WHY IS GRAMICIDIN VALENCE SELECTIVE?

A Theoretical Study

SHEN-SHU SUNG AND PETER C. JORDAN
Department of Chemistry, Brandeis University, Waltham, Massachusetts 02254

ABSTRACT Calculations contrasting the channel solvation energy for cesium ions and chloride ions associated with water in gramicidin-like channels are presented. The energy profile for the cation exhibits a deep well at the channel entrance; within the single file region the solvation energy is roughly constant. The anion exhibits a totally different energy profile. There is an energy barrier at the channel entrance; if the ion could surmount this barrier, it would be quite stable within the channel. At the channel entrance, the calculated solvation energy difference between anion and cation is $\approx$15 kcal mol^{-1}. This is completely consistent with the observation that chloride neither permeates nor blocks the channel since the estimated rate of ion entry would be ≈ 0.01–10^{-5} s^{-1}, far slower than the rate at which the channel dimer dissociates into monomers.

INTRODUCTION

Selectivity is a fundamental property of channel-forming molecules. For channels of known architecture, it should be possible to relate structure to specificity. Gramicidin A satisfies this condition; it is both simple and well characterized. The membrane bound dimer is ideally valence selective. It mediates the passage of alkali cations, Tl^+, H^+, NH_4^+, and small organic cations across lipid bilayer membranes (Andersen, 1984); it rejects halide ions even though the lumen is large enough (2.1-Å radius) to permit the passage of all but I^- (Koeppe et al., 1978). Not only do halide ions not permeate, they also do not block. They appear to be completely excluded from the channel. Why? The β-helical channel protein does not have negatively charged binding sites. The molecular dipoles of the CO and NH groups of the peptide linkage are nearly parallel to the channel axis (Urry, 1971; Koeppe and Kimura, 1984) and are alternately antiparallel to one another. The structure provides no obvious discrimination mechanism. Is anion exclusion a consequence of a large barrier to ion entry (kinetic control) rather than a low binding affinity (thermodynamic control) (Urry et al., 1981)? Molecular dynamics provides a tool for studying the details of the permeation process and discriminating between the two control mechanisms.

We present results, which have previously been abstracted (Sung and Jordan, 1986*a*), of calculations based on a model gramicidin-like pore. Our emphasis is upon the energy change in the reaction

$$A^{\pm}(H_2O)_n + \mathrm{Gram}(H_2O)_n = (H_2O)_n + \mathrm{Gram} \cdot [A^{\pm}(H_2O)_n], \quad (1)$$

where $A^{\pm}$ is an ionic species. This process involves partial dehydration (water is removed from the first hydration shell) and resolvation (the ion coordinates to gramicidin's polar moieties). It is termed "channel solvation energy" throughout the remainder of this paper. Rather than attempting to compute the energy of solvation for an ion interacting with the channel and bulk water (which would require incorporating a substantial number of water molecules to reasonably account for the presence of bulk water [King and Warshel, 1986]), we focus on the properties of a sequence of microclusters involving only a few water molecules. This simplifies the computation. If observed differences between anions and cations are fairly independent of the number of water molecules used in our simulation, we can feel reasonably confident that we have identified a property of the bulk system. Since the influence of the lipid membrane is also ignored, this approach provides a way to circumvent the computational difficulties attendant upon attempting to incorporate all features of the system in the calculation.

Because gramicidin is so well characterized, it is a favorite molecule for theoretical study (Fischer et al., 1981; Fischer and Brickmann, 1983; Kappas et al., 1985; Polymeropoulos and Brickmann, 1985; MacKay et al., 1984; Lee and Jordan, 1984; Sung and Jordan, 1987; Fornili et al., 1984; Kim et al., 1985; Pullman and Etchebest, 1983; Etchebest and Pullman, 1984; Etchebest et al., 1984). Various models have been used to describe the essential features of ion–gramicidin interaction, with and without the inclusion of water. While the gramicidin dimer is exceptionally simple when viewed from a biological perspective, it still comprises 548 atoms, a formidable computational problem. As such, most of the model calcu-

lations on this system describe the dimer–ion–water system in various highly approximate ways. The calculations fall into four major catagories: rigid framework models, models that exclude water, abstract gramicidin-like models, and fully interactive models. Each has its own limitations.

The encouraging feature of these diverse approaches to the theory of selectivity is that, where the models are comparable, the calculations are in good agreement with one another. The studies that have treated the properties of water (Fornili et al., 1984; Kim et al., 1985; MacKay et al., 1984; Lee and Jordan, 1984) all indicate that the water molecules in the channel line up single file forming a hydrogen-bonded chain. The number of water molecules that can be accommodated in the channel is consistent with the values determined experimentally (Rosenberg and Finkelstein, 1978*a, b;* Levitt et al., 1978). The effect of cation variation on the calculated values of energy barriers and/or diffusion constants (Fischer et al., 1981; Lee and Jordan, 1984; Kappas et al., 1985) are in qualitative agreement with the rate constants inferred experimentally for translocation across the channel (Urban et al., 1980).

In the following sections, we outline the features of our model. We then use it to establish differential binding energy profiles for water clusters in the channel. With these data and similar results for the binding of cesium ions and chloride ions associated with water in the channel, we determine channel solvation energy profiles for ions in the model system. We choose these ions in order to focus on the effect of valence. They have almost the same size and polarizability and their ability to solvate water in the gas phase is nearly identical (Kebarle, 1977). We believe our analysis suggests an unambiguous basis for the origin of the pronounced valence selectivity of gramicidin.

METHODS

We have provided a detailed description of our model for the gramicidin channel, of the interaction potential, and of the calculational method in other publications (Lee and Jordan, 1984; Sung and Jordan, 1986*b*). Except where we have revised our treatment, we only describe the method's main features. The channel has been constructed based upon the data obtained by Koeppe and Kimura using computer modeling of β-helical structures (Koeppe and Kimura, 1984). The CO and NH groups are modeled as point dipoles located at the centers of mass computed for these groups by Koeppe and Kimura. This model, in which the α-C's of the peptide linkage have been suppressed, is sufficiently restrictive to confine water and ions as large as (or larger than) Na^+ to the channel. The dipoles are mobile and reorientable. The force constants for deformation (k = 0.5 mdyn/Å) and reorientation ($K_T = 0.5 \cdot 10^{-18}$ J) are typical of bending and librational frequencies in proteins (Koyama and Shimanouchi, 1974); the values chosen correspond to frequencies in the 200–500 cm^{-1} range. The CO and NH groups have permanent dipole moments along their bonds with magnitudes determined by their compensated partial charges (Schultz and Schirmer, 1979); they are 2.26 and 0.86 Debye, respectively. Their respective polarizabilities are 1.82 and 1.44 $Å^3$ (Pethig, 1979).

