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Host/guest conformations of biological systems: valinomycin/alkali ions

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Abstract

Collision cross sections of gas phase valinomycin–alkali ion complexes were measured in helium using the ion mobility based ion chromatography technique. For the lithiated and sodiated species a value of 267 $Å^2$ was measured whereas the cross sections for the potassiated, rubidiated, and cesiated complexes were larger 272, 277, and 279 $Å^2$, respectively. The systematic increase with ion size indicates that the backbone folding of the cyclic valinomycin molecule is dependent on the choice of alkali ion. This result is in good agreement with theoretical cross sections of model structures obtained by molecular mechanics simulations. The model structures demonstrate that the valinomycin host completely encapsulates the alkali ion with five or six of the polar carbonyl groups in the first solvation sphere of the alkali ion. The polar core of the complex is shielded by the aliphatic valinomycin side chains, which were found to be predominant on the complex surface. The lithium ion is solvated by a fivefold carbonyl coordination sphere with at least four of the five carbonyls belonging to valine units. The sodiated species exhibits a five- to sixfold carbonyl coordination with highly excited O. . .Na^+ vibrations at 300 K. In the potassiated and cesiated complexes the alkali ion is coordinated by six valine carbonyl groups in a near octahedral arrangement causing the valinomycin backbone to fold in a quasi-S_6 symmetric fashion. These results demonstrate that the overall size and shape of the complex is not quite the same for different alkali ions, in contrast to conclusions made from solution salt extraction experiments and assumptions made in previous molecular mechanics calculations. However, our results were found to be in good agreement with earlier spectroscopic studies carried out on alkali salt valinomycin crystals and solutions thereof in organic solvents. Relative alkali ion–valinomycin binding energies extracted from the molecular mechanics data were able to qualitatively explain the experimentally observed preference of valinomycin for hosting potassium over lithium and sodium. (Int J Mass Spectrom 193 (1999) 143–152) © 1999 Elsevier Science B.V.

Keywords: Valinomycin; Ion mobility; Ion chromatography; Host/guest; Tertiary structure; Ion transport

1. Introduction

Understanding a phenomenon like the ion selectivity of antibiotics in membrane permeation processes on a molecular level, and on the basis of the thermochemistry involved in the complex system of reactions describing the phenomenon, is one of the central problems in biochemistry and biophysics. In order to solve such a problem the relevant molecular species have to be identified, their structures have to be analyzed, a reaction mechanism has to be proposed, and finally the thermochemistry for the individual steps in the mechanism have to be worked out. This

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latter step requires measurements of the individual elementary reactions, which for biological systems are generally not accessible. Although experiments on model systems often provide valuable insight, there is also the hope that computational chemistry will eventually be able to make significant contributions to the assessment of the energetics in biochemical reactions.

One active field of research is the investigation of the mechanism where neutral molecules, like the antibiotic valinomycin, make phospholipid bilayer membranes selectively permeable to cations [1–4]. The cation selectivity of the cyclodepsipeptide valinomycin, involving three repeats of the sequence (L-valine)–(D-α-hydroxyisovaleric acid)–(D-valine)–(L-lactic acid), has extensively been studied in solution revealing a remarkable 10^4–10^6-fold preference for formation of a K^+–valinomycin complex over the corresponding Na^+ species [5–8]. Also, the structure of valinomycin both as a neutral and as a cation host has been researched in numerous studies by x-ray analysis in crystals [9,10], by a number of methods including IR and ^{1}H-NMR in solution [7,11–18], and theoretically by molecular mechanics approaches [8, 19, 20]. These studies all agree that the structure of potassiated valinomycin is characterized by a near S_6 symmetric folding of the cyclic backbone, whereas there is some ambiguity about the structures of the lithiated and sodiated complexes. In some molecular mechanics studies [8,19] Gibbs free energy differences between sodium and potassium systems were calculated in an attempt to reproduce the experimentally observed cation selectivity and to fine tune the force field used in the calculations. The problem in these kinds of calculations to date is that there are too many unknowns, such that often important assumptions have to be made. For instance, in the studies mentioned previously [8,19], assumptions about the valinomycin structure were made which might be problematic as will be outlined in this article.

