

energy, 3-methyladenine, and 7-methyladenine, base fragment ions from [2-<sup>2</sup>H]adenosine and 1-methyladenosine, ion source produced adenine fragment ions *m/z* 67 and 82 and [per-<sup>15</sup>N]-adenine fragment ions *m/z* 113 and 123, protonated [1-<sup>15</sup>N]-adenine and [6-*amino*-<sup>15</sup>N]adenine under single-collision condi-

tions, and the odd-electron molecular ion (M<sup>•+</sup>) of adenine and tables of elemental compositions of ions produced by CID of protonated adenine and isotopic compositions of minor ions from CID of isotopically labeled adenines (8 pages). Ordering information is given on current masthead page.

## Molecular Dynamics Studies of Calixspherand Complexes with Alkali Metal Cations: Calculation of the Absolute and Relative Free Energies of Binding of Cations to a Calixspherand

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**Abstract:** We present molecular dynamics studies of the complexation of a macrobicyclic calixspherand host with guest metal cations (Na<sup>+</sup>, K<sup>+</sup>, and Rb<sup>+</sup>). By using a thermodynamic free energy perturbation method, the relative free energies of calixspherand complexation with alkali metal cations were determined. We tested two sets of van der Waals parameters, two methods of describing 1–4 nonbonded interactions and two extreme models of solvation for the calixspherand–alkali ion complex. Independent of model, the calculations correctly reproduced the tighter binding to the calixspherand of K<sup>+</sup> compared to the smaller Na<sup>+</sup> and the larger Rb<sup>+</sup>. Encouragingly, the “best model” (most highly refined ion parameters, most complete description of solvation) gave the best quantitative reproduction of the experimental free energies. We have also carried out the first example of the calculation of the absolute binding free energy of a macrobicyclic molecule–ion complex. The absolute binding free energy of the calixspherand·Rb<sup>+</sup> complex was calculated to be –11 to –13 kcal/mol, in good agreement with experiment (–12 to –13 kcal/mol). In addition to reproducing the observed K<sup>+</sup> selectivity and shedding light on the experimental free energy data, ion–oxygen radial distribution functions derived from molecular dynamics simulations on the ions, Na<sup>+</sup>, K<sup>+</sup>, and Rb<sup>+</sup>, both in water and in the calixspherand, allowed clear insight into the K<sup>+</sup> preference for this calixspherand. Only for K<sup>+</sup> does the first peak of the ion–oxygen radial distribution function in the host coincide with that in water; for Na<sup>+</sup>, the first peak is at a larger distance in the host than in water; for Rb<sup>+</sup>, the first peak is at a smaller distance in the host than in water. Both the free energy and structural results further emphasize the delicate balance between ion–water and ion–host interactions that lead to ion selectivity.

### Introduction

The ability of synthetic receptor molecules such as macrocyclic polyethers to complex cations or anions has received much attention during the last two decades.<sup>1</sup> The complexation properties of these hosts with a variety of guest molecules have been reported. Cram has reviewed two principles that govern the complexation process: complementarity and preorganization.<sup>2</sup> Complementarity involves the steric and electrostatic fit of host and guest; for complexation of macrocyclic polyethers with metal cations, complementarity is reflected by a cavity–shape cation–size relationship. Preorganization is defined as the absence of structural reorganization and desolvation of the host upon complexation; the more highly hosts organized for binding and for low solvation prior to their complexation, the more stable will be their complexes. Application of this preorganization principle has led to the synthesis of the spherands.<sup>3</sup>

Recently Reinhoudt et al. have presented the synthesis, thermodynamics, and kinetics of binding and X-ray crystal structures

of a series of calixcrowns and calixspherands and have demonstrated that calixspherand **1** (Chart I) forms the most stable complex with alkali metal cations of this class of structures.<sup>4</sup> X-ray and NMR studies on **1** indicate that the *m*-teranisyl moiety has an alternating arrangement of the three methoxy oxygen atoms, and the 1,3-dimethoxy-*p*-*tert*-butylcalix[4]arene moiety has a flattened partial cone conformation<sup>5</sup> both in free state and after complexation with alkali cations. For this reason, compound **1** can be considered a highly preorganized host molecule. The free energies of complexation of **1** with alkali picrates (in CDCl<sub>3</sub>) derived from the NMR studies<sup>4b</sup> at 298 K are –16.8 (1·Na<sup>+</sup>), –18.1 (1·K<sup>+</sup>), and –13.0 (1·Rb<sup>+</sup>) kcal/mol, respectively, while free energies of –13.6, –14.0, and –12.0 kcal/mol, respectively, were determined by the picrate extraction experiments.<sup>4a</sup> In both cases the selectivity of **1** for K<sup>+</sup> was observed.

A theoretical technique, the thermodynamic perturbation method,<sup>6</sup> has been applied to a variety of host–guest systems and

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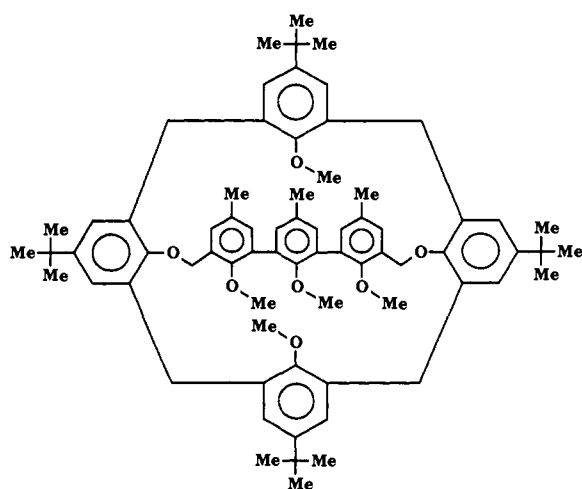
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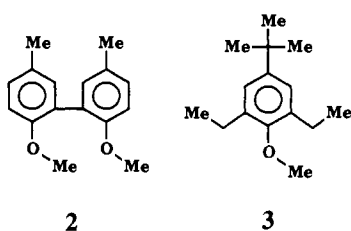
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Chart I



calixspherand 1



so far the results are very encouraging. This theory is based on the generation of ensembles by molecular dynamics or Monte Carlo simulations after which the statistical equilibrium averages such as free energy differences can be obtained. Lybrand et al. have simulated the complexation of the macrotricyclic cryptand SC24 with chloride and bromide anions in water using a united-atom force field in a molecular dynamics approach.<sup>7</sup> The interactions between a number of crown ethers and various cations in water or methanol solvent have been studied by several groups such as Feil,<sup>8</sup> McCammon,<sup>9</sup> and Kollman.<sup>10</sup> Very recently, Grootenhuys et al. have investigated crown ether-neutral molecule interactions by molecular mechanics, normal mode analysis, and free energy perturbation calculations.<sup>11</sup>