We include only the dipolar groups of the helix for computational simplicity. This approximation has been tested by incorporating the missing carbon atoms in a few instances. Inclusion of these groups has no significant effect upon the conclusions. Our model helix presumes a particular orientation of the OH group on the ethanolamine terminus. Rather than attempting to give the NH_2CH_2OH tail complete flexibility, we consider the three different low energy orientations (staggered with respect to the neighboring α-carbon atom) of the terminal OH group. While rotation does affect the channel solvation energy for an ion at the pore mouth, its influence on anions and cations is quite similar.

We continue to use the polarizable electropole model for water molecules (Barnes et al., 1979). Ions are described as polarizable spheres with point charges at their centers; the polarizabilities are those estimated by Gowda and Benson (1982).

In addition to the electrostatic terms, the various groups interact via Lennard-Jones 6–12 potentials. The parameters for water–water and water–ion interaction are established from microcluster properties (Sung and Jordan, 1986*c*). The water–water parameters are used to describe water–CO and water–NH interaction as well; cation–water parameters describe cation–CO and cation–NH interaction (Lee and Jordan, 1984). The same approximation is applied to chloride-NH interaction but not to chloride–CO. The reason, discussed elsewhere (Sung and Jordan, 1987), is that water and NH contain H-atoms that do not significantly affect group size (MacKay et al., 1984; Jorgensen and Swenson, 1985). A chloride ion interacting with a carbonyl group senses a rather larger group than one interacting with a water molecule. For Cl^-–CO interaction, size is determined by the C as well as the O; this is quite different from interaction with water where size is mainly attributable to interaction with O.

Our calculations focus upon the potential energy profile at 0°K, with particular emphasis on the locations and magnitudes of the energy minima. The total potential is the sum of five terms describing the contributions of electrostatic, polarization, Lennard-Jones, deformational, and orientational terms. We use the same notational scheme described previously. CO groups in the half helix occupied by an ion are numbered from 0 to 16; those in the other half helix are numbered 0″ to 16″. CO-0 is the formyl carbonyl; CO-16 is the COH group of the ethanolamine linkage at the channel mouth.

RESULTS AND DISCUSSION

General Considerations

One observation giving credence to our polarizable multipole picture is the total dipole moment of the groups in the channel. The bare dipole moments of the CO and NH moieties, determined from their uncompensated partial charges, were 2.26 and 0.86 Debye. In the fully interacting channel, the dipole moments are much larger due to the polarization interaction. Their respective average values are 2.65 and 1.30 Debye, just the values normally assigned in an interacting system (Pethig, 1979). Naturally, there are significant fluctuations about the mean, roughly ±10%, depending upon the local environment.

In the following sections, we first discuss the interaction between ions and the channel with no water present. Then we focus briefly on the water channel interaction before discussing the properties of the ion–water–channel system. The limitations of the model are then outlined and we conclude by describing what our results imply as to the origin of gramicidin's pronounced valence selectivity.

Ions in an Empty Channel

As illustrated in Fig. 1, the channel solvation energy profiles for Cl^- and Cs^+ in an empty channel differ substantially. For cesium there is a series of energy minima separated by ≈1.5 Å, the channel periodicity. For chloride

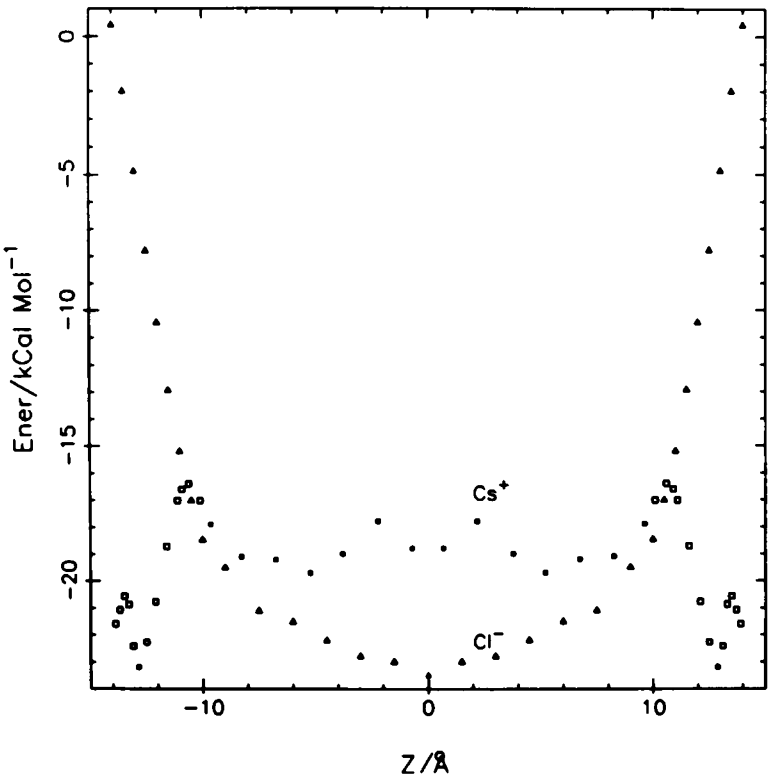

FIGURE 1 Channel solvation energy profiles for Cs^+ and Cl^- in a gramicidin-like channel with no water molecules present. *Solid squares*, relative minima in the cation potential surface. *Open squares*, the lowest energies for a cation constrained in a plane perpendicular to the axis (fixed z). *Solid triangles*, the only stable minimum on the anion surface. *Open triangles*, the lowest energies for an anion constrained in a fixed axial plane.

the picture is totally different. Compared with the cation, anion–channel solvation is relatively very unfavorable in the channel mouth; furthermore, there is only one energy minimum, the channel midpoint (a saddle point for the comparably sized cesium ion). Rather surprisingly, our calculations suggest that in the channel interior, the anion is more stable than the cation.

The general structure of the ion–channel potential energy profile is the most significant result of our work. As we shall see, the gross features are independent of whether or not water is included in the treatment even though the actual value of the channel solvation energy, Eq. 1, is quite dependent upon the presence of water. The dramatic differences between the two profiles reflect the differences in the way that the ions interact with the channel. The channel has a gross quadrupole moment, negative at both mouths and positive in the middle (Sung and Jordan, 1987); consequently, the cation is attracted to the channel while the anion is repelled by it (at 14 Å the anion's channel solvation energy is slightly positive). The large difference in channel solvation energy, Eq. 1, suggests that near the mouth of the channel anionic interaction is far less favorable than cationic interaction. Fig. 1 ignores the influence of ionic hydration. Since this must make the channel solvation process significantly less favorable energetically everywhere along the potential profile (as will be seen in the discussion of the ion–water–channel system), the energy differences in Fig. 1 translate into a substantial barrier to anion entry for a channel–electrolyte system.