In this study we will present both experimental and theoretical results about the gas phase structure of valinomycin hosting different alkali ions. These solvent free structures are expected to be very similar to those in nonpolar organic solvents or in lipid membranes. The valinomycin structure in all of these systems is determined by the strong electrostatic host–guest (valinomycin–alkali ion) interactions rather than by the weak van der Waals type of interactions between valinomycin and its environment (organic solvent/lipid). Thus, experimental gas phase data are very valuable both for comparison with results of in vacuo molecular mechanics calculations and for additional insight in structures of biomolecules in hydrophobic environments such as lipid membranes. Valinomycin has been studied by means of mass spectrometry before but these previous studies were not directed toward obtaining information about the three-dimensional structure of valinomycin–alkali ion complexes [21,22].

2. Experimental methods

The experimental setup for the ion mobility based ion chromatography technique has been described in detail elsewhere [23] as has been the matrix assisted laser desorption ionization (MALDI) source [24] employed here. Sample preparation was analogous to the peptide work published recently [25,26] with the only difference being that isopropanol was used to dissolve valinomycin instead of a water/methanol mixture. Alkali ions were added to the sample/matrix solution in the form of alkali halide salts LiCl, NaI, KI, RbI, and CsF in large excess to the valinomycin concentration.

Briefly, an ion pulse leaving the MALDI source was mass selected in a double focusing reversed geometry mass spectrometer, and injected into a 4 cm long drift cell filled with $\sim$3 Torr of helium. Ions were pulled through the cell by a weak electric field of 5–25 V/cm and detected after exiting the cell as a function of their arrival time. From the ion drift time, the drift velocity was obtained which is proportional to the ion mobility in helium and inversely proportional to the ion–helium collision cross section. Thus, relative cross sections are readily determined by the relative drift times, but absolute values are subject to systematic errors since such quantities as the effective cell length, effective temperature, and pressure are used in the calculation [27]. Those systematic errors

are estimated to be ±1%. Statistical errors based on the reproducibility of experiments are less than ±1%.

3. Theoretical methods

Molecular mechanics was employed to generate model structures of the valinomycin–alkali ion complexes $[VM + X]^+$. The AMBER 4 suits of programs [28] was used together with the standard AMBER force field [28,29]. Typically 100 model structures were created for each complex via a simulated annealing procedure described previously [25,26]. The structures thus obtained could be divided into groups, each group containing valinomycin conformations with similar structural features. Although each group covered a wide energy range overlapping with other groups, it was obvious that certain groups were lower in energy than others. Cross sections of optimized structures reported in the following refer to a typical value of a member of the lowest energy group.

Although the focus of this work was on structure rather than accurate energy values, some effort was made to locate particularly stable structures. For instance, in some cases it was observed, that a certain $[VM + X_i]^+$ structure was several kilocalories per mole more stable than the rest of the group members. Such structures found for one alkali ion X_i were paid particular attention, and it was checked whether those structures were also unusually stable for the other alkali ions X_j, $X_j \neq X_i$. Thus, the stable $[VM + X_i]^+$ structure was used as input for a $[VM + X_j]^+$ optimization. Energy values reported below correspond to the most stable structure found by any means for each alkali ion.

Molecular dynamics simulations at 300 K were performed in order to investigate the degree of flexibility of some of the lowest energy structures found with the annealing method. These dynamics calculations were based on 0.5 fs time steps whereas bonds involving hydrogens were constrained using the SHAKE algorithm [28].

For comparison with experiment both optimized geometries and snapshot structures from the dynamics calculation were used to determine theoretical cross sections. The procedure employed in this work was based on Monte Carlo integrations of many randomly chosen projections [30] using atomic collision radii, which were calculated from the ion–helium interaction. The Lennard-Jones parameters r and ϵ used for these interactions were 2.58 Å/0.340 kcal/mol for H–He, 2.98/0.357 Li–He, 3.28/0.370 C, N, O–He, 3.13/0.364 Na–He, 3.36/0.374 K–He, 3.58/0.383 Rb–He, 3.80/0.393 Cs–He. The well depths ϵ were the same as those used for ions composed of ~60 atoms [26,31], whereas the values for the radii reflect a slight scaling by 1.0846 to account for the increased interaction of valinomycin with 169 atoms compared to a molecule with 60 atoms (in analogy to work described in [26]). The value of this scaling factor was the result of an extensive study of the ion–helium interaction for ions composed of 10–170 atoms [32]. Statistical errors due to the numerical integrations were less than ±1% whereas systematic errors due to imperfections in the model are expected to be of the order of ±2%.

