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Quantitation and Nature of the Macrocyclic Effect: K^+ Complexation with 18-Crown-6 and Pentaglyme

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Abstract: Free energy perturbation calculations have been applied to calculate the absolute binding free energies of K^+ with macrocyclic ionophore 18-crown-6 in methanol and in water and its acyclic analogue, pentaglyme in methanol. For 18-crown-6, the free energies of binding with K^+ were evaluated as −1.2 kcal/mol in water and −5.2 kcal/mol in methanol, compared with experimental values of −2.9 kcal/mol in water and −8.4 kcal/mol in methanol. A simple force field model was used to estimate all available conformations and their relative energies for pentaglyme in solution. This allowed the estimation of the extra configurational free energy price pentaglyme has to pay in order to stay in the conformation optimal for binding K^+. In contrast, 18-crown-6 pre-exists in this conformation in solution. The total calculated free energies of binding in methanol were evaluated as −5.2 kcal/mol for 18-crown-6/K^+ and 0.5 kcal/mol for pentaglyme/K^+, compared with experimental values of −8.4 and −3.0 kcal/mol, respectively. The difference in binding free energies between 18-crown-6 and pentaglyme, which is usually described as the macrocyclic effect, is −5.7 kcal/mol from the calculations and −5.4 kcal/mol from experiments. Both the configurational free energy and the binding interaction contribute to this difference.

Introduction

The discovery of 18-crown-6 by Pedersen in 1967 marked the beginning of macrocyclic chemistry.[1,2] The primary goal of this exciting field is to design large cyclic molecules forming pre-existing optimized binding sites for specific ligands.[3−5] The attention it has received represents not only its importance per se but also the insight it can provide for the molecular recognition phenomena in general.

18-Crown-6, a cyclic ether, is a member of a series of crown ethers possessing host−guest complexation properties with cations. Both experimental and theoretical methods have been applied to study its structure and complexation properties.[6−14] In two previous publications concerning this molecule, we studied the conformational distribution of 18-crown-6 in vacuo and the effects of solvation on the relative free energies of its conformations.[15,16] The present work describes the study of its cation binding properties.

One of the key features of a macrocyclic molecule, its enhanced complexation affinity compared to an analogous acyclic molecule, is often described as the macrocyclic effect. That is, by forming a cyclic binding cavity prior to the association with a ligand, its binding affinity can be increased over its acyclic analogue. Although the usefulness and generality of the macrocyclic effect have been appreciated since the beginning,[1,2,11] no one has attempted to quantitatively simulate the free energy gain due to the macrocyclic effect. In this study we will try to do so using the cation K^+ binding properties of 18-crown-6 and its acyclic analogue pentaglyme as a typical representative of the macrocyclic effect. Among all the alkali metal ions, K^+ has the highest binding affinity with 18-crown-6. The free energies of binding with K^+ in methanol have been determined experimentally as −8.4 kcal/mol for 18-crown-6 and −3.0 kcal/mol for pentaglyme.[6] The binding free energy in water is −2.9 kcal/mol for 18-crown-6 and not available for pentaglyme, presumably due to its negligible association constant.

Methods

All simulations described in this paper were performed with the molecular mechanical simulation package Amber 4.1.[17] The potential energy function is of the following form:

$$U_{\text{total}} = \sum_{\text{bonds}} K_r(r - r_{\text{eq}})^2 + \sum_{\text{angles}} K_\theta(\theta - \theta_{\text{eq}})^2 + \sum_{\text{dihedrals}} \frac{V_n}{2}[1 + \cos(n\varphi - \gamma)] + \sum_{i<j} \left[\left(\frac{A_{ij}}{R_{ij}^{12}} - \frac{B_{ij}}{R_{ij}^{6}}\right) + q_iq_j/\epsilon R_{ij}\right] \quad (1)$$

Statistical mechanical free energy perturbation theory[18] allows for the calculation of free energy differences between two states of a system, A and B. The two states A and B are linked together with a coupling constant of λ. That is, the system is represented by a Hamiltonian

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[®] Abstract published in *Advance ACS Abstracts,* March 1, 1995.

(1) Pedersen, C. J. *J. Am. Chem. Soc.* **1967,** *89,* 7017.

(2) Pedersen, C. J. *J. Am. Chem. Soc.* **1967,** *89,* 2495.

(3) Cram, D. J. *Science* **1988,** *240,* 760−767.

(4) Lehn, J. M. *Angew. Chem., Int. Ed. Engl.* **1988,** *27,* 89−112.

(5) Izatt, R. M.; Pawlak, K.; Bradshaw, J. S.; Bruening, R. L. *Chem. Rev.* **1991,** *91,* 1721−2085.

(6) Frensdorff, H. K. *J. Am. Chem. Soc.* **1971,** *93,* 600−606.

(7) Pedersen, C. J.; Frensdorff, H. K. *Angew. Chem., Int. Ed. Engl.* **1972,** *11,* 16−25.

