MOLECULAR DYNAMICS SIMULATION OF CATION MOTION IN WATER-FILLED GRAMICIDINLIKE PORES

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ABSTRACT A model calculation is carried out to study the potential energy profile of a sodium ion with several water molecules inside a simplified model of the gramicidin ion channel. The sodium ion is treated as a Lennard-Jones sphere with a point charge at its center. The Barnes polarizable water model is used to mimic the water molecules. A polarizable and deformable gramicidinlike channel is constructed based on the model obtained by Koeppe and Kimura. Potential minima and saddle points are located and the static energy barriers are computed. The potential mimina at the two mouths of the channel exhibit an aqueous solvation structure very different from that at any of the interior minima. These sites are ~23.6 and 24.4 Å apart for binding of a sodium ion and a cesium ion, respectively. Ionic motion from these exterior sites to the first interior minimum requires substantial rearrangement of the waters of solvation; this rearrangement may be the hydration/dehydration step in ionic permeation through the channel. Based on these results, a mechanism by which the sodium ion moves from the exterior binding site to the interior of the channel is proposed. Our model channel accommodates about eight water molecules and the transport of the ion and water within the channel is found to be single file. Results of less extensive calculations for Cs^+ and Li^+ ions in a channel with or without water are also reported.

INTRODUCTION

Many biological processes are effected by ions moving across cell membranes via ion channels. One of the most extensively studied cation permeable channels is that formed by gramicidin A (1–8), which is a linear polypeptide containing 15 amino acids. The gramicidin A-Cs^+ complex may be crystalized from methanol. X-ray studies of such crystals by Koeppe et al. (8) have shown that the cesium complex crystal forms a cylindrical helical channel ~26 Å long with a radius of 3. 4 Å (measured from the axis of helix to the center of a carbonyl or NH group). These findings are compatible with the proposed and now widely accepted single stranded β-helical structure (4) with 6.3 residues/turn and a pitch of 4.85 Å/turn (8). The NMR experiments of Weinstein et al. (10) and the electrical measurements of Bamberg et al. (11) suggest that in lipid bilayers gramicidin A dimerizes by joining the N-formyl ends together. Although it is generally believed that gramicidin A in lipid bilayers has roughly the same conformation as that in the crystalline Cs^+ complex, there is no direct experimental evidence to support this view.

The prevailing view of the energy profile for ion translation through the channel is illustrated in Fig. 1 (12). The energy barrier at the mouth is attributed to the stripping of some of the hydration water when the ion enters the channel. There is a minimum near the channel entrance that is usually identified as the binding site. The central portion of the profile is an undulating potential superimposed on the well-known broad electrostatic barrier. This undulating part is due mainly to the interactions between the charge of the ion and the dipole moments of the carbonyl and the amine groups. We will examine this portion of the potential profile in the Results and Discussion section. Many potential profiles of slightly different shape have been proposed (see, for example, reference 7 and Fig. 4 B of reference 12).

The potential profile for ion translocation in a highly simplified model polypeptide channel was first calculated by Fischer et al. (13). These calculations are extremely idealized. They impose the following restrictions and simplifications: (*a*) The ion is constrained to move along the helix axis; (*b*) The carbonyl groups are constrained to oscillate in the plane formed by the CO bond and the helix axis; (*c*) It is assumed that at equilibrium, the orientation of all the CO bonds is at an angle of 20° with the helix axis; (*d*) The amine groups, which have dipole moments of ~1 Debye, are neglected. (The carbonyl groups of their model have dipole moments of 1.35 Debye.)(*e*) The effect of water molecules is not included; (*f*) End effects are ignored as periodic boundary conditions are imposed at the channel mouth. More recent work (14), designed to consider the diffusional process, eliminates the first three of these restrictions. The new model explicitly incorporates only the carbonyl groups; the resulting helix cannot constrain the ions within the channel. To circumvent this difficulty a repulsive wall is introduced to simulate the confining effect of the peptide linkages. The model that we are presenting has none of the above restrictions and represents a substantially more complete model for simu-

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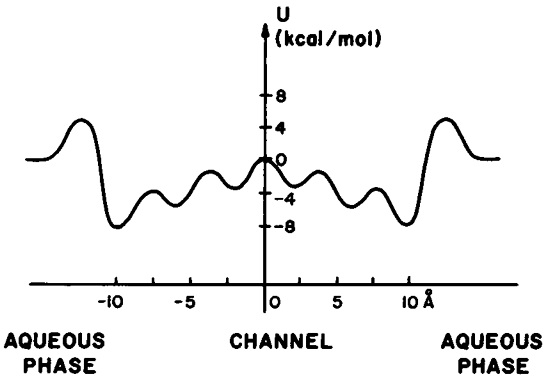

FIGURE 1 The potential profile for a cation translation through a gramicidin channel. The energy scale shown reflects only the order of magnitude, not the exact values.

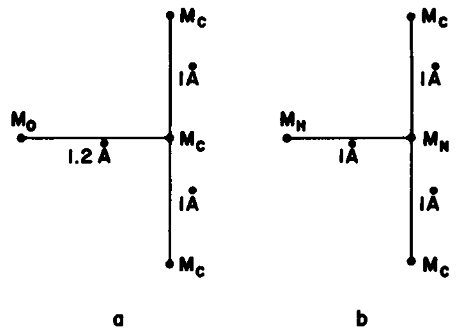

FIGURE 2 The rigid structures for calculating the effective moment of inertia tensor for (*a*) a CO group and (*b*) a NH group.

lating ion motion inside a protein channel in general and a gramicidin channel in particular.

While our model is more realistic than those analyzed by Fischer et al. (13, 14), it still incorprates only those interactions that we believe most significantly influence the structure of the potential profile for an ion in the water-filled gramicidin channel. A much more ambitious study has been carried out by Mackay et al. (15). They have performed complete molecular dynamics simulations for a series of cations in a water-filled gramicidin channel. Their model explicitly considers all nuclear degrees of freedom. Harmonic potentials describe stretching and bending; a standard torsional potential describes twisting; nonbonded atomic pairs are presumed to interact via the sum of Lennard-Jones and coulombic contributions. Polarization is, however, ignored. A wealth of information has been extracted from these calculations but they have been limited to considering the properties of the system when an ion is near a potential minimum located either near the center of the dimer or near the center of an individual monomer. Our calculations, being much simpler, permit studies to be carried out at many more points within the helix, both minima and saddle points. We are able to perform some didactic exercises to assess the sensitivity of the model calculations to the choice of force constants used to describe the motion of the helical backbone of the polypeptide. We can directly study how the presence of water molecules alters the properties of the ion-pore system. Finally we can see how inclusion of bond polarization effects the properties of the whole system.

METHOD

Model Gramicidin Channel

We construct a gramicidin channel based on the data obtained by Koeppe and Kimura (KK) by using computer modeling (9). This helix has a pitch of 4.85 Å/turn and has 6.3 residues/turn. The radius of the helix (measured from the helix axis to the centers of the carbonyl groups) is ~3.4 Å. This model helix is compatible with the unit cell of crystalline cation-bound gramicidin (8). We assume that the gramicidin channel in a lipid bilayer has the same conformation. We mimic the carbonyl and the NH groups by point dipoles located at the centers of mass computed for these groups by KK. These point dipoles will not move if the interactions between the residues are switched off. All point dipoles are assumed to be held in place by Hooke's law forces given by

$$\mathbf{F}_i = -k_e\,[\mathbf{r}_i(t) - \mathbf{r}_{oi}]\ i = i, \ldots, N_G, \tag{1}$$

where $\mathbf{r}_{oi}$ is the position of the center of mass of a carbonyl group or an NH group as given by KK, $\mathbf{r}_i(t)$ is the corresponding position at time t and $N_G = 66$ is the number of carbonyl and NH groups in our model helix. We emphasize that the displacement, $\mathbf{r}_i(t) - \mathbf{r}_{oi}$, represents the spatial deviation due to the bending deformation of the gramicidin backbone and we have, for the sake of convenience, modeled the force constant as a stretching one. We therefore have done most of our calculations by assuming $k_e = 0.5$ mdyn/Å, which is comparable to the size of most bending force constants. (For the HCH angle of C_2H_4 and the CCC angle of propane the bending force constants are equivalent to stretching force constants of 0.3 and 0.4 mdyn/Å, respectively. See also Table 8.2 of reference 16). We have also varied k_e to test our model, as will be described in the Results and Discussion section entitled An Empty Channel. According to the KK model, the carbonyl and the NH groups have well-defined orientations[1] that we denote by unit vectors $\mathbf{n}_{oi}$, $i = 1, \ldots, N_G$. We assume that whenever the ith group deviates from $\mathbf{n}_{oi}$ it will experience a restoring torque given by

$$\mathbf{J}_i = K_T\,\mathbf{n}_i(t) \times \mathbf{n}_{oi}, \tag{2}$$

where $\mathbf{n}_i(t)$ is the unit vector along the axis of the ith group at time t. In most of our calculations we have used $K_T = 0.5 \times 10^{-18}$ J, which is of the same order of magnitude as the skeletal deformation force constants (13). We assume that the effective mass of a carbonyl group is $m_O + 3m_C$, where m_O and m_C are the oxygen and carbon masses, respectively. This effective mass is chosen to account, in a very rough manner, for the α- and β-carbon atoms that are neglected in our model. The moment of inertia tensor of a carbonyl group is obtained assuming the rigid structure shown in Fig. 2 *a* and the masses are treated as point objects. This inertia tensor would, very roughly, account for the effects of the nearest neighbor carbon atoms on the angular motions of the CO group. Similarly, the effective mass of an NH group is taken to be $m_H + m_N + 2m_C$, where m_H and m_N are the masses of hydrogen and nitrogen, respectively. The moment of inertia tensor of an NH group plus the neighboring atoms is assumed to be that of the rigid unit in Fig. 2 *b*.

