

Free Energy Perturbation Calculations of the Relative Binding Affinities of an 8-Subunit Cavitand for Alkali Ions in Methanol

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Abstract: Experiments and molecular dynamics calculations have shown that cavitand I has a bimodal selectivity for alkali ions in water saturated CHCl_3 (experiment) and water (simulations). In this paper, molecular dynamics and free energy perturbation calculations are used to study the selectivity of I for alkali ions in methanol. The calculations predict that Cs^+ is bound tightest by I, the same as in water. As previously predicted, the bimodal selectivity disappears in methanol. This change in specificity is caused by a change in the number of stabilizing interactions in which solvent molecules that accompany the alkali ion into the cavity of I can participate.

Introduction

Host-guest chemistry provides the opportunity to study non-covalent interactions in relatively small simple systems that are important in larger biological systems.¹ Recently, Cram and co-workers synthesized a cavitand (I) that consists of eight anisole subunits.² I, shown in Figure 1, has a large cavity with two openings, one at the "top" and one at the "bottom" of the molecule. Eight anisole oxygens, four at each opening, are positioned to interact with the guest. The large cavity is complementary in size to Cs^+ , which binds tightest of the alkali ions. Usually the relative binding affinities of alkali ions to cavitands and other preorganized hosts, such as 18-crown-6 (18C6), are related to the size of the ion matching the size of the cavity of the host. In water-saturated CH_2Cl_2 , I has an unusual secondary binding preference for alkali ions. Na^+ binds to I more tightly than K^+ and Li^+ .² This is the first preorganized host to show a secondary binding preference.

Bayly and Kollman studied this system using free energy perturbation (FEP) molecular dynamics (MD) simulations.³ Their calculations qualitatively reproduced the primary and secondary binding preference of alkali ions to I in water. Examination of the structural information from the MD calculations led to the proposal of an explanation for the secondary binding preference of I. When the smaller alkali ions (Li^+ , Na^+ , K^+) bind to I, one or two molecules of water accompany the alkali ion into the cavity. The size of the cavity is complementary to the size of $\text{Na}^+ \cdot 2\text{H}_2\text{O}$ such that the waters interact strongly with the anisole oxygens as well as the alkali ion. Only one water accompanies K^+ into the cavity of I, lowering the intrinsic binding energy. $\text{Li}^+ \cdot 2\text{H}_2\text{O}$ is not large enough to form all the optimum hydrogen bonding interactions between the waters and the anisole oxygens. Consequently, in water Na^+ is bound by I tighter than Li^+ and K^+ . Bayly and Kollman predicted that the secondary binding preference would disappear in methanol, due to its larger size and inability to donate as many hydrogen bonds as water.

In this paper we present FEP-MD calculations on the relative binding affinities of I for alkali ions in methanol. FEP calculations have proven to be a useful tool in studying these types of host-

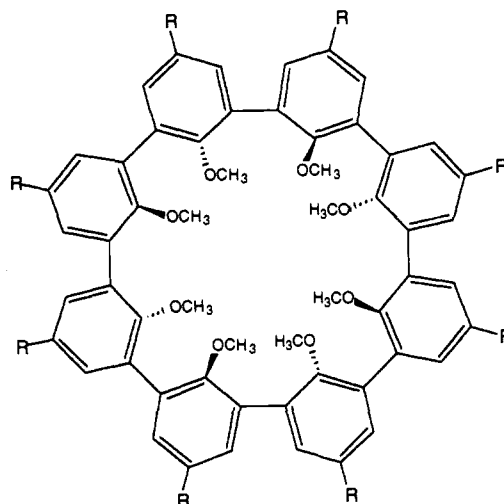


Figure 1. The chemical structure of I.

guest systems. The binding of alkali ions to 18C6 in water⁴ and in methanol⁵ has been studied using FEP calculations, as has the binding of larger guests to 18C6.⁶ The binding affinities of cavitands for alkali ions have also been examined using FEP calculations.⁷ In all cases, FEP calculations have qualitatively reproduced experimental binding preferences and have provided useful structural information.