The low energy anion configurations are along the channel axis; in no instance does Cl^- wander more than 0.15 Å from the center of the channel. For Cs^+, the results are dramatically different; over most of the length of the channel, the low energy configurations are 0.5–0.6 Å from the axis. In the channel mouth, where the cation can interact strongly and favorably with peptide-11, an observation already established experimentally (Urry et al., 1982*a*, *b*), its favored location is ≈3.0 Å from the channel axis. The total dipole moment of this CO group is anomalous, 3.6 Debye, about 35% larger than average. The CO groups in close proximity to a Cl^- are only slightly affected by the presence of the ion; their dipole moments usually differ insignificantly from the channel averaged values. For a Cl^- at the mouth of the channel (13.0 Å from the channel midpoint), the interaction with CO-11 is also anomalous. The dipole moment of this group is extremely small, only 2.24 Debye, less than its permanent moment; the local field opposes the bare dipole.

For Cs^+, the binding site at the mouth of the channel is by far the most stable location. For Cl^-, it is the channel center. These differences can be understood, in part, by contrasting local structures in these two regions. Fig. 2

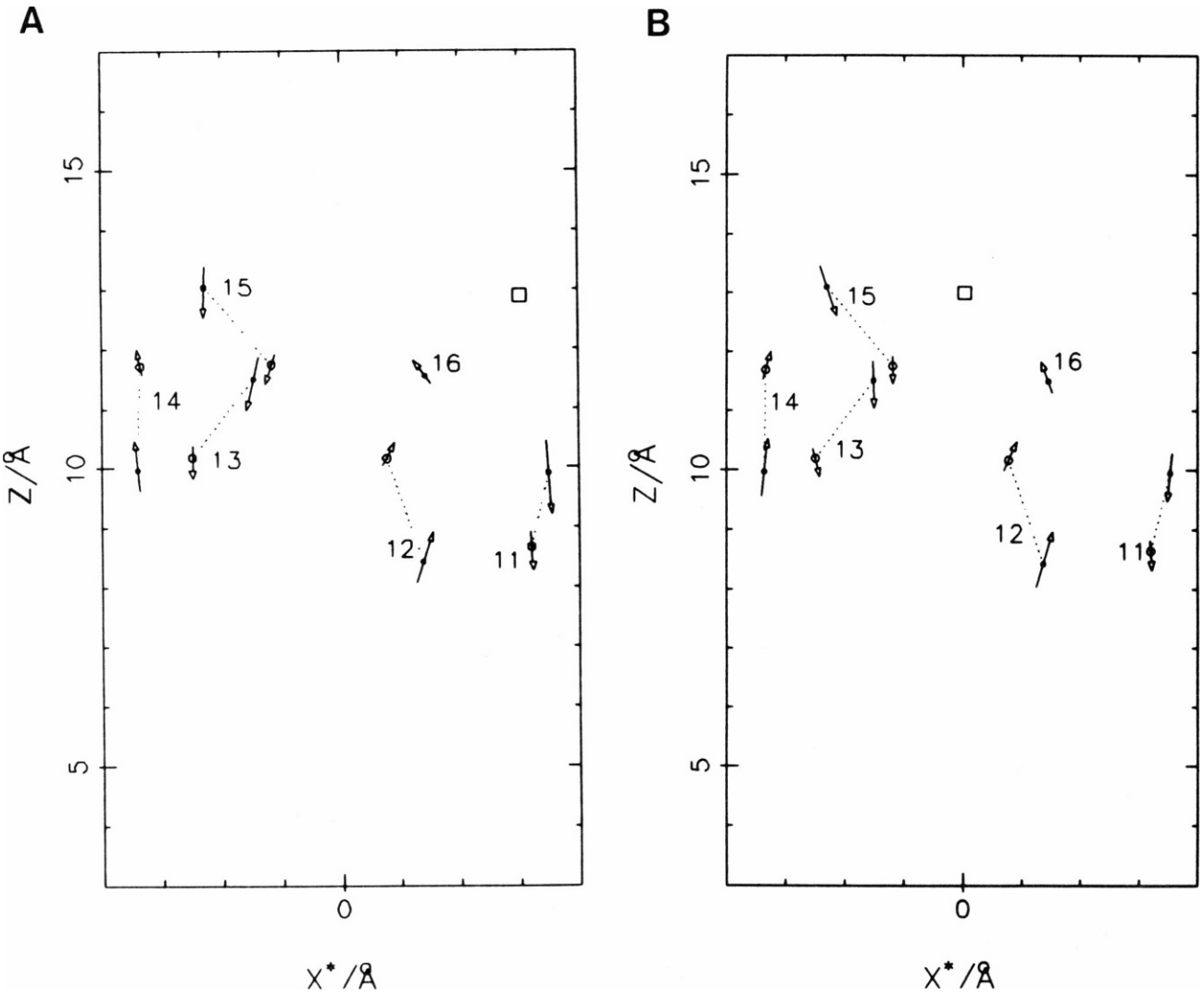

FIGURE 2 Projections of a bare ion and the CO and NH groups in the last turn of the protein helix. The projection planes are defined by the Z-axis and the cation (the axis in this plane is labelled X*). *Square, solid circle,* and *open circle* indicate the ion, the CO group, and an NH group, respectively. *Arrows,* the magnitude and the direction of the total dipole moment of the various groups; the arrowhead points toward the positive end of the dipole. A dipole with length equal to one division on the axis is 2.8 Debye. The numbers indicate which groups correspond to which peptide unit. For clarity, the CO groups are connected to their neighboring NH groups by dotted lines. The favorable relationship between the Cs^+ and CO-11 is shown in *A;* the significantly different orientation of the polar groups surrounding the Cl^- when it is 13.0 Å from the dimer junction is illustrated in *B.*

illustrates the interaction between the ion and the groups that form the last turn of the helix (the channel mouth). The projection plane is the same for both Cs^+ and Cl^-; it is defined by the channel axis and the cation. The differences between anion and cation interaction are striking. The cation approaches CO-11 closely and lines up directly along the dipolar axis. The anion cannot do this. Comparison of Fig. 2, *A* and *B* shows that the anion strongly repels groups 11, 13, 15; the attraction to groups 14 and 16 does not compensate and the anion remains close to the axis. This is consistent with the channel's local radial dipole moments (Sung and Jordan, 1987). While small, their negative poles are directed toward the axis almost everywhere in the channel forcing anions toward the axis.