(8) Dunitz, J. D.; Dobler, M.; Seiler, P.; Phizackerley, R. P. *Acta Crystallogr.* **1974,** *B30,* 2733−2738.

(9) Dale, J. *Isr. J. Chem.* **1980,** *20,* 3−11.

(10) Maverick, E.; Seiler, P.; Schweizer, W. B.; Dunitz, J. D. *Acta Crystallogr.* **1980,** *B36,* 615−620.

(11) Wipff, G.; Weiner, P.; Kollman, P. *J. Am. Chem. Soc.* **1982,** *104,* 3258−3262.

(12) Uiterwijk, W. H. M.; Harkeman, S.; Feil, D. *J. Chem. Soc., Perkin Trans.* **1987,** 721−731.

(13) Mazor, M. H.; McCammon, J. A.; Lybrand, T. P. *J. Am. Chem. Soc.* **1990,** *112,* 4411−4419.

(14) Ha, Y. L.; Chakraborty, A. K. *J. Phys. Chem.* **1993,** *97,* 11291−11299.

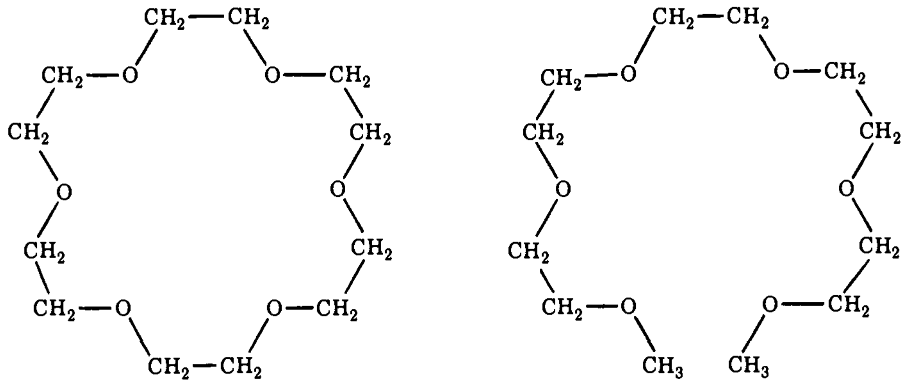
(15) Sun, Y.; Kollman, P. A. *J. Comp. Chem.* **1992,** *13,* 33−40.

(16) Sun, Y.; Kollman, P. A. *J. Chem. Phys.* **1992,** *97,* 5108−5112.

(17) Pearlman, D. A.; Case, D. A.; Caldwell, J. W.; Ross, W. R.; Seibel, G. L.; Singh, U. C.; Weiner, P.; Kollman, P. A. *AMBER4.1(UCSF);* University of California: San Francisco, 1993.

(18) Zwanzig, R. *J. Chem. Phys.* **1954,** *22,* 1420.

0002-7863/95/1517-3599$09.00/0



18-Crown-6

Pentaglyme

Figure 1. Structures of 18-crown-6 and pentaglyme.

$H(\lambda)$, such that $H(\lambda{=}0) = H_A$ and $H(\lambda{=}1) = H_B$, where H_A and H_B are the Hamiltonians of states A and B, respectively. The free energy difference between the states at λ and $\lambda + \Delta\lambda$ is

$$\Delta G_\lambda = -RT\ln\left\langle \exp\left(-\frac{H_{\lambda+\Delta\lambda} - H_\lambda}{RT}\right)\right\rangle_\lambda \qquad (2)$$

where R is the gas constant, T is the absolute temperature, and $\langle\,\rangle_\lambda$ denotes the ensemble average at state λ. The total free energy change between A and B is thus

$$\Delta G = \sum_{\lambda=0}^{\lambda=1} \Delta G_\lambda \qquad (3)$$

The AMBER all-atom force field,[19] supplemented with the parameters of Billeter et al.,[20] was used, as in previous studies.[15,16] The parameters for K^+ were from Aqvist.[21] The simulations were carried out at 300 K using explicit solvent in a cubic box at a constant pressure of 1 atm. Jorgensen's united-atom liquid methanol model[22] was used for the solvent. The box size was about 25 Å containing either 215 MeOH or 460 water molecules for K^+ and 30 Å containing either 400 MeOH or 900 water molecules for the ion/ionophore complexes. The SHAKE procedure[23] was employed to constrain all solute bonds involving at least one hydrogen atom and all solvent bonds. A long-range cutoff correction[24] was used for methanol but not water, in order to be consistent with the development of the liquid MeOH parameters. The simulations were carried out using a time step of 1 fs and a nonbonded interaction cutoff of 10 Å. The nonbonded pairs were updated every 10 steps. Prior to carrying out free energy perturbation calculations, all systems were equilibrated for at least 50 ps.