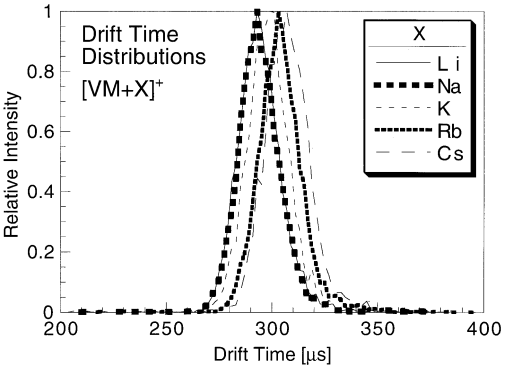

Fig. 1. Experimental drift time distributions of valinomycin–alkali ion complexes $[VM + X]^+$ recorded at a pressure of ~2.9 Torr of helium and an electric field of ~95 V across the 4 cm drift cell.

4. Results and discussion

Typical experimental ion drift time distributions obtained for the different valinomycin–alkali ion complexes $[VM + X]^+$ are shown in Fig. 1. Cross sections derived from typically about ten measurements for each ion are compiled in Table 1. It can be seen that the cross sections for $[VM + Li]^+$ and

Table 1
Experimental and theoretical cross sections[a] in square angstroms for valinomycin–alkali ion complexes

	Experiment	Theory	
		Rigid[b]	Dynamic[c]
Li^+	267.2	253.5	256.2
Na^+	266.9	252.3[d]	257.0
K^+	272.4	255.6	260.1
Rb^+	276.8	257.8	260.9
Cs^+	279.0	260.3	263.8

[a] Systematic errors are estimated to be ±1% for experimental and ±2% for theoretical values. The relative values are more accurate with statistical errors less than ±1% for both experiment and theory.

[b] Cross sections obtained from geometry optimized structures (Figs. 3, 5, and 6).

[c] Average cross sections obtained from 2000 structures of a 100 ps molecular dynamics calculation at 300 K.

[d] Same value (within ±0.5%) for both the lowest energy structure with sixfold Val >C=O coordination and the structure shown in Fig. 5.

$[VM + Na]^+$ are about equal, whereas those for $[VM + X]^+$, X = Na–Cs, increase monotonically in the order Na, K, Rb, Cs. This observation is direct experimental evidence that the folding of the host valinomycin is dependent on the choice of the guest alkali ion (Na^+–Cs^+). This result is perhaps not surprising, but it is an important result because there is some uncertainty in this respect in the molecular mechanics community where a fair amount of work has been done on valinomycin–alkali ion complexes in the absence of solvent [8,19,20].

Our molecular mechanics calculations clearly support the experimental cross section result. Geometry optimized structures obtained by simulated annealing procedures show that the valinomycin folding is different for each alkali ion complex. In the $[VM + Li]^+$ complex the Li^+ ion is solvated by five valine backbone carbonyl oxygens with Li–O bond lengths of the order of 2.0 Å. The Li–O bond length distributions of a ~50 ps dynamics simulation at 300 K are shown in Fig. 2(a). Five valine carbonyl oxygens are found at a distance of ~2 Å from Li^+, whereas another five lactic and hydroxyisovaleric acid oxygens are found between 3.5 and 5 Å. The remaining two carbonyl oxygens are in the 6–7 Å range. The

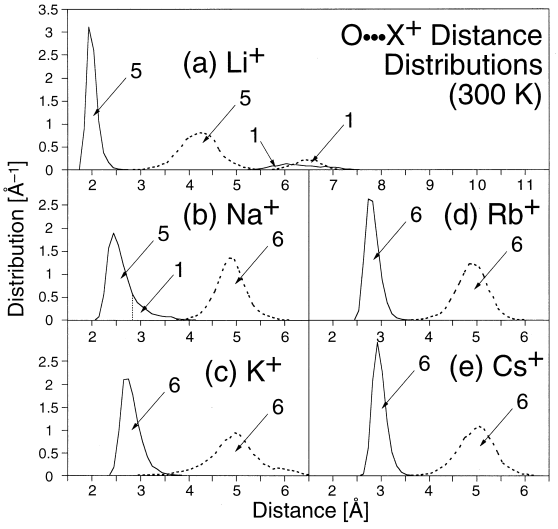

Fig. 2. Distribution functions of the distance between carbonyl oxygens and alkali ion obtained from 50 ps molecular dynamics runs of valinomycin–alkali ion complexes at 300 K. Distributions for the six carbonyl oxygens of D- and L-valine are shown as solid lines, those of D-α-hydroxyisovaleric and L-lactic acid as dashed lines. The numbers next to the arrows indicate the number of atoms expected within a given distance range (proportional to integrated area of the distribution function). Alkali ion is (a) Li^+, (b) Na^+, (c) K^+, (d) Rb^+, (e) Cs^+.

corresponding energy minimized structure with fivefold Val >C=O coordination is shown in Fig. 3. Some of the structures with somewhat (~2 kcal/mol) higher energy include coordination of one lactate >C=O in addition to a fourfold Val >C=O solvation.

