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Ion-Selective Properties of a Small Ionophore in Methanol Studied by Free Energy Perturbation Simulations

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The ion-selective properties of the cyclic depsipeptide molecule *valinomycin* in methanol are studied by free energy perturbation molecular dynamics simulations. The dependence of the alkali cation selectivity on the dipole moment of the backbone carbonyl groups that ligate the ion is examined and found to behave in a systematic way. Since available force fields have not been parametrized for this type of ion–carbonyl interaction, the results are used to determine carbonyl partial charges that reproduce the observed selectivity quantitatively. The optimal dipole moment is somewhat higher than that normally used in molecular mechanics force fields and indicates that there is a significant polarization of the carbonyl groups by the field from the ion. The simulations also suggest that the unloading of ions mainly would occur through the lactic acid face of the molecule since it does not provide as effective a shield against attack from the solvent as the more hydrophobic isohydroxyvaleric acid face.

Introduction

The principles underlying molecular recognition in membrane channels and ion carriers constitute an issue of fundamental interest in molecular biophysics. During the past years considerable progress has been made in advancing our understanding of recognition and selectivity phenomena both in biomolecular and synthetic organic systems.[1] With modern computational techniques it has also become possible to study these phenomena on a detailed microscopic level of description.[2] Unfortunately, we are still lacking accurate structural information for many of the biologically most interesting systems and in particular for transmembrane ion channels. However, there are several simpler systems which display nontrivial selectivity properties of essentially the same type as observed in biological channels and, from the viewpoint of theoretical chemistry, they can serve as useful test cases that allow us to judge the reliability of computational models and procedures. Needless to say, it is of great importance to be able to really gauge the accuracy of molecular mechanics force fields in cases where extensive experimental data is available. It is also necessary to consider both structure and energetics (and for some purposes also dynamics) when assessing the reliability of a given force field. Structural analysis based on molecular dynamics (MD) or Monte Carlo (MC) simulations provides a more stringent test than "static" energy minimization methods, since the former correspond to a more realistic situation in which the system has a certain (nonzero) thermal energy. This allows for a sampling of the available conformational space and the calculation of time- or ensemble-averaged structures. By employing free energy perturbation (FEP) simulations, it is also possible to make direct comparisons of energetics to experiments in solution.

In this paper we address the problem of ion binding and selectivity for the small ion carrier valinomycin. This molecule has the ability to bind small monovalent cations rather tightly in various solvents. For the alkali cation series it has the selectivity sequence Rb > K > Cs > Na > Li, which is nontrivial in the sense that an ion in the middle of the series is selected. We report FEP/MD simulations of the complexation of alkali ions with valinomycin in methanol, for which the most extensive set of experimental data is available. By varying key parameters of the force field we show that it is possible to identify the factors that give rise to particular selectivity patterns. The ion-selective properties of valinomycin are found to behave in a systematic way as a function of the ligand field strength (or dipole moment), as has been predicted earlier.[3]

Structure and Energetics of Valinomycin Complexes

The primary structure of valinomycin is cyclo-(-D-Hyl-D-Val-L-Lac-L-Val-)$_3$, where D-Hyl is D-hydroxyisovaleric acid, L-Lac is L-lactic acid, and Val denotes valine. In solvents of low dielectric

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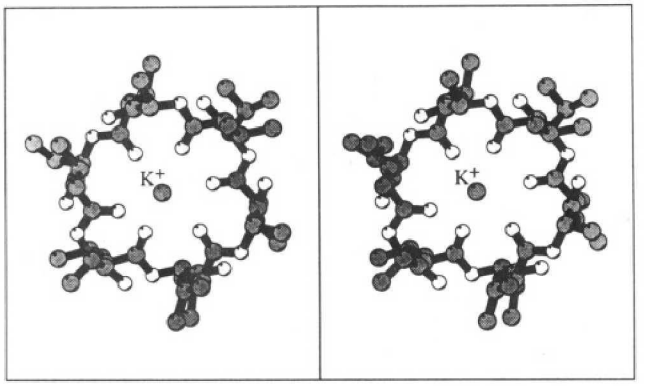

Figure 1. Stereoview of the crystal structure of the K^+–valinomycin complex[4a] (without iodides) seen from the Lac side of the molecule (white = oxygen, gray = carbon, black = nitrogen).

TABLE I: Observed Absolute and Relative (to Cs^+) Free Energies of Alkali Cation Binding to Valinomycin in Methanol[7] and Water[6] and Relative Free Energies of Ion Extraction from Water to Dichloromethane[5] (in kcal/mol)

ion	ΔG_{bind}^{MeOH}	$\Delta\Delta G_{bind}^{MeOH}$	ΔG_{bind}^{wat}	$\Delta\Delta G_{bind}^{wat}$	$\Delta\Delta G_{bind}^{CH_2Cl_2}$
Na^+	−0.9	+4.4	>+2.6	>+1.4	+6.9
K^+	−6.1	−0.8	−0.5	−1.8	−0.7
Rb^+	−6.5	−1.2	−1.1	−2.3	−1.2
Cs^+	−5.3	0.0	+1.3	0.0	0.0

constant, as well as in the crystalline cation complexes, the valinomycin molecule wraps around a cation to form a bracelet-shaped structure in which the ion is ligated by six ester carbonyl oxygens. The crystal structure of the K^+ complex with I_3^- and I_5^- as counterions has been determined by Neupert-Laves and Dobler[4a] (see also ref 4b and 4c) and is shown in Figure 1. The conformation is stabilized by six intramolecular H bonds between all the –NH groups and the amide carbonyl oxygens. One face of the structure is formed by D-Hyl and the other by L-Lac. The central cavity formed by this folded conformation of valinomycin is somewhat larger than a K^+ ion and the ion is shifted slightly off the central axis (perpendicular to the plane of the paper in Figure 1).