The carbonyl and the NH groups have permanent dipole moments along their bonds with magnitudes assigned as 2.17 and 0.96 Debye,

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[1] Koeppe and Kimura (15) do not give hydrogen atom coordinates. We assume the NH bond is 1 Å in length and that the HNC and HNCO angles are equal.

respectively (17).[2] The polarizabilities of the CO and NH groups are taken to be $\alpha_{CO} = 1.82$ Å^3 and $\alpha_{NH} = 1.44$ Å^3, respectively (17). The dipole moment of each group is the sum of its permanent dipole plus the induced dipole due to the other groups and molecules.

The nearest neighbor CO and NH groups are assumed to be noninteracting and have no inductive effect on one another because, as they are so close (~1.5 Å apart), the resultant-induced dipoles are unphysically large. If these interactions and inductive effects are incorporated into the model, the separation and the relative orientation of the nearest CO and NH group also deviate significantly from that of a realistic peptide unit. In a more complete model these rearrangements would be suppressed because they require significant bond stretching, a feature not included in our treatment. Furthermore, a quantum mechanical treatment is probably required if the groups are this close to one another.

The most significant difference between our gramicidin model and that of Mackay et al. (15) is the reduced number of degrees of freedom we consider. Their model is of a ~600 atom pore former; the corresponding number of degrees of freedom is ~1,800. Our model treats the helix as composed of 66 units; as each of these can both rotate and translate, there are ~400 degrees of freedom. This simplification reduces computational time by a factor of ~20. Another important difference is that we ignore the effect of bond stretching. As a consequence our picture is of a gramicidinlike channel; it can only be suggestive of the properties of the real pore former.

Water Model and Interactions between Molecules

We have used the polarizable electropole (PE) model for the water molecules as developed by Barnes et al. (18). In this model a water molecule is treated as a rigid body with the HOH angle taken to be 104.5° and the OH separation 1.0Å. The body coordinates of a water molecule are shown in Fig. 3. The z-axis is the symmetry axis of the molecule and the plane of the molecule is the zy-plane. The interaction between two water molecules is the sum of a Lennard-Jones interaction and an electrostatic one; the latter is due to a point dipole and a point quadrupole located at the center of mass of each molecule. The dipole moment is the sum of the permanent dipole of an isolated water and the induced dipole moment due to the surrounding molecules. The permanent dipole of the water molecules is the experimental value, 1.855 Debye along the positive z-axis (19). The quadrupole moments are $Q_{xx} = -4.844$ Debye Å, and $Q_{yy} = 5.060$ Debye Å, and $Q_{zz} = -0.216$ Debye Å, which are taken from the quantum mechanical calculations of Neumann and Moskowitz (20). The Lennard-Jones interaction is

$$V(r) = 4\epsilon\left[\left(\frac{\sigma}{r}\right)^{12} - \left(\frac{\sigma}{r}\right)^{6}\right].$$

The values of the parameters σ and ϵ for the interactions between pairs of molecules (or atom groups) are shown in Table I. We assume that the Lennard-Jones parameters for the H_2O-CO, H_2O-NH interactions are the same as the H_2O-H_2O interaction that was obtained by Barnes et al. (18). We also assume that the Lennard-Jones parameters for the ion-CO and ion-NH interactions are the same for the ion-water interaction; the latter are obtained by fitting the experimental data of Dzidic and Kebarle (21) using the procedure of Perez et al. (22). We assume that there is no Lennard-Jones interaction between the ligands of the gramicidin. That is, the CO and NH groups interact only via dipole-dipole forces. At this early stage of our investigation, we use this very simple set of Lennard-Jones parameters to deduce some qualitative feature from our model. It is well known that the Lennard-Jones parameters in the literature used by different workers vary substantially. (See, for example, Table Ia of reference 23). The ion is modeled as a Lennard-Jones sphere whose parameters are given in Table I. The cation also interacts electrostatically with its surroundings.

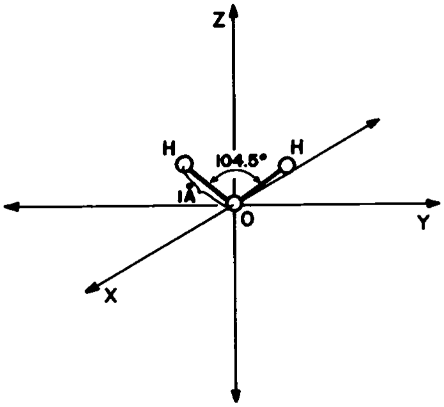

FIGURE 3 The model water molecule and its body coordinate system.

Computational Details

For a given configuration of the system, the resultant electric field at a given molecule (or group) induces a dipole moment in that molecule. The induced dipole moment is computed by an iterative method until self-consistency is obtained. Typically, three to five iterations are sufficient to achieve self-consistency of the order of 1%. The total dipole moment of a molecule is the sum of its permanent and induced dipoles. Once the iteration is completed, the energy, forces, and torques are evaluated. Expressions for the electrostatic interactions can be found in standard textbooks (24). The total potential energy of the system is given by $U_{tot} = U_{el} + U_{LJ} + U_p + U_K + U_T$, where U_{el} is the electrostatic interaction energy given by

$$U_{el} = -\frac{1}{2}\sum_{i,j=1}^{n}\left(\sum_{\nu=1}^{3}\mu_i^{\nu}E_j^{\nu} + \frac{1}{6}\sum_{\mu,\nu=1}^{3}Q_{\mu\nu}^{i}E_j^{\prime\mu\nu}\right).$$

TABLE I

LENNARD-JONES PARAMETERS

Interacting pairs	σ	ϵ
	Å	kcal/mol
H_2O-H_2O	3.02	0.75
H_2O-CO	3.02	0.75
H_2O-NH	3.02	0.75
Na^+-H_2O	3.20	0.10
Na^+-CO	3.20	0.10
Na^+-NH	3.20	0.10
Cs^+-H_2O	3.70	0.10
Cs^+-CO	3.70	0.10
Cs^+-NH	3.70	0.10
Li^+-H_2O	2.90	0.07
Li^+-CO	2.90	0.07
Li^+-NH	2.90	0.07

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[2] Because there is an H atom bonded to the CO group of ethanolamine we assign a smaller static dipole moment to this CO group, 1.0 Debye.

[3] For Cs^+ and Na^+ the barriers refer to a pentad, whereas for Li^+ the calculation refers to a triad. As the energy barrier changes by <1 kcal/mol upon addition of the third and fourth water molecules, we may still be confident that with more water present the translocation barrier is larger for Li^+ than for Na^+ or Cs^+.

i runs over all the labels of the groups; μ_i^ν is the ν component of the dipole moment of the ith group; E_j^ν is the ν component of the electric field due to molecule (group) j at the center of mass of molecule (group) i; $Q_{\mu\nu}^i$ is the $\mu\nu$ component of the quadrupole moment of molecule (group) i; $E_j^{\prime\mu\nu}$ is the $\mu\nu$ component of the electric field gradient tensor of molecule (group) j at the center of mass of molecule (group) i. U_{LJ} is the total Lennard-Jones potential and

$$U_p = -\frac{1}{2}\sum_{i=1}^{n}\sum_{\beta=1}^{3}(\mu_i^\beta - \mu_{0i}^\beta)^2\frac{1}{\alpha_i},$$

is the polarization energy; μ_{0i}^β and μ_i^β are the β components of the permanent and the total dipole moment of the group i, respectively; α_i is the polarizability of the ith molecule or group. The quantity $\mu_i^\beta - \mu_{0i}^\beta$ is quadratic in α_i, so that the polarization energy is zero in the limit $\alpha_i \longrightarrow 0$. U_K and U_T are the potentials due to the elastic force and the torque, Eq. 1 and Eq. 2, respectively.

In our calculations, the equations of motion are solved using the algorithm developed by Gear (25). The motion of the centers of mass is integrated in the same way as detailed by Rahman and Stillinger (26). In treating the rotational degrees of freedom, we have used the method of Evans and Murad (27, 28) who use the Cayley-Klein parameters. The time step used is 0.5×10^{-15} s. We have tested our computer program by reproducing the lowest energy configuration of the water dimer obtained by Barnes et al. (18).

To save computer time, the interaction between two entities is neglected when their separation is larger than a certain value of r_c. We typically use an r_c of 6.5 Å, which is large enough to obtain, to a good approximation, the equilibrium configurations of our system. After the system has been cooled down to zero temperature, the energy of the system is computed by using $r_c = \infty$. Immediately after increasing r_c to infinity, the energy increases ~4 kcal/mol when the ion is near $z = 0$ and ~1.0 kcal/mol near the mouth; the system then relaxes and the energy decreases ~0.1 kcal/mol from its new value. The change of r_c from 6.5 Å to infinity has no significant effect on the equilibrium configuration of the system.