The structures of $[VM + Na]^+$ found by simulated annealing exhibit a fivefold Val >C=O coordination

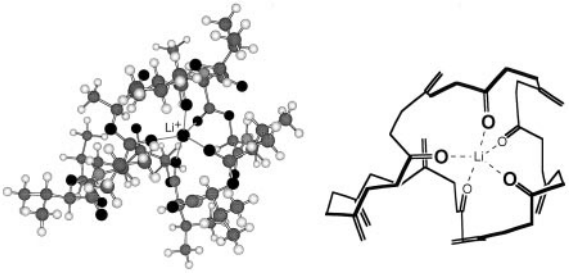

Fig. 3. Structure of a geometry optimized valinomycin–lithium complex. (a) Ball and stick model; (b) schematics of the backbone folding. The three-dimensional shape of the complex is a somewhat elongated (prolate) ellipsoid. In the schematic projection shown three of the oxygens bound to lithium are located in a plain above the lithium ion (bold), two in a plain below (light).

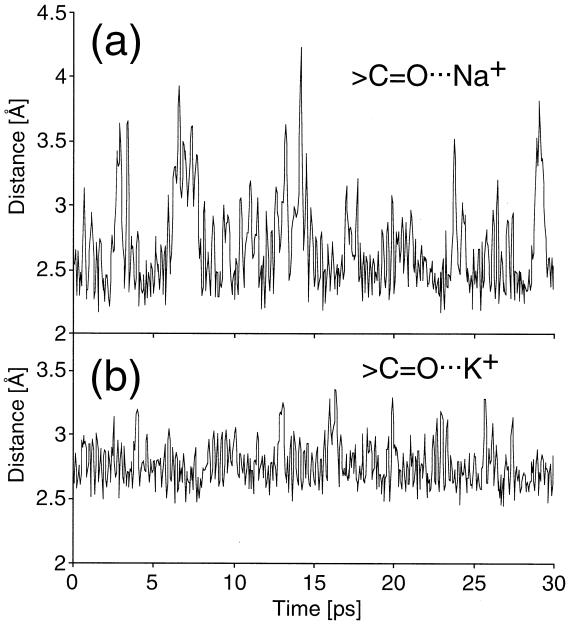

Fig. 4. Distance between alkali ion and Val[1] carbonyl oxygen as a function of time as observed in 300 K molecular dynamics simulations of alkali ion–valinomycin complexes. Alkali ion is (a) Na^+ and (b) K^+.

around Na^+ with an additional lactate or hydroxyisovalerate >C=O coordination in some cases. The Na–O distances for valine oxygens are ~2.3 Å, that for the hydroxyisovalerate oxygen ~2.5 Å. Simultaneous solvation of Na^+ by all six Val >C=O oxygens is not found by simulated annealing. However, when the coordinates of one of the highly symmetric $[VM + K]^+$ structures (see the following paragraph) are used as input for a straight $[VM + Na]^+$ geometry optimization, a stable valinomycin–sodium complex with sixfold Val >C=O coordination can be obtained (~2.5 Å Na–O bond lengths). In fact, this symmetric structure is ~3 kcal/mol more stable than any of the structures found by simulated annealing. It should be noted, however, that the symmetric $[VM + Na]^+$ structure is not stable at room temperature. Molecular dynamics simulations show the Na–O vibrations are highly excited at 300 K even when the complex is carefully heated from 0 to 300 K [Fig. 4(a)]. During those Na–O vibrations which occur rapidly on a 100 ps time scale the Na–O bonds can

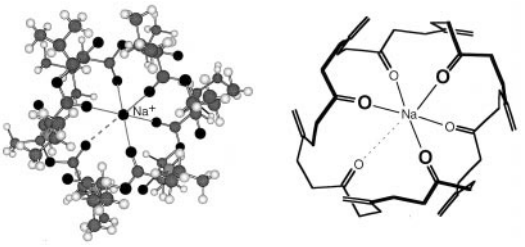

Fig. 5. Structure of a geometry optimized valinomycin–sodium complex. (a) Ball and stick model; (b) schematics of the backbone folding. Note, that the three-dimensional shape of the complex is not planar, but rather spherical. In the projection shown three of the oxygens bound to sodium are located in a plain above the sodium ion (bold), three in a plain below. The dashed line between Na^+ and one of the carbonyl oxygens indicates an elongated bond.

