Characterization of Self-Assembling Encapsulation Complexes in the Gas Phase and Solution

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Abstract: The interaction of quaternary ammonium ion guests with self-assembling hosts was examined by ^{1}H NMR and ESI-MS experiments. The hosts consist of four identical, self-complementary subunits, reversibly joined in a capsular assembly through hydrogen bonding. Both approaches show the ammonium ions to be encapsulated, held within the hollow shell of the capsule. Competition experiments with a series of different guest ions reveal a characteristic size selectivity. The NMR and MS methods are complementary: MS easily reveals the formation of heterotetramers from different subunits that could not be determined by NMR, while NMR allowed competitive encapsulations of neutral and ionic guests that were not possible to detect with MS. These competition experiments gave a lower limit of ca. 3.6 kcal/mol for the contribution of cation−π interactions involved in the encapsulation of the ionic guests.

Introduction

During the past few years, mass spectrometry has been widely used for the investigation of noncovalent interactions[1] in protein/protein,[2] enzyme/substrate, and enzyme/inhibitor complexes,[3] assemblies of DNA[4] with drugs, proteins, and oligonucleotides, supramolecular metal complexes,[5] knots and catenanes,[6] cerand/guest and cavitand/guest assemblies,[7,8] gas-phase micelles,[9] and carbohydrate complexes.[10] The examination of whole viruses[11] must rank as one of the highlights of these studies. In most of these assemblies the intermolecular interactions tend to be strong: multiple weak interactions of different kinds add up to large binding energies; nanomolar affinites are common, particularly in complexes involving biomolecules. Knots and catenanes are mechanically interlocked species, and the subunits are held together by strong covalent bonds, while metal complexes enjoy rather strong and numerous ligand−metal binding sites. In contrast, mass spectrometric studies of supramolecular assemblies[7] with hydrogen-bonded organic subunits,[12] where binding affinities are often in the micromolar or even millimolar range, are rare. After a number of unsuccessful efforts, we were recently able to observe host−guest

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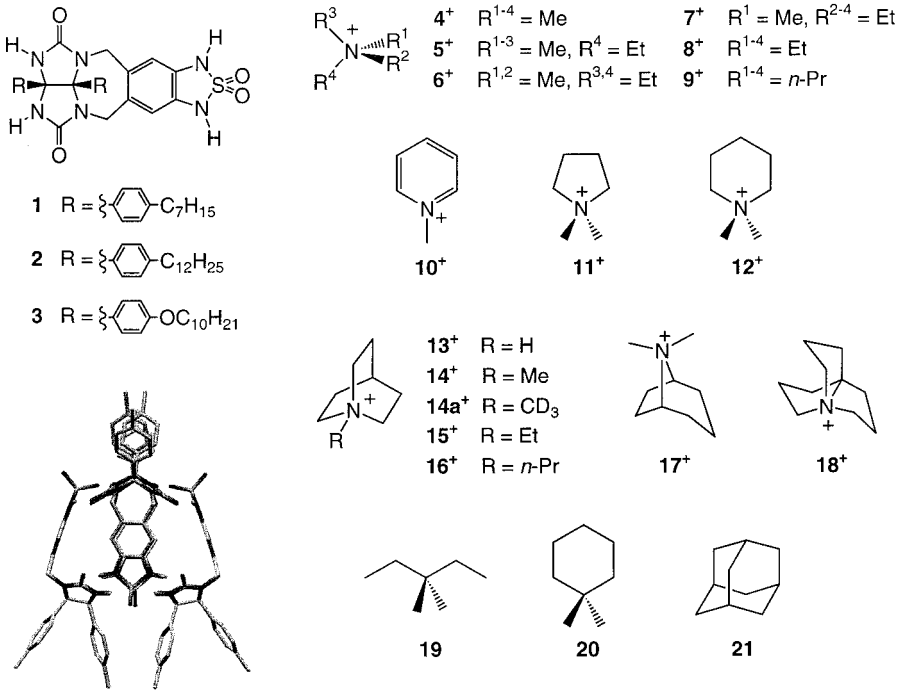
Chart 1



complexes of reversibly formed dimeric capsules—the softballs[13]—in the gas phase and characterize these by mass spectrometry.[14] In that study, quaternary ammonium ions served simultaneously as guests and ion labels. Evidence for the capsular ion structure in the gas phase came from size and shape selection of guests and collision-induced fragmentation experiments. Here, we apply a combination of NMR and electrospray ionization[15] (ESI) mass spectrometry to characterize the next generation of assemblies: the footballs. In these systems four identical subunits of the host and one guest molecule converge and then merge as the assembly. We determine the chemical compositions, exchange dynamics, and intermolecular forces involved in host−guest complexes derived from **1**−**3** (Chart 1).