Fig. 3 illustrates binding at the dimer junction. Here, the projection plane is chosen to emphasize the symmetrical nature of anion binding. The groups that interact most strongly with Cl^- are 2″, 0″, 0, and 2; those that interact most strongly with Cs^+ are 2″ and 4. To permit easier comparison, all five groups are included in each sketch. Cl^- is symmetrically situated between the equivalent groups; none is oriented especially favorably. Cs^+ is ≈0.6 Å from the axis (the projection used does not illustrate this) and ≈0.8 Å from the channel midpoint. It is asymmetrically located with respect to groups 2″, 0″, 0, and 2 and has moved much closer to group 4, with which it is strongly associated. Comparison of Fig. 3, *A* and *B* again illustrates the large influence the ion has upon dipole orientation. Just as at the channel mouth, the anion has no especially favorable local environment; as a consequence, it is most

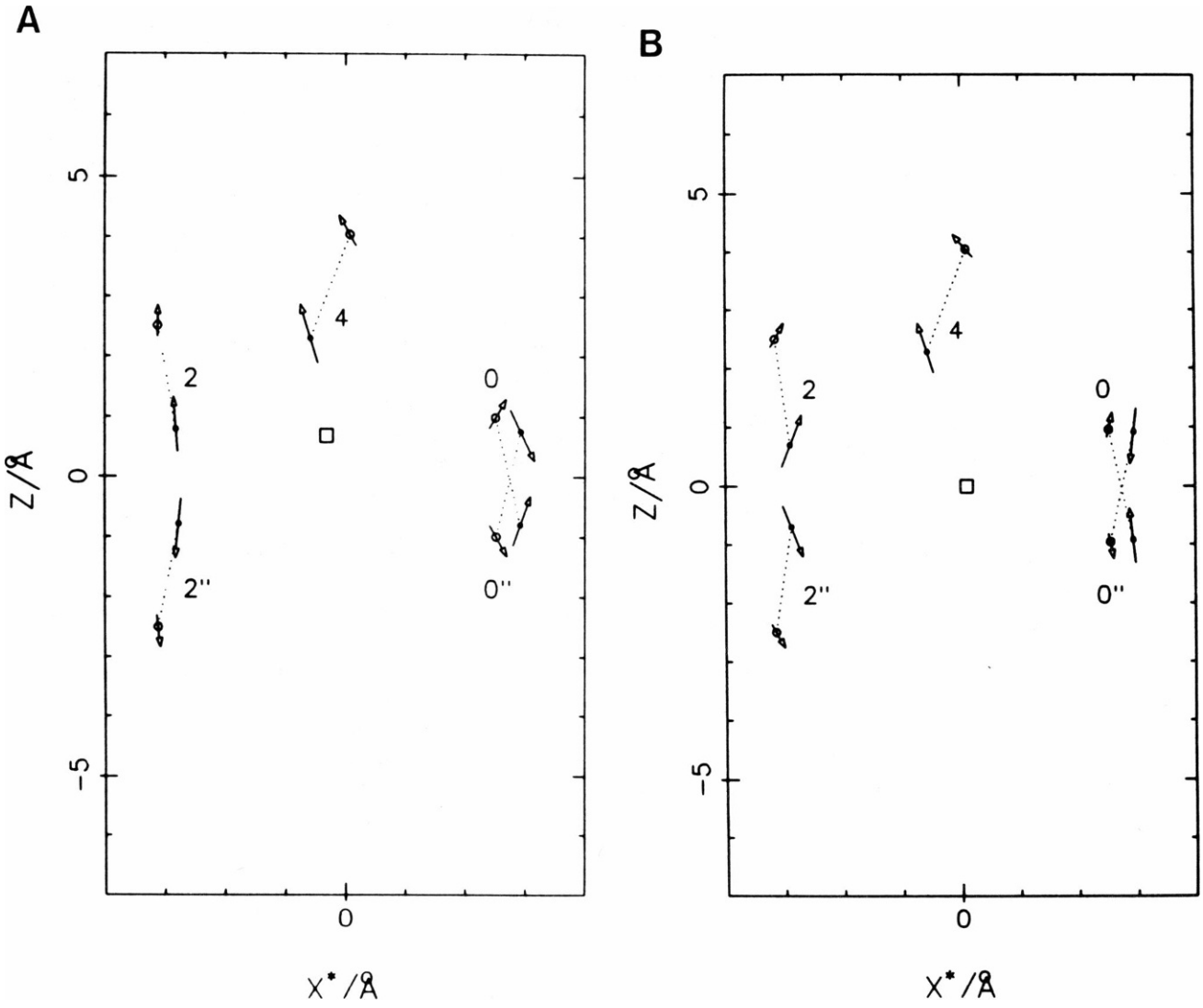

FIGURE 3 Projections of a bare (*A*) Cs^+ and (*B*) Cl^- and the polar groups with which they interact most strongly for the binding site near the dimer junction. The projection plane is chosen to emphasize the symmetry of the anion's local environment. The conventions are defined in the legend for Fig. 2. The cation is asymmetrically sited because it is strongly attracted to peptide groups 2″ and 4. The anion is bound symmetrically with respect to groups 2″, 0″, 0 and 2 as no local dipolar interactions favor binding.

stable near the channel axis. The cation associates with specific polar groups.

Local environmental differences account for preferential cation binding at the channel mouth and for the lack of structure in the anion binding profile. They do not explain the origin of the deep well in the anion energy profile. The most obvious reason is the gross charge distribution. The unpolarized channel has a quadrupole moment (Sung and Jordan, 1987); the total axial moment is negative in the channel mouths and positive at its midpoint. The axial dipole moment in the upper half of the channel is ≈0.5 Debye; that in the lower half of the channel is equal and opposite. The effect is augmented by self-polarization; in the empty channel, the half-channel dipole moments increase substantially to ≈1.7 Debye. As a result, the channel solvation energy profile is essentially flat once a cation has entered the channel; for an anion there is a substantial well. While cations bind to specific polar groups, it is the overall charge density in the interacting system that creates an anion binding site at the channel center.

The anion energy profile defines the potential energy along the permeation trajectory as Cl^- traverses a water-free gramicidin-like channel. In the channel interior, the cation profile does not define the potential energy as the calculations are for successive local minima. However, typical energy barriers for Cs^+ hopping in the favorable direction in the channel interior are ≈1 kcal mol^{-1} (Lee and Jordan, 1984). The complete profile, including saddle points and intermediate points, has only been established for cation permeation through the mouth of the channel. The differences between the low energy trajectories of cations and anions in the 10 to 14 Å region are simple and striking. The anion remains close to the axis while the

cation's radial location is a sensitive function of position. In the 13.5 to 14 Å region, the low energy trajectory places Cs^+ only ≈1.1–1.4 Å from the axis. As the cation approaches CO-11 the low energy path abruptly moves much further from the axis; then, as the ion moves further into the channel, it again moves much closer to the axis. It is ≈0.5 Å from the axis at the binding sites in the channel interior.