In this paper we apply free energy perturbation theory in order to simulate the cation selectivity of highly preorganized calixspherand 1. Because of the rigidity of the molecule, adequate convergence (generation of good ensembles) by molecular dynamics might be expected for the host-guest complexes, although the solvent system used in the experiments to estimate the free energies of complexation is not so straightforward to simulate.<sup>4</sup> Furthermore, the calculation of the absolute binding free energy of complexation for the calixspherand with Rb<sup>+</sup> was of interest, particularly because consistent experimental values of that free energy of complexation are available. Molecular dynamics simulations were also carried out to determine the radial distribution functions for oxygen atoms around metal cations. Those functions

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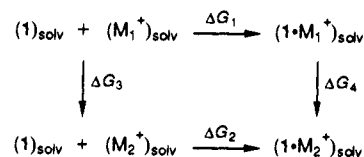
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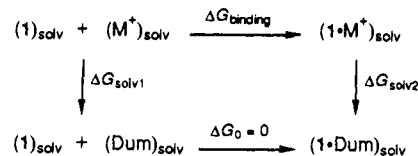
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Scheme I



Scheme II



were used to explain the reason for K<sup>+</sup> selectivity over Rb<sup>+</sup> and Na<sup>+</sup> by 1.

## Methods

For the calculation of free energy differences, the thermodynamic perturbation method was used.<sup>6</sup> This method is related to the thermodynamic cycle in Scheme I, in the case of complexation of two different cations M<sub>1</sub><sup>+</sup> and M<sub>2</sub><sup>+</sup> by calixspherand 1. The free energies of complexation, ΔG<sub>1</sub> and ΔG<sub>2</sub>, are experimentally measurable, but simulations of these processes require very long computation times.<sup>10c</sup> ΔG<sub>3</sub> is the difference in free energy upon changing guest M<sub>1</sub><sup>+</sup> into M<sub>2</sub><sup>+</sup> in the solvent. ΔG<sub>4</sub> is the difference in free energy for the same change, while the cation is bound to the host 1. Although physically unrealizable, these two perturbations can be simulated in manageable computer times. The free energy difference ΔΔG, defined as ΔG<sub>4</sub> - ΔG<sub>3</sub>, should be equal to ΔG<sub>2</sub> - ΔG<sub>1</sub> for this closed thermodynamic cycle because the free energy is a state function.

The use of free energy perturbation theory allows for the calculation of free energy difference between two states of a system, A and B. The perturbation is defined in terms of a coupling parameter λ, which gives an intermediate potential energy function V<sub>λ</sub>:

$$V_{\lambda} = \lambda V_A + (1 - \lambda) V_B$$

where V<sub>A</sub> and V<sub>B</sub> are the potential energy functions of states A and B. The free energy of perturbation can be expressed as the integral of the ensemble-averaged derivative of the potential energy with respect to λ.

$$\Delta G = \int \langle \delta V / \delta \lambda \rangle_{\lambda} d\lambda$$

In the so-called "slow growth" method, λ is changed slowly during a molecular dynamics simulation.<sup>12</sup> The integral is then replaced by a summation, using the instantaneous values of the derivative:

$$\Delta G = \sum (\delta V / \delta \lambda)_{\lambda} \Delta t$$

In the case of the absolute binding free energy calculation of complexation, the thermodynamic cycle in Scheme II is employed.<sup>13</sup> The simulations are carried out by perturbing M<sub>1</sub><sup>+</sup> into a dummy atom, Dum, which has no nonbonded interactions with other atoms (no charge, no van der Waals energy). When calculating solvation free energies of two species of different charges with a finite interaction cutoff, it is essential to include a correction due to long range effects of the solvent. This correction (kcal/mol) can be calculated in the following way from the Born formula if water is used as solvent:<sup>14</sup>

$$\Delta G_{\text{corr}} = -166z^2 / r_{\text{Born}} \quad (1)$$

where z is the charge of the ion and r<sub>Born</sub> is the cutoff radius in Å. The solvation energy ΔG<sub>solv</sub> is then expressed as the sum of a free energy change by perturbation, ΔG<sub>pert</sub> and ΔG<sub>corr</sub>:

$$\Delta G_{\text{solv}} = \Delta G_{\text{pert}} + \Delta G_{\text{corr}} \quad (2)$$

If the same cutoff value of the electrostatic interactions is used in both

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**Table I.** Additional Force Field Parameters Used in This Study with the AMBER 3.0A Package<sup>a</sup>

atom type	mass	atom type	mass		
CS	12.01	K	39.10		
Na	22.99	Rb	85.47		
bond	$K_r$ (kcal mol <sup>-1</sup> Å <sup>-2</sup> )	$r_{eq}$ (Å)	ref		
CA-CS	469.0	1.40			
CS-CS	300.0	1.51	17		
CS-CT	317.0	1.51			
CA-OS	300.0	1.40	18		
angle	$K_\theta$ (kcal mol <sup>-1</sup> rad <sup>-2</sup> )	$\theta_{eq}$ (deg)	ref		
CA-CA-CS	85.0	120.0			
CS-CA-CS	85.0	120.0			
CS-CA-HC	35.0	120.0			
CA-CA-OS	70.0	120.0	10a		
CS-CA-OS	70.0	120.0			
CA-CS-CA	85.0	120.0			
CA-CS-CS	85.0	120.0			
CA-CS-CT	70.0	120.0			
CA-CT-CA	70.0	109.5			
CS-CT-HC	35.0	109.5			
CA-CT-OS	54.0	111.0			
CS-CT-OS	54.0	111.0			
CA-OS-CT	46.5	113.0	18		
dihedral angle	idivf <sup>b</sup>	$V_n/2$ (kcal mol <sup>-1</sup> )	$\gamma$ (deg)	$n$	ref
X-CA-CS-X	4	5.3	180.0	2	
X-CA-OS-X	2	2.0	180.0	2	18
X-CS-CS-X	4	0.0	180.0	2	17
X-CS-CT-X	6	0.0	0.0	2	
VDW	$R^*$ (Å)	$\epsilon$ (kcal mol <sup>-1</sup> )	ref		
Na	1.6	0.010	10a		
K	2.0	0.030	10a		
Rb	2.2	0.035	10a		
Na	1.868	0.00277	19 <sup>c</sup>		
K	2.658	0.00033	19 <sup>c</sup>		
Rb	2.956	0.00017	19 <sup>c</sup>		

