External and Internal Guest Binding of a Highly Charged Supramolecular Host in Water: Deconvoluting the Very Different Thermodynamics

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Abstract. NMR, UV-vis and isothermal titration calorimetry (ITC) measurements probe different aspects of competing host-guest equilibria as simple alkylammonium guest molecules interact with both the exterior (ion-association) and interior (encapsulation) of the $[Ga_4L_6]^{12-}$ supramolecular assembly in water. Data obtained by each independent technique measure different components of the host-guest equilibria and only when analyzed together does a complete picture of the solution thermodynamics emerge. Striking differences between the internal and external guest binding are found. External binding is enthalpy driven and mainly due to attractive interactions between the guests and the exterior surface of the assembly while encapsulation is entropy driven as a result of desolvation and release of solvent molecules from the host cavity.

Introduction

Guest binding is a crucial property for the role of supramolecular catalysts. Supramolecular assemblies[1-5] can interact with multiple guests simultaneously and the driving forces for guest binding can include both specific interactions between the guest and host functional groups,[6] as well as non-specific, weak, supramolecular interactions such as CH-π, π-π, or cation-π interactions.[7-10] Solvent also frequently plays a critical role in molecular recognition: displacement of solvent molecules from a host cavity and guest desolvation must typically occur before encapsulation can take place.[11] All of these driving forces can generate different enthalpic and entropic contributions to the free energy of guest binding, making the determination of thermodynamic parameters for host-guest equilibria complicated and difficult. Such parameters have been measured by solution NMR or UV-vis spectroscopy, but each of these methods has inherent limitations due to their different time scales and observables. While NMR and UV-vis equilibrium measurements can be used to indirectly determine enthalpy and entropy values as a function of temperature, this makes these two values statistically correlated[12] and inaccurate if there is a significant change in heat capacity during the reaction.[13] Isothermal titration calorimetry (ITC) enables direct measurement of the heat change induced by guest binding at a constant temperature and can provide useful information about the nature of host-guest interactions.[14] This study uses the complementary techniques of NMR, UV-vis and ITC to untangle the thermodynamics ($\Delta G°$, $\Delta H°$, $\Delta S°$ value) of sequential internal and external guest binding to a highly charged supramolecular host.

We have reported a series of self-assembling, tetrahedral $[M_4L_6]^{n-}$ (L = 1,5-bis(2,3-dihydroxybenzamido)naphthalene) supramolecular assemblies[15] that act as chiral, nanoscale flasks for encapsulated guest catalysts or transient guest substrates and can carry out enzyme-like chemical transformations.[16-18] The $[Ga_4L_6]^{12-}$ assembly (Figure 1, **1**) has received the most attention and is used exclusively in the present study. The hydrophobic interior cavity of **1** can encapsulate a variety of hydrophobic monocationic[19] and neutral guest molecules.[20] The highly anionic exterior surface of **1** imparts solubility in water and other polar solvents, as well as an affinity for external ion-association of cationic molecules (Figure 2a); indirect observation of external ion-association has previously been observed in kinetic studies,[21,22] diffusion-based ^1H NMR experiments,[23] as well as solid-state structures.[24] The species distribution of these competing interior and exterior host-guest equilibria (Figure 2b), which cannot be deconvoluted by NMR, UV-vis or ITC alone, here has been elucidated by analyzing together the different observables measured by each technique.

Experimental Section

General synthetic procedures

$K_{12}[\mathbf{1}]$ was prepared as previously described[15] and stored under nitrogen. Ammonium salts NEt_4Cl and NMe_4Cl were purchased from Sigma-Aldrich and recrystallized from ethanol prior to use. All solvents were degassed with nitrogen prior to use.