All FEP were run with electrostatic decoupling, i.e., separately perturbing the changes of electrostatic charges and vdw parameters. Not only that additional information is obtained through the decoupling, direct perturbations of ion to nothing without decoupling often have been found to be unstable. Each perturbation consisted of 200 windows, with 1500 steps of equilibration/2500 steps of data collection per window for electrostatic perturbations and 1000 steps each for equilibration/data collection for vdw perturbations. The total simulation time was 800 ps for each electrostatic FEP and 400 ps for each vdw FEP. All FEP were run in both the forward and the backward directions.

Experimentally, 18-crown-6 is found to have the same D_{3d} conformation in solution and in its complex with potassium in solution.[25,26] In this conformation, all the C−O−C−C torsion a angles are trans and all the O−C−C−O torsion angles are gauche, which are energetically favorable positions for both torsions. Since the similar "D_{3d}" conformer is also one of the lowest free energy conformations of pentaglyme, it is expected that this conformation is also the conformation with which pentaglyme binds with potassium in solution. Thus, the starting conformations used in the simulations for both 18-crown-6 and pentaglyme are the D_{3d} (or quasi D_{3d}) conformations, with the C−O−C−C trans (including the CH_3−O−C−C) and the O−C−C−O gauche. To avoid any dihedral transitions during the simulations, especially as K^+ disappears, a weak dihedral restraint of 2 kcal/rad^2 was applied to all the dihedrals at their minimum energy values, 180° for all the C−O−C−C dihedrals and +65° and −65° for all the O−C−C−O dihedrals. In addition, a harmonic distance restraint of 0.5 kcal/Å^2 was used between the ion and the oxygen atoms at 3-Å separation to keep K^+ from drifting away from the ionophore when its charge becomes too small at the end of the disappearing process. The later kind of restraint is necessary for almost all the absolute free energy FEP calculations, where ligands are perturbed to nothing. As a ligand disappears, it can no longer bind its host strong enough to stay at the binding site. Fortunately, the effects of these restraints can be evaluated and a correction added to the final result. This is done using the same FEP formula,

$$\Delta G_{\text{rest}} = -RT\ln\langle\exp(-H_{\text{rest}})\rangle_{H_0} \qquad (4)$$

where all the notations are the same as in the regular FEP eq 2, except that $H_1 - H_0$ in eq 2 is now the restraint function H_{rest}.

One important note is that the free energy of restraints should be calculated for not only $(\text{18-crown-6}/K^+)_{\text{rest}}$ and $(\text{pentaglyme}/K^+)_{\text{rest}}$ but also (18-crown-6/dummy) and (pentaglyme/dummy), since restraints applied to dummy atoms increase the free energies of the states as well.

The following thermodynamic cycle illustrates the scheme of the simulations (Figure 2). ΔG_1 and ΔG_2 are the free energies of perturbing K^+ to dummy atoms in solution and in the complex with an ionophore. ΔG_3 and ΔG_4 are the free energies of restraints for dummy and K^+. The free energy of binding $\Delta\Delta G_2$ can be obtained experimentally and is used to compare with the calculated value $\Delta\Delta G_1 + (\Delta G_3 - \Delta G_4) = (\Delta G_1 - \Delta G_2) + (\Delta G_3 - \Delta G_4)$.

Results and Discussion

Free Energy Perturbation Calculations in Methanol. The free energy perturbation results are in Table 1. Growing the vdw (with zero charge) of potassium in the cavity of 18-crown-6 increased the free energy by 5.0 kcal/mol, compared to the 3.5

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(19) Weiner, S. J.; Kollman, P. A. *J. Comput. Chem.* **1986**, *7*, 230−252.

(20) Billeter, M.; Howard, A. E.; Kuntz, I. D.; Kollman, P. A. *J. Am. Chem. Soc.* **1988**, *110*, 8385−8391.

(21) Aqvist, J. *J. Phys. Chem.* **1990**, *94*, 8021−8024.

(22) Jorgensen, W. L. *J. Phys. Chem.* **1986**, *90*, 1276−1284.

(23) Ryckaert, J.; Ciccotti, G.; Berendsen, H. J. C. *J. Comput. Phys.* **1977**, *23*, 327−341.

(24) Allen, M. P.; Tildesley, D. J. *Computer Simulation of Liquids*; Oxford University Press: New York, 1987.

(25) Takeuchi, H.; Arai, T.; Harada, I. *J. Mol. Struct.* **1986**, *146*, 197−212.

(26) Fukushima, K.; Ito, M.; Sakurada, K.; Shiraishi, S. *Chem. Lett.* **1988**, *1988*, 233.

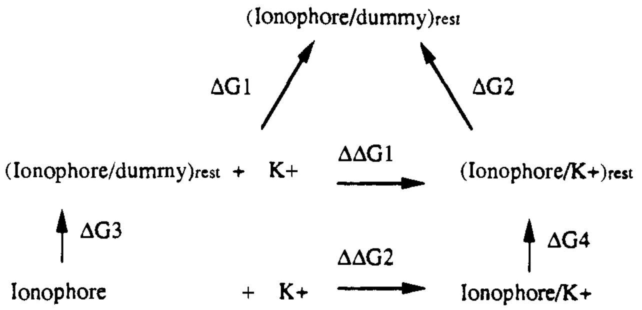

Figure 2. Thermodynamical cycle used in simulations.