<sup>a</sup> Although most of the values were previously reported, all additional parameters to AMBER 3.0A standard data base are listed in order to make it easy to use them. <sup>b</sup> The factor by which the torsional barrier is divided. It corresponds to the number of torsions associated with the central bond, which is the product of the number of explicit substituents on the two central atoms. <sup>c</sup> Aqvist's parameters derived for GROMOS force field were converted to AMBER force field parameters.

of the two perturbations, it can be assumed that correction terms cancel out each other. The binding free energy, therefore, is given by

$$\Delta G_{\text{binding}} = \Delta G_{\text{pert1}} - \Delta G_{\text{pert2}} \quad (3)$$

where  $\Delta G_{\text{pert1}}$  and  $\Delta G_{\text{pert2}}$  are free energy changes by perturbations in a pure solvent and in a solvated host, respectively.

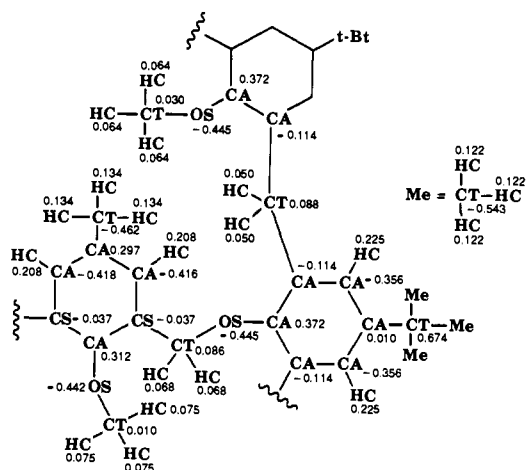
Before each perturbation calculation, the system was energy minimized through a molecular mechanical calculation followed by an equilibration at the proper temperature using a molecular dynamics. The molecular mechanics, dynamics, and free energy perturbation calculations were performed with the AMBER package.<sup>15,16</sup> In all cases the all-atom force field was used. Most of the force field parameters have been previously published.<sup>10a,17,18</sup> A complete list of all additional parameters used in the present study is given in Table I. The force constant and the equilibrium bond angle of CA-CT-OS came from analogous parameters provided in the AMBER 3.0A standard data base. As for the van der Waals (VDW) parameters of alkali metals, new parameters have been proposed recently by Aqvist.<sup>19</sup> We, therefore, planned to carry out

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**Chart II****Table II.** Free Energy Changes ( $\Delta G_3$ ) for Perturbing  $M_1$  into  $M_2$  in Aqueous Solution with Grootenhuys's VDW Parameters<sup>10a</sup>

$M_1$	$M_2$	time (ps)	$\Delta G_3$ (kcal/mol)			
			$\lambda = 1 \rightarrow 0$		$\lambda = 0 \rightarrow 1$	
			forward	backward	forward	backward
K <sup>+</sup>	Rb <sup>+</sup>	20	7.4	-7.4	-7.4	7.4
		30	7.4	-7.4	-7.4	7.4
K <sup>+</sup>	Na <sup>+</sup>	30	-23.4	23.4	22.4	-22.4
		40	-23.0	23.0	22.9	-22.9

calculations using two sets of those VDW parameters. Since Aqvist's parameters were derived with SPC water<sup>20</sup> using the GROMOS,<sup>21</sup> they were converted to the AMBER force field parameters, which are shown in Table I in addition to the former Grootenhuys's parameters.<sup>10a</sup> With regard to the 1-4 nonbonded interactions, two scale factors of 0.5 and 1.0 for 1-4 VDW and 1-4 electrostatic energies were examined. Throughout all calculations a constant dielectric with  $\epsilon$  equal to 1.0 was used.

Because of the dominant role of electrostatic interactions in the systems under study, much attention was paid to the derivation of partial charges at the calixspherand atoms. In order to obtain electrostatic point charges for the molecule, the following procedure was employed. The geometries of a couple of acyclic model systems, i.e., anisole derivatives 2 and 3, were taken from the X-ray structures of spherand<sup>3a</sup> and calixspherand 1.<sup>4</sup> After the geometry optimization of the hydrogens with AMBER, a single-point ab initio calculation was carried out with a 3-21G basis set to determine the partial atom charges by electrostatic potential fitting<sup>22</sup> of the molecules using GAUSSIAN 80 UCSF.<sup>23</sup> The partial charges and atom types of the fragments are found in Chart II. A 3-21G basis set was used because this basis set leads to enhanced polarity for molecules compared to their gas phase polarity, just as found for TIP3P water.

## Results

**Calculation of  $\Delta G_3$ .** In order to calculate  $\Delta G_3$ , the system with a potassium cation and 189 water molecules was used. That is, K<sup>+</sup> was placed in the center of a TIP3P<sup>24</sup> water box with dimension of 18.6 Å. The size of the box was chosen so as to give two complete solvation layers around the central solute and to allow a residue-based cutoff distance of 8.0 Å for the calculation of the nonbonded interactions. Even though this cutoff might seem short for ionic interactions, it should be adequate for studying free energy changes from one ion to another. After the removal of water molecules with short contacts to the cation, the system was energy minimized using AMBER with periodic boundary conditions. The

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**Table III.** Free Energy Changes ( $\Delta G_3$ ) for Perturbing M<sub>1</sub> into M<sub>2</sub> in Aqueous Solution with Aqvist's VDW Parameters<sup>19,31</sup>

M <sub>1</sub>	M <sub>2</sub>	time (ps)	$\Delta G_3$ (kcal/mol)			
			$\lambda = 1 \rightarrow 0$		$\lambda = 0 \rightarrow 1$	
			forward	backward	forward	backward
K <sup>+</sup>	Rb <sup>+</sup>	30	5.6	-5.6	-5.6	5.6
K <sup>+</sup>	Na <sup>+</sup>	40	-17.8	17.8	17.7	-17.7

energy-minimized system was warmed up and equilibrated at 298 K for 10 ps before starting the perturbation simulations. This period appeared to be sufficient to reach plateau values for the potential and kinetic energies of the system. The SHAKE algorithm was applied to constrain all bonds to their equilibrium lengths.<sup>25</sup> Thus a time step of 0.002 ps was used. The molecular dynamics simulations were of the isothermal-isobaric type.<sup>26</sup> The temperature was maintained at 298 K by velocity scaling using a relaxation time of 0.1 ps, whereas for constant pressure, position scaling with a time constant of 0.6 ps was applied.