Isothermal titration calorimetry (ITC)

Data for determination of the thermodynamic parameters were obtained using a nano-isothermal titration calorimeter (Nano-ITC III CSC 5300) at 25 °C in water with 0.1 M KCl. Since accurate determination of the enthalpy of reaction requires concentrations to be precisely known, the effective molecular weight of K_{12}[**1**] was determined *via* thermogravimetric analysis (TGA, Figure S1). The first decrease observed in the TGA curve (up to 130 °C) accounts for the adsorbed residual solvent that amounts to 5-8% of the total host weight. The final weight % at 800 °C is consistent with the expected value based on the inorganic components (potassium and gallium oxides) of the host. The concentration of the hygroscopic NEt_4Cl and NMe_4Cl was obtained indirectly by determining the chloride concentration according to the Mohr procedure.[25]

ITC measurements were carried out by titration of an aqueous guest solution (in 0.1 M KCl) into a 1 mM host solution (in 0.1 M KCl). ^{1}H NMR studies have previously shown that the encapsulation process can be relatively slow.[26] As such, preliminary ITC experiments were run with different time intervals between guest addition, ranging from 300 to 1200 seconds; complete equilibration of the guest encapsulation process was achieved only at the longer time intervals. Accordingly, the time interval between each of the first 8-9 additions was set at 1200 sec. Six and twelve independent ITC experiments were run to explore the 0.2 – 0.8 equiv. and the 0.2 – 20 equiv. regime, respectively. These experiments totaled 120 and 300 points, respectively. The heats of dilution were determined in separate experiments by titration of the solution of the guest (in 0.1 M KCl) into a solution containing 0.1 M KCl. The net heat obtained was fit using two different computer programs: HyperΔH[27] and BindWorks (TA Instruments, New Castle, DE). HyperΔH allows for the simultaneous fitting of data from multiple titrations. The results obtained with both software packages are consistent with one another and fits for a typical ITC titration are shown in Figure S2.

UV-vis titrations

Spectrophotometric measurements (Agilent 8453 diode-array spectrophotometer) were carried out at 25 °C in aqueous 0.1 M KCl. Increasing amounts of the guest were added with a precision burette (Hamilton, 1.00 mL) into the measuring cell containing a host solution having the same concentration investigated *via* ITC. The solution was allowed to fully equilibrate before absorbance values were recorded. Equilibration and data reading and storage were controlled with homemade software. For each independent titration run, 30-40 scans were recorded. Four independent runs were collected for the NEt_4^+-**1** system exploring the 435-800 nm range which leads to a total of more than 50000 absorbance *vs.* volume data points. Typical absorbance changes in the visible region resulting from the addition of NEt_4^+ to a solution of **1** are shown in Figure S3. Data, corrected for dilution, were analyzed with two different software packages (Specfit[28] and Hyperquad[29]) that make use of a multi-wavelength and multivariate treatment of spectral data but use different data-fitting algorithms. Hyperquad is also able to refine data from multiple titrations. The fit for a typical UV-vis titration is reported in Figure S4.

^{1}H NMR titrations

NMR titrations were performed by combining the guest and host **1** in varying ratios (0 – 20 equiv. guest) in separate NMR tubes with 0.1 M KCl in D_2O. The NMR tubes were prepared under nitrogen and allowed to equilibrate overnight. All ^{1}H NMR spectra were acquired on a Bruker AV-500 NMR spectrometer with an inverse TBI probe. The chemical shifts corresponding to the CH_2 and CH_3 protons of exteriorly bound NEt_4^+ were simultaneously fit using HyperNMR[30] in the 2-20 equiv. guest regime to yield the external binding constant K_{ext}. A typical fit obtained with HyperNMR is shown in Figure S5. Attempts to analogously evaluate the K_{int} value using the chemical shifts of encapsulated NEt_4^+ between 0 and 1 equiv. of added guest failed due to negligible changes in the chemical shifts of these resonances.

Result and Discussion

The encapsulation equilibrium (K_{int}) of the strongly binding guest NEt_4^+ with **1** was first examined (Figure 2b). Due to slow exchange between interior and exterior guest on the NMR time scale, NMR experiments clearly show that the internal binding affinity of NEt_4^+ is large and the first equiv. of added NEt_4^+ is exclusively bound to the host interior. Therefore, examination of guest binding equilibria below 1 equiv. of NEt_4^+ allows almost complete isolation of the interior encapsulation equilibrium from exterior guest binding. ITC experiments were accordingly carried out by titrating NEt_4^+ (0.2 – 0.8 equiv.)[31] into an aqueous solution of **1** (1.0 mM in 0.1 M KCl) while monitoring the heat evolved.[32] The interior binding constant of NEt_4^+ as determined by ITC is $\log(K_{int}) = 4.4(7)$, which is consistent with previous ^{1}H NMR experiments ($\log(K_{int}) = 4.55$).[26b]