Several experimental studies of the energetics and selectivity of ion binding to valinomycin have been reported. These include measurements of two-phase salt extraction equilibrium constants in different hexane/dichloromethane mixtures,[5] complexation constants in water[6] and methanol,[7] permeability ratios in bilayers,[8] conductance ratios in bilayers,[9] and bulk-phase electrode selectivities.[10] As pointed out earlier,[5] the selectivity ratios obtained from the different experiments in low-dielectric solvents agree very closely with each other. This type of behavior in which the difference in binding energy for different ions is not affected by the solvent is expected only for isosteric complexes.[5,11] The fact that the differential binding energies in water differ considerably from those in solvents of lower dielectric constant thus indicates that the conformation of the ion–host complexes changes as the polarity of the solvent becomes large. The absolute values of the free energies of binding in water are also much smaller than those measured in methanol, as can seen from Table I, and the Na^+ and Cs^+ complexes are in fact unstable in water.[6] Table I also gives the relative free energies for ion extraction by valinomycin from water to dichloromethane.[5] It can be seen that, with the exception of Na^+, these free energies are virtually identical to the relative complexation energies in methanol, which is the expectation for perfectly isosteric complexes. For the present calculations we have chosen to use methanol as the solvent.

Methods

The starting structure in all the simulations discussed below is the K^+ complex of valinomycin determined by Neupert-Laves and Dobler.[4a] Each FEP/MD simulation starts with 30 ps of equilibration with K^+ bound to valinomycin. The ion parameters are then slowly perturbed so that the ion gradually is changed from K^+ to Na^+ and from K^+ to Cs^+. In order to assess the convergence errors in the FEP procedure, the Na^+ and Cs^+ ions are then perturbed back to K^+. Altogether, each simulation covered a total time span of 180 ps using an MD time step of 0.002 ps. The interaction parameters used for the alkali ions are those reported in ref 12a, and the potential function used for the valinomycin molecule is that of the GROMOS program[13] (which represents polar hydrogens explicitly, while nonpolar ones are incorporated into the heavy atoms to which they are bound). The atoms of the host molecule were subjected to a very weak harmonic potential with a force constant of $k_c = 0.05$ kcal/mol that restrains them to the crystallographic positions. This was done in order to prevent possible spontaneous larger scale conformational transitions that could be associated with ion expulsion from the host. One might expect this type of events to be possible during such long simulations, in particular for Na^+ for which the observed absolute binding energy is only of the order of kT. This choice of a weak harmonic force constant was found not to have any significant effect on the rms coordinate deviation from the X-ray structure and the average total energy of these restraints was only about 1 kcal/mol (which is less than 1% of the ion–host interaction). Increasing the harmonic force constant by a factor of 10, however, was found to reduce the rms deviation from the X-ray structure by about 30%. All the calculations were performed with a modified version[12] of the MOLARIS program[14] that incorporates bond length and angle constraints for the solvent with the SHAKE algorithm.[15] The methanol molecules are represented by three atoms since the CH_3 group is an extended atom, and the corresponding Lennard-Jones (LJ) parameters were also taken from the GROMOS library.[12] The partial atomic charges on the methanol molecule are +0.19 on CH_3, −0.60 on O, and +0.41 on H. The C–O and O–H bond-lengths and the intramolecular angle are 1.43 Å, 0.99 Å, and 109.5°, respectively, and this geometry was constrained by using SHAKE. Spherical boundary conditions that include radial harmonic restraints for the molecules in the surface layer of the sphere[16] were used and a total of 151 methanol molecules were included in the calculations, corresponding to a sphere of about 14 Å radius. No interaction cutoff radii were used in the calculations.

The free energy associated with perturbing a given ion, Me_a^+, into another, Me_b^+, is calculated from

$$\Delta G_{FEP}(Me_a^+ \rightarrow Me_b^+) = -RT \sum_{m=0}^{m=n-1} \ln \langle \exp[-(V_{m+1} - V_m)/RT] \rangle_m \quad (1)$$

where V_m denotes the effective mapping potential which is of the form $V_m = (1 - \lambda_m)V_a + \lambda_m V_b$, and λ is the mapping parameter that controls the transformation from the potential V_a to V_b (for a recent review of FEP methods, see ref 17). The total number of mapping points, n, from Na^+ to Cs^+ was 38 and these were interspaced for optimal sampling efficiency (viz., closer spacing for smaller ions).

Results and Discussion

Energetics of Alkali Ions in Methanol. Before turning to the calculations of valinomycin complexes it is important to examine the consistency of the present set of interaction parameters for the case of "bare" ions solvated in methanol. The ion interaction potentials derived in ref 12a were designed to reproduce hydration free energies and radial distribution functions in connection with the SPC water model,[18a] and they also seem to give very similar results with the TIP3P[18b] model. Furthermore, their performance has been examined in the context of catalysis of proton transfer in water and its was found that these parameters also reproduce the pK_a's of solvated aquo cations in satisfactory way.[12b,c] However, the present ion potentials have not been tested in methanol, a test which is necessary in order to provide a meaningful comparison with the results for valinomycin. We therefore calculated the relative solvation energies in methanol by using the same perturbation procedure as for the valinomycin complexes (see above) and these results are shown in Table II and compared to experimental data from ref 19 (calculations similar to these have

