulfonatocalixarenes show an order of binding ability as C4AS > TC4AS > C5AS at pH 2.0, but this order changes to C4AS > C5AS > TC4AS at pH 7.2. That is, C4AS gives stronger binding ability toward pyridine guests than TC4AS and C5AS in either an acidic or a neutral environment. For example, C4AS gives a K_a value for 2,6-mPY up to 2.41×10^4 M^{-1} at pH 2.0, that is, 24 times higher than the corresponding value of C5AS. These results should be reasonable, because the strength of electrostatic, hydrogen bond, π-stacking, and van der Waals interactions between sulfonatocalixarenes and guests is closely related to the distance and the contacting surface area between host and guest. Therefore, a good host−guest size-fit will unambiguously lead to the strong binding. CPK molecular model studies demonstrate that, possessing the smallest cavity, C4AS has the best size-fit relationship to guest pyridines among the sulfonatocalixarenes examined, which consequently results in the strong noncovalent interactions between host and guest.

It is also interesting to compare the binding ability of TC4AS and C5AS at different pH values. Possessing a smaller cavity, TC4AS has a better size-fit with pyridine guests than C5AS, which consequently leads to the stronger intermolecular interactions, especially the electrostatic interactions, between host and guest at pH 2.0. However, these two sulfonatocalixarenes give an opposite order of binding ability at pH 7.2, that is, C5AS > TC4AS. It is documented that the π electron density of thiacalixarene is lower than that of classical calixarene.[17] This subsequently rationalizes that TC4AS shows the weaker binding ability because of the weakened π···π and C−H···π interactions with pyridine guests.

Conclusion

In summary, the binding modes, binding ability, molecular selectivity, and thermodynamic origins of three water-soluble sulfonatocalixarenes upon complexation with some pyridines at different pH values have been investigated by ITC and NMR spectroscopy. The results show that sulfonatocalixarenes afford the stronger binding ability at pH 2.0 but the higher molecular selectivity at pH 7.2. Significantly, the π-stacking, especially the C−H···π interactions, play an important role in the inclusion complexation of calixarenes, and their strength can be efficiently controlled through a judicious adjustment of the protonation/deprotonation status and the electron density of both host and guest, such as, by altering the pH value of the environment. These conclusions may be useful for the design of functional water-soluble calixarenes with high binding ability and molecular selectivity.

Experimental Section

Materials. Calix[4]arene tetrasulfonate (C4AS),[18] thiacalix[4]arene tetrasulfonate (TC4AS),[19] and calix[5]arene petasulfonate (C5AS)[20] were synthesized and purified according to the literature reports. Pyridine (PY), 2-picoline (2-mPY), 4-picoline (4-mPY), and 2,6-dimethylpyridine (2,6-mPY) were commercially available and 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 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. The pH value of buffer solution was verified on a pH meter calibrated with two standard buffer solutions. At pH 2.0, every sulfonate group of sulfonatocalixarenes is in anionic form, and all of phenolic hydroxyl groups are protonated according to the reported pK_a values.[20,21] At pH 7.2, the sulfonate groups of sulfonatocalixarenes are still in anionic form, but one of phenolic hydroxyl groups becomes deprotonated. On the other hand, all of the guest pyridines exist as pyridinium cations at pH 2.0 but as neutral molecules at pH 7.2.[22]

Measurement. ^{1}H NMR spectra were recorded at pD 2.0 and 7.2 (adjusted by DCl or NaOD) on a spectrometer using 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) as an external reference, and the host and the guest were mixed in an 1:1 stoichiometry. A thermostated and fully computer-operated isothermal calorimetry (VP−ITC) instrument 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 shown to be in good agreement (error < 2%) with the literature data. All microcalorimetric titrations between water-soluble sulfonatocalixarenes 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 titration experiment. Twenty-five successive injections were made for each titration experiment. A constant volume (10 μL/injection) of guest (or host) solution (9.91−19.83 mM) in a 0.250 mL syringe was injected into the reaction cell (1.4227 mL) charged with host (or guest) molecules solution (0.49−1.02 mM) in the same buffer solution.

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