The perturbation experiments consisted of molecular dynamics runs of 20, 30, or 40 ps in length in which  $\lambda$  changed linearly over the runs (slow growth). Although Mitchell and McCammon<sup>27</sup> have noted the slow convergence of slow growth for a thr  $\rightarrow$  ala mutation and Pearlman and Kollman<sup>28</sup> have suggested "dynamically modified windows" to be an efficient approach to study "disappearance" of noble gas atoms in water, this method and the length of the simulations employed here should be adequate for the mutations studied here, in which no atoms disappear, only change in size. Results for those runs with Grootenhuus's VDW parameters for metals are given in Table II. First, the K<sup>+</sup> cluster was changed into Rb<sup>+</sup> cluster, yielding a value for  $\Delta G_3$  of 7.4 kcal/mol. It seems that the 20-ps run for a complete perturbation is satisfactory since it gives the same results as the 30-ps run without hysteresis. The value of 7.4 kcal/mol for the free energy difference of solvation of a potassium and a rubidium ion is in qualitative accord with the difference of 5.1 kcal/mol in experimental solvation free energies.<sup>29</sup> For the larger perturbation K<sup>+</sup>  $\rightarrow$  Na<sup>+</sup>, 30-ps and 40-ps runs were carried out similarly. The 30-ps run was not considered satisfactory because the hysteresis amounted to 1.0 kcal/mol. From the 40-ps run a relative free energy of -23.0 kcal/mol was estimated for the solvation of K<sup>+</sup> and Na<sup>+</sup>, whereas the experimental value of -17.6 kcal/mol is reported.<sup>29</sup> In both cases the interaction between the cation and the water molecules is somewhat overestimated with respect to the experimental solvation free energies. A likely reason for this is that the nonbonded van der Waals parameters for alkali cations were derived from the interaction not with bulk water but with a single water molecule.<sup>30</sup>

In Table III results of two runs using Aqvist's VDW parameters are shown.<sup>31</sup> The calculated values of 5.6 kcal/mol for the perturbation K<sup>+</sup>  $\rightarrow$  Rb<sup>+</sup> and of -17.7 kcal/mol for K<sup>+</sup>  $\rightarrow$  Na<sup>+</sup> are in excellent agreement with observed values for  $\Delta G_3$  of 5.1 and -17.6 kcal/mol, respectively. This is because those parameters were derived in order to reproduce hydration free energies.<sup>19</sup>

**Calculation of  $\Delta G_4$ .** The free energies of complexation of **1** with alkali metal cations were determined by NMR studies in a homogeneous CDCl<sub>3</sub> solution which was saturated with D<sub>2</sub>O<sup>4b</sup> and by the two phase (water, chloroform) extraction method,<sup>4a</sup> independently. The solvent system around the calixspherand

**Table IV.** Free Energy Changes ( $\Delta G_4$ ) for Perturbing M<sub>1</sub> into M<sub>2</sub> in Host Calixspherand **1** Using Grootenhuus's VDW Parameters<sup>10a</sup>

M <sub>1</sub>	M <sub>2</sub>	time (ps)	$\Delta G_4$ (kcal/mol)			
			$\lambda = 1 \rightarrow 0$		$\lambda = 0 \rightarrow 1$	
			forward	backward	forward	backward
K <sup>+</sup>	Rb <sup>+</sup>	20	9.8	-9.8	-9.8	9.8
K <sup>+</sup>	Na <sup>+</sup>	20	-13.9	13.9	14.6	-14.6
		30	-14.1	14.1	14.5	-14.5

**Table V.** Free Energy Changes ( $\Delta G_4$ ) for Perturbing M<sub>1</sub> into M<sub>2</sub> in Host Calixspherand **1** Using Aqvist's VDW Parameters<sup>19,a</sup>

M <sub>1</sub>	M <sub>2</sub>	solvent	time (ps)	$\Delta G_4$ (kcal/mol)			
				$\lambda = 1 \rightarrow 0$		$\lambda = 0 \rightarrow 1$	
			forward	backward	forward	backward	
K <sup>+</sup>	Rb <sup>+</sup>	vacuo	20	6.7	-6.7	-6.9	6.9
				(6.6)	(-6.6)	(-6.6)	(6.6)
		water	20	7.6	-7.6	-7.5	7.5
K <sup>+</sup>	Na <sup>+</sup>	vacuo	20	-13.5	13.5	13.8	-13.8
		vacuo	30	-13.6	13.6	13.8	-13.8
		water	30	(-12.9)	(12.9)	(12.7)	(-12.7)
		water	30	-14.9	14.9	14.7	-14.7

<sup>a</sup>Free energy values derived using a scale factor of 1.0 for 1-4 nonbonded interactions in parentheses.

complexes, therefore, is rather difficult to model while most of the solvent molecules surrounding them may be chloroform due to the highly hydrophobic collar around the molecular cavity. Because of this complexity and ambiguity, we first took the simplest approximation, i.e., no solvent model, since the interaction between the complex and chloroform would be expected to be smaller than that of water.

As a starting geometry for the minimization on the **1**-K<sup>+</sup> complex, the X-ray data of **1** and sodium picrate were used. After the replacement of Na into K, the geometries of the **1**-K<sup>+</sup> complex were optimized with AMBER. The conformation of **1** showed only minor changes by minimization; 10 ps of equilibration of the system was performed prior to the start of the perturbation simulations. A time step of 0.001 ps was used since no SHAKE was applied. Results of the slow growth perturbation at 298 K are found in Tables IV and V. The simulations with the former VDW parameters gave the free energy change of 9.8 kcal/mol for  $\Delta G_4$  of the smaller perturbation K<sup>+</sup>  $\rightarrow$  Rb<sup>+</sup>. Then K<sup>+</sup> was changed into Na<sup>+</sup> in the complex with **1**. The 30-ps run produced better values than the 20-ps run with respect to hysteresis, resulting in the relative free energy of 14.3 kcal/mol for  $\Delta G_4$ .