Water in the Channel

Channel solvation energy profiles for chains of two and four water molecules in the channel are illustrated in Fig. 4. The points indicate the z-coordinate of the center of mass of the water molecules in the low energy configurations. The most significant feature is the occurrence of local minima reflecting the channel's helical periodicity. It is also notable that the symmetric configuration at the channel center is not a local energy minimum; the stable arrangements have z-components of their centers-of-mass ≈0.5 Å to either side of the channel midpoint, a feature that has been observed by others (Fornili et al., 1984)

Ions and Water in a Channel

Channel solvation energy profiles for Cs^+ and Cl^- associated with two and four water molecules in the channel are illustrated in Fig. 5, *A* and *B*. The ion has been sandwiched by water, with which it forms an essentially linear chain, as shown in Figs. 6 and 7 (for the case of four water molecules). The channel solvation energy profiles, which are similar in overall shape to those without water present (Fig. 1), depend upon the total energy profiles of both the ion–water–gramicidin complex and the water–gramicidin complex. As can be seen from Fig. 4, the energy of the gramicidin · $(H_2O)_n$ complex is different at each ion binding site. The value used is the energy of the water chain for which the center-of-mass z-component of the water molecules is roughly the same as for the ion–water chain at the binding site of interest. Our energy calculations do not treat the influence of bulk water, which is presumably most significant as the ion–water chain nears the channel mouth. The trend, comparing Figs. 1 and 5, suggests that by neglecting bulk water we overestimate the stability of the hydrated ion-gramicidin complexes. However, as our main interest is the difference between anion and cation properties, we believe that the errors are roughly the same for both species. Certainly Figs. 1 and 5 suggest that this is true for the systems we are studying.

Comparison of Figs. 1 and 5 shows that the existence and location of minima in the channel solvation energy profile for the cation are not sensitive to the presence of water; the spacing is again ≈1.5 Å, reflective of the helical periodicity. For the anion, there is now a succession of minima; these demonstrate the importance of the water

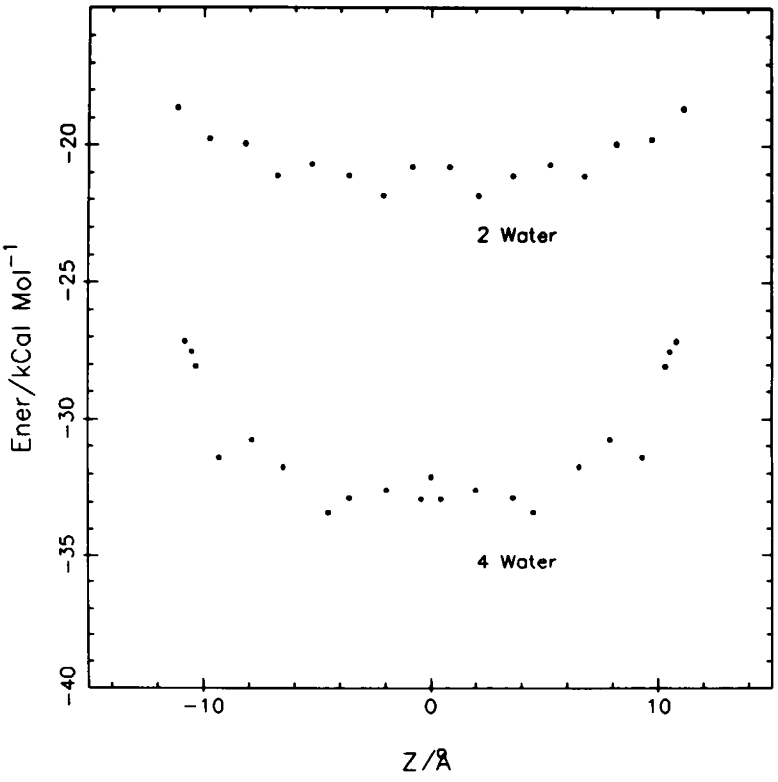

FIGURE 4 Channel solvation energy profiles for two and four water molecules in the model helix. The points denote relative mimina on the potential energy surface.

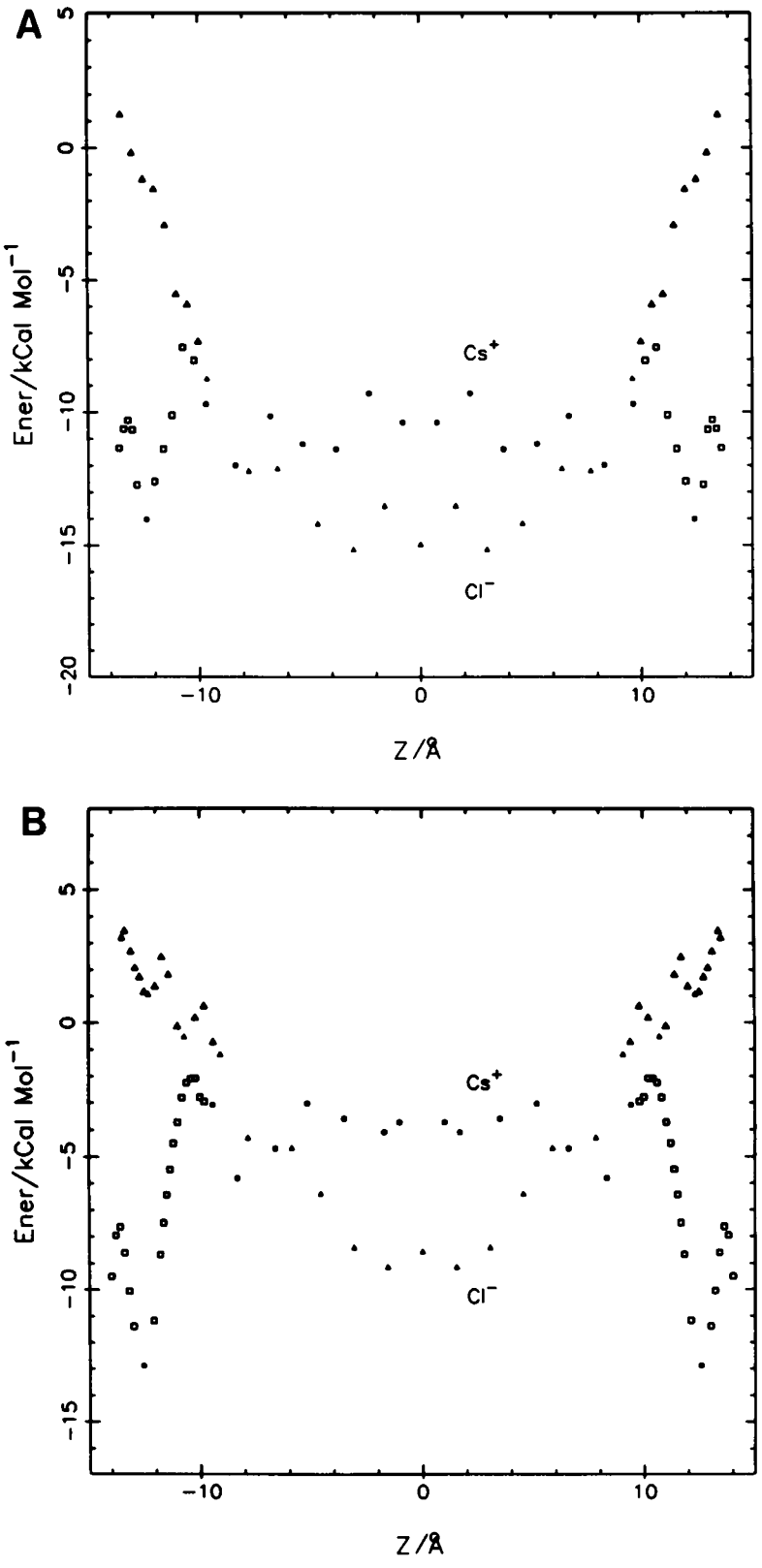

FIGURE 5 Channel solvation energy profiles for Cs^+ and Cl^{-1} in gramicidinlike channels with (*A*) two and (*B*) four water molecules present. The conventions are those of Fig. 1. The qualitative similarity between these curves and those of Fig. 1 is self-evident.