TABLE II: Calculated and Observed Relative (to Cs^+) Free Energies of Solvation (in kcal/mol) in Methanol for the Alkali Cations[a]

ion	$\Delta\Delta G_{sol}^{calc}$	$\Delta\Delta G_{sol}^{obs}$
Na^+	−30.6 ● 0.1	−30.7
K^+	−13.5 ± 0.2	−12.7
Rb^+	−7.8 ± 0.2	−7.6
Cs^+	0.0 ± 0.0	0.0

[a] Experimental data are from ref 19 and the calculated errors denote the FEP hysteresis errors.

been reported by a number of authors[2e,f,j,20]). It can be seen that the calculated $\Delta\Delta G_{sol}$'s are in good agreement with the observed numbers. This quantity is, however, not as sensitive to the dipole moment of the solvent molecules as one might think. For instance, one can note that the observed relative solvation energies of the alkali ions in water and methanol are very similar[19] and this is also the case for the corresponding calculated free energies of ref 12a and Table II. This reemphasizes the point[12a] that calculations of relative ion solvation free energies are not sufficient to demonstrate that a given potential function is reliable.

In order to assess the reliability of the methanol model in a more objective way we therefore also calculated the *absolute* solvation free energy of a Na^+ in methanol. This calculation was done in exactly the same manner as those reported in ref 12a and the resulting value (which includes the necessary "Born energy" correction due to the finite size of the system) was found to be $\Delta G_{sol}(Na^+) = -94.4 \pm 4.0$ kcal/mol. The FEP hysteresis errors for $\Delta G_{sol}(Na^+)$ in methanol are considerably larger than those in water,[12a] but the resulting value is, nevertheless, in reasonable agreement with the experimental estimates of ref 19 which range between −92 and −96 kcal/mol.

Energetics and Selectivity of Valinomycin Complexes. The main motivation for the present study was to examine the factors that give rise to a particular selectivity optimum for the binding of a series of ions to a host molecule, that, at least superficially, looks like a protein binding site or an ion-channel "filter". A theory for how such selectivity optima can arise was developed by Eisenman and co-workers some 30 years ago[3] with particular emphasis on transport through membrane channels. In this model, it is mainly the *field strength* (viz., dipole moment) of the ligands surrounding the ion and the *ion radius* that, together with the *solvation free energy* of the ion in the surrounding medium, determines the selectivity pattern. The model also predicts that, as the ligand field strength is varied over a wide range, the selectivity pattern will pass through a number of well-defined sequences (the so-called Eisenman selectivity sequences).[3] These sequences are depicted schematically in Figure 2. The limiting cases are the "low field strength" sequence (with selectivity Cs > Rb > K > Na > Li) which is the inverse of the sequence of (absolute) solvation energies and the "high field strength" sequence (with selectivity Li > Na > K > Rb > Cs) which corresponds to the opposite ordering of the ions. In the former case, the interactions with the host binding site are so small that the selectivity is completely determined by the cost of extracting the ions from solution. The high field strength sequence, on the other hand, corresponds to a situation where the interactions with the binding site are strong enough to overshadow the differences in solvation energies. The model does, however, not take into account any factors relating to strain or conformational energy of the binding site or the possibility that the number of solvent molecules that (possibly) accompany the ion into the binding site may differ between different ions and ligand field strengths.

To examine the ion-selective properties of valinomycin we carried out a number of FEP/MD calculations (spanning the $Na^+ \leftrightarrow Cs^+$ range, as described above) for different values of the dipole moment of the six ligand carbonyl groups. The results from these simulations are summarized in Figure 3a, where each curve represents one value of the carbonyl partial charge and the free energies are all normalized with respect to Cs^+. The experimental data from ref 7 are also shown in the figure. The FEP convergence errors range from typically about 0.2 kcal/mol for $\Delta\Delta G(Cs^+ \rightarrow Rb^+)$ to about 0.8 kcal/mol for $\Delta\Delta G(Cs^+ \rightarrow Na^+)$ Figure 3b

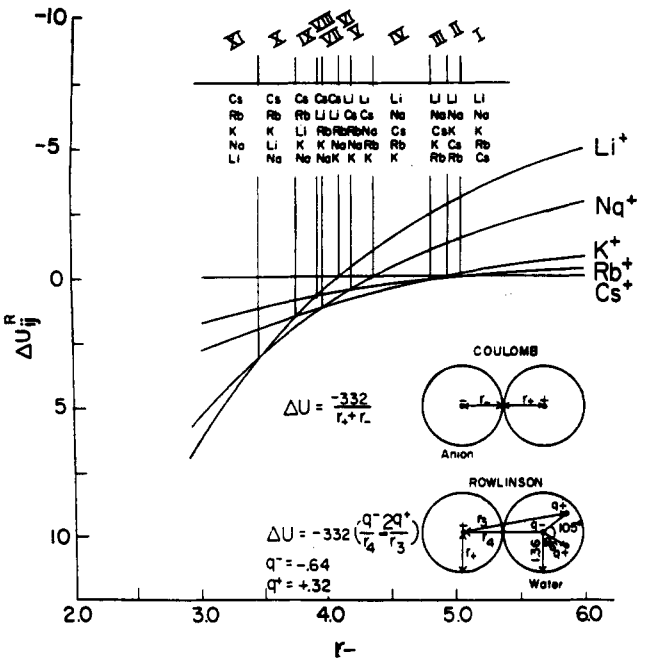

Figure 2. The atomistic asymmetry underlying the cationic selectivity rule as illustrated by the hypothetical exchange between monopolar anion and a single polar water molecule (represented by the early Rowlinson model[18c]). ΔU_{ij} denotes the energy of exchanging ligand relative to that for Cs^+ (units in kcal/mol and Å). Above the graph are tabulated the cationic sequences (increasing specificty downwards), corresponding to the 11 rank order designations, which in their particular progression as a function of r_- (increasing anionic field strength) comprise the selectivity rule.