Earlier NMR studies[16] indicated that **1** and **2** assemble to form pseudo-spherical host capsules in CD_2Cl_2 when suitable guest species are present. The instructions for assembly are written into the hydrogen-bonding preferences and the curvature of the subunits through chemical synthesis, but only the presence of a guest nucleates the formation of the assemblies $\mathbf{1}_4$−$\mathbf{2}_4$. The subunits have the urge; the system is on the verge, but only a guest which fills it the best will make the assembly emerge.

Experimental Section

Syntheses. The tetramers were synthesized as previously reported.[16] The ammonium salts $\mathbf{4}^+PF_6^-$, $\mathbf{8}^+PF_6^-$, and $\mathbf{9}^+PF_6^-$ as well as the neutral guests **19**−**21** were used as received (Aldrich). $\mathbf{18}^+BF_4^-$ was prepared from its chloride (Aldrich) by anion exchange with $AgBF_4$. All other ammonium salts were synthesized by reacting an ethereal solution of the corresponding tertiary amine with an excess of an appropriate alkyl iodide (1−2 h, room temperature) followed by filtration. The ammonium iodide was dissolved in acetone/methanol (ca. 1:1), and the anion was exchanged by addition of 1 equiv of $AgBF_4$. The mixture was stirred at room temperature for 2 h. Filtration to remove AgI and evaporation of the solvents gave the ammonium tetrafluoroborate salts in >95% yield. All compounds were characterized by ^1H NMR (CD_2Cl_2; 600 MHz). All NMR experiments were performed with a Bruker DRX 600 spectrometer using $\delta(CH_2Cl_2)$ 5.32 ppm as internal standard.

Mass Spectrometry. The ESI-MS experiments were performed on a single-quadrupole Perkin-Elmer API-100 Sciex (mass range <3000 amu) and a Finnigan MAT LCQ ion trap instrument (mass range <4000 amu). The samples were introduced as 100 μM solutions of monomeric **1**−**3** with 0.5 equiv (i.e. 50 μM) of the guest salt in CH_2Cl_2 at a flow rate of 4 μL/min. Monomer **3** does not completely dissolve upon addition of 0.5 equiv of the guest. Therefore, the solutions containing **3** were filtered prior to injection into the mass spectrometer in order to remove remaining precipitate. Addition of ca. 2.5 equiv of guest $\mathbf{14}^+$ gave almost complete formation of the capsule $\mathbf{[14^+@3_4]}$. The mass spectrum was recorded again and did not change when compared to that of the standard solution containing 0.5 equiv of the guest. The ion intensities increased with the ion spray and the orifice potentials, which were set to 4−5 kV and 100−200 V, respectively. In contrast, the intensities did not vary substantially with the flow rate or changes in nebulizer and curtain gas streams. To improve the signal-to-noise ratio, 100−200 scans were accumulated. Due to the lower mass resolution of the LCQ ion trap instrument, meaningful isotope patterns could only be recorded on the API-100 Sciex mass spectrometer for complexes with masses lower than 3000 amu. Analogously, the heterotetramer experiments were performed with CH_2Cl_2 solutions 50 μM in each monomer.

For competition experiments, 100 μM solutions of **1** in CH_2Cl_2 with 0.5 equiv (50 μM) of each guest salt were prepared. These experiments were performed with the API-100 instrument (ion spray and orifice potentials set to 5000 and 200 V, respectively), and 900−1000 scans of the 2800−2900 amu region were averaged. As the mass difference for any combination of two capsules with different guests is smaller than 100 amu, mass discrimination effects have been neglected in the evaluation of the relative intensities. Neither the solvent (monomer **1** is insoluble in CH_2Cl_2 and dissolves only upon addition of a guest) nor the anions (no signals for anions encapsulated inside a tetrameric capsule were observed in the negative mode of the instruments) act as guests for the capsules. Accordingly, the relative intensities of the encapsulated ammonium ions can be used to determine relative apparent binding constants (see below). The solution-phase binding constants are likely to be affected by the electrospray process, and the MS may