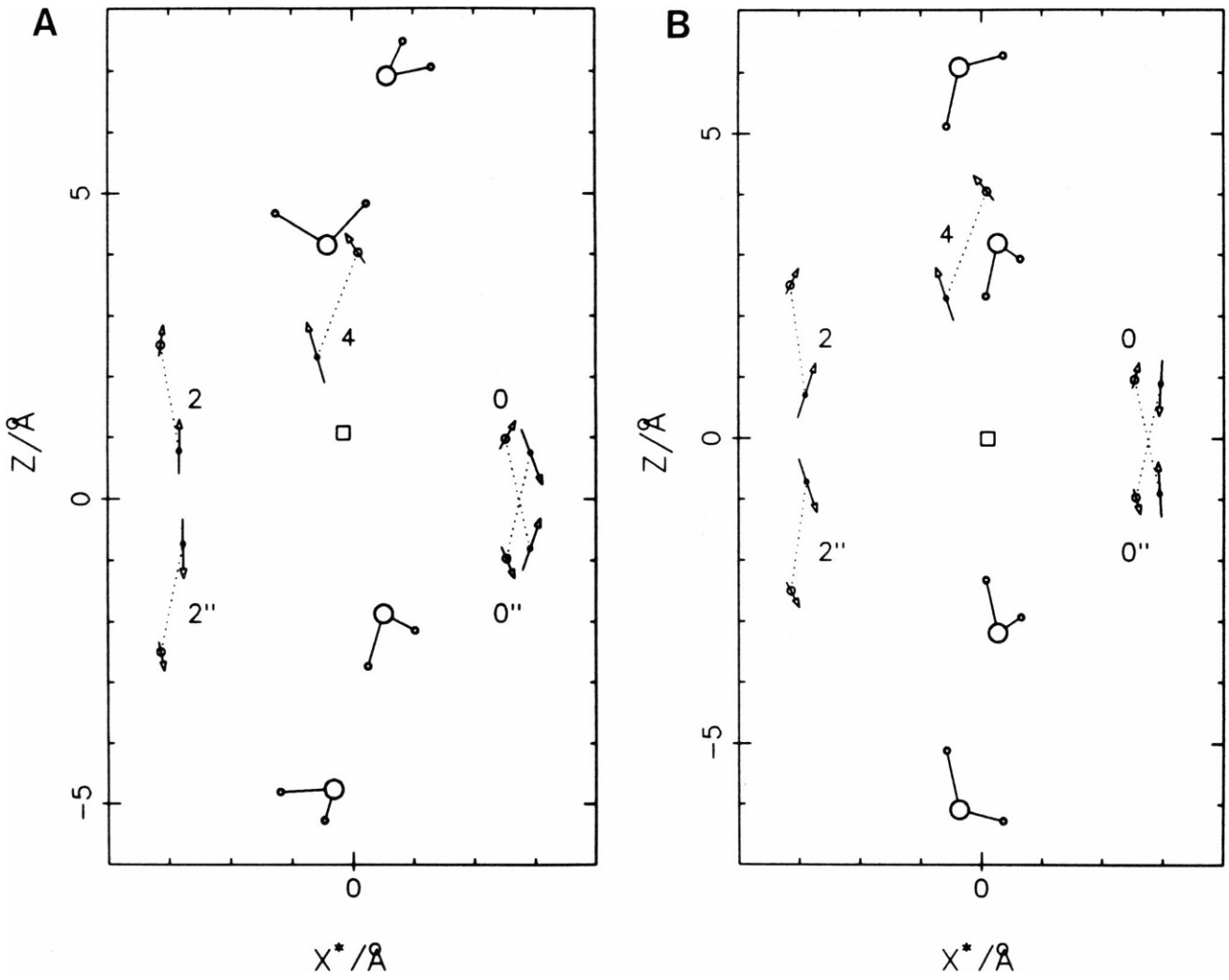

FIGURE 6 Projections of (*A*) Cs^+ and (*B*) Cl^{-1}, the nearest CO and NH groups and four water molecules for ion binding near the dimer junction. The projection is the same as that of Fig. 3. The influence of the ion on peptide and water orientation is evident.

molecules. The occurrence of the intermediate binding sites is due to water-channel interaction; the overall shape of the profile reflects chloride–channel interaction.

Two distinct structural domains can be identified. There is a single file region extending ≈10 Å to either side of the channel midpoint. Here, the channel water molecules form linear dipolar chains, oriented by the ion, as shown in Fig. 6 for the binding site nearest the dimer junction. Ion–peptide binding is quite similar to what is found when no water is present (see Fig. 3). The water molecules hydrogen-bond to one another and the channel constrains the ions to the vicinity of the axis. Comparison of Figs. 3 and 6 indicates that the interaction with water has had negligible effect on the structure of the ion-gramicidin complexes.

Ion binding near the ethanolamine terminus is completely different (Lee and Jordan, 1984; Kim et al., 1985). Comparison of Figs 2 *A* and 7 *A* illustrate that water significantly affects cation binding. Because of the openness of the helix, Cs^+ still binds quite far from the channel axis (Fig. 7 *A*). However, the interaction with water causes it to be less favorably oriented with respect to CO-11; instead of binding ≈3.0 Å from the axis, the binding site is now ≈1.6 Å from the axis. As a result the dipole moment of CO-11, while still 15% larger than the channel average value, is much smaller than when no water is present. Using the differential channel solvation energy as a guide, it would appear that the binding site is not in the single file region of the channel but rather in the mouth, an identification different from that made by only considering the total energy profile (Lee and Jordan, 1984).

Water also significantly influences anion solvation at the mouth. In the absence of water, Cl^- does not bind. The solvation structure of the last turn of the helix is shown in Fig. 7 *B*. It is very different from that shown in Fig. 7 *A* (for Cs^+ in the mouth). However, the ion–peptide arrangement is again nearly identical to that found in the water-free channel (Fig. 2 *B*).