Table 1. Free Energy Perturbation Results of ($K^+ \rightarrow$ Dummy Atom) (kcal/mol)

		electrostatic			vdw		
solvent	system	$\lambda =$ 1 → 0	$\lambda =$ 0 → 1	av	$\lambda =$ 1 → 0	$\lambda =$ 0 → 1	av
methanol	K^+	73.92	73.74	73.8	−3.25	−3.73	−3.5
	K^+/18C6	80.80	81.64	81.2	−5.22	−4.79	−5.0
	K^+/glyme	78.46	78.89	78.7	−4.39	−4.71	−4.6
water	K^+	77.16	76.59	76.9	−4.90	−5.02	−5.0
	K^+/18C6	80.24	80.10	80.2	−6.49	−6.35	−6.4

kcal/mol increase for growing the vdw of K^+ in solution. With the grown vdw, perturbing the charge of K^+ from 0.0 to 1.0 in the middle of 18-crown-6 decreased the free energy by −81.2 kcal/mol, compared to the −73.8 kcal/mol decrease obtained for K^+ in solution. The results for pentaglyme were in between those in solution and in 18-crown-6, probably reflecting the partially opened nature of pentaglyme as an ionophore, compared with the closed chain topology of 18-crown-6. Figure 3b shows that pentaglyme avoids the steric contact between the two ending CH_3 groups and thus achieves its "D_{3d}" binding conformation by small shifts in dihedrals within the molecule. The resulting difference in FEP calculated binding free energies with K^+ is 1.9 kcal/mol between the crown D_{3d} conformation and the pentaglyme "D_{3d}" conformation.

The free energy effects of restraints are listed in Table 2. With K^+ as a ligand, the free energy increases due to the restraints are 0.7 kcal/mol for both 18-crown-6/K^+ and pentaglyme/K^+. With a dummy atom as a ligand, the free energies of restraints are 1.4 kcal/mol for 18-crown-6/dummy and 2.2 kcal/mol for pentaglyme/dummy. Interestingly, the free energies of restraints for the dummy atom are larger than the free energies of restraints for K^+. This is because the strong electrostatic attraction between K^+ and the oxygen atoms of ionophores reduces the freedom of the ion and the vibrational motion of the ionophores. With a dummy as a ligand, the deviation of positions for both the ligand and the ionophores increases, resulting in an increase in the free energies of restraints. Likewise, the deviation from the minimum energy structure should be more pronounced for pentaglyme/dummy compared to 18-crown-6/dummy, again due to its open chain nature, resulting in the free energies of restraints of 1.4 kcal/mol for 18-crown-6/dummy and 2.2 kcal/mol for pentaglyme.

Free Energy Perturbation Calculations in Water. The free energy perturbation results for K^+ and 18-crown-6/K^+ in water are also in Table 1. The free energy of $K^+ \rightarrow$ dummy atom was calculated as 71.9 kcal/mol in water, compared with 70.3 kcal/mol in methanol. The free energy of transfer from water to methanol is therefore calculated as 1.6 kcal/mol, compared with the experimental value of 2.4 kcal/mol.[27] Small free energies of transfer have been observed experimentally for a wide range of solvents, but the good agreement between the calculations and the experiment is still encouraging because it involves the difference between two large numbers.

The absolute free energies of binding between 18-crown-6 and K^+ were calculated as −1.2 kcal/mol in water and −5.2 kcal/mol in methanol, compared with experimental values of −2.9 kcal/mol in water and −8.4 kcal/mol in methanol. The agreement is again very good since these are absolute binding free energies.

The relative difference between the free energy of binding of 18-crown-6/K^+ in methanol and in water is −4.0 kcal/mol theoretically and −5.5 kcal/mol experimentally. In the simulation result, the difference between the K^+ desolvation in the two solvents is −1.6 kcal/mol, while surprisingly, the rest (−2.4 kcal/mol) came from the more attractive interaction between K^+/18-crown-6 in methanol than K^+/18-crown-6 in water. The vdw contributions to the total binding free energies are about the same in water (+1.4 kcal/mol) and in methanol (+1.5 kcal/mol) while the electrostatic contributions are very different, −3.3 kcal/mol in water and −7.4 kcal/mol in methanol. Interestingly, the electrostatic free energy is more favorable for the interaction of K^+/18-crown-6 in methanol than the interaction of K^+/18-crown-6 in water. It is not clear why the K^+ interaction with 18-crown-6 is stronger in methanol than in water. We speculate that the weaker solvent−solvent interactions in methanol allow the first shell methanol molecules to align better and interact more favorably with the K^+ inside the crown than what happens in water.