To facilitate the description of our method and results, we introduce some notation. We denote the carbonyl and amine groups of the gramicidin monomer, the coordinates of which are given in reference 2, by CO-i' and NH-j', where $i' = 0', \ldots, 16'$ and $j' = 1', \ldots, 16'$. CO-0′ and CO-16′ are the carbonyl of the formyl end and the ethanolamine end, respectively. NH-16′ is the amine group of the ethanolamine. Our labeling scheme is identical to that of Table I of KK, except that we have added primes to our labels. The carbonyl and NH groups of the other strand of gramicidin are denoted correspondingly with unprimed labels. The coordinates of the unprimed groups are related to the primed ones by

$$x = -x'\cos(2\phi) - y'\sin(2\phi)$$
$$y = -x'\sin(2\phi) + y'\cos(2\phi)$$
$$z = -z',$$

where $\phi = 144.8°$, and (x, y, z) and (x', y', z') are the coordinates of the centers of mass of the groups labeled by the unprimed and primed groups, respectively. The majority of the z-coordinates of the unprimed groups are positive. The axis of the helical dimer coincides with the z-axis. Since the dimer has a reflection symmetry with respect to the xy-plane, we have done our calculations by placing the cation at positions above the xy-plane. We denote the water molecules by Wi, $i = 1, 2, \ldots, n_W$, and their corresponding center of mass coordinates by $\mathbf{r}_{Wi} = (\rho_{Wi}, \theta_{Wi}, z_{Wi})$ in cylindrical coordinates. The position of the cation is denoted by $\mathbf{r}_I = (\rho_I, \theta_I, z_I)$.

To start a calculation, we put the ion at a certain position close to the helix axis and two water molecules at $z = z_I \pm 3$ Å, with their dipole moments pointing away from the cation. The system is then allowed to move according to the equations of motion assuming zero initial velocities and angular momenta. As time goes on, the kinetic energy increases and the potential decreases. The linear and angular velocities are occassionally set to zero so that the system drifts towards a potential minimum that is characterized as a stationary value of the potential energy. (In practice, there is a small fluctuation of ~0.05 kcal/mol around the minimum potential energy.) After we have located two adjacent potential minima, we place the $M^+(H_2O)_2$ triad between the two minima and try to locate the position of a saddle point in the potential. It is a saddle point because the potential energy is a minimum if the ion travels along the radial direction and is a maximum as the ion travels along a helical path with the same pitch as the gramicidin helix. The static energy barrier is taken to be the difference between the potential energies (of the entire system) with the ion at the minimum and the saddle point. When the ion is placed close to the saddle point, the $M^+(H_2O)_2$ triad is in unstable equilibrium, therefore occasional adjustment of the position of the triad is required to determine the approximate location of the saddle point.

With the $M^+(H_2O)_2$ triad located at a potential minimum (or saddle point), we add two water molecules, one at $z_{W1} + 3$ Å and the other at $z_{W2} - 3$ Å (with their dipole moments pointing away from the cation) to study the effect of the additonal water molecules. As we will see, except near the channel mouth, the additional water molecules only marginally affect the location of a minimum or a saddle point. However, they significantly alter the energy barrier.

Minimum Local Steepness

To characterize some salient features of the low energy pathways connecting minima on the potential energy surface for the cation-water complexes, we adopt a criterion of minimum local steepness. This way of identifying trajectories insures than as the hydrated ion moves in the channel, it always follows the path of least resistance. It is an essentially static method of analyzing ionic motion. Furthermore, it is clear that this approach need not identify the lowest energy pathway since, just as in mountain climbing, always choosing a locally favorable trail does not ensure that a hiker finds his way through the lowest pass.

RESULTS AND DISCUSSION

An Empty Channel

As a test of the physical reasonableness of the model, we compute the deviation of the structure of our model of gramicidin from the structure given by KK. Such displacement occurs since in our treatment the carbonyl and the NH interact electrostatically. We introduce the following notations

$$\Delta r_i = |\Delta \mathbf{r}_i| = |\mathbf{r}_i(t) - \mathbf{r}_{0i}|, \tag{3a}$$

$$\Delta\theta_i = \cos^{-1}(\mathbf{n}_i(t) \cdot \mathbf{n}_{0i}), \tag{3b}$$

which measure the positional and angular deviation from the KK values. The notation has been defined in the Methods. At 0°K, $\mathbf{r}_i(t)$ and $\mathbf{n}_i(t)$ are temporally invariant. The permanent dipole moments and polarizabilities are those given in the Model Gramicidin Channel section of Methods. At present we do not have a good criterion for choosing the forces constants k_e and K_T. We only know that K_T should be roughly equal to skeletal deformation force constants that are on the order of 10^{-18} J (29). The force constant k_e is expected to be much smaller than the C-C stretching force constant and is likely to be of the same order as bending force constants that are roughly 0.5 mdyn/Å. In our model calculations, we tolerate a maximum Δr_i of ~0.15 Å (even with a cation inside the channel), which already results in a significant distortion of the bond lengths and bond angles. In most of our calculations, we have used $k_e = 0.5$ mdyn/Å and $K_T = 0.5 \times 10^{-18}$ J, which we will call the standard force constants. Using these parameters we found that Δr_i varies from 0.01 to 0.06 Å (with the majority of these ~0.06 Å) and $\Delta\theta_i$ varies between 1.0° and 1.8°. (If we set the polarizibilities to zero, then Δr_i varies between 0.01 and 0.04 Å and $\Delta\theta_i$ varies between 1.0° and 1.8°.) These results suggest that the choices of k_e and K_T are reasonable because (*a*) the gramicidin helix is only slightly disturbed and (*b*) such deviations from $\mathbf{r}_{oi}$ and θ_{oi} are well within the error limit of computer modeling of the structure of molecules. We found that the $\Delta\theta_i$ are roughly proportional to $1/K_T$ for a fixed k_e and the Δr_i are roughly proportional to $1/k_e$ for a fixed K_T. For $k_e < 0.1$ mdyn/Å, large distortion of the helical structure was observed ($\Delta r_i \sim 0.3$ to 0.5 Å). Our model is considered to have broken down for these values of k_e.

The resultant dipole moments of the carbonyl groups and the NH groups have magnitudes of 2.55 and 1.40 Debye, respectively. (Their corresponding permanent values are 2.17 and 0.96 Debye.) The total potential energy of the helix at 0°K, computed using the standard force constants is −151.79 kcal/mol. The contributions to the total energy are listed in Table II. The total energy of the helix (U_{tot}) is sensitive neither to k_e nor to K_T. If we halve the value of K_T and keep $k_e = 0.5$ mdyn/Å, U_{tot} decreases by only 2 kcal/mol. If we halve the value of k_e and keep K_T fixed at 0.5×10^{-18} J, U_{tot} decreases by 7 kcal/mol. If we halve both k_e and K_T, U_{tot} decreases by ~9.5 kcal/mol, suggesting that the effects that k_e and K_T have on the energy are roughly independent.

One Bare Na$^+$ Ion Inside the Channel

By placing one bare Na$^+$ ion in the channel (i.e., no water molecule), we have found seven potential minima in the region $z > 0$ and one minimum at $z = 0$. Because of the symmetry of the channel there should be seven minima for $z < 0$. We shall refer to these minima as the minima at $\mathbf{r}_{mK} = (\rho_{mK}, \theta_{mK}, z_{mK})$, or minima at $z_I = z_{mK}$, $K = 0, \ldots, 7$, where $\mathbf{r}_{mK}$ is the position vector of the ion at the Kth potential minimum, with $z_{mK} < z_{m(K+1)}$. That is, $K = 0$ and $K = 7$ are the potential minima at $z = 0$ and the channel mouth, respectively. The locations of the potential minima are summarized in Table III. The saddle points are roughly midway between adjacent minima with $\rho_{mK} \sim 1.1 \pm 0.1$ Å; at a saddle point an ion is ~0.4 closer to the axis than it is at a minimum. The gramicidin helix has a pitch of 4.85 Å/turn and has 6.3 residues/turn. Because two residues form a basic building block, we expect the system to have a period of 4.85 Å(2/6.3) = 1.54 Å in the z-direction and