Computational Method

All molecular dynamics calculations were performed using the AMBER force field and the AMBER 4.0 molecular dynamics programs.⁸ All atom parameters and charges for the anisole subunits of the cavitand

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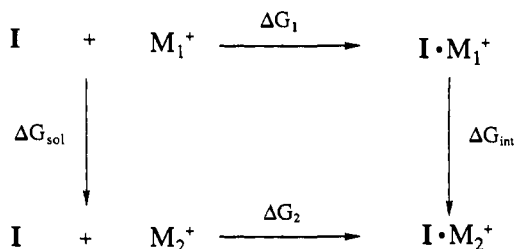


Figure 2. The thermodynamic cycle to determine the relative free energy of binding of two complexes of I-alkali ion.

were taken from the work of Kollman et al.¹⁰ The alkali ion parameters were adopted from work by Åqvist.¹¹ Methanol was represented as a three point model, with a united atom methyl group and explicit oxygen and hydroxyl proton. The parameters for methanol were taken from the OPLS force field.¹² The united atom methyl group was shrunk, in accord with the work of DeBolt and Kollman using OPLS methanol parameters with the AMBER force field.¹³ In order to keep the methanol rigid, an extra bond was defined connecting the united atom methyl group with the hydroxyl proton.

During the simulations, all bond lengths were constrained using the SHAKE⁹ algorithm with a tolerance of 0.005 Å, allowing a time step of 2.0 fs. Periodic boundary conditions were employed. A constant pressure bath was employed with a coupling constant of 0.4 ps⁻¹. Solute and solvent were coupled to a constant temperature heat bath with a coupling constant of 0.2 ps. A residue based cutoff of 9.0 Å was employed. Pairlists were generated every 25 fs. All initial boxes were minimized to a RMS gradient of less than 0.1 kcal/mol before any MD simulations were performed.

The thermodynamic perturbation method was used to calculate the free energy differences.¹⁴ The thermodynamic cycle relevant to this study is shown in Figure 2. The free energies of complexation, ΔG_1 and ΔG_2 , of M_1^+ and M_2^+ to the cavitand (I) are experimentally determinable values, but are difficult to calculate. ΔG_{sol} corresponds to changing M_1^+ into M_2^+ in methanol, while ΔG_{int} corresponds to changing M_1^+ into M_2^+ bound to I in methanol. Since the thermodynamic cycle is closed and the free energy is a state function, $\Delta \Delta G_{bind} = \Delta G_2 - \Delta G_1 = \Delta G_{int} - \Delta G_{sol}$. While ΔG_{sol} and ΔG_{int} represent physically unrealizable processes, these two simulations are computationally manageable.

Li^+ was solvated with 125 methanols using the program LEAP.¹⁵ The initial box size was $\sim 21 \text{ \AA} \times 21 \text{ \AA} \times 21 \text{ \AA}$. The box was minimized and equilibrated for 50 ps. Initially, Li^+ was perturbed into Na^+ over 21 windows with 250 steps of equilibration and 500 steps of data collection, resulting in a total simulation time of 31.5 ps. The final coordinates of this run were used as the starting point for the perturbation of $Na^+ \rightarrow K^+$. The box was equilibrated for 50 ps before performing the FEP calculations. This procedure was used for the remaining perturbations of $K^+ \rightarrow Rb^+$ and $Rb^+ \rightarrow Cs^+$. In order to obtain a rough estimate of the error involved in these calculations, the hysteresis for each perturbation was determined by running the calculations in the reverse direction. Cs^+ was solvated in a box of methanol using the program LEAP.¹⁵ The same procedure of minimization, equilibration, and free energy calculations was followed until all perturbations had been performed.