When the new parameters were employed, the free energy difference of 6.8 kcal/mol for the change of K<sup>+</sup> into Rb<sup>+</sup> was obtained as shown in Table V. The relative free energy of 13.7 kcal/mol for the perturbation K<sup>+</sup>  $\rightarrow$  Na<sup>+</sup> was estimated from the 30-ps run. This value is almost the same as that produced by the former parameters. Figure 1a illustrates the energy minimized structure of the complex. In the above simulations, a scale factor of 0.5 for the 1-4 VDW and 1-4 electrostatic interactions (SCNB = SCEE = 2.0) was used in analogy with the simulations on crown ethers<sup>10,11</sup> and calixarenes.<sup>18</sup> The effect of the reduction of the scale factor from 1.0 to 0.5, however, needs to be investigated. The simulations of 20-ps and 30-ps runs were performed for the perturbations of K<sup>+</sup>  $\rightarrow$  Rb<sup>+</sup> and K<sup>+</sup>  $\rightarrow$  Na<sup>+</sup>, respectively, using the scale factor of 1.0 in order to check the conformational and energetical features. The energy minimized structure of the **1**-K<sup>+</sup> complex was almost the same as that shown in Figure 1a with the rms deviations of 0.12 Å for nonhydrogen atoms and of 0.16 Å for all atoms. The differences of  $\Delta G_4$  derived from two scale factors were about 1 kcal/mol or less for the both perturbations as shown in Table V. Since the structural and energetical changes were reasonably small, the factor of 0.5 for 1-4 nonbonded interactions was also used in the following calculations.

We carried out the calculations of  $\Delta G_4$  in water for the next approximation of the solvent system. As mentioned above, in the experimental system, most of the solvent molecules around the complexes are considered to be chloroform, which would be ex-

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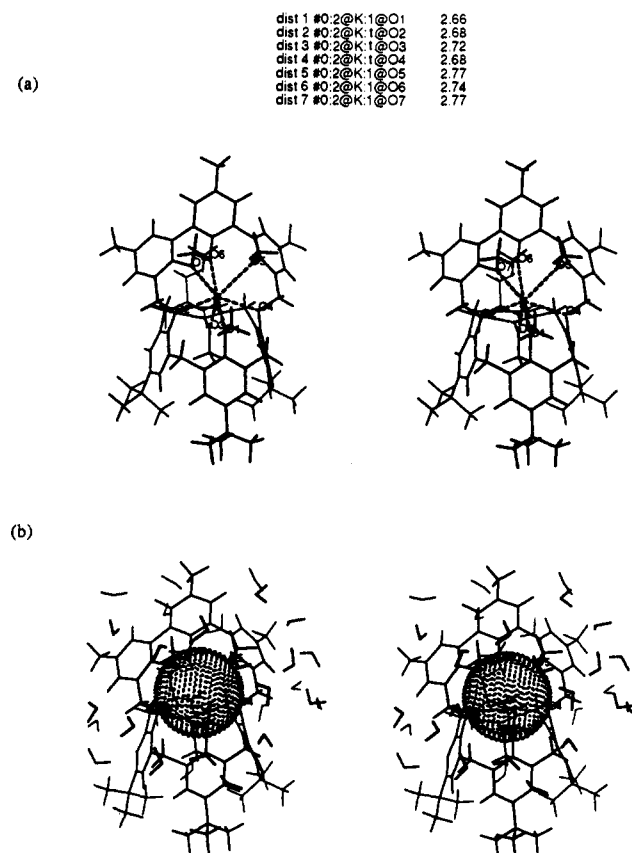
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Table VI. Results of Relative Free Energy Calculations (kcal/mol)<sup>a</sup>

M <sub>1</sub>	M <sub>2</sub>	$\Delta\Delta G$									
		$\Delta G_3$		$\Delta G_4$			$\Delta G_4 - \Delta G_3$			$\Delta G_2 - \Delta G_1$	
		former <sup>b</sup> aq <sup>f</sup>	new <sup>c</sup> aq <sup>f</sup>	former <sup>b</sup> vac <sup>g</sup>	new <sup>c</sup> vac <sup>g</sup>	new <sup>c</sup> aq <sup>f</sup>	former <sup>b</sup> vac - aq <sup>h</sup>	new <sup>c</sup> vac - aq <sup>h</sup>	new <sup>c</sup> aq - aq <sup>i</sup>	ext <sup>d</sup>	NMR <sup>e</sup>
K <sup>+</sup>	Rb <sup>+</sup>	7.4	5.6	9.8	6.8	7.6	2.4	1.2	2.0	2.0	5.1
K <sup>+</sup>	Na <sup>+</sup>	-23.0	-17.7	-14.3	-13.7	-14.8	8.7	4.0	2.9	0.4	1.3

<sup>a</sup> See the thermodynamic cycle for an explanation of the symbols employed. <sup>b</sup> Former Grootenhuys's VDW parameters were used. <sup>c</sup> New Aqvist's VDW parameters were used. <sup>d</sup> Calculated from the experimental data determined by the two phase extraction method, ref 4a. <sup>e</sup> Calculated from the experimental values determined by NMR studies, ref 4b. <sup>f</sup> Simulations in aquo (water). <sup>g</sup> Simulations in vacuo. <sup>h</sup>  $\Delta G_4$  in vacuo -  $\Delta G_3$  in aquo. <sup>i</sup>  $\Delta G_4$  in aquo -  $\Delta G_3$  in aquo.