Because the mouth is so capacious, the hydration structure differs significantly from that in the single file region. Water molecules in the endo-region still form a linear

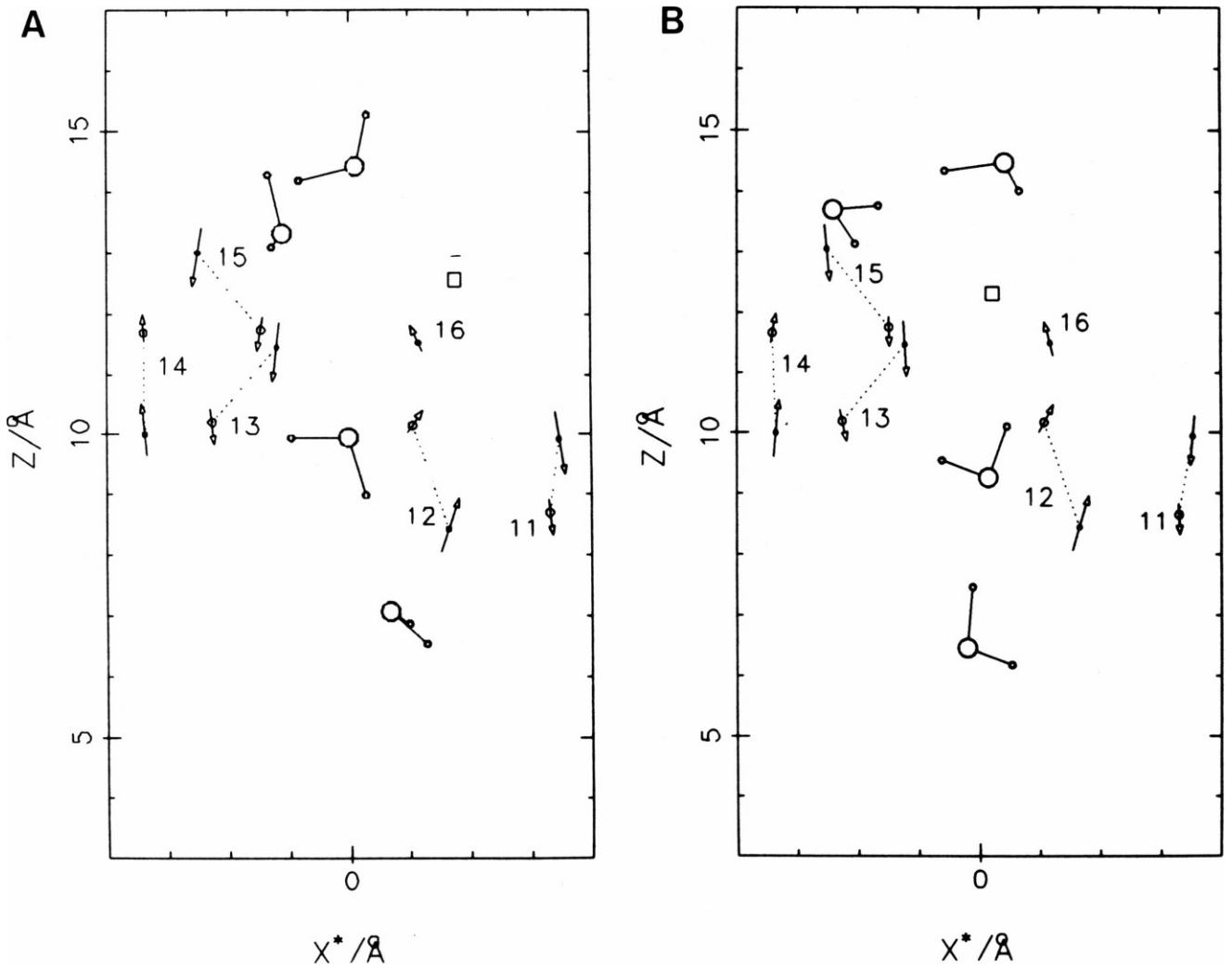

FIGURE 7 Projections of (*A*) Cs^+ and (*B*) Cl^{-1}, the CO and NH groups of the last turn of the helix and four water molecules for ion binding near the mouth of the channel. The projection convention is the same as in Fig. 2; because the cation binding site is at a slightly different location, the projection plane is not the same as that of Fig. 2. The effect of the ion on peptide and water orientation is again obvious.

chain; exo- to the channel ion hydration strongly resembles that of bulk water, a feature that has been observed previously for cations (Fornili et al., 1984; Lee and Jordan, 1984). Even though there is partial bulk hydration at this site, ion motion through the channel would still have to be single file and coupled to the motion of the water chain.

The channel solvation energy profiles of Fig. 5 establish isolated points on the permeation trajectories for coupled ion–water motion in the channel interior. The energies at intermediate points, which have only been approximately determined because substantial water reorientation is required as ions move across the saddle points (Lee and Jordan, 1984), are included for ions in the channel mouth. Qualitatively, ionic motion is similar to that found when no water is present. Cl^- remains close to the channel axis throughout its permeation. Cs^+ oscillates. It moves away from the axis and close to the CO groups as it approaches a binding site; it moves closer to the axis as it approaches a saddle point. As Cs^+ moves toward the exterior binding site from outside the channel it is $\approx$1.6–2.0 Å from the channel axis. It then moves rapidly towards the axis as it passes into the channel interior; at the saddle point it is only $\approx$0.35 Å from the axis.

Effects due to Model Primitiveness

Our calculations suffer from some severe oversimplifications. The α-C atoms of the helix have been ignored; the ethanolamine group at the mouth is not treated as flexible chain. We have investigated the consequences of these approximations.

Within the single file region of the channel, inclusion of the α-carbon atoms has only a minor effect, even for ions as small as lithium (Jordan, P. C., manuscript in preparation). With water present, the location of the binding sites varies insignificantly when the α-carbons are incorporated into the model.

In the mouth of the channel and at the dimer junction,

α-carbons must be included. Because the mouth is quite open, the α-carbon atoms influence both the solvation structure and the channel solvation energy. The channel solvation energy is substantially less negative (or more positive, for the anion) for the complete helix than for the punctuated one. Similar changes occur at the junction. Comparisons of calculated channel solvation energies for the full and the punctuated helices are given in Table I at the three binding sites most sensitive to the absence of the α-carbon atoms: the site nearest the dimer junction, the first single file site, and the mouth site. Within the rest of the single file region of the channel the differences are $\approx$0.5 kcal mol^{-1}.

We have not attempted to give the ethanolamine tail complete configurational flexibility. Instead, we have treated reorientation of the terminal OH group by carrying out three different calculations in which the OH group is staggered with respect to the neighboring CH_2NH_2 group. Throughout the single file region, OH orientation has essentially no influence on the channel solvation energies. In the channel mouth, reorientation influences stability. The effect is more pronounced for cesium than for chloride, as seen from the entries in Table I.