The exterior binding (K_{ext}) of NEt_4^+ to **1** was explored using NMR, UV-vis and ITC experiments. The ^{1}H NMR chemical shifts corresponding to the unencapsulated NEt_4^+ resonances were monitored as a function of added guest (2 – 20 equiv. relative to **1**). The observed chemical shifts of the unencapsulated guest are the average of the external ion-associated and non-associated species, due to rapid exchange of these species on the NMR time scale. Chemical shift changes are observed upon exterior association of NEt_4^+ to **1** and these can be accurately fit to afford an external binding affinity of $\log(K_{ext}) = 1.8(1)$.[33] Here again, due to the large interior binding constant of NEt_4^+ in **1**, the observed equilibria past 1 equiv. of added NEt_4^+ correspond almost exclusively to external host-guest interactions.[34]

We also examined UV-vis spectroscopy under the same conditions. External host-guest interactions[35] induce small red shifts of the host charge transfer bands in the visible region of the spectrum (Figure S3).[36] These signals have been accurately analyzed with two different software packages which use factor (multi-wavelength) analysis of all the spectrophotometric data.[37] Both clearly showed that the spectral changes were ascribable to one absorbing complex species only ($NEt_4[NEt_4\subset\mathbf{1}]^{10-}$) and gave a binding affinity of $\log(K_{ext}) = 2.04(1)$. Analogous ITC experiments (Figure 3) afforded a similar value for external guest association of $\log(K_{ext}) = 1.96(5)$, which is consistent with the external binding affinities determined by both ^{1}H NMR and UV-vis. Despite the small changes observed in both the

NMR and UV-vis spectra, the combination of these with ITC observations provides a clear picture of guest external association.

The binding affinities and thermodynamic parameters obtained by NMR, ITC and UV-vis for internal and external equilibria (Figure 2b) with NEt_4^+ are summarized in Table 1. These data show that the encapsulation of NEt_4^+ into **1** is an entropically driven process. Desolvation of the cationic guest and release of solvent from the interior of the empty host assembly account for the large entropic gain observed in this process.[38] Here "empty" refers to the host cavity with no encapsulated guest, which is presumably instead occupied by solvent molecules. Previously measured cavity volumes (~250 Å^3) suggest 8-10 water molecules can occupy the host interior.[24] The weight loss observed in TGA is consistent with this estimate of the number of encapsulated solvent molecules. Despite the enthalpic cost of host and guest desolvation, the overall encapsulation equilibrium is an enthalpically favored process. We attribute this enthalpic gain to a combination of the highly exothermic association of the positively charged guest to the "empty" 12- host (similar to the K_{ext} step of Figure 2b and Table 1) and the endothermic encapsulation (due to desolvation) of that ion-associated NEt_4^+ into the host cavity.

In marked contrast, exterior association of NEt_4^+ is an enthalpically driven, but entropically disfavored, process. The exothermic external association of NEt_4^+ is attributed to enthalpically favorable attractive forces, including Coulombic, cation-π and CH-π, between the guest and the aromatic host exterior. These attractive interactions have been previously observed in solid-state crystallographic[24] and diffusion NMR studies.[23] The diffusion NMR experiments also demonstrated that external association of NEt_4^+ is favored over K^+, used in this study to keep the ionic strength constant.[39] The higher cost for the desolvation of the K^+ cation also accounts for the preferential exterior association of the NEt_4^+ species. Furthermore, control ITC experiments carried out in the absence of KCl resulted in a similar amount of heat released as when titrations were performed in the presence of 0.1 M KCl. Values of K, $\Delta H°$ and $\Delta S°$ obtained from data collected in the absence of KCl are the same as those reported in Table 1; this rules out any possible effect of KCl on the binding of the investigated guests with **1**.

Since external association is highly exothermic and requires only partial desolvation of the NEt_4^+ cation, the overall process is observed to be entropically disfavored. Both encapsulation and ion-association involve a loss of degrees of freedom upon internal or external binding. However, in the case of encapsulation, the loss of degrees of freedom (negative entropy) is more than compensated by the desolvation of the guest and release of solvent (entropy gain) from the "empty" (*i.e.*, solvent filled) host cavity, resulting in a process with an overall positive entropy. For external binding, only partial desolvation of the guest is required and this does not counterbalance for the loss of degrees of freedom, resulting in an overall negative entropy change. This is commonly observed for enthalpically driven host-guest interactions.[32,40-42] Preliminary NMR experiments have also shown the presence of higher-order, externally-associated, guest-host stoichiometries with formation constants that are lower than that of the first association, as expected for a host with decreasing charge and some occupied external binding sites.