shows the same data plotted in the manner of Figure 2, where each curve represents one ion and the abscissa denotes the partial charges of the carbonyl groups (the free energies are again taken relative to Cs^+). It can be seen that the valinomycin complexes indeed seem to obey the schematic relationships suggested by Figure 2. For low values of the C=O partial charge ($|q| < 0.45$) the selectivity corresponds to sequence I of Figure 2 which is simply the inverse of the sequence of solvation energies (viz., Cs > Rb > K > Na). As the C=O dipole moment is increased, the system passes through the Eisenman sequences II, III, and IV, where IV corresponds to K > Rb > Cs > Na. The systematic nature of the dependence of the selectivity of the ligand field strength indicated by Figure 3 suggests that, for even higher values of the partial charge, the system would continue through the sequences V, VI, and VII, if not limited by steric factors (see below). It should also be noted here that most standard force fields have their carbonyl dipole moments parametrized for non-ionic interactions (i.e., with other dipolar groups), wherefore an adequate representation of the interaction with ions is yet to be determined.

The experimentally observed values for the selectivity of valinomycin in methanol (sequence III) are, within the convergence errors of the calculations, quantitatively reproduced by a carbonyl partial charge of about ±0.58. This value for the C=O dipole moment not only agrees best with the observed energetics, but it also minimizes the structural deviation from the crystal conformation. Table III shows the time-averaged ion–ligand distances and angles for the K^+ complex as a function of the C=O *partial charge*, as well as the same quantities as function of the *ion species* for the optimal value of the C=O partial charge (±0.58). The first entry in the table gives the corresponding geometries of the crystal structure.[4a] It can be seen from Table III that for $q(C{=}O) = \pm 0.58$ the average K^+–O distance and the K^+–O–C angle are 2.77 Å and 155.1°, respectively, which is very close to the experimentally observed values of 2.76 Å and 156.4°. As the C=O partial charge is increased to 0.66 the average structure "tightens" a bit with the average ligand distance dropping to 2.73 Å and the K^+–O–C angle becoming slightly less favorable. When the partial charge is increased to 0.74, the average distance to the ligands does not decrease further (but actually rises to 2.75 Å) possibly

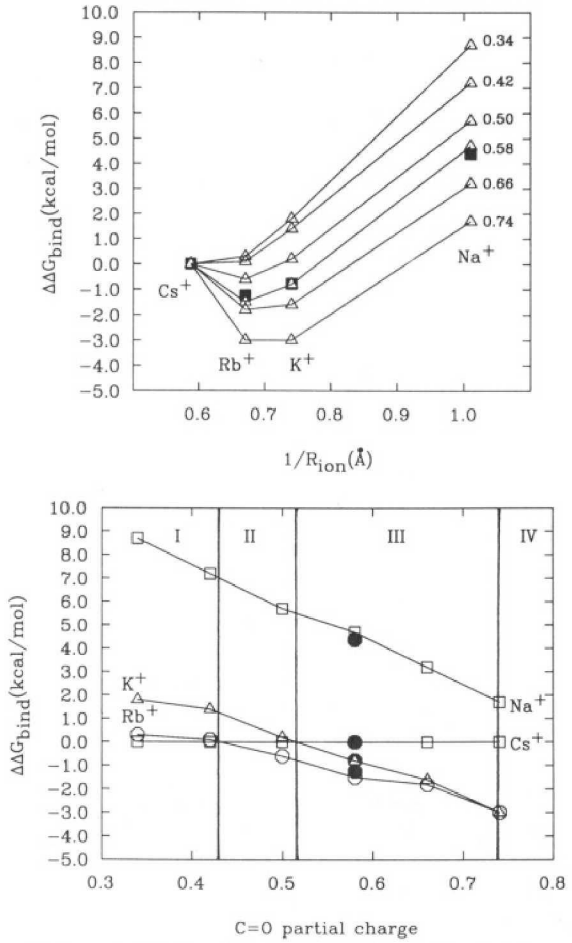

Figure 3. (a, top) Calculated free energies of ion binding to valinomycin relative to Cs^+, for different values of the carbonyl ligand partial charges. The abscissa denotes the inverse of the Pauling ion radius. The C═O partial charges are (counting the curves from top to bottom) ±0.34, ±0.42, ±0.50, ±0.58, ±0.66, ±0.74. Experimental results[7] are denoted by solid squares. (b, bottom) The same data as in Figure 3a, but plotted as a function of the C═O partial charges where each curve represents one ion (open squares = Na^+, open triangles = K^+, open circles = Rb^+, and open squares = Cs^+). The experimental results[xx] are denoted by solid circles. The different selectivity sequences are indicated at the top of the figure.