**Figure 1.** Stereoviews of the complexes of **1** with K<sup>+</sup>. (a) Energy-minimized structure in vacuo. Distances between K<sup>+</sup> and oxygen atoms are shown. (b) A snapshot of the equilibrated structure in water. Solvent molecules having their oxygen atoms within 8 Å of the K ion are shown. In this case the numbers of water molecules having their oxygen atoms in distances of 6 and 7 Å from the cation are 0 and 12, respectively. A VDW sphere for K<sup>+</sup> is illustrated using the radius of 2.658 Å.

pected to have smaller interactions with the complexes than water.  $\Delta G_4$  derived in an aqueous solution, therefore, may represent the upper bound of the solvent effect. Simulations were performed in a manner similar to that of the calculation of  $\Delta G_3$ . The energy-minimized **1**·K<sup>+</sup> complex was placed in a water box, which contained 1110 water molecules. In this case only Aqvist's parameters were used because of the heavy demand of the computational time. The whole system was optimized under periodic boundary conditions followed by the equilibration at 298 K. A snapshot of the equilibrated structure of the complex in water is shown in Figure 1b. Two runs of 20 ps and 30 ps were carried out for the perturbations K<sup>+</sup> → Rb<sup>+</sup> and K<sup>+</sup> → Na<sup>+</sup>, respectively. After every forward and reverse perturbation as well as the equilibration, the structure was examined to find no oxygen atoms of water within 5 Å of the metal cation. In most cases only a dozen of solvent molecules were found within a distance of 7 Å from the cations, as seen in Figure 1b. This suggests that there is no coordination or strong interaction between the cation in **1** and H<sub>2</sub>O. Although this phenomenon should be expected owing to the small

cavity of the host and the highly hydrophobic nature around it, the confirmation of the structural feature leads to the idea that, even in aqueous solvent, the direct effect on the central ion is rather small. It was also found that during the simulations the conformation of the calixarene did not change from its starting flattened partial cone conformation, which indicated the rigidity of the complex.

The relative free energies of 7.6 and 14.8 kcal/mol for the change of K<sup>+</sup> into Rb<sup>+</sup> and Na<sup>+</sup> were obtained in the solvated complexes, as shown in Table V. These values are not so different from those in vacuo, while the absolute values (magnitudes) of  $\Delta G_4$  are about 1 kcal/mol larger than those without solvent for both perturbations. These small differences in  $\Delta G_4$  appear to be related to the geometric features found in the simulation of the solvated complexes. That is, because of the small direct effect of water on the perturbed ions in addition to the rigidity of the complexes, the calixspherand complexes might be little influenced by the aqueous solvent in terms of free energy, resulting in the small differences in  $\Delta G_4$  between the perturbations in vacuo and in aquo (water). The very little hysteresis observed in the all calculations of  $\Delta G_4$ , as well as the rigidity of the complexes, suggests either that conformational space was searched (good ensembles were generated) by molecular dynamics or that a large barrier prevented sampling of some regions.

**Calculation of  $\Delta\Delta G$ .** Combining the results of the slow growth perturbation shown in Tables II and IV, and III and V, gives free energy differences,  $\Delta G_4 - \Delta G_3$ . Those values are collected in Table VI with the experimental free energy changes of  $\Delta G_2 - \Delta G_1$ . If the Grootenhuys's parameters are used,  $\Delta\Delta G$  of 2.4 kcal/mol is obtained for the perturbation K<sup>+</sup> → Rb<sup>+</sup>, in favor of K<sup>+</sup> complexation. This value compares reasonably with the experimental values of 2.0 and 5.1 kcal/mol. The relative free energy for the complexation by **1** of Na<sup>+</sup> versus K<sup>+</sup> is now calculated to be 8.7 kcal/mol, which is in only qualitative agreement with the experimental values of 0.4 and 1.3 kcal/mol. With respect to the Aqvist's parameters,  $\Delta G_4 - \Delta G_3$  of 1.2 and 4.0 kcal/mol are found for the changes of K<sup>+</sup> into Rb<sup>+</sup> and Na<sup>+</sup>, respectively, when the  $\Delta G_4$  in vacuo are employed. The experimental tendency toward selection of K<sup>+</sup> by **1** was successfully reproduced in both cases using the former and the new VDW parameters. As a whole Aqvist's parameters seems to be better since the differences of calculated and observed  $\Delta\Delta G$  are within 4 kcal/mol for the two thermodynamic cycles.

The solvated complexes apparently give calculated relative free energies for  $\Delta\Delta G$  of 2.0 and 2.9 kcal/mol, closer to the experimental values than the nonsolvated complexes. If the  $\Delta\Delta G$  values derived from the simulations of the solvated complexes are compared to the average numbers of the experimental  $\Delta\Delta G$  values, the differences are within 2 kcal/mol for the two thermodynamic cycles. This might indicate that the solvent effect is taken into account in the right direction although a water model was used instead of chloroform/water solution. In view of the uncertainty of the solvent system and the fact that the present force field parameters were not fully optimized for this kind of calculation, the agreement is qualitatively satisfactory.

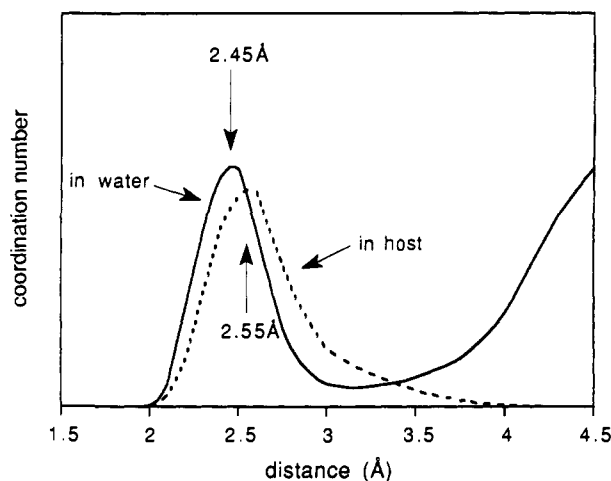
**Calculation of the Absolute Binding Free Energy of Complexation.** The good agreement obtained above between the calculations and the experiments led us to carry out the simulations for estimating the absolute binding free energy of complexation.