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not reflect solution properties in a quantitative sense. Therefore, we draw only qualitative conclusions from these MS data. Nevertheless, the relative binding constants were evaluated quantitatively in order to compare the MS data with analogous NMR experiments. Any two pairs of competition experiments were cross-checked; e.g., the equilibrium constants for $\mathbf{4}^+$ vs $\mathbf{5}^+$ and $\mathbf{5}^+$ vs $\mathbf{6}^+$ must lead to the same result as measured using $\mathbf{4}^+$ vs $\mathbf{6}^+$. The error of these competition experiments was quite high (ca. ±25% for K_{rel}), but several repetitions of these experiments showed the ranking of guests to be reproducible.

Determination of Apparent Relative Binding Constants. For the following equilibrium, the equilibrium constant K is defined as shown in eq 1:

$$[\mathbf{G_1}^+@\mathbf{1_4}] + \mathbf{G_2}^+ \overset{K}{\rightleftharpoons} [\mathbf{G_2}^+@\mathbf{1_4}] + \mathbf{G_1}^+$$

$$K = \frac{[\mathbf{G_2}^+@\mathbf{1_4}]}{[\mathbf{G_1}^+@\mathbf{1_4}]}\frac{[\mathbf{G_1}^+]}{[\mathbf{G_2}^+]} \quad (1)$$

The ammonium ions are the only possible guests (see above), and the concentrations of free and encapsulated guests can be expressed as follows, provided that no precipitated monomer is present and the amount of monomeric **1** consumed for tetramer formation is equal to the total amount of **1** added (I_1^0 and I_2^0 are the relative intensities obtained from the mass spectrum and normalized to $I_1^0 + I_2^0 = 1$; n represents the molar excess of the guest with respect to the tetrameric capsule, here $n = 2$):

$$[\mathbf{G_1}^+@\mathbf{1_4}] = I_1^0[\mathbf{1_4}]_{total} \quad (2)$$

$$[\mathbf{G_2}^+@\mathbf{1_4}] = I_2^0[\mathbf{1_4}]_{total} \quad (3)$$

$$[\mathbf{G_1}^+] = (n - I_1^0)[\mathbf{1_4}]_{total} \quad (4)$$

$$[\mathbf{G_2}^+] = (n - I_2^0)[\mathbf{1_4}]_{total} \quad (5)$$

With these equations, one derives eq 6 for K, which only depends on the measured intensities and the molar excess of the guest with respect to the tetramer:

$$K = \frac{I_2^0}{I_1^0}\frac{n - I_1^0}{n - I_2^0} \quad (6)$$

The relative binding constants used in Figure 4 are based on the definition of $K_{rel}(\mathbf{14}^+) = 1$ as the anchor point.

Computational Details. The geometries of the empty tetramer and guests $\mathbf{4}^+$–$\mathbf{18}^+$ were optimized using the Amber* force field as implemented in the MacroModel 5.5 program.[17] The calculations of the volume of the cavity and the guests were performed with the GRASP program,[18] as described in detail previously.[19] Briefly, the calculation of the cavity volume involves rolling a spherical probe along the interior surface. A small probe can easily fall out of the holes, while a large probe fails to define the smaller dimples of the concave inner surface. The default size of the probe in the GRASP software package (1.4 Å radius) is suitable. It has also been used for the calculation of other capsule volumes before and thus ensures comparability with earlier results.[19] The volumes of the guests were determined as those enclosed by their van der Waals molecular surfaces.

Results and Discussion

Rationale. For ion generation, electrospray ionization was chosen, because it is a gentle ionization method and it has been reported to reflect, at least qualitatively, solution phenomena.[12e,20] Common ESI solvents such as methanol and water, which protonate species during the ESI process, disrupt the hydrogen bonds that hold the capsules together; therefore, only noncompetitive organic solvents such as $CHCl_3$ and CH_2Cl_2 can be used. Now protonation by these solvents is not likely and some other means of ion labeling is required; the simplest means is through the encapsulation of an ionic guest. Accordingly, quaternary ammonium ions[21] $\mathbf{4}^+$–$\mathbf{18}^+$ with sizes close to those of known neutral guests (Chart 1) were chosen. In addition, counterions (BF_4^- and PF_6^-) were used to impart solubility of the salts in organic solvents without interfering with the seam of hydrogen bonds.