Limitations of the Model

Our calculations place the cation binding site in the channel mouth, preferentially associated with CO-11, in accord with the interpretation of nuclear magnetic resonance (NMR) data (Urry et al., 1982*a, b*). For Cs^+, this site is $\approx$12.5 Å from the dimer junction; for Na^+, similar calculations locate it $\approx$11.8 Å from the junction (Jordan, P. C., manuscript in preparation). These distances are larger than the value of $\approx$10.5 Å, suggested by kinetic analysis (Andersen et al., 1981). This may reflect the fact that in the interpretation of the kinetics "electrical distance" is equated to physical distance. The total voltage drop is presumed to be confined to the length of the channel. If, as is likely (Jordan, 1982), the field extends outside the channel, the binding sites would be further from the dimer junction. The identification of binding sites at 10.5 Å from the channel center (Koeppe et al., 1979) was for a dimer crystallized from organic solvent; this probably forms antiparallel double stranded helices, not head-to-head dimers (Wallace, 1986).

However, the discrepancy may be due to our model. The Lennard-Jones parameters for ion and water interaction with the CO and NH groups have simply been assigned; unlike the ion-water and water-water parameters, they are not optimized. Another source of uncertainty is the force constants. Ours permit relatively small displacement of the peptide groups. With water present, CO-11 only tilts $\approx$14° from its original orientation. Other calculations on the Cs^+-gramicidin system have suggested that the angular deviations could be as large as 60° (MacKay et al, 1984). Finally, our calculations focus upon channel properties at 0°K. Thermal motion contributes to CO flexibility.

TABLE I
SOLVATION ENERGIES (IN KCAL MOL^{-1}) FOR Cs^+ AND Cl^- AND VARIABLE NUMBERS OF WATER MOLECULES AT SELECTED SITES IN THE CHANNEL*

Model system	Cs^+			Cl^-		
	A	G	H	A	G	H
Four waters						
Punctuated helix	−3.7	−3.1	−12.9	−8.6	−1.2	+1.0
Complete helix	−5.1	−2.4	−11.6	−8.8	−0.4	+3.6
Reorientable terminal OH	−4.8	−3.3	−13.3	−8.7	−2.2	+1.4
No water						
Punctuated helix	−18.8	−17.9	−23.2	−23.5	‡	‡
Complete helix	−18.8	−17.9	−23.1	−23.5	‡	‡
Reorientable terminal OH	−18.7	−18.4	−25.0	−23.5	‡	‡

*Site A is the site nearest the dimer junction; site G is the single file site nearest the channel mouth; site H is the binding site in the mouth.
‡These are not binding sites for Cl^- in the absence of water.

Valence Selectivity

The differences in channel solvation energy between Cs^+ and Cl^- at the channel mouth are large. The values are listed in Table II for the various approximate calculations we have carried out. Regardless of how many water molecules are included in the simulation, of whether or not the α-carbon atoms are considered, or if the OH group of the ethanolamine terminus is mobile, the conclusions are the same. The channel solvation energy for a chloride ion at the channel's mouth is $\approx$13–17 kcal mol^{-1} more positive than for a cesium ion. If this is a reliable measure of the difference in the energy barrier to ion entry, kinetic factors of $\approx 10^9$–10^{12} are to be expected. A more direct (but less accurately calculated) estimate, given by the difference in saddle point energies for passage over the barrier at the channel mouth, indicates that this is not unreasonable. Using a two or four water molecule model, the results for the punctuated helix imply that the energy barrier to ion entry is $\geq$11.5 kcal mol^{-1} more positive for chloride than for cesium.

The rate constant for channel dissociation into mono-

TABLE II
SOLVATION ENERGY DIFFERENCES (IN KCAL MOL^{-1}) BETWEEN Cl^- AND Cs^+ FOR BINDING IN THE MOUTH OF THE GRAMICIDIN CHANNEL*

Number of water molecules	Punctuated helix	Complete helix	Reorientable terminal OH
0	12.7	12.7	13.5
2	15.6	16.3	16.8
4	13.9	15.1	14.7

*For the cases with zero and two water molecules, Cl^- does not bind in the mouth of the channel; the solvation energy is computed for an anion clamped 12.0 Å from the dimer junction.

mers is in the range of 1–100 s^{-1} (Bamberg and Benzy, 1976; Kolb and Bamberg, 1977). Permeation of a cesium ion through the channel requires 0.1 μs (Urban et al., 1980); a chloride ion would need roughly 100–10^5 s to enter the channel during which time it would have long since dissociated.

All the calculations show that, in the channel center, Cl^- is 4–5 kcal mol^{-1} more stable than Cs^+. This indicates that valence selectivity is a consequence of the large barrier to anion entry; if the anion could surmount this obstacle, it should be able to bind in the channel. Put differently, we feel that the process of ion entry is kinetically, not thermodynamically, controlled.[1] This is consistent with the viewpoint outlined by Hille (1975) and by Armstrong (1975) that emphasizes the importance of energy barriers along the potential profile in determining relative ionic permeabilities.

Our emphasis differs from that of Urry and his coworkers (Urry et al., 1981; Venkatachalem and Urry, 1984). They note that libration of the CO groups forming the mouth of the channel tends to create a binding site peculiarly favorable to cation coordination. The conformational stabilization associated with this deformation is only $\approx$4 kcal mol^{-1} (Venkatachalem and Urry, 1984), by itself not enough to account for Cl^- impermeability. Implicit to this picture is the observation that an anion is naturally repelled by the local charge distribution created by these groups (Urry et al, 1981). Our calculation suggests that CO-11 interacts especially strongly with cations, while anions are rejected at the channel mouth because of the dipolar orientation. In this respect, the two viewpoints are similar. However, our analysis also suggests that, if the entrance barrier could be overcome, anions might contribute to the conductance of gramicidin. A mechanism for reducing this barrier would be the presence of a cation at the binding site at the other end of the channel. There might then be enough interaction across the channel to permit anion entry. Some evidence for such cooperativity at high salt concentration has been reported (Eisenman et al., 1977). Double occupancy calculations are being carried out to provide a theoretical estimate of the size of this effect.

Summary

Channel solvation energy profiles for Cs^+ and Cl^- associated with water in gramicidin-like channels have been contrasted. There is a large barrier to anion entry that precludes either blocking or permeation during the lifetime of a gramicidin dimer. If a chloride ion could enter the channel, it would be $\approx$4–5 kcal mol^{-1} more stable at the channel center than a cesium ion. Cations bind preferentially to the exposed CO-11 group in the open channel mouth. Anions are stabilized at the channel midpoint by the quadrupolar nature of the dimer; it is negative in both mouths and positive at the dimer junction.

This work has been supported by grant GM-28643 from the National Institutes of Health. We are most grateful for the valuable critiques given by the referees.

Received for publication June 20 1986 and in final form 4 December 1986.

[1]Thermodynamic control is exemplified by the stability of water with respect to molecular hydrogen and oxygen; $\Delta G \gg 0$ for this reaction and it is thermodynamically forbidden. At room temperature, the reverse reaction, if uncatalyzed, does not occur even though $\Delta G \ll 0$; it is kinetically forbidden.

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