As shown by Jorgensen et al.,<sup>13a</sup> this can be accomplished by mutating the ion to a dummy atom both in water and in the ligand binding site. The simulations were performed with respect to the complexation of **1** and Rb<sup>+</sup>, for which rather consistent experimental values of -12.0 and -13.0 kcal/mol were reported for the binding free energy. In order to calculate  $\Delta G_{\text{pert1}}$  and  $\Delta G_{\text{pert2}}$ , we used solvated systems and procedures similar to those for the calculation of  $\Delta G_3$  and  $\Delta G_4$  described above. First, the perturbations between Rb<sup>+</sup> and the dummy atom solvated by 189 water molecules were carried out. The 50-ps runs in the both forward ( $\lambda = 1 \rightarrow 0$ ) and reverse ( $\lambda = 0 \rightarrow 1$ ) directions gave free energy changes of  $64.7 \pm 0.1$  and  $-61.0 \pm 0.1$  kcal/mol, respectively. Since the hysteresis of 3.7 kcal/mol was not ideal, we doubled the simulation time. The 100-ps runs produced much better values than those of the 50-ps runs with respect to hysteresis; that is, the free energy differences of  $62.2 \pm 0.1$  and  $-62.5 \pm 0.1$  for  $\Delta G_{\text{pert1}}$  were obtained for the forward and reverse perturbations, respectively. Although it is not our current objective to calculate the accurate  $\Delta G_{\text{sol}}$ , it might be helpful to know how the calculated value compares with the experimental value. Since the  $r_{\text{Born}}$  is not well defined in the case of the periodic boundary conditions,<sup>19</sup> it is difficult to compute the exact  $\Delta G_{\text{corr}}$  of our system. By using  $r_{\text{Born}} = \text{cutoff} = 8 \text{ \AA}$ , however, we can estimate the approximate  $\Delta G_{\text{corr}}$ , which seems to serve the present purpose. After the estimation of  $\Delta G_{\text{corr}}$  of -20.8 kcal/mol from eq 1, the hydration free energy is computed as -83.1 kcal/mol based on eq 2, while the experimental value is -75.5 kcal/mol.<sup>29</sup> Considering the ambiguity of the  $r_{\text{Born}}$ , the agreement is at least qualitatively satisfactory.

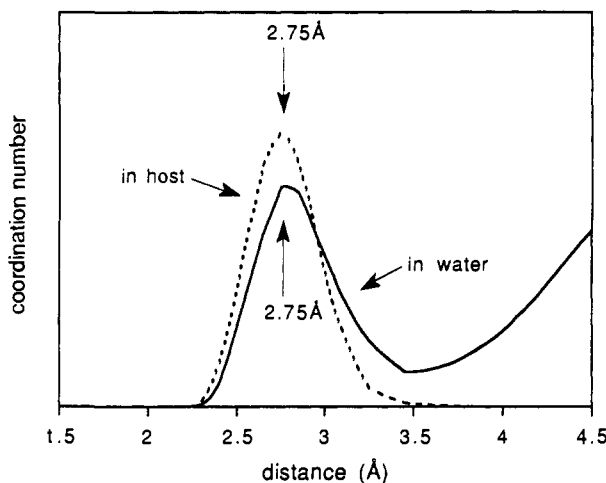
For the simulations in the solvated calixspherand, the perturbation in the reverse direction ( $\lambda = 0 \rightarrow 1$ ) is hard to perform because the position of the dummy atom changes significantly near  $\lambda = 0$ , resulting in the movement of Rb<sup>+</sup> to outside the host. In the calculations of the relative hydration energies of  $\Delta G_3$  and  $\Delta G_4$ , very small hysteresis values of  $\Delta G_4$  were observed in shorter simulation times than those of  $\Delta G_3$ . We could, therefore, assume that a 100-ps forward ( $\lambda = 1 \rightarrow 0$ ) perturbation for  $\Delta G_{\text{pert2}}$  would give the reasonable value since the hysteresis of  $\Delta G_{\text{pert1}}$  was small enough. The free energy change for the perturbation Rb<sup>+</sup>  $\rightarrow$  Dum,  $\Delta G_{\text{pert2}}$  in **1** solvated by 1110 water molecules, was then estimated to be  $75.4 \pm 0.1$  kcal/mol from a 100-ps run. We also carried out the additional simulations of 100-ps runs in both pure water and the solvated host on a different machine, CRAY Y-MP, while the above mentioned calculations were done on FPS264 and FPS500. The free energy differences of  $63.0 \pm 0.1$  and  $-61.1 \pm 0.1$  for  $\Delta G_{\text{pert1}}$  were obtained for forward and reverse perturbations, respectively, and that of  $73.3 \pm 0.1$  was determined for  $\Delta G_{\text{pert2}}$ . By substitution of  $\Delta G_{\text{pert1}}$  and  $\Delta G_{\text{pert2}}$  with the numbers obtained above into eq 3, absolute binding free energies of the **1**-Rb<sup>+</sup> complex,  $\Delta G_{\text{binding}}$ , were calculated as -13.0 and -11.2 kcal/mol. These values are in quite good agreement with the observed values of -12.0 and -13.0 kcal/mol.

The final structure of the perturbation ( $\lambda = 0$ ) corresponds to the solvated free host. Those structures of **1** obtained by 100-ps simulations were examined to find an arrangement of the aromatic rings similar to that of the initial complex, as expected from the experimental studies.<sup>4</sup> The simulated free host **1** had the flattened partial cone conformation of the calixarene moiety with the alternating arrangement of the three methoxy groups of *m*-teranisyl fragment. One water molecule was located at the entrance of the cavity, hydrogen bonding to two methoxy oxygens. One of these methoxy group was the outer *m*-teranisyl OCH<sub>3</sub> while the other came from the "endo-positioned" methoxy group of the calixarene moiety. Because of the binding of the water molecule, a flip of the methyl group of the "endo-positioned" methoxy moiety was not observed although such a rotation of the methoxy group was suggested by the NMR studies.<sup>4b</sup>

**Calculation of Radial Distribution Function.** We carried out molecular dynamics simulations to determine the radial distribution functions for oxygen atoms around metal cations in order to understand the reason for K<sup>+</sup> selectivity over Na<sup>+</sup> and Rb<sup>+</sup>. In these simulations Aqvist's parameters was used and the solvent



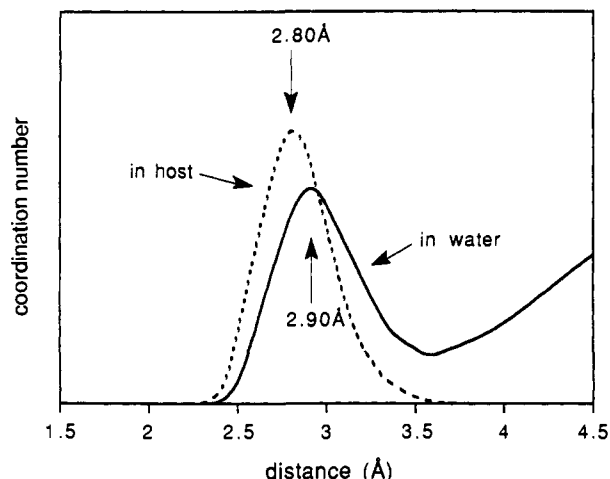
**Figure 2.** Radial distribution functions of oxygen atoms around Na<sup>+</sup>, in water (—) and in **1** (---). The peak of the function for water represents the first solvation shell. The distance corresponding to the peak top is 2.45 Å. The distance of 2.55 Å is found as the peak top in the case of the host-guest complex.