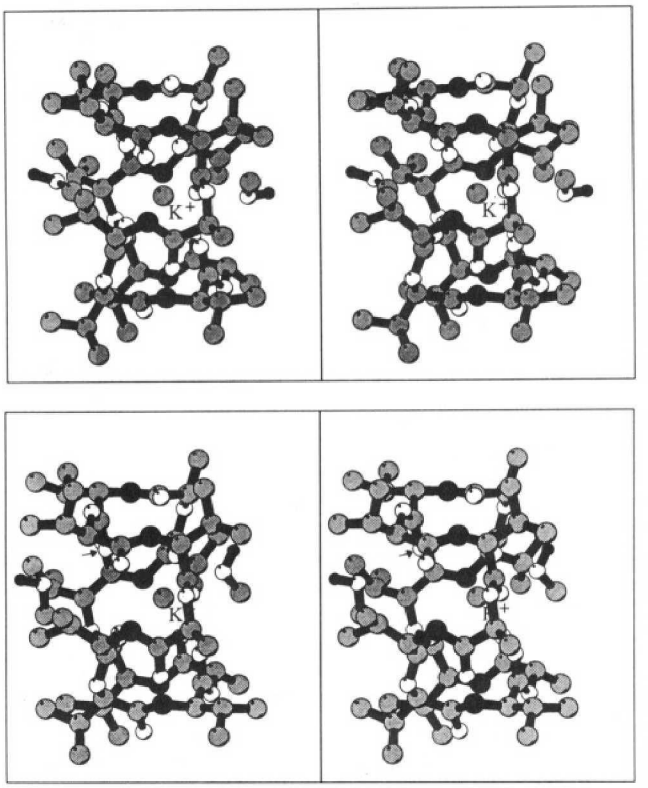

Figure 4. (a, top) Stereo snapshot of the K^+ complex using a partial $C\bar{\bar{O}}$ charge of ±0.58. The two methanol molecules closest to the ion are also shown. The Lac and Hyl faces of valinomycin are on the right and left side, respectively, of the picture (white = oxygen, gray = carbon, black = nitrogen, and methanol hydrogens). (b, bottom) Same as Figure 4a, but from the simulations with a C═O partial charge of ±0.42. In this case a methanol molecule replaces one of the carbonyl groups in the inner ligand sphere of the ion.

reflecting an increased electrostatic repulsion between the ligands themselves. It is also interesting to note that for the lower values of the C═O partial charge (0.34, 0.42, and 0.50) one of the carbonyl ligands becomes replaced by a methanol molecule. In these cases the structure also apparently becomes "looser" as evidenced by the average K^+—O═C distance which increases as the dipole moment of the C═O ligands decreases.

The average ion–ligand distances for the optimal value of the C═O partial charge (0.58) as a function of the *type of ion* bound to valinomycin behaves as expected. That is, the ring of carbonyls has some ability to adjust to the size of the complexed ion. This ability is, however, limited due to steric factors as becomes most evident in the case of Na^+. Here, the optimal Na^+–O distance expected from the sum of Pauling radii is about 2.4 Å, and such a short distance can only be fulfilled for two of the ligands while the remaining Na^+–O distances are clearly unfavorable. This behavior would thus also contribute to the discrimination against Na^+; i.e., in this case the difference between the ion radius and the size of the cavity is too large for the structure to be able to wrap tightly around the ion. Steric factors are therefore bound to modulate the simple relationship between field strength and selectivity discussed above, as is the mutual electrostatic repulsion between the ligands. One can also note that, for a value of the C═O partial charge that is sufficiently high to exclude solvent molecules from the inner ligand sphere of the ion, there appears to be a correlation between the ion size and the distance to the nearest solvent molecule. The data in Table III for q = ±0.58 shows a nearest ion–methanol distance that increases monotonically with the radius of the ion, which could be interpreted as an overall "swelling/shrinking" type of behavior of the host molecule.

In this context, it should be pointed out that the reversibility of the FEP simulations appears quite satisfactory, not only with respect to energetics (see above) but also in the structural sense. For example, comparing the average K^+ structure (q(C═O) = ±0.58) in the beginning of the "mutation cycle" and the corresponding one after mutation to Na^+ and back to K^+ gives rms coordinate deviations for all (host) atoms with respect to the crystal structure of 0.46 and 0.34 Å, respectively. The rms deviation between these two average MD structures are 0.62 Å (all host atoms) and 0.43 Å (backbone only). These deviations are thus rather small and mainly reflect slightly different side-chain orientations that do not involve any dihedral angle transitions.

It is also of interest to note that the ion–solvent interactions always appear to be stronger through the "Lac face" of valinomycin than through the "Hyl face". This is most evident in the low field strength cases where the attack from the solvent, which leads to the replacement of carbonyl ligand, invariably comes from Lac face. Figure 4, a and b, shows two snapshots of the K^+ complex for C═O partial charges of ±0.58 and ±0.42, respectively. It can be seen that the penetration of a methanol molecule into the first coordination shell of the K^+ ion is also accompanied by a translation of the ion toward the Lac face of valinomycin (the lost C═O ligand is marked by an arrow in Figure 4b). The finding that the Lac face is more susceptible to solvent attack can probably be rationalized in terms of the difference in hydrophobicity or "bulkiness" between the Hyl and Lac faces. That is, the Hyl face with its longer hydrophobic side chains apparently provides a more efficient shield against the surrounding medium. We would therefore predict that the actual unloading of ions

TABLE III: Time-Averaged Ion–O Ligand Distances (r) and Ion–O–C Angles (θ) from the Simulations for Different Values of the C=O Partial Charges and for the Different Ions[a]