Configurational Free Energy for Pentaglyme. There is an extra free energy associated with pentaglyme in order to stay in the optimum potassium binding conformation, "D_{3d}". Although this conformer is also one of the lowest energy conformations for pentaglyme, there are also a large number of other low-energy conformations available. The decrease of freedom results in an increase of the free energy of the system.

The total free energy of an ensemble of conformers (G_{total}) can be expressed as follows:

$$\exp\left(-\frac{G_{\text{total}}}{RT}\right) = \exp\left(-\frac{G_0}{RT}\right) + \exp\left(-\frac{G_1}{RT}\right) + \exp\left(-\frac{G_2}{RT}\right) + \ldots$$

where G_0, G_1, G_2, ..., are the free energies of conformer 1, 2, 3, ... The difference between total free energy and free energy of one conformation (G_0) is

$$G_{\text{total}} - G_0 = \Delta G = -RT \ln N^*$$

where N^* is the Boltzmann weighted effective number of conformations,

$$N^* = 1 + \exp\left(-\frac{\Delta G_1}{RT}\right) + \exp\left(-\frac{\Delta G_2}{RT}\right) + \ldots$$

$$\Delta G_i = G_i - G_0$$

Pentaglyme has a total of 15 dihedrals, 10 of the C−C−O−C type and 5 of the O−C−C−O type. We used a macroscopic dielectric constant of $\epsilon = 4$ to represent the effect of solvent. This is partly supported by a previous work, in which we calculated the relative free energies of four 18-crown-6 conformations in solution and found using $\epsilon = 4$ without solvent compared reasonably well with the free energies calculated using $\epsilon = 1$ with explicit solvent.[16] We compared the molecular mechanical minimized energies of a variety of conformations

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(27) Burgess, J. *Metal Ions in Solution*; Ellis Horwood: Chichester, England, 1978.