TABLE II

TOTAL POTENTIAL ENERGY U_{tot} AND THE VARIOUS CONTRIBUTIONS TO U_{tot} FOR n_I Na$^+$ IONS AND n_W WATER MOLECULES AT SELECTED POINTS IN THE CHANNEL ($k_e = 0.5$ mdyn/Å and $K_T = 0.5 \times 10^{-18}$ J)*

n_W	n_I	K	z_I	U_{tot}	U_{el}	U_p	U_{LJ}	U_K	U_T	Character‡
			Å	*kcal/mol*	*kcal/mol*	*kcal/mol*	*kcal/mol*	*kcal/mol*	*kcal/mol*	
0	0	—	—	−151.79	−213.08	54.42	0.00§	5.77	1.10	–
0	1	0	0.00	−187.45	−305.05	85.33	14.13	12.59	5.56	min
0	1	1	0.86	−180.47	−268.46	71.85	4.25	7.15	4.75	sad
0	1	1	1.70	−184.44	−291.12	79.43	11.14	9.70	6.41	min
0	1	4	6.63	−183.98	−286.46	79.83	7.58	8.73	6.34	min
0	1	5	7.38	−178.06	−266.93	73.11	2.87	6.88	6.00	sad
0	1	5	8.19	−183.04	−286.88	80.13	7.18	9.67	6.85	min
0	1	7	12.17	−185.58	−280.13	77.03	6.68	7.17	3.65	min
2	1	0	0.00	−245.12	−328.13	79.28	−8.20	7.22	4.71	min
2	1	1	0.87	−240.94	−321.72	78.27	−8.84	6.67	6.68	sad
2	1	1	1.65	−245.00	−326.18	79.28	−9.49	6.81	4.82	min
2	1	4	6.41	−244.29	−327.88	81.46	−10.20	7.06	5.26	min
2	1	5	7.82	−242.59	−327.76	81.79	−9.16	7.08	5.46	min
2	1	6	8.67	−240.53	−327.29	81.93	−7.45	6.97	5.24	sad
2	1	6	9.27	−241.76	−331.52	82.97	−6.12	7.21	5.70	min
2	1	7	11.37	−238.36	−328.00	81.45	−3.59	7.14	4.65	min
4	1	0	0.00	−287.00	−359.66	86.81	−26.48	7.53	4.79	min
4	1	1	0.88	−283.70	−355.47	86.19	−25.53	6.74	4.38	sad
4	1	1	1.64	−284.80	−362.61	87.00	−21.12	7.12	5.01	min
4	1	4	6.30	−283.66	−358.68	87.29	−24.69	7.22	5.19	min
4	1	5	8.12	−283.56	−368.84	88.25	−15.37	7.21	5.18	min
4	1	7	11.78	−280.54	−363.34	84.84	−13.53	7.26	4.24	min

*K is the label of a potential minimum or a saddle point.

‡Min stands for the potential minimum, sad, the potential saddle point.

§In our model there is no Lennard-Jones interaction between the ligands of the gramicidin.

$360° (2/6.3) = 114°$ in the azimuthal angle, which corresponds almost exactly to the periodicities listed in Table III. Except for the minima near $z_1 = 0$ and at the channel mouth, the stable sites reflect the helical periodicity.

A potential minimum occurs whenever the Na^+ ion is located at a position where it is strongly attracted by two carbonyl groups. The configurations of the ion and the ligands to which it is strongly attracted are shown in Fig. 4 *a*, *b* for the minima at $z_1 = 0$ Å ($K = 0$) and 3.63 Å ($K = 2$). Note the difference between the arrangements of the CO and NH groups in the two *XY*-plane projections. In Fig. 4 *a*, there is symmetry about the dashed line, whereas there is no such symmetry in *b*. This difference reflects the fact that z_{m0} and z_{m1}, as is clear from Table III, are not established by the periodicity. In fact, the symmetry shown in Fig. 4 *a* is unique due to the joining of the two monomers at $z = 0$; z_{m1} is close enough to $z = 0$ that the symmetry is more important than the periodicity.

At a saddle point, the Na^+ ion is also attracted strongly by two CO groups that are farther apart than those two at the minimum. This fact is illustrated in Fig. 4*c*, *d*. The two CO groups that are closest to the Na^+ ion have the following properties. They tilt ~10° towards the helix axis. For a fixed k_e, $\Delta\theta_i$ is again roughly proportional to $1/K_T$, and $\Delta r_i \sim 0.15$ Å. The resultant dipole moments are ~3.3 Debye, ~50% larger than that of an isolated CO group.

The potential energies at selected minima and saddle points are listed in Table II. There is little regularity in the minimum energy as a function of *K*. The minima at the center of the channel ($K = 0$) and at the mouth ($K = 7$) are the most stable; the energies at the intermediate minima vary between −183.0 and −185.0 kcal/mol. There are no clear trends in the variation of the individual contributions to the U_{tot} as a function of *K*. However, the various terms in U_{tot} are quite different at $K = 0$ than at any other minimum; this reflects the differences in structure already mentioned. The barriers to surmounting the saddles, measured from the lower of the adjacent minima (to be described as the unfavorable direction), vary between 6 and 7 kcal/mol. The individual contributions to U_{tot} at the saddle points are very different from their values at the minima, which reflects the changes in ρ. As the ion is further from the ligands, the magnitudes of the electronic, polarization, and Lennard-Jones contributions to U_{tot} are all smaller than at the minima. Finally, by comparison with the empty channel, we see that the binding energy for a bare Na^+ in the channel is ~35 kcal/mol.

We now consider the potential minimum at the channel mouth with coordinates

$$\mathbf{r}_{m7} = (2.70\ \text{Å},\ -47.7°,\ 12.17\ \text{Å}). \tag{4}$$

Here the Na^+ ion is farther away from the *z*-axis than it is at the other minima. This is due to the termination of the gramicidin helix; there is less spatial hindrance at the mouth than in the interior of the channel. These observations describe one bare Na^+ ion, i.e., the effects of water, which will be described later, are neglected.

At the mouth, there are three CO groups, namely CO-11, CO-13, and CO-15, which are not hydrogen bonded to any NH group and are therefore capable of strongly attracting the Na^+. There exist three sites S_1, S_2, and S_3 (open triangles in Fig. 5) at which such strong attractions and hence possible minima may occur. However, an ion located at S_2 or S_3 is not in stable equilibrium because at either location the ion is only strongly attracted by one CO group, at S_2 by CO-13, and at S_3 by CO-15. On the other hand, an ion located at S_1 may be in stable equilibrium because it is attracted by both CO-11 and

TABLE III

POSITIONS OF THE POTENTIAL MINIMA FOR A BARE Na^+ IN THE CHANNEL ($n_W = 0$) FOR THE $Na^+ (H_2O)_2$ TRIAD ($n_W = 2$) AND FOR THE $Na^+ (H_2O)_4$ PENTAD ($n_W = 4$)*

n_W	K	ρ_{mK}	θ_{mK}	z_{mK}
		Å	*degrees*	*Å*
0	0	1.78	54	0.00
0	1	1.65	−62	1.70
0	2–6	1.52 ± 0.02	117 − 114(K − 2) ± 2	3.62 + 1.52(K − 2) ± 0.03
0	7	2.70	−48	12.17
2	0	1.30	55	0.00
2	1	1.21	−62	1.64
2	2–6	1.18 ± 0.03	117 − 112(K − 2) ± 2	3.46 + 1.46(K − 2) ± 0.04
2	7	1.41	−50	11.37
4	0	1.34	55	0.00
4	1	1.34	−61	1.64
4	2, 3, 5, 6	1.33 ±0.03	169 − 113(K − 2) ± 1	3.54 ± 1.52(K − 2) ± 0.06
4	4	1.21	−52	6.30
4	7	1.50	−47	11.78

*The stable sites are identified by $K = 0$ to 7 (see text).