ion, $\pm q$		CO2	CO4	CO6	CO8	CO10	CO12	MeOH	⟨ ⟩
K^+ (X-ray)	r	2.66	2.69	2.83	2.92	2.84	2.62		2.76
	θ	148.5	158.3	157.2	159.3	156.6	158.6		156.4
K^+, 0.34	r	2.85	3.09	2.83	3.58	2.82	2.84	2.79	2.89
	θ	142.0	159.4	149.9	145.3	162.7	153.2		153.4
K^+, 0.42	r	2.76	2.98	2.78	3.39	2.78	2.82	2.77	2.82
	θ	143.4	159.7	156.7	144.7	156.1	157.4		154.7
K^+, 0.50	r	3.30	2.77	2.79	2.98	2.68	2.73	2.69	2.79
	θ	143.3	157.8	145.2	148.1	153.3	157.3		152.3
K^+, 0.58	r	2.81	2.75	2.75	2.81	2.68	2.83	3.85	2.77
	θ	149.6	157.1	154.9	153.6	155.7	159.6		155.1
K^+, 0.66	r	2.74	2.71	2.65	2.76	2.86	2.66	4.73	2.73
	θ	144.8	158.6	154.9	150.8	147.2	156.4		152.1
K^+, 0.74	r	2.70	2.70	2.77	2.78	2.80	2.77	3.98	2.75
	θ	148.3	147.0	154.0	159.1	142.2	146.2		149.5
Na^+, 0.58	r	2.66	2.97	2.65	2.94	2.37	2.47	3.61	2.68
	θ	157.7	151.5	155.9	148.7	161.9	160.0		156.0
Rb^+, 0.58	r	2.95	2.92	2.80	2.86	2.78	2.85	4.44	2.86
	θ	145.2	150.4	146.2	152.3	155.8	147.1		149.5
Cs^+, 0.58	r	2.94	3.02	2.88	2.98	2.99	2.93	5.05	2.96
	θ	146.1	145.1	152.8	146.2	150.2	155.3		149.3

[a] q denotes the C=O partial charge, MeOH denotes the nearest methanol oxygen and ⟨ ⟩ denotes the average taken over the carbonyl ligands in the innermost coordination sphere of the ion (distances are in Å). The first entry lists the crystallographically observed geometries.[4a]

TABLE IV: Minimum Interaction Energies and Distances (in kcal/mol and Å) in Vacuum for a K^+ Ion (Given by the Parameters of Ref 12a) Interacting with a Carbonyl Group for Different Force Fields[a]

force field	(A_C, B_C)	(A_O, B_O)	$\pm q$ (C=O)	$R^{i\text{-}O}_{min}$	E_{min}
GROMOS[13]	(898.0, 23.65)	(550.0, 23.25)	0.383	2.67	−13.1
GROMOS	(898.0, 23.65)	(550.0, 23.25)	0.580	2.56	−21.1
MOLARIS[14]	(1956.0, 32.00)	(793.3, 25.00)	0.550	2.67	−18.7
AMBER[21]	(888.8, 24.81)	(480.2, 20.72)	0.500	2.57	−18.2
CHARMM[22]	(1901.5, 36.30)	(428.3, 18.48)	0.550	2.52	−20.6

[a] A and B denote the LJ 6/12 parameters and the subscripts C and O refer to the atoms of the C=O group). The AMBER[21] peptide carbonyl group is not electroneutral in itself and the value of $q = \pm 0.50$ is used to approximate the actual charge distribution.

proceeds through the Lac face rather than the Hyl face.

Since the effect of a more polar solvent such as water may be interpreted as a relative reduction of the C=O field strength compared to that from the solvent, one might also expect the valinomycin complexes in water to have the ion partially hydrated. This would thus correspond to a situation that is similar to our low field strength cases and may be relevant for the observed difference of the energetics in water compared to solvents of lower dielectric constant.

Comparison of Force Field Parameters for Carbonyls. The optimal value of $q = \pm 0.58$ for the C=O partial charge found above, is quite different from the standard value of the GROMOS potential which is ± 0.383.[13] It turns out that the standard GROMOS C=O parameters actually differ substantially from those used in other force fields. Table IV compares the vacuum interaction energy between a K^+ ion (given by the LJ parameters of ref 12a) and a carbonyl group for the force fields of GROMOS,[13] MOLARIS,[14] AMBER,[21] and CHARMM[22] (param19). It can be seen that the other force fields as well as GROMOS with a C=O partial charge of ±0.58 give rather similar values for this interaction energy (~−20 kcal/mol), although the latter clearly corresponds to the strongest interaction. The optimal K^+—O=C interaction energy in vacuum can be compared to that between K^+ and a methanol molecule (given by the parameters above) which is −15.1 kcal/mol (the corresponding K^+–H_2O minimum energy in vacuum is −17.8 and −18.2 kcal/mol for SPC[18a] and TIP3P[18b] water, respectively, at an ion–oxygen separation of 2.64–2.65 Å). It thus appears that in order to reproduce the experimental energetics for valinomycin one needs a ion–carbonyl interaction that is larger than the corresponding one with methanol (it is 5–6 kcal/mol larger for all of the ions).

From the above discussion one might be tempted to draw the conclusion that it is the standard GROMOS carbonyl parameters that are deficient for describing interactions with positively charged ions. However, it may be noted that the experimental values[23] for the dipole moments of "related" carbonyl compounds such as acetone and acetaldehyde range between 2.5 and 2.7 D and these values fall somewhere in between those used by GROMOS[13] and AMBER[21] for peptide carbonyl groups. The optimal value of ±0.58 for the C=O partial charge found above corresponds to a dipole moment of 3.48 D and one would therefore expect this value to be inadequate for acetone or acetaldehyde. In order to examine the transferability of the C=O parameters we also calculated the free energy of solvation (as described above) for a Na^+ ion in acetaldehyde using the optimal C=O partial changes from the valinomycin simulations and the standard GROMOS LJ parameters. This is, of course, a rather crude model for CH_3CHO since the entire dipole moment is ascribed to the C=O group. However, the calculated value of ΔG_{sol} comes out almost 10 kcal/mol more negative than in water, while the experimental free energy of transfer of a Na^+ ion from water to acetaldehyde is expected to be rather small and positive[19] (this estimate is based on comparison with acetone for which the ΔG_{tr} value is given in ref 19). Using the standard GROMOS parameters (C=O partial charge of ±0.383) for acetaldehyde, on the other hand, gives a calculated value of $\Delta G_{tr}(Na^+)$ from water of about +5 kcal/mol, which appears to be in better agreement with experiment (although maybe somewhat too positive). One would thus conclude that the standard GROMOS parameters do not seem to work well in the valinomycin case while their performance for ion solvation by a liquid carbonyl compound appears more satisfactory. There is, of course, no a priori reason for the existence of a universal set of carbonyl parameters and, in particular, the assumption made above that the C=O group in itself constitutes an electroneutral entity is probably a rather crude approximation. It may well be the case that ester carbonyls (as in valinomycin) differ considerably from peptide (or amide) and keto carbonyl groups with respect to their polarization, although this effect is unlikely to account for such large differences as those discussed above. Furthermore, it is quite possible that there is a particularly strong polarization of the carbonyl groups in the type of arrangement encountered in valinomycin. That is, the field from the central ion can be expected to induce a substantial polarization of the six C=O ligand groups, in particular since the hydrophobic surface of the molecule to some extent "protects" the complex from the surrounding high dielectric medium. Hence, it may turn out that the inclusion of induced dipoles (e.g., through atomic or group polarizabilities)[24] in this type of calculations is important and that the unusually high C=O dipole moment suggested by Figure 3 represents an effective value that in some sense accounts for such