stretch to 4 Å and beyond. Thus, in average the complex exhibits five tighter Na–O bonds (~2.5 Å) and one looser bond [Fig. 2(b), solid line]. Some side chain orientations at higher energy result in asymmetric $[VM + Na]^+$ structures with five tight Na–O bonds (~2.5 Å) and one loose one (~3.3 Å), which are actual minima on the potential energy surface (Fig. 5) with energies below that of the corresponding symmetric backbone folding.

The valinomycin conformation in the $[VM + K]^+$ complex shown in Fig. 6 is characterized by its highly symmetric folding of the backbone in near S_6 symmetry. The K^+ ion is solvated by all six Val >C=O oxygens in a near perfect octahedral arrangement. The K–O bond lengths are ~2.7 Å. There is also a family

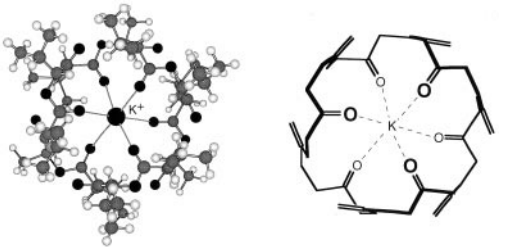

Fig. 6. Structure of a geometry optimized valinomycin–potassium complex. (a) Ball and stick model; (b) schematics of the backbone folding. Potassium is bound to six amid carbonyl oxygens in a octahedral arrangement yielding a near spherical valinomycin shape. In the projection shown three of the oxygens bound to potassium are located in a plain above the potassium ion (bold), three in a plain below.

of fivefold Val >C=O coordination structures that is less favorable and ~10 kcal/mol higher in energy. Some of these latter structures have an additional lactate >C=O coordination, some do not. Structures with sevenfold coordination are also found but are also much less stable (by >10 kcal/mol) than the structures with the symmetric $(>C=O)_6K^+$ coordination. The S_6 type of structure was also found in the $VM \cdot KI_4$ and $VM \cdot KAuCl_4$ crystals by x-ray [9,10] analysis and by a number of molecular mechanics studies on $[VM + K]^+$ [8,19,20]. Spectroscopic studies of $[VM + K]^+$ in organic solvents [7,11–18], including ORD, IR, CD, and ^{1}H-NMR techniques, supported a highly symmetric crystal-like structure in solution with six equivalent >C=O ester, six equivalent >C=O amide, and six equivalent >N–H units. Numerous salt extraction equilibrium measurements [8], measurements of complexation constants in methanol [7], permeability ratios in bilayers [8], conductance ratios in bilayers [5], and bulk-phase electrode selectivities [6] indicate that $[VM + X]^+$ complexes, X = Na–Cs, are "isosteric," that is "the size, shape, and chemical properties of the complex are virtually independent of the particular cation bound" [33]. However, the degree of structural similarity has to be put in perspective on the basis of solution spectroscopic studies. In particular, solution IR results show distinct differences between the spectra of $[VM + Li]^+$, $[VM + Na]^+$, and $[VM + K]^+$ obtained in organic solvents, which clearly indicate that the $[VM + Li]^+$ and $[VM + Na]^+$ structures are less symmetric than $[VM + K]^+$ [7]. This result is in very good qualitative agreement with our molecular mechanics structures and with our experimental cross section observations.

All of the $[VM + Rb]^+$ and $[VM + Cs]^+$ structures that we found by molecular mechanics in the lowest 15 kcal/mol range exhibit a sixfold Val >C=O coordination with no other additional coordinations. The backbone in these structures is folded in a similar manner to the lowest energy $[VM + K]^+$ structure with near S_6 symmetry (Fig. 6). However, superposition of the $[VM + K]^+$, $[VM + Rb]^+$, and $[VM + Cs]^+$ structures shows that the backbone dihedral angles are somewhat different in all cases yielding slightly more expanded shapes for the complexes with larger alkali ions compared to potassium. Fig. 7 demonstrates that the average distance of the backbone atoms from the alkali ion increases monotonically with increasing alkali ion size Li^+–Cs^+.