The cavitand calculations were also performed using the same procedure described above. The cavitand-ion complex was solvated with 679 methanols, yielding an initial box size of $\sim 38 \text{ \AA} \times 38 \text{ \AA} \times 29 \text{ \AA}$. During the initial Li^+ to Na^+ free energy perturbation, the ion drifted out of the cavity of the cavitand. To eliminate this problem, a restraint of 0.1 kcal/mol was placed on the ion to stay at the center of the cavitand cavity. The constraint energy was always less than 1% of the potential energy and allowed the ion to move about the cavity. The use of a constraint has been successfully employed in FEP simulations of a host-guest

system.¹⁶ All FEP simulations were performed over 31.5 ps. In order to test the convergence of these calculations, all ion and cavitand/ion FEP simulations were rerun with 500 steps of equilibration and 1000 steps of data collection for a total simulation time of 63.0 ps. All structural data were obtained from the 50 ps equilibration runs performed between FEP simulations.

Results and Discussion

The calculated structures of the various host-guest complexes are very different. With Cs^+ bound to I, the ion is centered in the middle of the cavity of I, though slightly displaced toward an opening of the cavity. The eight anisole oxygens interact with ion. The ion moves around the cavity, causing the $Cs^+ - O_{(anisole)}$ distances to range from 2.8 to 4.3 Å over the course of the 50 ps equilibration simulation. The displacement of Cs^+ toward the opening of the cavity allows a stronger interaction with a methanol, which can interchange with other solvent molecules during the simulation. The $I \cdot Rb^+$ complex is very similar in structure to the $I \cdot Cs^+$ complex. Rb^+ is also centered in the middle of the cavity of I, but its smaller size allows Rb^+ to move around the cavity more than Cs^+ . The $Rb^+ - O_{(anisole)}$ distances range from 2.6 to 4.8 Å. Like Cs^+ , Rb^+ is displaced toward one opening of I, allowing the coordination of a methanol.

The complexes of I with the smaller ions, shown in Figure 3, are different from the complexes of I with the larger ions; one or two solvent molecules accompany the ion in the cavity. One methanol can fit into the cavity with K^+ , which causes K^+ to shift off-center in the cavity. Four anisole oxygens form strong dipole-dipole interactions with K^+ , along with the methanol, which also forms a hydrogen bond to an uncomplexed anisole oxygen. The methanol methyl group sticks through a cavity opening toward bulk solvent. The $I \cdot Na^+$ and $I \cdot Li^+$ complexes generally have two methanols in the cavity, similar to these complexes in water. Three to four anisole oxygens interact strongly with the ions, along with the two methanols. Both methanols are hydrogen bonded to uncomplexed anisole oxygens. One methanol methyl group sticks out one cavity opening and the second methanol methyl group sticks out the other cavity opening. Unlike crown ethers, which can curl around the smaller alkali ions to obtain the optimum number of crown-ion interactions, the more rigid cavitands require solvent molecules to obtain the optimum number of interactions with the smaller alkali ions. In each complex of I with the smaller alkali ions, the ion and solvent molecule(s) move about the cavity. Thus the anisole oxygens that bind the ion are changing over the course of the simulation.

The relative free energies of solvation (ΔG_{sol}) of alkali ions in methanol were obtained from the FEP calculations of one alkali ion into another alkali ion in a box of methanol. These values are given in Table 1. In all cases, the ΔG_{sol} reported here are in good agreement with experimental¹⁷ and calculated^{5a,b,16} values. The ΔG_{sol} and hysteresis are relatively insensitive to simulation time for all perturbations. The largest difference between values of ΔG_{sol} for the 31.5- and 63.0-ps simulations is ~ 1.0 kcal/mol for the $Li^+ \rightarrow Na^+$ perturbation. The other differences in ΔG_{sol} are on the order of 0.1 kcal/mol. The hysteresis for the 31.5- and 63.0-ps simulations are small, though overall, the longer simulations provide the better results. This provides confidence that the length of the simulations is adequate to obtain proper sampling. Li^+ is the best solvated alkali ion and Cs^+ is the worst solvated alkali ion in methanol.