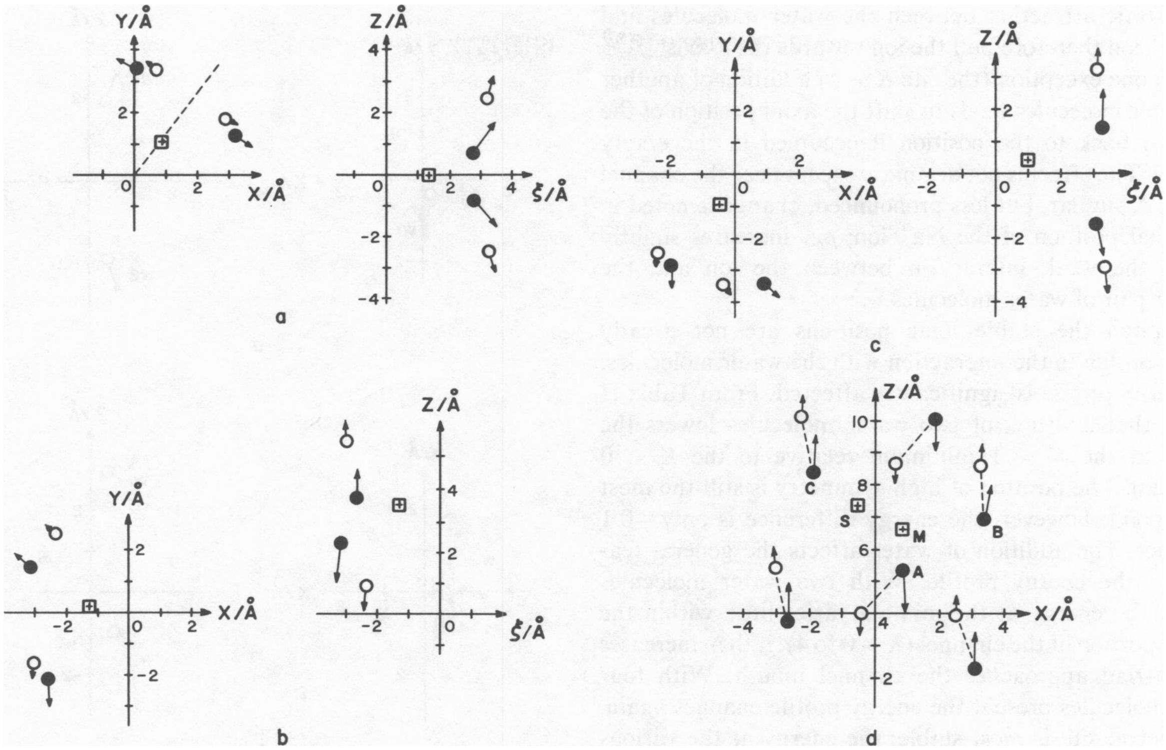

FIGURE 4 Projections of the bare Na$^+$ ion and the nearest CO and NH groups. Diagrams on the left are projected on the *xy*-plane while those on the right (except *d*) are projected on a plane containing the *z*-axis and the ion (the axis in this plane is labeled as ξ). The square, the solid circles, and the open circles represent the Na$^+$ ion, the CO groups, and the NH groups, respectively. The arrows indicate the magnitude and the direction of the total dipole moment of the groups. The arrowhead points toward the positive end of the dipole. A dipole with length equal to one division on the axis is two Debye. (*a*) The minimum at $z_1 = 0$; (*b*) the minimum at $z_1 = 3.62$ Å; (*c*) the saddle point at $z_1 = 7.38$ Å; (*d*) an ion located at *M* is strongly attracted to the CO groups A and B and is a minimum, $z_1 = 6.63$ Å. An ion located at S is strongly attracted to the CO groups A and C, and is a saddle point ($z_1 = 7.38$ Å). For clarity, the CO groups are connected to their nearest neighbor NH groups by a dashed line.

CO-16 (the carbonyl group of the ethanolamine); this is confirmed by our simulation.

Ion and Water in a Channel

Starting from configurations with one bare ion inside the channel, we have added water molecules to our model as described in the Computation Details section of Methods. The calculations suffer from a significant limitation. Long-range interactions between polar groups and the surrounding dielectrics (phospholipid membrane and bulk water) are ignored. The consequences of ignoring image effects are not great when comparing the energy at adjacent minima; they vary from ≤0.2 kcal/mol near the center of the dimer to as much a ~1 kcal/mol near the center of each monomer (30). We may thus expect that these long-range interactions have little effect on the position of the minima and the relative stability of adjacent sites. They may, however, significantly effect the overall shape of the potential.

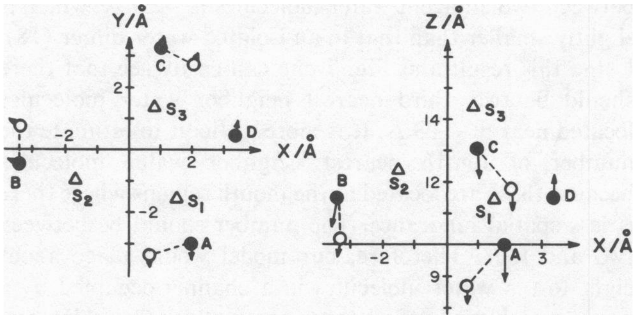

FIGURE 5 The three sites (indicated by the open triangle) at which a Na$^+$ may be strongly attracted by a CO group or CO groups. *A* = CO-11, *B* = CO-13, *C* = CO-15, *D* = CO-16. See also the legend of Fig. 4. (*a*) The projection on the *xy*-plane; (*b*) projection on the *xy*-plane. *z*-distance is measured from the middle of the channel. The dipole moments shown here are those of an isolated helix.

The addition of water does not greatly perturb the axial location of potential minima well within the channel. The results summarized in Table III indicate that with two additional water molecules, z_{mK} usually shifts by <0.2 Å; however, ρ_{mK} decreases substantially, ~0.3 to 0.4 Å. This decrease occurs because the combined effect of the repulsive Lennard-Jones interactions and the relatively weak electrostatic attractions between the water molecules and the nearby ligands of the helix keep the water molecules fairly close to the *z*-axis (ρ_{Wi} ~ 0.4 to 0.7 Å). The electrostatic attraction between the water molecules and the Na^+ ion therefore pull the ion towards the z-axis.

With one exception (the site $K = 4$) addition of another two water molecules tends to shift the axial position of the Na^+ ion back to the position it occupied in the empty channel. The effect is not dramatic except near the channel mouth. A similar, but less pronounced, change is noted in the radial position of the Na^+ ion; ρ_{mK} increases slightly due to the weak interaction between the ion and the exterior pair of water molecules.

Although the stable ionic positions are not greatly perturbed due to the interaction with the water molecules, the energy profile is significantly affected. From Table II we see that addition of two water molecules lowers the energy at the $K = 1$ minimum relative to the $K = 0$ minimum. The position of high symmetry is still the most stable point; however, the energy difference is only ~0.1 kcal/mol. The addition of water affects the general features of the energy profile. With two water molecules present the energy at the minima varies little within the central portion of the channel ($K = 0$ to 4); it then increases as the triad approaches the channel mouth. With four water molecules present the energy profile changes again. The central site is most stable; the energy at the various minima does not change monotonically. There is an intermediate maximum for $K = 2$; the least stable site is again that with $K = 7$. There are few trends to be seen among the various contributions to U_{tot}. However, with four waters present, the magnitude of U_{LJ} is greatest at the channel center; with the exception of the anomalous site, $K = 4$, it drops steadily as the pentad moves toward the mouth. The structural differences for $K = 4$ are reflected in a very different energy partitioning; the electronic energy is sharply higher and the Lennard-Jones contribution is sharply lower.

With $n_W = 2$ or 4, the central site ($K = 0$) is more stable than the mouth site ($K = 7$) by ~6 kcal/mol. This is, of course, just the opposite of the experimental energy profile illustrated in Fig. 1 (12), and reflects limitations already mentioned. We will discuss these at greater length later.

Addition of two and four water molecules substantially lowers the barrier to translocation over the first saddle point (the saddle at $z_I \sim 0.85$ Å) to ~4.5 and 3.5 kcal/mol (in the unfavorable direction), respectively. This decrease is due in part to the fact that with water present the Na^+ ion cannot get as close to the CO groups as can the bare ion and in part to the fact that contributions to the total potential change in a complex fashion when water molecules are added.

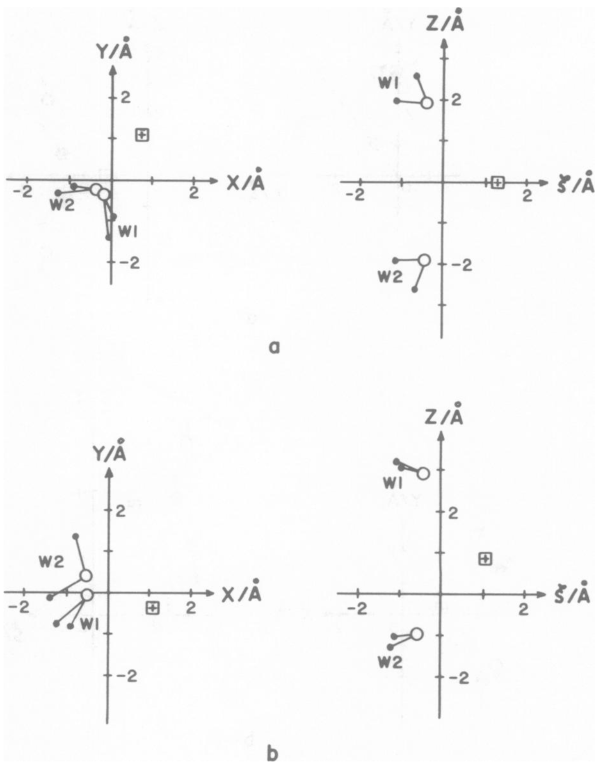

FIGURE 6 The configurations of water molecules ($n_W = 2$) for (a) the minimum at $z_I^{(2W)} = 0$, and (b) saddle at $z_I^{(2W)} = 0.87$ Å. The square represents the Na^+ ion, lengths are in Ångströms. The right-hand figures are again projections in the plane of the ion and the z-axis (the ξ-z plane).

Arrangement and Motion of Water Molecules

An isolated $\mathrm{Na}^+(\mathrm{H_2O})_2$ triad has a linear structure (22), which is slightly distorted by the channel as illustrated in Fig. 6, that contrasts the configurations of the water molecules for the minimum at $z_I = 0$ and the saddle point at $z_I = 0.87$ Å. This figure also shows that the arrangement of the water molecules depends somewhat on the location of the ion. If two more water molecules are put into the channel, the position of the original water molecules is only slightly perturbed as can be seen by comparing Figs. 6 and 7; however, there is considerable reorientation at the saddle point. The distances between the Na^+ and the nearest neighbor water molecules remain ~2.6 Å. (In an isolated $\mathrm{Na}^+\,[\mathrm{H_2O}]_2$ triad, the value is 2.57 Å [22].)The separation between two adjacent water molecules is ~2.9 Å, which is slightly smaller than that in an isolated water dimer (18). Using this result and Fig. 7 one can easily see that there should be two, third nearest neighbor water molecules located near $z = \pm 8$ Å. It is more difficult to estimate the number of fourth nearest neighbor water molecules because these are located at the mouth regions where there is less spatial hindrance. The number should be between two and four. Therefore, our model would place about eight to ten water molecules in a channel occupied by a single ion; this is in good agreement with the experimental result of Dani and Levitt (31) but larger than the value found by Rosenberg and Finkelstein (5).