Internal and external binding interactions of the smaller guest NMe_4^+ with **1** were also investigated. Since NMe_4^+ is weakly bound and rapidly exchanging, a direct determination of the internal and external binding affinities with **1** is difficult. In order to isolate the exterior guest binding equilibrium in this system, NMR experiments were carried out in which NMe_4^+ was titrated into a solution of $[NEt_4 \subset \mathbf{1}]^{11-}$ so that the interior cavity is blocked by the strongly bound guest NEt_4^+. Fitting the ^1H NMR chemical shifts corresponding to external NMe_4^+ gives an external binding affinity of $\log(K_{ext}) \approx 1$. Combining these NMR data with preliminary ITC experiments allowed for separation of exterior and interior binding equilibria and showed that NMe_4^+ is weakly bound to both the host exterior and interior ($\log(K_{int}) \approx 2$). This is consistent with previous experiments[15] in which NEt_4^+ readily displaces encapsulated NMe_4^+ from the cavity of **1**.

Conclusion

We have used a combination of NMR, UV-vis and isothermal titration calorimetry to definitively separate and evaluate multiple guest binding to the interior and exterior of this highly charged supramolecular assembly. There are dramatic differences between the internal and external binding events of the simple alkylammonium cations NEt_4^+ and NMe_4^+. Encapsulation of NEt_4^+ into **1** is entropically driven, while external ion-association is enthalpically driven; the encapsulation requires guest desolvation while releasing many solvent molecules, while the external association involves ion-association and loss of degrees of freedom. The binding affinities determined by all three techniques are in good agreement with one another and show that NEt_4^+ binds more strongly to both the host interior and exterior than NMe_4^+. This study illuminates, and for the first time quantifies, the very different internal and external host-guest interactions of the $[Ga_4L_6]^{12-}$ assembly that are a consequence of its high charge and hydrophilic outer space contrasted by its hydrophobic inner space.

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Supporting Information Available: Thermogravimetric analysis, UV-vis spectra and fits of ITC, UV-vis and NMR titration data. This material is available free of charge via the Internet at http:/pubs.acs.org.

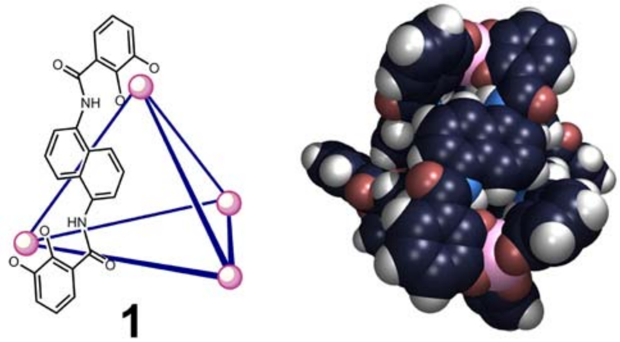

Figure 1. Schematic of the $[Ga_4L_6]^{12-}$ framework, only one ligand is shown for clarity (left). Space-filling model of **1** as viewed down the 2-fold axis (right).