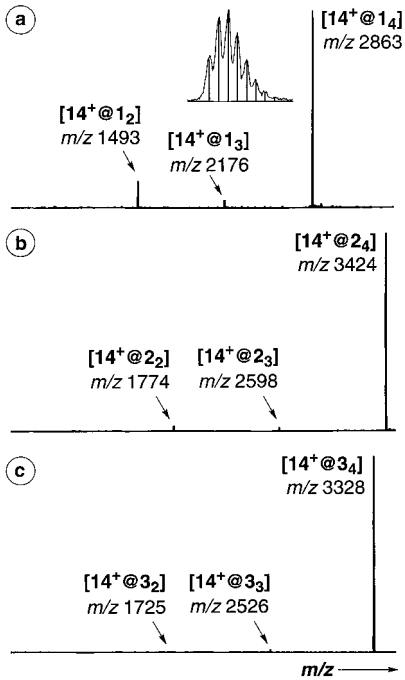

Figure 1. ESI mass spectra (m/z 600–3450) of CH_2Cl_2 solutions of $\mathbf{14}^+$ (50 μM) and (a) **1** (inset: measured and calculated isotope patterns for [$\mathbf{14}^+@\mathbf{1_4}$]), (b) **2**, and (c) **3** (each 100 μM).

The ESI mass spectra of $\mathbf{1_4}$–$\mathbf{3_4}$ with encapsulated $\mathbf{14}^+$ (Figure 1) each show three signals corresponding to dimeric, trimeric, and—as the base peaks—tetrameric complexes of **1**–**3** with the guest. The monomer–guest ion is not detectable; cooperativity[22] probably favors the assembly of complete capsules over their fragments. The measured isotope pattern for [$\mathbf{14}^+@\mathbf{1_4}$] nicely matches the calculated abundances (Figure 1a) and confirms the elemental composition and the monocationic nature of the complexes. Further evidence for the correct composition comes from a mass shift of $\Delta m = 3$ upon inclusion of [D_3]-labeled $\mathbf{14a}^+$: a monocation which gives rise to this mass shift must contain only one guest cation. The mass difference between the complex and the guest cation corresponds to four subunits of

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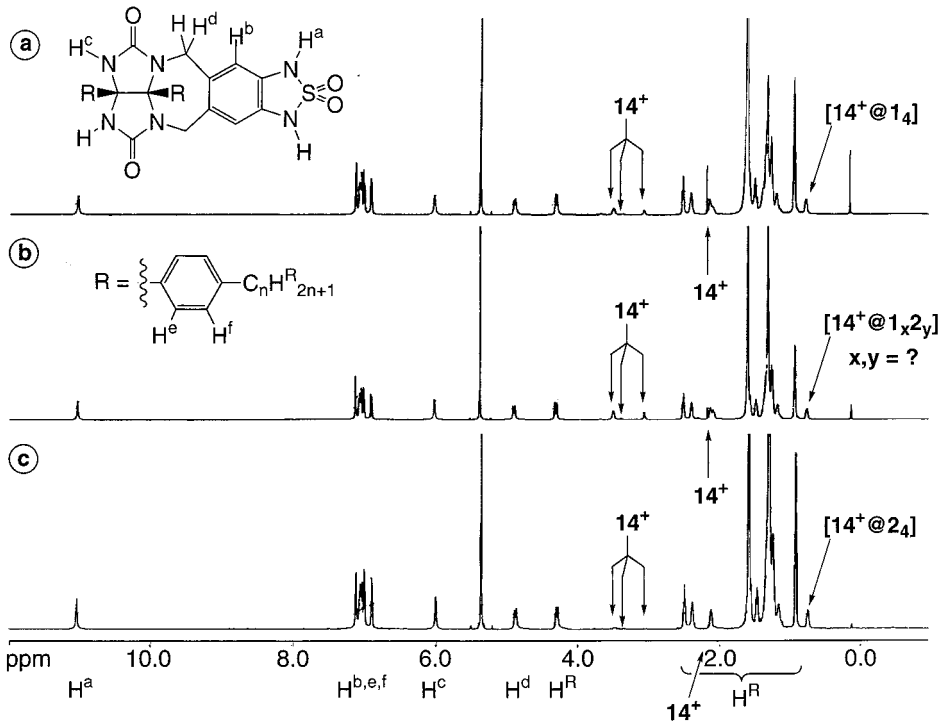

Figure 2. ^{1}H NMR spectra (600 MHz, CD_2Cl_2) of (a) **1**, (b) **1** and **2**, and (c) **2** with $\mathbf{14}^+BF_4^-$ as the guest salt (0.25 equiv with respect to the monomers). The bottom line provides information about the assignment of the signals to the protons of capsules and guest.