**Figure 3.** Radial distribution functions of oxygen atoms around K<sup>+</sup>, in water (—) and in **1** (---). The peak of the function for water represents the first solvation shell. The distance corresponding to the peak top is 2.75 Å, which is also the distance of the peak top for the host-guest complex.

was not incorporated into the complex calculations because no water molecules were considered to coordinate with metal cations bound to the host. Following the same procedure for the calculation of  $\Delta G_3$ , Na<sup>+</sup> and Rb<sup>+</sup> solvated by 189 water molecules were equilibrated at 298 K. Three 10-ps MD runs of water boxes with Na<sup>+</sup>, K<sup>+</sup>, and Rb<sup>+</sup>, respectively, were performed and the coordinates were saved every 0.04 ps for further analysis. Three other 10-ps MD runs were carried out starting with the calixspherand complexes of ions which were equilibrated for 10 ps at 298 K in similar fashion to that shown in the calculation of  $\Delta G_4$ . The coordinates were saved every 0.02 ps. Based on those coordinate sets, radial distribution functions were calculated to give Figures 2-4 for each of the metal ions with oxygens either from the host or from water.

The peak of the function for water represents the first solvation shell in each Figure. The curves for the host roughly fit those peaks, but a close perusal of the functions reveals the following. It is found from Figure 2 (Na<sup>+</sup>) that the distribution peak is moved outward in the case of the host-guest complex, resulting in a weaker interaction between Na<sup>+</sup> and oxygen atoms in the host than in water. The two peaks are almost overlapped with respect to K<sup>+</sup>, as seen in Figure 3. Potassium ion in **1**, therefore, feels as "comfortably solvated" as in water. Figure 4 illustrates that the peak for the host complex is shifted closer toward the Rb ion



**Figure 4.** Radial distribution functions of oxygen atoms around  $\text{Rb}^+$ , in water (—) and in **1** (---). The peak of the function for water represents the first solvation shell. The distance corresponding to the peak top is 2.90 Å. The distance of 2.80 Å is found as the peak top in the case of the host-guest complex.

**Table VII.** Coordination Numbers of Oxygen Atoms in the Span of 0.4 Å

metal		inner	center <sup>a</sup>	outer
$\text{Na}^+$		1.85–2.25 Å	2.25–2.65 Å	2.65–3.05 Å
	in water	0.1	4.9	0.9
	in host	0.1	4.0	2.1
	diff <sup>b</sup>	0.0	–0.9	1.2
$\text{K}^+$		2.15–2.55 Å	2.55–2.95 Å	2.95–3.35 Å
	in water	0.2	4.5	1.8
	in host	0.4	5.6	1.0
	diff <sup>b</sup>	0.2	1.1	–0.8
$\text{Rb}^+$		2.30–2.70 Å	2.70–3.10 Å	3.10–3.50 Å
	in water	0.5	4.4	1.8
	in host	1.4	5.0	0.6
	diff <sup>b</sup>	0.9	0.6	–1.2

<sup>a</sup> Middle of the center span is the distance corresponding to the peak of the radial distribution function for water. <sup>b</sup> Coordination number difference between in host and in water (=coordination no. in host – coordination no. in water).

than the peak in water. It means that oxygen atoms are pushed inward by the host compared to the cation–oxygen distances found in water. Table VII may be helpful to further understand the above observations. It gives the coordination numbers in the span of 0.4 Å centered at the peak of the radial distribution function for water. In the case of  $\text{K}^+$ , an increase of 1.1 is found for the number in the middle span by changing from water to the host.

On the contrary, the corresponding value decreases by 0.9 for  $\text{Na}^+$ . The larger increase is seen not in the center span but in the inner section for  $\text{Rb}^+$ , which has much more unfavorable VDW interactions with the oxygen atoms within **1**. The above structural observations appear to explain why complexation of **1** with  $\text{K}^+$  is most favorable in free energy terms.

#### Discussion and Conclusions

This study has shown that molecular dynamics simulations with the thermodynamic perturbation method can qualitatively reproduce the experimental trends with respect to binding preferences of highly preorganized calixspherands to alkali metal cations. These results are consistent with previous studies by Feil, Grotenhuis et al., and McCammon et al., but, in addition, differ from them in a number of important ways. First, we have studied a calixspherand, a chemically more complex and encapsulating host than the simple crowns studied in refs 8, 9, and 10a. We are able to reproduce the experimental preference of a given ion over the next larger and smaller ion, as done previously only in ref 8 and 10a. Furthermore, to our knowledge, the work presented here represents the first computational study on the absolute binding free energy of complexation of a host macrobicyclic molecule and a guest cation, resulting in reasonable agreement with the experimental data. Our study is among the first to examine potential function model dependence on ionophore binding; encouragingly, we find that the “best model” (Aqvist’s ion parameters empirically fit to solvation free energies and full inclusion of solvent of the ionophore) leads to the best agreement with experiment. The best calculated  $\Delta\Delta G$  ( $\text{K}^+$  versus  $\text{Na}^+$ ) is not in as quantitative agreement with experiment (2.9 calculated versus 0.4–1.3 kcal/mol experiment) as for  $\Delta\Delta G$  ( $\text{K}^+$  versus  $\text{Rb}^+$ ), suggesting subtle defects in our potential functions or in the additive approximation in this and most other force fields (Dang, Caldwell, et al.<sup>32</sup>).

In addition to a semiquantitative reproduction of the experimental  $\Delta\Delta G$  values, the qualitative insights afforded by analyzing the ion–oxygen radial distribution functions of ion–water and ion–host systems have been exciting and instructive. This suggests that one might use molecular dynamics simulations per se in suggesting selective designs of ionic hosts, prior to engaging in the more time consuming free energy calculations. For the most rigid hosts, of course, Cram has been doing this exercise more impressively simply with CPK models. But when there is ambiguity in molecular conformation or cavity size in complex hosts, molecular dynamics can be a useful qualitative tool.

**Acknowledgment.** We thank Dr. C. Bayly for valuable discussions. P.A.K. is glad to acknowledge research support from the NIH (GM-29072) and the NSF (CHE-91-13472) and we are glad to acknowledge the use of the UCSF Computer Graphics Lab, supported by RR-1081, R. Langridge, P. I.

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