effects. In this context, it is also interesting to note that a similar value of the C=O partial charge was found to be required for maintaining the crystallographically observed ligand sphere for Ca^{2+} and Mg^{2+} in the "5-fold binding-site" of satellite tobacco necrosis virus.[25a]

Related Calculations of Selectivity and Molecular Recognition. Early energy minimization calculations of ion complexation with valinomycin and the macrotetralide actins have been carried out by Pullman and co-workers (for a review, see ref 2i). Although these studies were done without solvent and with rigid host molecules, the interplay between different energy components underlying selectivity was described in a qualitative way. With the emergence of FEP methods a number of interesting studies of molecular recognition in synthetic ion-binding hosts have been reported.[2a,c,e,f,j] Lybrand et al.[2a] have evaluated the relative binding affinity of Cl^- and Br^- to the macrotricyclic receptor SC24 and the corresponding hydration free energy difference between the two ions. This case represents an example of anion recognition by $-NH^+$ ligands and the computed results were found to be in excellent agreement with experiment. A similar study addressing the cation affinity of 18-crown-6 in methanol has also been reported.[2e] Here the Na^+/K^+ selectivity was calculated by FEP simulations and both the differential solvation free energies and relative binding energies were reasonably well reproduced.

Obviously, calculations of ion selectivity that involve more than two ions provide a more stringent test of the computational models, particularly if the system has nontrivial (nonmonotonic) selectivity properties. Such studies have been reported by van Eerden et al.,[2c] Grootenhuis and Kollman,[2f] and by Auffinger and Wipff.[2j] The work of ref 2c evaluated the $Na^+/K^+/Rb^+$ selectivity of 18-crown-6 in water using FEP methods. While the K^+/Rb^+ selectivity was reasonably well modeled, the calculations of Na^+ vs K^+ binding appeared more problematic and yielded different free energies depending on the starting structure. The differential hydration energy of Na^+ vs K^+ was also overestimated by as much as 4 kcal/mol. The calculations reported in ref 2f on the alkali cation selectivity of dibenzo-18-crown-6 and dibenzo-30-crown-10 is another example that illustrates the difficulties with obtaining quantitatively correct selectivity properties for a series of ions. The simulations of $Na^+/K^+/Rb^+$ complexation with the 222 cryptand[2j] appears to be an example of rather successful selectivity calculations in synthetic host systems. In this case, the correct $K^+ > Rb^+ > Na^+$ sequence was obtained, although the observed free energy differences were not quantitatively reproduced. The study by Auffinger and Wippf also addressed the problems associated with conformational sampling by performing calculations from different initial structures of the 222 cryptand.[2j]

We have also reported earlier calculations on valinomycin[5] that were carried out in vacuum using both the MOLARIS[14] and GROMOS[13] force fields. The reason for not including solvent in these calculations was the experimental evidence for isostericity in low-dielectric solvents.[5] The results reported in the preliminary work of ref 5a with the MOLARIS potential are in qualitative agreement with the present study, although the two force fields used are rather different. Furthermore, it is interesting to note that the vacuum calculations using the GROMOS potential[5b] give nearly quantitative agreement with the present study in methanol. This is in accord with the experimentally observed "invariance" of the relative complexation constants to the solvent composition (for low-dielectric solvents).[5]

Concluding Remarks

The present study has elucidated several important aspects of ion selectivity in a simple ionophore system and demonstrated the influence of the main factors that govern the selectivity pattern. The two key elements determining the selectivity sequence for a series of ions such as the alkali metals are the free energy of solvation in the surrounding medium and the strength of the electrostatic interaction with the closest ligands. As predicted earlier by Eisenman's selectivity theory,[3] it is essentially the balance between these two factors that give rise to a set of well-defined selectivity sequences related to each other in a systematic way. However, the calculations also show that steric factors such as the flexiblity of the ion binding site, or rather the ability of the ligands to pack tightly around a given ion, contribute significantly to the overall selectivity properties of the host molecule.

The simulations further indicate that the ion–valinomycin complexes are more susceptible to attacks from the solvent from the Lac face of the host. This asymmetry with respect to the solvent interactions suggests that the pathway for ion unloading is directed through the Lac face rather than the Hyl face on the opposite site of the complex.

We have found that the experimental results for ion complexation with valinomycin in methanol can be quantitatively reproduced by using ligand carbonyl partial charges of about ±0.58. As discussed above, the fact that this value is somewhat higher than that normally expected for carbonyl groups in proteins or liquid compounds may be indicative of a significant polarization of the carbonyls by the field from the ion.