1−**3**, respectively. Furthermore, the complexes are held together by hydrogen bonds: when methanol is added, signals for the assemblies disappear in favor of the protonated monomers and, with sharply decreasing intensities, proton-bridged dimers, trimers, and tetramers.

Structures and Equilibria. Evidence for encapsulation rather than unspecific binding of the charged guests comes from ^{1}H NMR experiments (Figure 2). For example, Figure 2a shows the ^{1}H NMR spectrum of a CD_2Cl_2 solution of **1** with $\mathbf{14}^+BF_4^-$ as the guest salt. Without addition of the guest, **1** is insoluble in CD_2Cl_2 and the NMR spectrum cannot be recorded. Upon addition of 0.25 equiv of guest (1 equiv with respect to the tetrameric capsule $\mathbf{1}_4$), the NMR spectrum shows formation of a discrete species. Some **1** is still present as a precipitate but dissolves completely upon addition of another 0.25 equiv of the guest (a total of 2 equiv relative to $\mathbf{1}_4$). All signals can then be assigned to the tetramer and the guest as indicated in Figure 2. The glycoluril N−H signals are shifted upfield (δ 5.98 ppm) as compared to a DMSO-d_6 solution (δ 8.09 ppm), a solvent in which **1** exists as a monomeric species. The complex formed is highly symmetrical, and only sharp singlets are observed for the glycoluril and sulfamide N−H resonances. Evidence that the guest cation is inside the capsule is provided by the signal at δ 0.75 ppm. The other signals for encapsulated $\mathbf{14}^+$ are probably buried under the peaks for the *n*-heptyl side chains of the tetramer. The stoichiometry obtained from integration is roughly four molecules of **1** per encapsulated guest. A very similar pattern is observed if, for example, $\mathbf{5}^+$, $\mathbf{6}^+$, $\mathbf{11}^+$, $\mathbf{12}^+$, and $\mathbf{13}^+$ are used as the guests. Shielding by the anisotropic environment of the aromatic capsule surfaces causes characteristic upfield shifts of the signals for encapsulated guests; some enjoy a $\Delta\delta$ value of −1.6 ppm.

The formation of heterodimers from different monomers has been useful in characterizing noncovalent, dimeric assemblies by NMR,[23] and we attempted to apply this method to the more complicated disproportionation of tetramers $[\mathbf{14}^+@\mathbf{1}_4]$ and $[\mathbf{14}^+@\mathbf{2}_4]$. In addition to the two homotetramers, formation of heterotetramers $[\mathbf{14}^+@\mathbf{1}_3\mathbf{2}_1]$, $[\mathbf{14}^+@\mathbf{1}_2\mathbf{2}_2]$ (two possible isomers), and $[\mathbf{14}^+@\mathbf{1}_1\mathbf{2}_3]$ is possible. Unfortunately, the ^{1}H NMR spectrum of **2** with $\mathbf{14}^+BF_4^-$ as the guest salt (Figure 2c) is almost superimposable on that of $\mathbf{1}/\mathbf{14}^+BF_4^-$ (Figure 2a); the only significant difference is in the integration of the alkyl protons. A mixture of equal amounts of both solutions (Figure 2b) shows no evidence for the formation of heterotetramers; instead, the spectrum appears to be the superpositioning of the spectra of the two homotetrameric species.