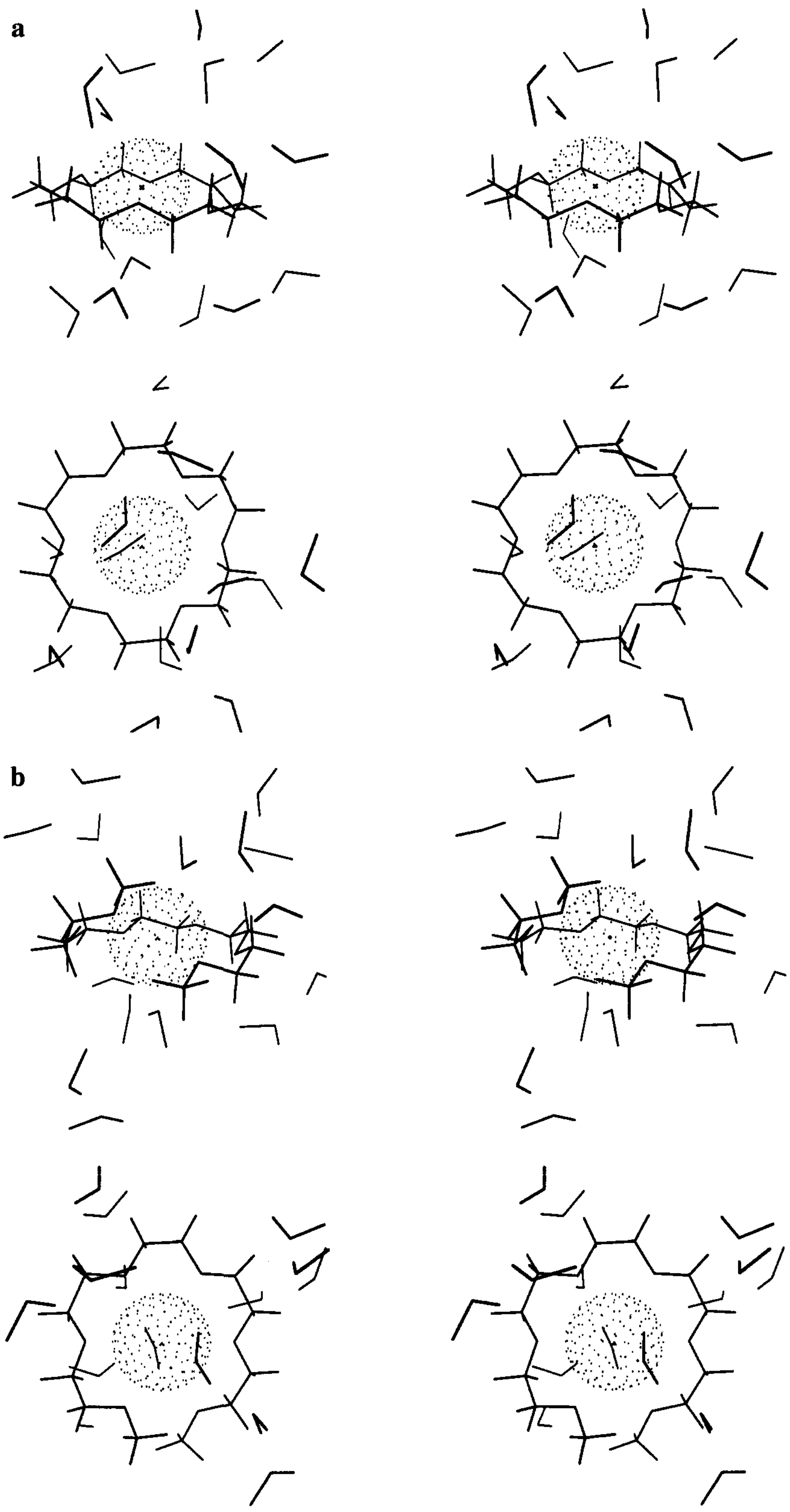

Figure 3. (a) Stereoview of a snapshot (two views) of 18-crown-6/K^+ in methanol. (b) Stereoview of a snapshot (two views) of pentaglyme/K^+ in methanol.

with such a model. To a good approximation, for every O—C—C—O dihedral angle that was trans rather than gauche, the energy was higher by 0.4 kcal/mol, and for every C—O—C—C dihedral angle that was gauche rather than trans, the energy was 1.3 kcal/mol higher. The energy changes due to the variations of O—C—C—O and C—C—O—C dihedrals can also be treated approximately independently. We then counted the number of possibilities having a certain number (0, 1, 2, 3, ...) of dihedral angles in the higher energy states for O—C—C—O dihedral and C—O—C—C dihedral, respectively, and the average energies of these conformations. (The D_{3d} conformation has all of the dihedrals in the low-energy states, i.e., all the O—C—C—O gauche and all the C—C—O—C trans.) Table 3 lists the results. The Boltzmann factor weighted total number of conformations is $51 \times 3.6 = 184$. The free energy of configuration for pentaglyme is $\Delta G = G_{\text{total}} - G(D_{3d}) = -RT \ln N^* \approx 3$ kcal/mol.

Table 2. Free Energies of Restraints (kcal/mol)

ligand	18-crown-6 in MeOH	18-crown-6 in H_2O	pentaglyme in MeOH
K^+	0.7	0.7	0.7
dummy	1.4	1.4	2.2
$\Delta\Delta G(\Delta G_3-\Delta G_4)$	0.7	0.7	1.5

Table 3. Boltzman-Weighted Number of Conformations for Pentaglyme

O−C−C−O

no. of trans dihedral angles	no. of combinations $(N)^a$	$\Delta E(\mathrm{av})^b$	$N^*(N\ \exp(-\Delta E/RT))$
0	16	0.0	16
1	40	0.4	21
2	40	0.8	11
3	20	1.1	3
			total 51

C−C−O−C

no. of gauche dihedral angles	no. of combinations $(N)^a$	$\Delta E(\mathrm{av})^b$	$N^*(N\ \exp(-\Delta E/RT))$
0	1	0.0	1.0
1	10	1.3	1.1
2	90	2.7	0.7
3	480	4.0	0.6
4	1680	5.4	0.2
			total 3.6

[a] *N* is the total number of all possible combinations of conformations with specific number of trans (O−C−C−O) or gauche (C−C−O−C) dihedral angles. [b] ΔE is the average energy of the conformations with this specific number of higher energy dihedrals calculated from five randomly chosen ones.

It is important to note that our goal is to qualitatively estimate the Boltzmann factor weighted number of conformations. The result is only intended to show that this number is in the range of hundreds, instead of tens or several thousand. Fortunately the conformational free energy is not very sensitive to the value of N^*. For example, for N^* = 10, 100, 1000, and 10000, ΔG = 1.4, 2.8, 4.1, and 5.5 kcal/mol. As a result, the accuracy of ΔG is much higher than the accuracy of N^*.

It is important to note that the optimum K^+ binding conformation "D_{3d}" also happens to be one of the sixteen (16) lowest free energy conformations in solution, as calculated by the simple model of using macroscopic dielectric constant of $\epsilon = 4$. If the binding conformation were not among the ensemble of lowest free energy conformations in solution, the "macrocyclic effect" would be increased by approximately ΔG^*, with ΔG^* being the difference in free energies between the true lowest free energy conformation and the optimum binding conformation. Another possible source of error in our analysis comes from the possible multiple binding conformations available to either 18-crown-6 or pentaglyme, i.e., other low free energy conformations that could also bind with K^+. For example, 10 possible binding conformations for pentaglyme that are of the same free energy and only one for 18-crown-6 would change the relative binding free energy by 1.4 kcal/mol. However, since the "D_{3d}" conformer is the only conformation among the low free energy conformations that has all six oxygen atoms coordinated to the K^+ and uniformly favorable torsion angles, other conformers are unlikely to bind K^+ as effectively as this conformation. Even a small difference in free energies of binding of these alternative conformations (such as 1.4 kcal/mol) would reduce their contribution to the conformational ensemble to 10% relative to the D_{3d} conformation.