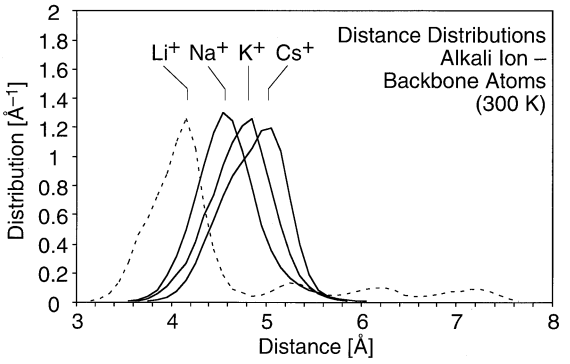

Fig. 7. Distribution functions of the distances between the backbone atoms and the alkali ion obtained from 50 ps molecular dynamics runs of valinomycin–alkali ion complexes at 300 K. The backbone atoms included are the 6 amid nitrogens, the 6 ester linkage oxygens, the 12 α-carbon atoms, and the 6 carbonyl carbons of D-α-hydroxyisovaleric and L-lactic acid. Data for rubidium is very similar to that of potassium and cesium and is omitted for clarity.

The cross sections calculated for the different structures discussed previously are given in Table 1 along with the experimental data. It can be seen that $[VM + Li]^+$ and $[VM + Na]^+$ have about the same cross section and that the size of the structures increases continuously with increasing alkali ion size from Na^+ to Cs^+ in perfect agreement with the experimental pattern. However, the theoretical structures exhibit ~6% smaller cross sections than those observed experimentally at 300 K. This discrepancy could partly be due to shortcomings in the model used for cross section calculations of theoretical structures. In our model geometrical cross sections are evaluated rather than momentum transfer collision integrals, the quantity describing the cross section of a scattering process. However, it is not surprising that the theoretical cross sections are smaller than the experimental since the rigid energy minimized model structures are expected to be more compact than ions undergoing molecular motion at room temperature. In an attempt to quantify this dynamic effect, 100 ps molecular dynamics calculations were carried out at 300

K starting from the energy minimized structures. The averages of the cross sections calculated at 50 fs intervals during those molecular dynamics runs are indicated in Table 1 as well. It can be seen that the 0 K energy minimized structures expand by ~2% when allowed to undergo molecular motion at 300 K. Thus, these increased cross sections agree better with experiment bringing the values within the range of the combined error limits of experiment and theory. It is possible that more extended motion is actually occurring during the experimental time scale of several 100 μs and that the motion of the 100 ps simulation is trapped in the local minimum of the starting structure. For instance rotation of the isopropyl Val and hydroxyisovalerate side chains are generally not observed during 100 ps simulations at 300 K, because these rotations are hindered by barriers of several kcal/mol [34]. Interconversions between structures with different alkali ion coordination is only observed in a few limited cases on a 100 ps time scale.

One example for such interconvertions has been mentioned above for $[VM + Na]^+$, where rapid $>C{=}O\ldots Na^+$ vibrations are observed [Fig. 4(a)]. The $O–Na^+$ distances to all six valine carbonyl oxygens oscillate in a very similar manner as the one shown in Fig. 4(a) for Val^1 and there exists no obvious synchronization between the six degrees of freedom. The structures of valinomycin hosting other alkali ions than sodium appear more rigid on a 100 ps time scale. As shown in Fig. 4(b) for Val^1, typical $O–K^+$ vibrations are much less vigorous than the corresponding $O–Na^+$ oscillations. However, at any given time interval of several tens of picoseconds one out of the six $O–K^+$ bonds appear to be more likely to undergo larger excursions, up to 4 Å in a few short instances. But in contrast to the sodiated complex, which is asymmetrical most of the time, the potassiated species is near symmetrical most of the time [see Fig. 2(c)]. Obviously any one of the six valine residues can be the one with higher probability for larger excursions, and we observe multiple bond stretching beyond 3.4 Å for Val^2 at the beginning, for Val^3 and Val^6 in the middle, and for Val^4 during the second half of our 100 ps dynamics simulation. Another example for structural changes observed on a 100 ps time scale is found in lithiated complexes. Any one of the five $O–Li^+$ bonds is observed to loosen up (3 Å) or even break (5 Å), but reformation of the same bond occurs in each case. However, interconvertion between a structure with coordination of the Val^i $>C{=}O$ oxygens, $i = 1, 3, 5, 7, 9$, and one with Val^j coordination, $j = 3, 5, 7, 9, 11$, does not take place in any of the 100 ps simulations performed starting with different $[VM + Li]^+$ structures.