In contrast, MS readily reveals the heterotetramers invisible to NMR: mixing solutions of $[\mathbf{14}^+@\mathbf{1}_4]$ and $[\mathbf{14}^+@\mathbf{2}_4]$ (Figure 3a) gives rise to signals for an almost statistical 1:4:6:4:1 distribution of all possible capsules.[24] The equilibrium is reached in less than 1 min (the exchange of monomeric and/or dimeric subunits is fast on the human time scale). Under the same conditions, experiments with **3** showed some anomalies. The ESI mass spectra (Figure 3b,c) of mixtures of $[\mathbf{14}^+@\mathbf{3}_4]$ with $[\mathbf{14}^+@\mathbf{1}_4]$ or $[\mathbf{14}^+@\mathbf{2}_4]$, respectively, revealed a distinct underrepresentation of those peaks expected for capsules containing **3**. Apparently, the equilibrium between the tetrameric capsule $[\mathbf{14}^+@\mathbf{3}_4]$ and precipitated **3** plus free guest lies more toward the precipitate, and similarly, this holds true for the heterotetramers containing **3**. Addition of a 2.5-fold excess of $\mathbf{14}^+$ drives the equilibria toward the capsules and gives spectra much closer to the pattern expected for a statistical distribution. We can only guess what the reasons are for the lower solubility of **3** that hampers the formation of capsules containing **3** as a building block. The additional oxygen in the (*n*-decyloxy)phenyl side chains may be involved in hydrogen bonds within the solid state that stabilize the precipitate. Two such oxygens from different monomers come near one another at each end of the assembly

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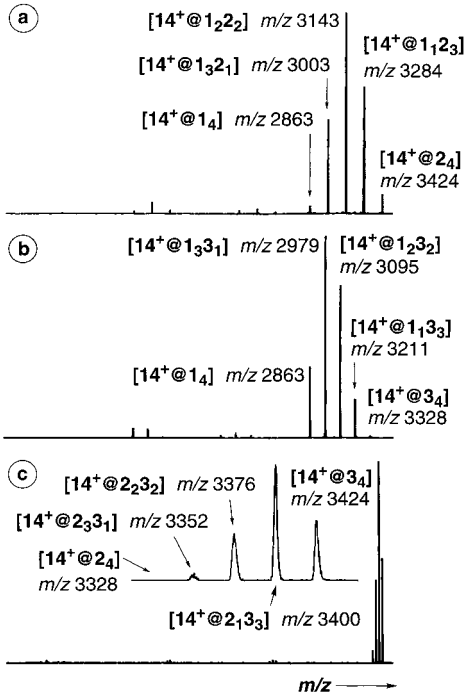

Figure 3. ESI mass spectra (*m*/*z* 600−3450) of CH_2Cl_2 solutions of **14**$^+$ (50 μM) and (a) **1** and **2**, (b) **1** and **3**, and (c) **2** and **3** (each 50 μM) (inset: expanded heterotetramer region, *m*/*z* 3300−3450).

(Chart 1). Unfavorable positioning of their local dipoles may further destabilize the assembly with respect to the precipitated aggregate.

Do the complexes observed by ESI-MS retain the capsular structure in the gas phase? In our previous study of the softballs,[14] collision experiments demonstrated the ammonium ions to be encapsulated. This was concluded from the observation that covalent bond cleavage competed with guest release, and a weakly bound structure such as an ammonium ion on the outer surface of an empty capsule would not likely exhibit such behavior. It is tempting to extrapolate this behavior to the tetrameric capsules as well, but collision experiments with [**14**$^+$@**1**$_4$], [**14**$^+$@**2**$_4$], and [**14**$^+$@**3**$_4$] did not yield any significant information about the gas-phase structure. It seems unlikely that the capsular structure, which according to the NMR experiments is present in solution, is transformed into a nonspecific aggregate during the electrospray process, but this possibility cannot be rigorously excluded. However, the good agreement between NMR and MS data discussed below suggests that the MS results do mirror the properties of the species in solution.

Energetics. Competition experiments with **1** and equimolar amounts of two different guests were staged to examine the selectivtiy of the tetramer for guests of appropriate sizes and shapes with both MS and NMR methods. The intensities of signals for the two different capsules in either spectrum give relative binding constants and correlation of these with the fraction of the cavities' space occupied by the guests is expected to give a clear-cut preference for guests of optimal sizes. In contrast, unspecific binding—for example, on the outer surface of a capsule—is not expected to result in such a correlation. The volumes of the guests and the cavity (178 Å^3) were determined by first optimizing the geometry of guests and tetramer (Chart 1) with the Amber* force field as implemented in MacroModel 5.5[17] and then calculating the volumes using the GRASP software package.[18]

Figure 4 displays the correlation of K_{rel} obtained from MS intensities with the packing coefficients (ratio of guest to cavity volumes) of guests **4**$^+$−**18**$^+$.[25] The packing coefficients for the best guests of each series of ammonium ions—acyclic (**5**$^+$), monocyclic (**11**$^+$), and bi-/tricyclic (**13**$^+$)—were found to be in the range of ca. 0.55−0.65 (Figure 4). This is close to the size selectivity determined empirically for encapsulated neutral guests in solution.[19] In that study, statistical evaluation resulted in an optimal packing coefficient of 0.55 for neutral guests of a shape congruent with that of the cavity. The observation of a distinct size selectivity for the case at hand indicates that the encapsulation occurring in solution is also monitored by the mass spectrometric probe.