The relative free energies of solvation of the host-guest complexes (ΔG_{int}) are given in Table 2. These calculations describe the relative intrinsic binding affinity of I for alkali ions in methanol. The ΔG_{int} for the larger ion perturbations in the cavitand ($K^+ \rightarrow Rb^+$, $Rb^+ \rightarrow Cs^+$) are insensitive to the simulation time and have very small hysteresis. In contrast, the ΔG_{int} and

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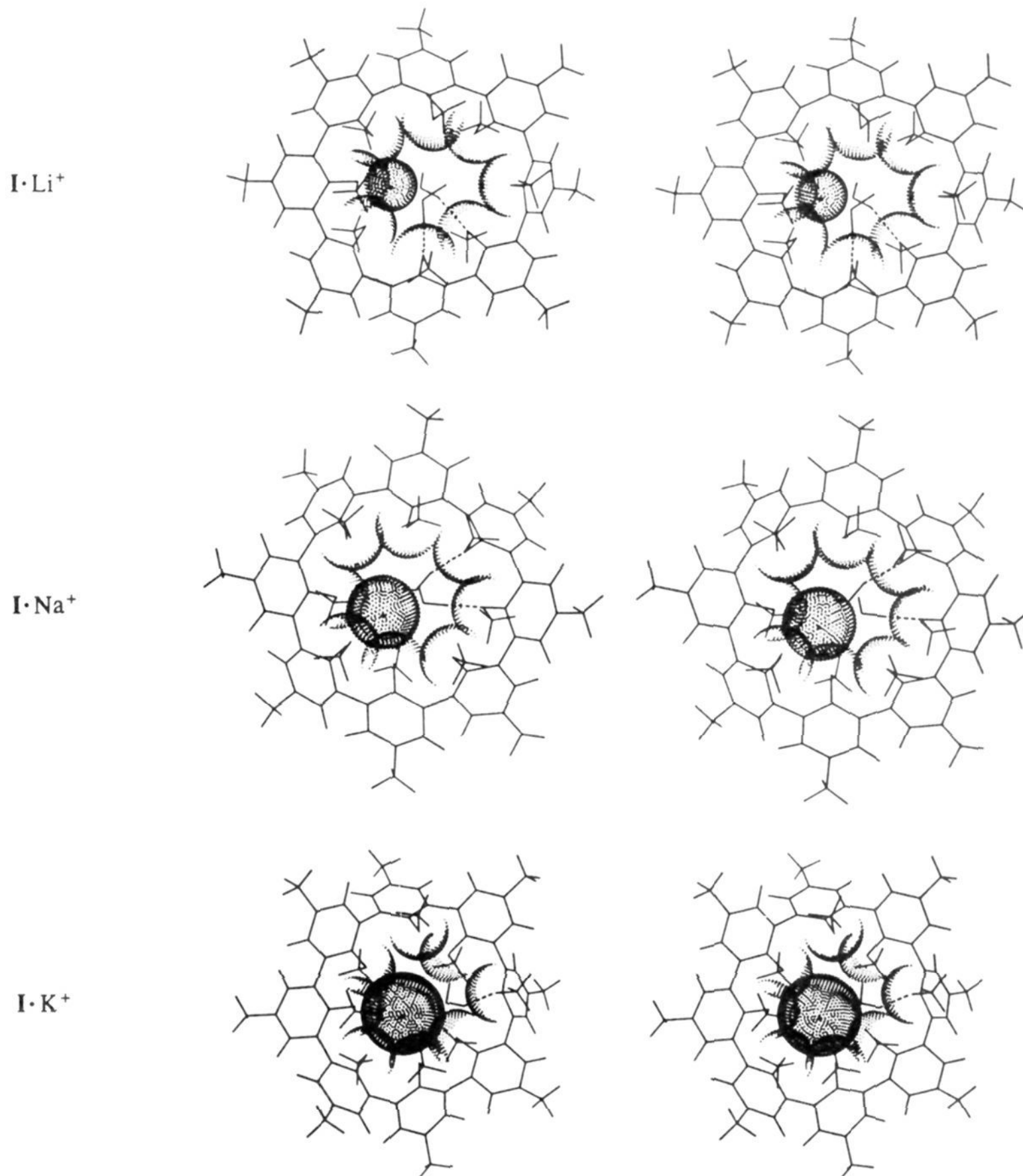


Figure 3. Stereoviews of the complexes $I \cdot Li^+$, $I \cdot Na^+$, and $I \cdot K^+$, including any methanol(s) that reside in the cavity. The van der Waal's surfaces of the ions and the anisole oxygens are shown, as are the hydrogen bond(s) between the methanol hydroxyl proton and the anisole oxygen. The methanol methyl groups are in a united atom representation.