Table 2
Relative alkali ion–valinomycin binding energies $\Delta\Delta E$ obtained from molecular mechanics calculations, relative alkali ion hydration enthalpies $\Delta\Delta H_{hydr}$, and selectivity ratios $r = \exp[-(\Delta\Delta E - \Delta\Delta H_{hydr})/RT]$

	$\Delta\Delta E$ (kcal/mol)	$\Delta\Delta H_{hydr}$ [a] (kcal/mol)	r
Li^+	−38.7	−46.3	1/340 000
Na^+	−13.5	−20.2	1/72 000
K^+	0	0	1
Rb^+	6.5	5.9	1/2.7
Cs^+	18.2	13.8	1/1600

[a] [35].

In order to speed up molecular motion and make it visible on a 100 ps time scale simulations at a temperature of 500 K were carried out for $[VM + Li]^+$ and $[VM + Cs]^+$. As expected, the average cross section increases in both cases by about an additional 1% due to the significantly increased molecular motion. This additional motion might well take place in the experiment even at 300 K, but the 300 K simulations are too short to detect it.

Although the emphasis in this study is on structural aspects of alkali ion–valinomycin complexes, the molecular mechanics results can be used to determine relative alkali ion binding energies in an attempt to explain the extreme preference of valinomycin for hosting K^+ (and Rb^+/Cs^+) rather than Li^+ or Na^+. The theoretical binding energies obtained for the lowest energy $[VM + X]^+$ structures (X = Li–Cs) relative to $[VM + K]^+$ are given in Table 2. Not surprisingly it can be seen that the strongest bond is formed in $[VM + Li]^+$ and the weakest in $[VM + Cs]^+$. However, the ligand valinomycin has to compete with the solvent water for hosting an alkali ion. In fact, to account for entropic effects free energy

Table 3
Selectivity ratios[a] for the preference of valinomycin for hosting K^+ relative to other alkali ions obtained by molecular mechanics calculations (this work) and by a variety of experimental methods

	This work ($\Delta\Delta E - \Delta\Delta H_{hydr}$)	This work[b] ($\Delta\Delta E - \Delta\Delta G_{hydr}$)	Salt extraction equilibria[c]	Complexation constants[d]	Bilayer permeability[c]	Lipid membrane conductance[e]	Liquid membrane electrode selectivity[f]
Li^+	1/340 000	1/110	1/5 300 000		1/210 000	1/400	1/5000
Na^+	1/72 000	1/940	1/400 000	1/6400	1/28 000	1/280	1/4000
K^+	1	1	1	1	1	1	1
Rb^+	1/2.8	1/10	2.1	2.2	1.8	2.3	1.9
Cs^+	1/1600	1/8500	1/3.5	1/3.8	1/1.3	1/1.9	1/2.5

[a] Selectivity ratios calculated in this work using the formula $r = \exp[-(\Delta\Delta E - \Delta\Delta H_{hydr})/RT]$ or $r = \exp[-(\Delta\Delta E - \Delta\Delta G_{hydr})/RT]$. All other selectivity ratios were experimentally determined.
[b] Values for $\Delta\Delta G_{hydr}$ relative to K^+ are −41.5 kcal/mol (Li^+), −17.7 (Na^+), 5.1 (Rb^+), 12.8 (Cs^+) [35].
[c] [8].
[d] [7].
[e] [5].
[f] [6].

(ΔG) quantities should be compared. However, ΔG values for $[VM + X]^+$ are not available from this study and it has to be assumed that entropy effects are negligible. It could be argued that entropy rather favors the unsymmetrical $[VM + Li]^+$ and $[VM + Na]^+$ complexes compared to the symmetric $[VM + K]^+$, $[VM + Rb]^+$, and $[VM + Cs]^+$. Thus, K^+/Na^+ selectivity ratios obtained from $[VM + X]^+$ energy (rather than free energy) quantities may unduly favor K^+ coordination. Entropic effects of hydrated alkali ions, on the other hand, can be accounted for by using free energy of hydration data available in the literature instead of hydration enthalpies [35]. Using Table 2 it can be seen that $[VM + K]^+$ complexes are indeed preferably formed compared to $[VM + Na]^+$ by a factor of 70 000 (or 900 when $\Delta\Delta G_{hydr}$ values are used; see Table 3). Many different experiments have been carried out to measure this K^+/Na^+ selectivity ratio covering a large range of values from 280 to 400 000 [5–8,19]. The most precise values appear to be obtained by the salt extraction equilibrium experiments [8,19,33]. In this method the ratio of amount of alkali ions dissolved in aqueous solution to amount of alkali ions hosted by valinomycin in an organic solvent is determined spectroscopically and compared for the different alkali ions Li^+ through Cs^+. The salt extraction experiments indicate a 400 000-fold preference of valinomycin for hosting K^+ compared to Na^+ and a 5×10^6 preference compared to Li^+. This is in qualitative agreement with our calculated values of 900–70 000 for K^+/Na^+ and 100–300 000 for K^+/Li^+ (Table 3). For the larger alkali ions K^+–Cs^+ all experimental techniques agree that Rb^+ is slightly preferred over K^+ by a factor of ~2, whereas Cs^+ is disfavored compared to K^+ by a factor of 2–4. This is not in agreement with our calculations which indicate a K^+ preference of 3–10 over Rb^+ and 2000–9000 over Cs^+.