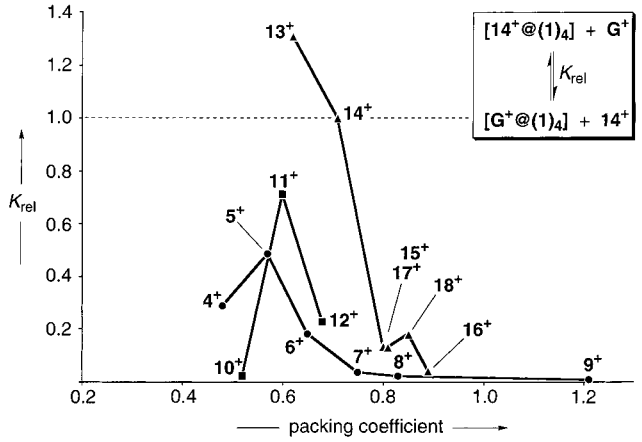

Figure 4. Dependence of the apparent relative binding constant K_{rel} on the guest size represented by the packing coefficient. Lines connect three different series of guests: acyclic (●), monocyclic (■), and bi-/tricyclic (▲) guests.

Nonetheless, the MS results could be compromised by some yet unknown aspect of the electrospray process; therefore, several combinations of guests were also examined by NMR. The same trends were observed by NMR in solution as for MS in the gas phase: good guests were in the same range of packing coefficients. However, the relative binding constants obtained by NMR differ significantly (and not systematically) from those measured with MS. Accordingly, we feel that the MS results cannot be interpreted with the same confidence as solution-phase binding constants. Several effects may account for the deviations of NMR and MS data. (i) Although the mass spectrometer used for the competition experiments operates with the electrospray capillary at room temperature, the droplets are cooled by evaporation of the solvent and the temperature is not well-defined. (ii) The concentrations of the solutions used for MS and NMR experiments are quite different: evaporation of the solvent during the electrospray process leads to an increase of the concentrations for short periods of time and it is not clear which concentrations should be used for the calculation of the binding constants from MS data. (iii) A third, entropic effect may account for the nonsystematic deviations. Imagine an empty capsule in solution surrounded by guest molecules. The empty space inside the capsule can be considered as exclusion volume that decreases the entropy compared with that of capsules containing a guest; i.e., in solution the filled capsules are entropically more favorable. In contrast, a filled capsule in the vacuum of a mass spectrometer surrounded by empty space is no longer favorable in entropic terms. The guest is restricted to the small volume of the cavity, and release into the gas phase is favored. Depending on the particular geometric properties of

(25) We are aware of the drawbacks of this modeling approach: e.g., the low-quality parameters for the SO_2 groups. Therefore, the packing coefficients should be considered as a rough estimate rather than exact numbers.

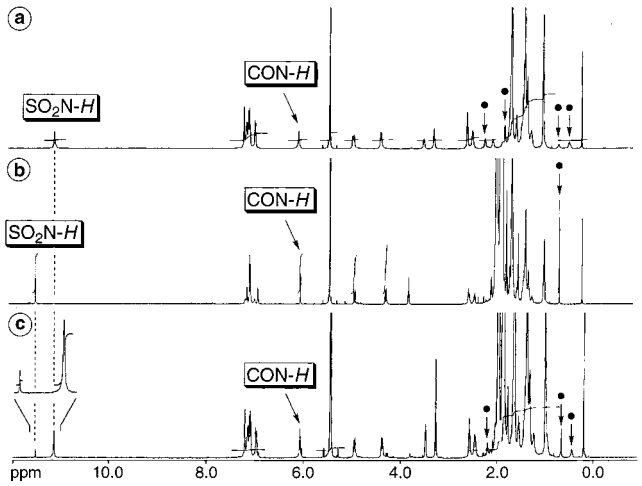

Figure 5. ^{1}H NMR spectra (600 MHz, CD_2Cl_2) of **1** (2.28 mM) with (a) $\mathbf{12}^+$ (0.5 equiv), (b) **21** (excess), and (c) $\mathbf{12}^+$ (0.5 equiv) and **21** (10 equiv). The dots mark signals of the encapsulated guests.

the guests, their release can proceed at different rates, and the relative abundance of the two capsules may be altered by the transfer from solution to the gas phase.