In a relatively simple ion-binding system as valinomycin it is possible to carry out rather extensive FEP/MD simulations in a systematic way, as shown here. This is quite important in order to examine the performance of molecular mechanics force fields in detail and provides a useful basis for studies of more complicated systems, such as protein sites.[25] Valinomycin may also be a good test case for the study of actual ion loading/unloading processes since the corresponding rate constants have been determined experimentally.[7]

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Registry No. Rb, 7440-17-7; K, 7440-09-7; Cs, 7440-46-2; Na, 7440-23-5; Li, 7439-93-2; valinomycin, 2001-95-8.

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Excess Partial Molar Free Energies, Enthalpies, and Entropies in 2-Butanone–H_2O Mixtures: Solute–Solute Interactions

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The excess partial molar enthalpies, $H_m^E(i)$, in 2-butanone (BUT)–H_2O mixtures were measured at 4.20, 20.00, and 25.00 °C (i = BUT or H_2O). The vapor pressures were also measured by a static method at 20.00 and 25.00 °C. From the latter data, the partial pressures and hence the excess partial molar Gibbs free energies, $G_m^E(i)$, were calculated by the Boissonnas method. The excess partial molar entropies, $S_m^E(i)$, were then calculated at 20.00 and 25.00 °C. From the composition derivatives of these partial molar quantities, the nature of the solute–solute interactions was discussed in relation to the global information about the mixture: existence of phase separation, the way in which the phase boundary slants, and existence of azeotropy and addition compound.

Introduction

The *partial* molar enthalpy of component i, $H_m(i) = (\partial H/\partial n_i)_{n_j}$, or the excess *partial* molar enthalpy, $H_m^E(i) = (\partial H^E/\partial n_i)_{n_j}$, is the actual enthalpic contribution of the i-th species in the mixture at a given condition. It is evaluated by changing the amount of the i-th species, n_i, infinitesimally keeping the amounts of other components, n_j, constant and measuring the response, in terms of enthalpy, of the entire system toward such a perturbation. Consider next the derivative

$$(\partial H_m^E(i)/\partial n_j) = \{(1 - x_j)/N\}(\partial H_m^E(i)/\partial x_j)$$

where x_j is the mole fraction of the j-th species in the mixture. This derivative signifies the effect of an additional j-th species on the excess partial molar enthalpy of the i-th species, or the influence of the j-th species upon the i-th species in terms of enthalpy. Thus, it is an indicator of the i–j interaction in terms of enthalpy. If accurate values of $H_m^E(i)$, within 0.5% say, are available in small enough increments in concentration, then the derivatives can be evaluated within a reasonable accuracy, within several percent, and the information about the i–j interactions in terms of enthalpy is obtained. Exactly the same argument is applicable for other partial molar quantities: excess Gibbs free energy, $G_m^E(i)$, entropy, $S_m^E(i)$, volume, $V_m^E(i)$, etc.

In previous papers from this laboratory, such quantities have been reported for the mixtures of H_2O–D_2O,[1] *tert*-butyl alcohol (TBA)–H_2O,[2-6] 2-butoxyethanol (BE)–H_2O,[7-13] and isobutyric acid (IBA)–H_2O.[14] Using these data, a deeper insight into the nature of each mixture, the intermolecular interactions in particular, became more apparent than before.

In the BE (2-butoxyethanol)–H_2O system, for example, it was pointed out that there are three concentration regions within a single-phase domain, in each of which the mixing scheme is qualitatively different from those in the other regions. Moreover, the crossover from one scheme to the next is associated with anomalies in the thermodynamic quantities that are proportional to the third or the fourth derivatives of the Gibbs free energy. While the situation for TBA (*tert*-butyl alcohol)–H_2O is quite similar to that of BE–H_2O, it was found to be completely different for IBA (isobutyric acid)–H_2O. That is, the enthalpic IBA–IBA interaction at low concentration of IBA is more weakly repulsive than the cases for BE–H_2O and TBA–H_2O. In the range $x_{IBA} > 0.03$, however, the IBA–IBA interaction becomes attractive in terms of enthalpy, which naturally leads to phase separation with the UCST (upper critical solution temperature) at a higher concentration. The crossover at about $x_{IBA} = 0.03$ is associated with a peak anomaly in the fourth derivative of the Gibbs free energy.

This work is a study on the 2-butanone (BUT)–H_2O system using the same methodology.

Experimental Method

BUT (2-butanone) of Aldrich, HPLC, 99.7%, was used without further purification. H_2O was freshly distilled immediately before use.

The excess partial molar enthalpies, $H_m^E(i)$ (i = BUT or H_2O), were measured by using an LKB 8700 Bromma Precision Calorimeter. We used the titration method, the details of which were described elsewhere.[3]

The vapor pressures were measured by a static method and analyzed by the Boissonnas method described in detail in the previous papers.[5,6,9] From the resulting values of the excess partial molar Gibbs free energies, $G_m^E(i)$, and those of $H_m^E(i)$, the values of $S_m^E(i)$ were obtained.

As ancillary information, the freezing points of the mixtures were determined by cooling curve. The temperature was followed on a chart recorder by a copper–constantan thermocouple within ±1 °C.

Results, Discussion, and Conclusion

All the data about the phase diagram available in literature, as well as our observation, are shown in Figure 1. The fact that there is an eutectic at −88.8 °C and $x_{BUT} = 0.996$[17] and the fact that the liquidus curve seems to have the maximum at about $x_{BUT} = 0.7$[17] hint existence of a solid addition compound at about $x_{BUT} = 0.7$. Indeed, in the concentration range $0.15 < x_{BUT} < 0.7$, our

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