Table 1. The Relative ΔG of Solvation for the Perturbation of Alkali Ions in Methanol

perturbation	no. of windows	total time, ps		$\Delta G_{for.}$	$\Delta G_{rev.}$	ΔG_{sol}
		equil	av			
Li \rightarrow Na	21	10.5	21.0	22.40	-23.34	22.87 ± 0.47
	21	21.0	42.0	24.31	-23.37	23.84 ± 0.03
Na \rightarrow K	21	10.5	21.0	18.32	-18.39	18.36 ± 0.03
	21	21.0	42.0	18.25	-18.36	18.31 ± 0.06
K \rightarrow Rb	21	10.5	21.0	5.79	-5.90	5.85 ± 0.05
	21	21.0	42.0	5.98	-5.68	5.83 ± 0.15
Rb \rightarrow Cs	21	10.5	21.0	8.31	-7.83	8.07 ± 0.24
	21	21.0	42.0	8.34	-8.06	8.20 ± 0.14

hysteresis for the small ion perturbations are more sensitive to the simulation time. ΔG_{int} differ by ~ 1.1 kcal/mol for $Li^+ \rightarrow Na^+$ and $Na^+ \rightarrow K^+$. The hysteresis is > 2.0 kcal/mol for the 31.5-ps simulations compared to < 0.1 kcal/mol for the 63.0-ps

Table 2. The Relative ΔG for the Perturbation of Alkali Ions Bound to the 8-Membered Unit Cavitand in Methanol

perturbation	no. of windows	total time, ps		$\Delta G_{for.}$	$\Delta G_{rev.}$	ΔG_{int}
		equil	av			
Li \rightarrow Na	21	10.5	21.0	19.15	-23.51	21.33 ± 2.18
	21	21.0	42.0	22.34	-22.42	22.38 ± 0.04
Na \rightarrow K	21	10.5	21.0	12.00	-16.23	14.12 ± 2.12
	21	21.0	42.0	15.45	-15.34	15.39 ± 0.06
K \rightarrow Rb	21	10.5	21.0	2.31	-3.10	2.70 ± 0.40
	21	21.0	42.0	2.68	-2.90	2.79 ± 0.11
Rb \rightarrow Cs	21	10.5	21.0	4.35	-4.46	4.41 ± 0.06
	21	21.0	42.0	4.36	-4.28	4.32 ± 0.04

simulations. Again the 63.0-ps simulations provide the more reliable results. The large ions (Cs^+ , Rb^+) fit well in the cavity of the cavitand. Solvent molecules can interact with the ion from outside the cavity through the two openings. The time needed

Table 3. The Relative $\Delta\Delta G$ of Binding for Alkali Ions to I in Methanol

perturbation	total simulation time, ps	$\Delta\Delta G_{\text{binding}}$
Li \rightarrow Na	31.5	-1.5 ± 2.6
	63.0	-1.5 ± 0.1
Na \rightarrow K	31.5	-4.2 ± 2.2
	63.0	-2.9 ± 0.1
K \rightarrow Rb	31.5	-3.2 ± 0.5
	63.0	-3.0 ± 0.3
Rb \rightarrow Cs	31.5	-3.7 ± 0.3
	63.0	-3.9 ± 0.2

to obtain proper sampling is reduced for the large ions because the large ions have less room to move about the cavity of the cavitand. With the smaller ions, one or two solvent molecules can fit into the cavity with the ion. The ion and solvent can move about the cavity, requiring longer simulation times to obtain proper sampling. Li^+ intrinsically binds tighter to I and Cs^+ intrinsically binds weakest to I, even though its size is complementary to the cavity of I.