Encapsulation of these quaternary guests surely involves cation$-\pi$ interactions[26] between the ammonium ions and the aromatic rings and other π bonds that line the capsule's interior.[27] To evaluate these forces, ionic ($\mathbf{6}^+$, $\mathbf{12}^+$) and neutral (**19**, **20**) guests of the same sizes and shapes were compared in NMR competition experiments. Unfortunately, neither of the neutral species serves as a guest, even when 80 equiv of **20** competes with 2 equiv of the corresponding ionic guest $\mathbf{12}^+$. From the signal-to-noise ratio in the NMR spectrum of this solution we estimate that the intensity of the sulfamide signal of [**20**@$\mathbf{1_4}$] is less than ca. 5% of the corresponding signal for the [$\mathbf{12}^+$@$\mathbf{1_4}$] complex. This results in a factor of >1600 in equilibrium constants of and $\Delta\Delta G > 4.3$ kcal/mol attributable to the cation$-\pi$ interactions of the ionic guest with the capsule walls. However, one might argue that the sulfamide signal for [**20**@$\mathbf{1_4}$] might be much broader than that for [$\mathbf{12}^+$@$\mathbf{1_4}$], in which case the estimate may be overstated. A competition experiment was also performed with $\mathbf{12}^+$ and adamantane **21**, the weakest neutral guest found so far.[16] Since **20** is no guest at all, the energy difference for encapsulation of **21** instead of $\mathbf{12}^+$ is bound to be smaller than that for the **20**/$\mathbf{12}^+$ pair.

The ^{1}H NMR spectrum of [$\mathbf{12}^+$@$\mathbf{1_4}$] (Figure 5a) is an ideal example of encapsulation, as all four signals for the encapsulated guest can be identified. Similarly, the NMR spectrum of [**21**@$\mathbf{1_4}$] (Figure 5b) exhibits a signal for encapsulated adamantane at $\delta \sim 0.75$ ppm. The most important feature of these two spectra is the chemical shift difference between the two sulfamide N$-$H protons. These differ by ca. 0.5 ppm and permit facile integration. A direct competition of $\mathbf{12}^+$ (0.5 equiv) and **21** (10 equiv relative to monomeric **1**) showed (Figure 5c) that the affinity of the cation was 450-fold greater than that of adamantane ($\Delta\Delta G > 3.6$ kcal/mol). Accordingly, this is a more conservative lower limit for the cation$-\pi$ interaction operating for $\mathbf{12}^+$.

Conclusions

From the perspective of physical organic chemistry, the encapsulation of cations (apart from their anions) establishes the existence of capsule-separated ion pairs, the supramolecular counterparts of solvent-separated ion pairs. That quaternary ammonium ions are such good guests for tetrameric assemblies derived from **1**$-$**3** underlines the significance of cation$-\pi$ interactions in molecular recognition phenomena. The estimate of the affinity due to this interaction is some 2 times that obtained from a recent biological example[28] and may reflect the multiple and exquisitely positioned π surfaces available to the cation and the capsule walls. From the analytical perspective, the observation of noncovalent complexes in the gas phase furthers the applicability of mass spectrometry for characterizing supramolecular sytems with low binding constants.[29] The NMR and MS provide complementary information: MS easily revealed the formation of heterotetramers from different subunits that were invisible by NMR, while NMR allowed competitive encapsulations of neutral and ionic guests that were not observable with MS. A combination of both methods gives a more complete picture of the structure, dynamics, and energetics of encapsulation phenomena.

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(27) Other forces, e.g. ion$-$dipole interactions, may play a role. However, none of the dipoles in the cavity walls are directed with the negative partial charge toward the encapsulated cation; rather, they are tangential to the capsule walls. Therefore, we believe these interactions to be small and the cation$-\pi$ interaction to be the major contribution.

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(29) Binding constants for neutral guests in $\mathbf{2_4}$ have been reported to range from 19 M^{-1} for adamantane to 3200 M^{-1} for adamantanedione. For $\mathbf{1_4}$ the binding constant of adamantanedione was determined to be 600 M^{-1}.[16]