The solvation structure within the channel clearly reflects the confining nature of the helix. At the local energy minima both the triad and the pentad form essen-

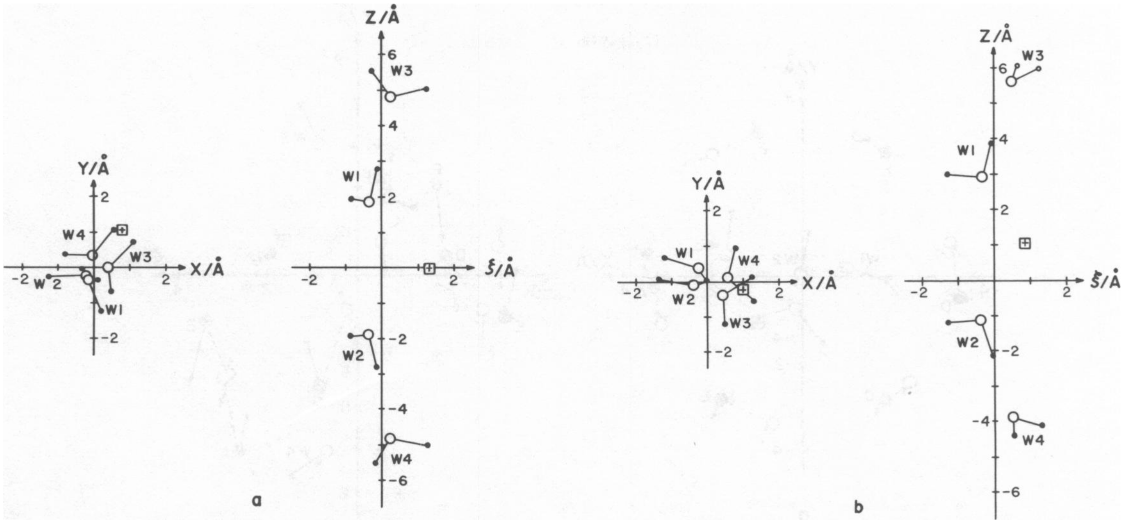

FIGURE 7 The configurations of water molecules ($n_W = 4$) for (*a*) the minimum at $z_I = 0$ and (*b*) the saddle point at $z_I = 0.89$ Å. The square represents the Na^+ ion; lengths are in Ångstroms. The projections are similar to those at Fig. 6.

tially linear arrays with the negative end of the water dipoles pointing towards the ion (Figs. 6 *a* and 7 *a*). The orienting effect of the helix alone is illustrated in calculations with only water in the channel; in the most stable arrangements the water molecules are aligned linearly with their dipoles in the same direction. Both observations are identical to those of Mackay et al. (15). The arrangement and the orientation of the water molecules are also maintained near the saddle points of the potential energy surface (Figs. 6 *b* and 7 *b*), which indicates that motion within the channel must be highly correlated.

Except near the channel mouth ($z_W \geqslant 12$ Å), all the water molecules are within 0.8 Å of the helical axis; this strongly suggests that molecular and ionic motion in the channel interior is single file. To test the validity of this assertion we carried out two complementary dynamical experiments. (*a*) An ion and two (or four) water molecules were positioned near a saddle point and the ensemble released after which the motion toward the potential minimum was observed. (*b*) An ion and two (or four) water molecules located near a potential minimum were each given the same initial z component of velocity and the motion of this ensemble observed.

In all cases tested the motion was found to be single file. When analogous calculations were carried out for Cs^+ and Li^+ ions, the motion remained single file. While it is obvious that this should be the case for the large Cs^+, in the case of Li^+ (with water) it is a dramatic indication of the confining nature of the helical structure. This is again consistent with observations made previously (15).

Exterior Binding Site

As indicated in the Results and Discussion section entitled One Bare Na^+ Ion Inside the Channel, the binding site at the mouth of the channel is anomalous. For an Na^+ ion in a bare channel the ion is much further from the axis than it is at any of the interior minima. It is also much further up the channel than would be expected if helical periodicity were still governing its position. In fact, as has already been discussed, binding at site 7 is strongly influenced by the ethanolamine carbonyl group.

The presence of water does not eliminate the special structure at site 7. When two water molecules are added, one on either side of the Na^+ ion, using the procedure described in the Water Model and Interactions between Molecules in Methods, the Na^+ $(H_2O)_2$ triad moves towards the interior of the channel. After the temperature is cooled to zero, the triad assumes a stable configuration with the Na^+ located at

$$\mathbf{r}^{(2W)}_{m7} = (1.41\ \text{Å}, -50°, 11.37\ \text{Å}). \qquad (5)$$

The superscript 2W, which means two water molecules, is used to distinguish this position vector from that of a bare ion. The water molecules (especially the one below the ion, i.e., the one in the channel) have the effect of pulling the ion closer to the z-axis by 1.29 Å and further into the channel by 0.80 Å.

An important feature of this minimum is found when two more water molecules are added, one from above (W3) and one from below (W4). The stable ionic position is again perturbed; the Na^+ position is now

$$\mathbf{r}^{(4W)}_{m7} = (1.50\ \text{Å}, -47°, 11.78\ \text{Å}). \qquad (6)$$

The molecule W3 is very close to the Na^+ ion (only 2.84 Å away, comparable with the Na^+-W1 distance of 2.60 Å); the arrangement of the water molecules is illustrated in Fig. 8. At this minimum the Na^+ has at least three nearest neighbor water molecules (W1, W2, and W3), whereas there are only two when the Na^+ is at any other minimum, an obvious consequence of the spatial hindrance due to the

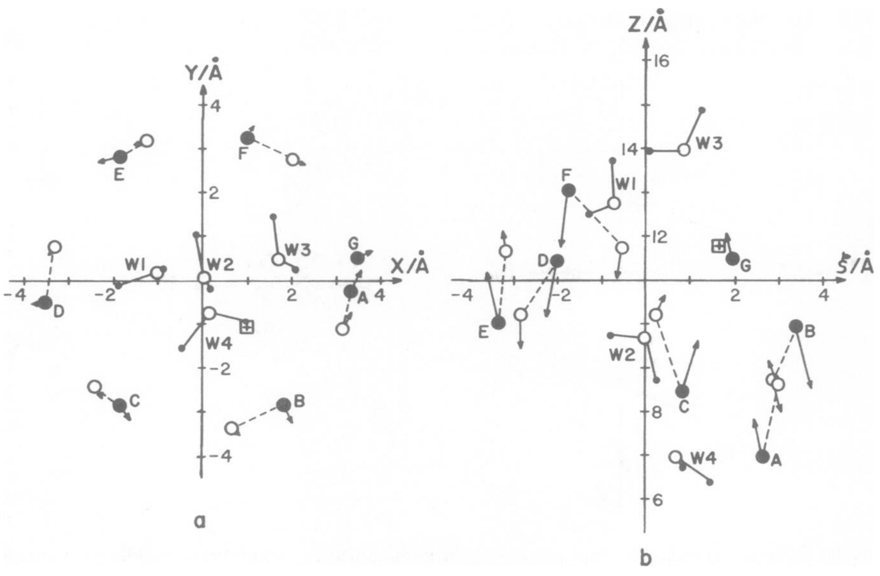

FIGURE 8 The configurations of water molecules with the Na^+ located at the minimum at the mouth ($K = 7$). The ligands of last turn of the gramicidin near the mouth are also shown. See the caption of Fig. 4 for the explanations of the symbols. The solid circles labeled by *A–G* are CO-10–CO-16, respectively. (*a*) Projection on the *xy*-plane, (*b*) projection on the ξz-plane. For clarity, the CO groups are connected to their nearest neighbor NH groups by dashed lines.

ligands of the channel interior. At site 6 the Na^+-W3 distance is already 4.02 Å; this separation remains roughly the same for $K = 2$ to 5. At the channel center, it increases further, to 5.07 Å. Thus in moving from the exterior minimum to the channel interior, the ion has to shed one of its nearest neighbor water molecules. The exterior site is thus qualitatively different from any of the sites, $K = 0$ to 6; it might possibly be identified with an exterior binding site such as that proposed by Eisenman on kinetic grounds (32, 33).

The dipolar orientation of the water molecules is also very different at this minimum. The two interior water molecules are aligned linearly with the negative end of the dipoles pointing toward the ion (see Fig. 8). The exterior water molecules solvate the ion in a totally different way, reminiscent of part of the sixfold coordination typical of bulk water. This provides a further reason to identify this minimum as an exterior binding site.

As already illustrated in Fig. 5 the ion is strongly attracted to two carbonyl groups, CO-16 (*G* in Fig. 8) and CO-11 (*B* in Fig. 8); these are only 2.87 and 2.68 Å from the Na^+ ion. All other CO groups in the last turn of the helix are between 4.56 and 5.42 Å from the ion; the ion-dipole interaction with these groups is thus much weaker. As ion-dipole energies are proportional to $1/r^2$, the interaction between the ion and CO-11 and CO-16 is from 2.5 to 4 times stronger than with any other nearby ligands.