Table 4. Comparisons of Experimental and Theoretical Free Energies of Binding (kcal/mol)

	FEP	restraints	config	theory	exp
18C6/K^+ in methanol	−5.9	0.7	~0	−5.2	−8.4
glyme/K^+ in methanol	−4.0	1.5	~3	0.5	−3.0
18C6/K^+ in water	−1.9	0.7	~0	−1.2	−2.9

The Macrocyclic Effect. The free energy results, including restraint corrections and free energies of configurations, are in Table 4. The calculated free energies of binding are −5.2 and 0.5 kcal/mol for 18-crown-6 and pentaglyme, respectively, compared with the experimental values of −8.4 and −3.0 kcal/mol. The macrocyclic effect for 18-crown-6, which is often described by the relative difference between the binding free energies of 18-crown-6 and pentaglyme, is −5.4 kcal/mol experimentally, compared with the theoretical value of −5.7 kcal/mol. There are three contributions to this free energy: firstly, there is the interaction free energy, which is more favorable in 18-crown-6 than pentaglyme by 1.9 kcal/mol; secondly, there is the restraint free energy, which can be viewed as the difference in the entropic contribution to the free energy *within the binding conformation*, which is more favorable in 18-crown-6 than pentaglyme by 0.8 kcal/mol; and finally, there is the *configurational* free energy, which can be viewed as the entropic contribution to the free energy needed to drive the pentaglyme into its binding conformation, which is more favorable for 18-crown-6 than pentaglyme by ~3.0 kcal/mol.

It should be noted that such a good agreement with experiment for the "macrocyclic effect" could well be fortuitous, since some of the uncertainties and approximations with the individual 18-crown-6 and pentaglyme calculations may cancel out and, due to the fact that our conformational free energies of pentaglyme are approximate.

The calculated binding free energies for 18-crown-6/K^+, pentaglyme/K^+ in methanol, and 18-crown-6/K^+ in water are all too positive, by 1.7 to 3.5 kcal/mol. The electrostatic partial charges, affecting directly the binding free energies of the ionophores, are the most likely cause of this, since making the charges of oxygen atoms more negative will make the binding more favorable. Of course, an increase in favorable electrostatic interactions when cation binding takes place could be handled more "naturally" with non-additive force fields, as we have described elsewhere for spherand cation association.[28]

We would also like to comment on some recent experimental work on the gas-phase macrocyclic effect on crown ethers.[29,30] The idea is that there are two factors related to the macrocyclic effect, configurational entropy and desolvation. By doing experiments in the gas phase, it was hoped that the role of solvent could be eliminated and the "intrinsic" effect could be observed. What is missing in this analysis is that the configurational effect is highly solvent dependent. The configurational free energies are totally different in the gas phase and in solution, because solvation often completely alters the relative free energies of conformations. For pentaglyme, the energy difference between the conformer with the O−C−C−O dihedrals all +65° and the conformer with the O−C−C−O alternating between +65° and −65° ("D_{3d}" like) is 0.0 kcal/mol with $\epsilon = 4$ and 2.1 kcal/mol with $\epsilon = 1$. As a result, the gas-phase macrocyclic effect should be at least 2.1 kcal higher for pentaglyme than in solution. There may be intrinsic interest in

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(28) Sun, Y.; Caldwell, J. W.; Kollman, P. A. In preparation.

(29) Zhang, H.; Dearden, D. V. *J. Am. Chem. Soc.* **1992**, *114*, 2754–2755.

(30) Chu, I. H.; Zhang, H.; Dearden, D. V. *J. Am. Chem. Soc.* **1993**, *115*, 5736–5744.

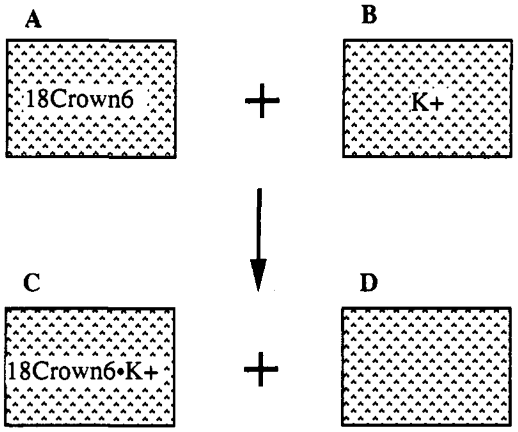

Figure 4. Schematic representation of 18-crown-6 and K^+ association.