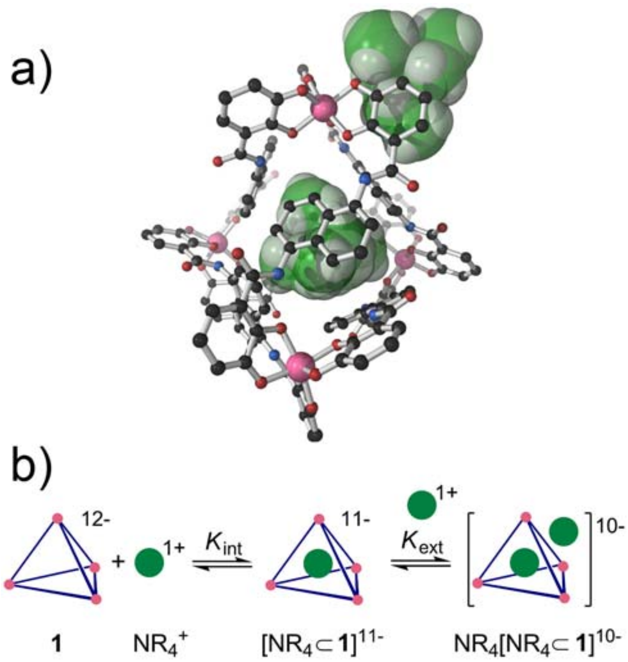

Figure 2. (a) Internally and externally bound NEt_4^+ to **1**, adapted from the crystal structure of $K_5(NEt_4)_6[NEt_4 \subset Fe_4L_6]$.[15] (b) Schematic equilibria for internal (K_{int}) and external (K_{ext}) NEt_4^+ guest binding with **1**. The symbol $\subset$ denotes encapsulation.

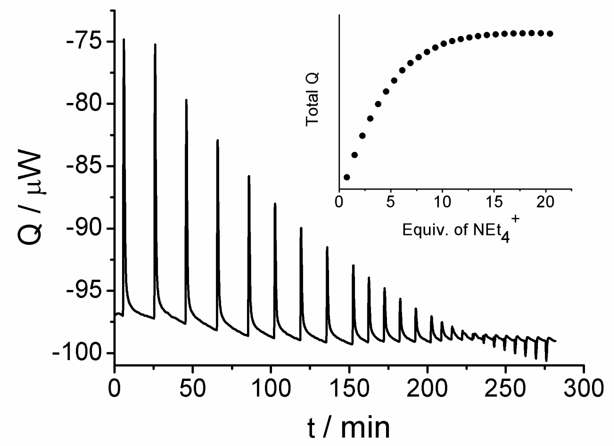

Figure 3. ITC data for the addition of a 90 mM solution of NEt_4^+ into a 1 mM solution of **1**. Inset: total heat *vs.* equiv. NEt_4^+.

Table 1. Thermodynamic parameters for interior (K_{int}) and exterior (K_{ext}) binding of NEt_4^+ with $[Ga_4L_6]^{12-}$ (**1**) at 25 °C in water (0.1 M KCl).[a]

Reaction	log K			$\Delta H°$ (kJ mol^{-1})	$\Delta S°$ (J deg^{-1} mol^{-1})
	NMR	UV-vis	ITC		
$NEt_4^+ + \mathbf{1} \overset{K_{int}}{\leftrightarrows} [NEt_4{\subset}\mathbf{1}]^{11-}$	4.55(6)	n.d.	4.4(7)	-4.1(8)	70(10)
$NEt_4^+ + [NEt_4{\subset}\mathbf{1}]^{11-} \overset{K_{ext}}{\leftrightarrows} NEt_4[NEt_4{\subset}\mathbf{1}]^{10-}$	1.8(1)	2.04(1)	1.96(5)	-27.6(1)	-56(3)

[a] $\Delta H°$ and $\Delta S°$ values were calculated by holding $\log(K_{int}) = 4.4$ constant, as determined by ^1H NMR and ITC measurements.

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(31) In these titrations, the initial data points in the 0.1 – 0.2 region were excluded due to possible host templation which is currently under investigation.

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(33) The observed chemical shift changes are modest but since the exterior guest resonances are sharp their chemical shifts can be precisely measured and accurately fit.

(34) To further explore these exterior binding interactions we carried out similar experiments with $NBut_4^+$ which, due to its larger size, cannot be encapsulated. However, because $NBut_4^+$ is both more hydrophobic and more strongly bound to the host exterior than NEt_4^+, this results in precipitation of the host-guest complex after about 6 equiv. of guest added.

(35) Since encapsulation is complete within the first 2-3 points of the vis titration, only the exterior binding was monitored here.

(36) Catechol and naphtalene absorbance bands in the UV region do not change upon guest addition.

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(39) The exterior interactions between NEt_4^+ and **1** cannot be exclusively attributed to simple Coulombic attractions since K^+ would show similar, if not larger, electrostatic attractive forces toward the anionic exterior of the host. If NEt_4^+ binding were due to Coulombic attraction only, a large excess of KCl would remove any interaction with **1**; this was clearly not the case and additional attractive forces have to be involved.

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