This identification of site 7 as qualitatively distinct is further substantiated by experiments designed to locate the minimum energy pathway between neighboring sites. Unlike the pathways between interior binding sites, ionic motion over the barrier between sites 6 and 7 requires major rearrangement of the water molecules, which is most clearly seen by contrasting the adiabatic pathways between site 5 and site 6 with those between sites 6 and 7. These paths are found as outlined in the Minimum Local Steepness section in Methods. Tables IV and V list the coordinates of the Na^+ ion in an $Na^+(H_2O)_2$ triad as the group moves between sites 5 and 6 and between sites 6 and 7, respectively.

Table IV contrasts the adiabatic track leading away from site 5 with that leading away from site 6. Two points should be noted. The pathways differ from one another. There are two separate low energy tracks between these sites; at zero temperature the triad cannot make the transition between them. A triad starting from site 5 moves

TABLE IV
COORDINATES OF THE Na^+ ION FOR PATHS OF MINIMUM LOCAL STEEPNESS BETWEEN SITES 5 AND 6 FOR A $Na^+(H_2O)_2$ TRIAD

Site 5 to site 6			Site 6 to site 5	
z_I	ρ_I	θ_I	ρ_I	θ_I
Å	*Å*	*degrees*	*Å*	*degrees*
7.82	1.17	−167	1.17	−167
8.12	1.28	−172	1.27	169
8.42	1.11	159	1.32	150
8.57	1.03	131	1.19	145
8.67	1.11	116	1.13	142
8.77	1.04	102	1.00	118
8.97	1.06	89	1.06	89
9.27	1.16	84	1.16	84

in one trough; in starting from site 6, another (quite parallel) pathway is used. Associated with the two channels are somewhat different solvation structures for the water molecules. However, the peak energies for motion along them differ only trivially, <0.2 kcal/mol. Furthermore, as the triad approaches minimum number 6 along the pathway from 5, the 5 to 6 path becomes a tributary of the 6 to 5 path. A similar phenomenon is observed in reversing direction. The consequences for motion at normal temperatures should be straightforward; thermal motion would insure that the two paths are no longer distinct. A triad would move comfortably from one to the other. The associated barrier for motion in the unfavorable direction (site 5 to site 6) is ~2 kcal/mol.

The potential energy surface between sites 6 and 7 is of a totally different character. The adiabatic troughs leading away from site 6 and away from site 7 differ substantially in structure as indicated in Table V. The paths do not come close to one another. The favorable ionic positions are almost 90° apart at some of the intermediate z_I; the associated radial positions also differ substantially as does the solvation structure.

Fig. 9 compares the solvation structure along the two trajectories for $z_I = 10.17$ Å 7(roughly the pass in the path leading away from site 7). The structures exhibit both a distinct memory of their origin and an indication of the restraining effect of the helix on the motion of the water molecules. In each instance the water molecules are closer to their sites of origin than would be expected if the arrangements at the minima were translated to the point $z_I = 10.17$ Å. Except for W1 along the path from site 7 to site 6, the lag is ~0.2 Å; for this water molecule it is almost 0.7 Å.

Furthermore, adiabatic motion inward from site 7 does not lead directly to site 6; rather it leads to a local minimum at $z_I = 9.56$ Å, which is ~1 kcal/mol less stable than site 6. At this local minimum, the Na^+, W1, and W2 are, respectively, 0.38 Å, 0.48 Å, and 0.65 Å away from their site 6 locations. Similarly, motion outward from site 6 leads not to site 7 but to a local minimum with $z_I = 11.22$ Å, which is ~1 kcal/mol less stable than site 7. At this local minimum the Na^+, W1, and W2 are 0.45, 0.04, and 0.21 Å away from their site 7 locations.

TABLE V

COORDINATES OF THE Na^+ ION FOR PATHS OF MINIMUM LOCAL STEEPNESS BETWEEN SITES 6 AND 7 FOR A Na^+ $(H_2O)_2$ TRIAD

Site 6 to site 7			Site 7 to site 6		
z_I	ρ_I	θ_I	z_I	ρ_I	θ_I
Å	*Å*	*degrees*	*Å*	*Å*	*degrees*
9.27	1.16	84	—	—	—
9.57	1.24	74	9.56	1.34	77
9.87	1.29	68	9.87	1.12	29
10.17	1.25	54	10.17	0.89	−23
10.47	0.93	46	10.47	0.94	−43
10.77	0.71	8	10.77	1.07	−46
11.07	0.87	−24	11.07	1.30	−45
11.22	1.14	−36	11.37	1.41	−50

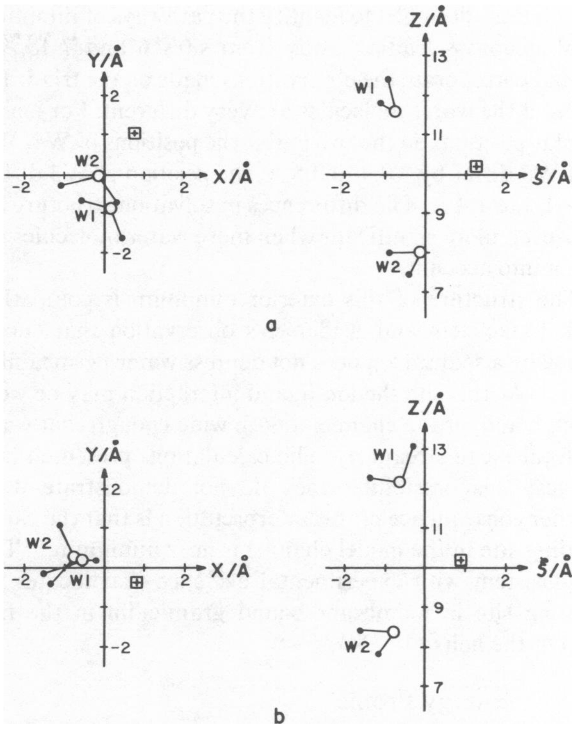

FIGURE 9 The configurations of the particles in the $Na^+(H_2O)_2$ triad at $z_I = 10.17$ Å for the adiabatic pathways leading from (*a*) site 6 to site 7 and (*b*) from site 7 to site 6. The position illustrated is near the saddle point in the adiabatic pathway leading away from site 7. The projection planes are those of Fig. 6.

The energy barriers associated with the two trajectories are also very different. The path leading inward from site 7 has a barrier of ~2 kcal/mol; that leading outward from site 6 has a barrier of nearly 10 kcal/mol. The actual paths connecting sites 6 and 7 must require motion along some locally unfavorable trajectory during which the solvation structure changes radically. Under thermal conditions these would no longer be distinct. However, in contrast to translocation between interior sites, major restructuring of the waters of solvation is required in this process.

The calculations summarized in Tables IV and V and in Fig. 9 relate to the motion of a $Na^+(H_2O)_2$ triad in the channel. While detailed calculations for the $Na^+(H_2O)_4$ ensemble have not been carried out, it seems unquestionable that the adjustment of the solvation shell would be even more of a hindrance in moving from the exterior site to the inner sites due to the presence of more water molecules. In particular, at the exterior site the two water molecules in the channel mouth are both near neighbors of the ion. At site 6, the solvation structure of the exterior waters has been radically changed. One is a near neighbor of the ion (~2.56 Å away); the other is ~4.03 Å away. Preliminary calculations designed to identify the pathways of minimum local steepness leading away from sites 6 and 7 for the pentad corroborate the observations made on the triad. The paths of the water molecules are very different. For ions at similar positions on the two paths, the positions of W1, W2, and W3 differ by ~0.4 to 0.5 Å; the position of W3 differs by ~1.3 to 1.4 Å. The differences in solvation structure are thus even more significant when more water molecules are taken into account.

The structure of this exterior minimum is compatible with Finkelstein and Andersen's observation that "occupancy by a sodium ion does not depress water permeability (12). " At this site the ion-ligand interaction may be weak enough and/or the channel mouth wide enough that water molecules can stream by. The calculations presented here suggest this possibility; they do not demonstrate it. A further consequence of this interpretation is that the cation binding site in the model channel is near minimum 6. This is consistent with experimental evidence that locates the binding site in membrane-bound gramicidin in the first turn of the helix (12, 34).

Energy Profile

It is clear from Table II that the energy of the Na^+ ion tends to increase as it approaches the mouth of the channel, regardless of how many water molecules are present. For $n_W = 2$ or 4, the energy difference between site 1 and site 7 is ~6.5 kcal/mol. The energy profile is not monotonic but the energy is basically decreasing as the ion moves into the channel. This is at first troubling since the energy profile deduced from experiments has a maximum near the channel center (see Fig. 1). However, as already pointed out in the Ion and Water in a Channel section in Results and Discussion, a number of factors that have been neglected in our calculations must substantially affect the energy in a way that stabilizes sites near the channel mouth.

At the mouth the ion probably has two or three second nearest neighbor water molecules in the external solution. At the interior sites, W3 and W4 are the second nearest neighbors. The associated energy lowering, estimated as the direct interaction of the ionic charge and the water dipole is approximately -4 kcal/mol.

Interaction with bulk water also lowers the energy of the ion in the channel. The effect will be most pronounced at the exterior sites. The electrostatic image forces due to interaction with the lipid interior are more destabilizing the closer the ion is to the center of the channel (35, 36). These effects and the influence of the membrane dipole potential (37) suggest that long-range interactions could add from 4–6 kcal/mol to the energy at the channel center relative to that at the mouth (30, 38).