Table 5. Energy Components of 18-Crown-6 and Potassium Association (kcal/mol)[a]

	K−18C6	K−H_2O	18C6−H_2O	H_2O−H_2O
A			−33.0 ± 0.8	−3870 ± 10
B		−156.3 ± 0.8		−3854 ± 12
A + B		−156.3 ± 0.8	−33.0 ± 0.8	−7724 ± 16
C	−53.9 ± 1.6	−93.1 ± 2.4	14.1 ± 0.3	−3852 ± 19
D				−3937 ± 19
C + D	−53.9 ± 1.6	−93.1 ± 2.4	14.1 ± 0.3	−7789 ± 27
(C + D) − (A + B)	−53.9 ± 1.6	63.2 ± 2.5	47.1 ± 0.9	−65 ± 31

[a] Each system (A, B, C, D) contains 443 water molecules solvating the solute in an approximately 24 Å cubic box. Results shown are means and standard deviation of the average energy components of three 15-ps simulations.

the study of the gas-phase macrocyclic effect, but it does not seem to be directly relevant to the nature of the effect in solution.

Balance of Energy Components. Free energies provide only the overall information about molecular association. The satisfactory agreement reached between the calculated free energy of binding with the experimental value provided us with a foundation for further analysis. To assist the understanding of the physical basis of the binding process, we proceeded to study the components of the interaction energies during the 18-crown-6 and K^+ association. Experimentally it has been determined that for 18-crown-6 and K^+ binding, $\Delta G = -2.9$ kcal/mol, $\Delta H = -5$ to -7 kcal/mol, and $-T\Delta S = 2{\sim}5$ kcal/mol.[5] This is an "enthalpy driven" process. The question is how the interaction energies are balanced and where the excess enthalpy comes from.

To study these questions, we used a model scheme to schematically represent the 18-crown-6 and K^+ binding process (Figure 4). There are four water boxes with solvated solutes (box A, 18-crown-6; box B, K^+; box C, 18-crown-6/K^+ complex; and box D, pure water) all with the same number of water molecules. The binding process is the equivalent of transforming boxes A + B into boxes C + D. Molecular dynamics were run for all boxes and the average energies of four interaction components, K^+ with 18-crown-6, K^+ with water, 18-crown-6 with water, and water with water were calculated. In Table 5, A + B represents the interaction energies prior to the association, C + D is the interaction energy after the association, and (C + D) − (A + B) is the change in interaction energies during the binding process.

The most interesting feature of these results is that the changes in all four energy components upon binding are of a similar magnitude. The K^+−18-crown-6 interaction energy decreased (became more favorable) by 53.9 kcal/mol, while the K^+−H_2O interaction energy increased by 63.2 kcal/mol. The large contribution from the interaction energy of 18-crown-6−H_2O was unexpected, which became 47.1 kcal/mol less favorable upon the K^+ association with the crown. That is, the interaction energy between water and an ion-binding 18-crown-6 is 47 kcal/mol higher than that between water and an isolated 18-crown-6, changing from −33 kcal/mol before the binding to +14 kcal/mol after the binding. The strong polarizing effect of K^+ changes the structure of water molecules surrounding 18-crown-6 and thus makes the interaction of water with 18-crown-6 far less favorable. Freeing up water during the molecular association is always important, and in this case the water−water interaction energy decreased −65 ± 31 kcal/mol. Summing up the changes of all four components, the total binding energy is about −9 ± 31 kcal/mol. Although it contains a large error bar, the result is qualitatively consistent with the experimental value of enthalpy change −5 to −7 kcal/mol. This type of analysis, while very approximate in nature, has provided us useful insight into the physical nature of the binding process.

Conclusions

We have quantitatively studied the properties of potassium association with ionophore 18-crown-6 and its acyclic analogue pentaglyme.

1. Solvent Effect. The difference in solvation free energies of K^+ in water and in methanol was calculated as 1.6 kcal/mol, compared with the experimental value of 2.4 kcal/mol. The difference in binding free energies of 18-crown-6 with K^+ in water and in methanol was calculated as −4.0 kcal/mol, compared with the experimental value of −5.5 kcal/mol. Both the desolvation of K^+ and the interaction of K^+/18-crown-6 in the two solvents contributed to the total difference.

2. Macrocyclic Effect. Using a simple force field model, the configurational free energy of pentaglyme was estimated semiquantitatively as 3 kcal/mol, equivalent to about 200 conformations. The difference in binding free energies with K^+ in methanol between 18-crown-6 and pentaglyme is evaluated as −5.7 kcal/mol, compared with the experimental value of −5.4 kcal/mol.

3. Interaction Energies. For 18-crown-6 association with K^+, the changes in four interaction energies—K^+ with 18-crown-6, K^+ with water, 18-crown-6 with water, and water with water—are of similar magnitude, −54 ± 2, 63 ± 3, 47 ± 1, −65 ± 31 kcal/mol, respectively. The small change in the total energies (−9 ± 31 kcal/mol) is consistent with the experimental value of enthalpy change −5 to −7 kcal/mol. The results demonstrated the complicated and delicate balance in energies even in such a simple molecular association.

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