The $\Delta\Delta G_{\text{binding}}$ values are given in Table 3. In contrast to the primary and secondary binding preferences of I in water, I has only a primary binding preference for alkali ions in methanol. The loss of the secondary binding preference in methanol can be explained by the size of methanol and its hydrogen bonding ability compared to water. Waters that reside in the cavity with M^+ (Li^+ , Na^+ , K^+) can form three strong interactions, one oxygen dipole-dipole interaction with M^+ and two hydrogen bonds to anisole oxygens, which can strengthen the intrinsic binding of M^+ to I. In addition, there are no significant steric interactions between the water and the cavitand. $\text{Na}^+ \cdot 2\text{H}_2\text{O}$ happens to fit perfectly in the cavity, yielding a secondary binding preference. Methanol can only form two strong interactions inside the cavity, one oxygen dipole-dipole interaction with M^+ and one hydrogen bond to an anisole oxygen. $\text{I} \cdot \text{Li}^+$ and $\text{I} \cdot \text{Na}^+$ complexes lose two hydrogen bonds each going from water to methanol versus one hydrogen bond for $\text{I} \cdot \text{K}^+$. Also, the methyl group of methanol interacts sterically with the cavitand. Consequentially, the additional methanols in the cavity of I with M^+ do not increase the intrinsic binding energy to the degree that water does and the extra methanol that accompanies Na^+ into the cavity cannot compensate for the difference in the free energies of solvation of Na^+ and K^+ in methanol. Thus the secondary binding preference does not exist in methanol.

Examination of the $\Delta\Delta G_{\text{binding}}$ leads to the notion that the primary binding preference is dictated by how closely the size of the ion matches the size of the cavity, with Cs^+ binding tightest and Li^+ binding weakest. Analysis of the different components of the $\Delta\Delta G_{\text{binding}}$ present a different picture. The free energy of binding of a guest to a host is the difference between the free energy of solvation of the guest and the intrinsic free energy of binding of the guest to the host in solvent. Li^+ is intrinsically bound by I in methanol better than any of the other alkali ion, but it also is better solvated by the methanol than any of the alkali ions. Since solvent does a better job of solvation than the host, Li^+ binds weakest of all the alkali ions. The idea that

desolvation of the ion dictates the binding preference of certain host-guest systems has been presented previously.^{10b,18} Izatt and co-workers studied the binding of ions to a variety of crown ethers experimentally and concluded that the binding preference for smaller ions was dictated by the desolvation of the ion.¹⁸ This is consistent with these and other FEP calculations.^{5-7,10b} In general, the relative binding affinities of alkali ions to preorganized guests is dictated by desolvation of the ion if the size of the ions of interest is equal to or less than the size of the host. When the ions get larger than the cavity of the host, van der Waal's interactions also became important in determining the relative binding affinities.^{10b}

Conclusion

MD/FEP calculations have become a very important tool in studying host-guest systems. In particular, these calculations can yield strikingly accurate results, even for more complex host and guests. In a recent study, Jorgensen and Nguyen demonstrated that FEP calculations could very accurately reproduce and predict the relative binding affinities of a group of aromatic compounds to a water soluble cyclophane.¹⁹ In studies such as presented here, only qualitative agreement can be expected due to the fact that additive two-body molecular mechanics potentials would be expected to be less accurate for charged systems than neutral systems. There is some evidence that more quantitative results can be expected with the incorporation of non-additive effects into the force field.²⁰

This study predicts that the specificity of I for alkali ions is different in methanol than it is in water. In water a bimodal specificity exists, with the primary specificity for Cs^+ and a secondary specificity for Na^+ . In methanol only a primary specificity exists for Cs^+ . This change in specificity is caused by a change in the number of stabilizing interactions in which solvent molecules that accompany the alkali ion into the cavity of I can participate. This represents a relatively unique example where the results of a computer simulation have suggested a new qualitative result for an experiment. The theoretical study presented here represents a theoretical "proof of concept" for this expected new qualitative result. Hopefully, an experimental test will follow.

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