While the calculations were never designed to establish the total energy of the ion-water-gramicidin-membrane ensemble, which they clearly do not, it might have been expected that they could locate the ion binding site that has been experimentally established to occur at $z \sim 10.5$ Å (34, 39). Presumably site 6, at which the solvation structure is that of channel water, i.e., only two nearest neighbor water molecules, represents the binding site in our calculations. The same factors that may account for the differences between the calculated and the observed energy profile are significant here too. All would tend to stabilize configurations closer to the channel mouth and would quite possibly make one (or more) of the subsidiary minima between $z = 9.67$ Å and $z = 11.78$ Å more stable than the site 6 configuration.

In addition to the limitations imposed by not considering bulk lipid and water, the model we are using is crude in other ways. The Lennard-Jones parameters have been assigned arbitrarily and the constraining effect of the α-carbon atoms have been neglected. The major conclusion to be drawn is that the details of the energy profile and the precise location of the binding sites are determined by a very complicated interplay of local and long-range interactions.

Cesium Ion in Channel

We have studied the energy minima and saddle points by placing a bare Cs^+ ion near $z = 0$ Å, $z = 7$ Å, and $z = 12$ Å (channel mouth); the results are listed in Table VI. The Lennard-Jones parameters used are those of Table I; the Cs^+ ion is assumed to be larger than the Na^+ ion by 0.5 Å, while the interaction strength is assumed to be the same as that of the Na^+ ion. For $z_I \sim 7$ Å, the potential minimum occurs at roughly the same z coordinate as in the case Na^+. However, for Cs^+ the point $z_I = 0$ is a saddle point; the first minimum occurs at $z_I = 0.8$ Å. At the minimum and the saddle point, the Cs^+ ions are ~0.6 and 0.5 Å from the helix axis, respectively; for the minimum at the mouth, the Cs^+ ion is 1.44 Å from the helix axis. The reason for the qualitative differences between Cs^+ and Na^+ at the channel center is because the distinction between a minimum and a saddle point is determined by the balance between the electrostatic and the Lennard-Jones forces. In the case of Cs^+, the ion is close enough to the helical axis that neither of these quantities varies greatly with z or ρ; hence the roles of the saddle point and minima may be easily interchanged according to the detailed balance of the forces involved. The energy barriers for motion of a bare Cs^+ ion are <1 kcal/mol, much smaller than those for a bare Na^+, typically ~6 or 7 kcal/mol. The difference is due to the fact that the Cs^+ ion must be farther away from the ligands of the gramicidin. When two water molecules are added to the channel, the energy barrier increases to ~2 kcal/mol. This is attributable to the interactions between the water molecules and the gramicidin. In fact, if only one water molecule is put into the channel, the energy barrier is ~2 kcal/mol. When two more water molecules are added to the channel so that four water molecules are present, the energy barriers reduce to ~1 kcal/mol. These results suggest that for Cs^+ translocation in a channel, the water-

TABLE VI

TOTAL POTENTIAL ENERGY U_{tot} AND THE VARIOUS CONTRIBUTIONS TO U_{tot} FOR A Cs^+ ION WITH n_W WATER MOLECULES AT SELECTED POINTS IN THE CHANNEL (k_e = 0.5 mdyn/Å AND K_T = 0.5 × 10^{-18} J)

n_W	z_I	U_{tot}	U_{el}	U_p	U_{LJ}	U_K	U_T	Character*
	Å	*kcal/mol*	*kcal/mol*	*kcal/mol*	*kcal/mol*	*kcal/mol*	*kcal/mol*	
0	0.00	−171.13	−251.36	67.18	2.08	6.49	4.47	sad
0	0.83	−171.79	−251.39	66.65	1.56	6.44	4.93	min
0	6.73	−171.61	−255.93	70.03	1.70	6.69	4.89	min
0	7.40	−171.78	−254.04	69.64	0.80	6.64	5.19	sad
0	12.23	−173.02	−254.61	69.03	2.00	6.49	4.06	min
2	0.00	−218.48	−291.53	71.77	−9.67	6.61	4.34	sad
2	0.71	−220.94	−297.24	71.88	−10.38	5.88	4.25	min
2	12.18	−216.72	−299.36	75.23	−3.28	6.63	4.06	min
4	0.00	−258.76	−314.98	76.23	−31.81	6.72	4.07	sad
4	0.79	−259.92	−315.17	76.11	−30.94	6.10	3.97	min
4	12.18	−249.17	−323.73	76.24	−12.10	6.65	3.77	min

*Min stands for the potential minimum, sad, the potential saddle point.

gramicidin interactions play an important role in determining the energy barriers.

The water molecules in the $Cs^+(H_2O)_2$ and $Cs^+(H_2O)_4$ groups are aligned and oriented similarly to their respective Na^+ analogues (see Fig. 6 and 7). The structures at the minimum and at the saddle point do not differ greatly, except that $\rho_{Cs^+} < \rho_{Na^+}$. Water again forms linear arrays within the channel consistent with the observations of Mackay et al. (15).

The effect that water has on the position of the energy minima is much less striking for Cs^+ than for Na^+, a direct reflection of the increased ionic size. In the absence of water, Cs^+ is constrained to be much closer to the helical axis. The added water molecules have little additional effect since they cannot pull the ion closer to the axis without themselves being forced into unfavorable proximity with the ligands of the helix.

The position of the Cs^+ at the potential minimum near the channel mouth is essentially unaffected by the presence of water molecules; for the $Cs^+(H_2O)_4$ pentad it is located at

$$\mathbf{r}_I^{(4W)} = (1.41\ \text{Å}, -68°, 12.18\ \text{Å}), \tag{7}$$

<0.1 Å away from the position of Cs^+ in the absence of any water. Here too both exterior water molecules are near neighbors of the Cs^+; the separation of W1 and W3 from Cs^+ is 3.16 and 3.07 Å, respectively. That the Cs^+-W3 distance is less than the Cs^+-W1 distance reflects the fact that W3 hardly interacts with any of the helix ligands. Well inside the channel the Cs^+-W3 separation is ~6 Å.

As in the case of Na^+, the exterior minima for the $Cs^+(H_2O)_2$ and $Cs^+(H_2O)_4$ units are less stable than the minima near the channel center. For the triad the energy difference is ~4 kcal/mol; for the pentad it is ~10 kcal/mol. The increased energy difference when four water molecules are present reflects the fact that the Cs^+ is so large that W3 is really not a channel water molecule at all; the closest W3 approaches any of the channel ligands is 4.88 Å, its distance from CO-16 (the ethanolamine carbonyl). The situation for Na^+ is very different; W3 interacts with the channel ligands. It is located only 3.10, 3.05, and 3.23 Å from the CO-15, CO-16, and NH-16 groups, respectively. The arguments presented in the Energy Profile section of Results and Discussion, which account for the qualitative differences between the calculated and experimental energy profiles, are equally applicable to Cs^+.

Lithium Ion in Channel

The properties of a Li^+ ion with water in the channel are qualitatively very similar to those of a Na^+ ion. For a bare Li^+ ion, due to its small size and relatively weak interactions with the ligands (Table I), its equilibrium position at the minimum at $z_I \sim 3.6$ Å is ~3.2 Å from the helix axis. In other words, it is extremely close to the boundary of the channel since the CO and NH groups are located ~3.4 Å from the helix axis. At these positions the interactions between the Li^+ ion and the α-carbon atoms, the β-carbon atoms and even the atoms of the membrane lipids, which have been neglected in our model, are probably significant. For this reason we did not carry out further calculations with a bare Li^+. However, when two water molecules are added, the Li^+ ion moves much closer to the helix axis. Under these conditions it is only 1.7 Å from the helix axis, the corresponding energy barriers in the channel interior are ~6 kcal/mol.

CONCLUSION

We have constructed a flexible and polarizable model gramicidin channel in which we can study the properties of cations and water molecules by using molecular dynamics. Comparison of our model with those presented previously (13–15) indicates that the qualitative features of the channel, ion, and water ensemble are not very dependent on the specific features of the interaction potentials assumed. The translocation energy barriers near the channel center for Cs^+, Na^+, and Li^+ ions are ~1.0, 3.5, and 6.0 kcal/mol, respectively,[3] which is compatible with the observed trend of the conductivity of these ions in gramicidin A channels (1) and calculations for ions in a bare channel (14). We have found that in the interior of the channel the water molecules do not greatly affect the location of either the potential minima or the saddle points. They do affect all the energy barriers and for small ions, the location of energy minima near the channel mouth. Furthermore, the arrangement of the water molecules for the potential minimum at the channel mouth is very different from that for the minima inside the channel. At both saddle points and local energy minima inside the channel, the waters line up with the negative end of the dipoles pointing toward the cation, as has been seen previously only near local minima (15). At the outer minimum, which we identify with the exterior binding site suggested by Eisenman (32, 33), the channel water molecules maintain the linear arrangement while the exterior water molecules appear similar to bulk water of solvation. The motions of the ions with water in the channel are found to be single file, which is in agreement with the experiment of Rosenberg and Finkelstein (5) and the calculations of Mackay et al. (15).

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