Of course there arise problems when comparing energies across different systems ($[VM + Li]^+$ versus $[VM + Na]^+$). The rather large number of degrees of freedom in these systems makes it impossible to find the global minima on the potential energy surfaces. This is not a real problem when the interest is focused on structure because a simulated annealing procedure allows sampling of typical structures which are expected to be similar to the global minimum. However, for comparison of energies across systems it is important to get precise energy values for each system because an error of 1 kcal/mol is already significant. In an attempt to anneal the isopropyl side chains in $[VM + K]^+$ when the backbone is constrained by harmonic potentials in its S_6-like folding we found energy spreads of several kilocalories per

mole between structures that differed only in their side chain conformations.

As mentioned previously, ΔG values should be compared rather than ΔE. In other molecular mechanics studies [8,19] on the valinomycin–alkali ion system free energy perturbation calculations yielded excellent agreement with alkali ion selectivity experiments [5–8]. However, in those studies [8,19] the conformation of valinomycin was confined into the $[VM + K]^+$ x-ray structure by a weak harmonic potential centered at each atom for all of the $[VM + X]^+$ complexes (X = Na–Cs). This method eliminates any troubles with side chain annealing because any nonideal side chain orientation is equally nonideal for all alkali ion complexes. However, since the valinomycin folding in this $[VM + K]^+$ x-ray structure is optimum for the potassium complex and not necessarily favorable for the other alkali ion complexes there is an automatic bias towards favoring K^+ built into the model. This is potentially dangerous as demonstrated below. Both experiment and theory show that neither the $[VM + Na]^+$ nor the $[VM + Cs]^+$ structure is the same as that of $[VM + K]^+$. Although it is true that the room temperature $[VM + Na]^+$ structure with its four- to sixfold Val coordination is somewhat similar to the $[VM + K]^+$ structure (see the above mentioned $[VM + Na]^+$ molecular dynamics result) a least square fit to overlap the two types of structures yielded a root mean square atomic deviation of 0.38 Å and a potential energy due to the constraining harmonic potential (0.1 kcal/mol/Å^2 [8]) of 1.2 kcal/mol. This leads to almost an order of magnitude error in the selectivity which is substantial in view of the fact that the study [8] was an attempt to fine tune the force field used. Although we are not claiming that our study is more precise in terms of energy, our data certainly suggests one must use caution when making assumptions for boundary conditions in molecular mechanics calculations.

5. Conclusions

Ion–helium collision cross sections measured for valinomycin–alkali ion complexes indicate that the valinomycin folding is dependent on the choice of alkali ion (Na^+–Cs^+). The size of the complex is the same for Li^+ and Na^+ and it increases steadily from Na^+ to Cs^+. This result is in perfect agreement with the cross section trend found in our molecular mechanics calculations.

The calculations indicate that the valinomycin–lithium ion complex exhibits a fivefold, the sodium complex a five- to sixfold, and the potassium, rubidium, and cesium complexes a sixfold >C=O oxygen–alkali ion coordination sphere. At 0 K, the Na^+, K^+, Rb^+, and Cs^+ complexes show a highly symmetric folding of the macrocyclic backbone in near S_6 symmetry. At 300 K, the Li^+ and Na^+ complexes are unsymmetrical, with the K^+, Rb^+, and Cs^+ on average symmetrical, in agreement with spectroscopic studies carried out in solutions of organic solvents.

Relative alkali ion–valinomycin binding energies obtained from molecular mechanics data are in fair, but not quantitative agreement with the experimentally observed extreme preference of valinomycin for hosting K^+ (or Rb^+ or Cs^+) over Na^+ (or Li^+).

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