Valence Selectivity of the Gramicidin Channel: A Molecular Dynamics Free Energy Perturbation Study

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ABSTRACT The valence selectivity of the gramicidin channel is examined using computer simulations based on atomic models. The channel interior is modeled using a gramicidin-like periodic poly (L, D)-alanine β-helix. Free energy perturbation calculations are performed to obtain the relative affinity of K^+ and Cl^- for the channel. It is observed that the interior of the gramicidin channel provides an energetically favorable interaction site for a cation but not for an anion. Relative to solvation in bulk water, the carbonyl CO oxygens can provide a favorable interaction to stabilize K^+, whereas the amide NH hydrogens are much less effective in stabilizing Cl^-. The results of the calculations demonstrate that, as a consequence of the structural asymmetry of the backbone charge distribution, a K^+ cation can partition spontaneously from bulk water to the interior of the gramicidin channel, whereas a Cl^- anion cannot.

INTRODUCTION

Understanding the selectivity of biological transmembrane ion channels at the molecular level is of fundamental interest. Electrophysiological flux measurements have demonstrated that for most biological ion channels a narrow region along the permeation pathway acts as a molecular sieve, or filter, able to select specific ions (Hille, 1992). Evidence from sequence analysis and site-directed mutagenesis on biological ion channels suggests that the selectivity filter is constituted by highly conserved residues (Catterall, 1988; Heineman et al., 1992; Hille, 1992; Sather et al., 1994). Nevertheless, little information is available about the functional molecular unit enabling a biological ion channel to discriminate between different ions. Charge specificity may be considered the crudest form of ion selectivity. In a simplified view, residues carrying a net charge provide an obvious mechanism for discriminating between ions with different charges (e.g., anions versus divalent or monovalent cations). For example, the presence of a number of specific residues carrying a negative charge, such as aspartic and glutamic acid, appears to be correlated with the selectivity of the sodium and calcium channels (Heineman et al., 1992); calcium-selective channels appear to possess more negatively charged residues in the selectivity filter region than the sodium channel, which is otherwise very similar. An obvious reason is to provide the stabilizing interactions needed to dehydrate a divalent cation. Residues carrying a positive charge such as arginines and lysines might play a similar role in the case of anionic channels (Galzi et al., 1992). Nonetheless, even though the charge of residues

located along the permeation pathway certainly plays an important role, a number of neutral but polar groups can also contribute to the selectivity of an ion channel. For example, it has been suggested that backbone carbonyl oxygens could be involved in the selectivity filter of potassium channels, although such a mechanism cannot be easily tested with site-directed mutagenesis experiments (Lipkind and Fozzard, 1995). An interesting puzzle for that matter is the marked valence specificity exhibited by the gramicidin A dimer channel (GA). Experimentally, it is observed that the GA channel is ideally selective for cations while it is virtually impermeable to anions (for a recent review, see Andersen and Koeppe, 1992). In fact, anions do not penetrate into the channel at all, because no blocking by anions is observed. Because GA carries no net charge, no obvious discrimination mechanism is suggested based on the structure of the channel.

Because it is so well characterized, the GA channel offers a unique model system for addressing questions concerning the valence specificity at the molecular level. As originally formulated by Eisenman (1962), selectivity results from a free energy difference between the hydrated and bound states. Permeating ions translocating through the GA channel interact mostly with the backbone atoms and the water molecules disposed in single file along the channel axis. The molecular origin of the valence specificity must therefore lie in the detailed interactions with the backbone and single-file waters involved in the permeating process. A permeating ion in GA is mostly stabilized by its interactions with the atoms of the backbone peptide group. Amides such as formamide, acetamide, N-methylacetamide (NMA), and dimethylformamide (DMF) are thus good models for understanding the details of ion-channel interactions. In addition, comparison of the free energy of transfer of cations and anions from water to amide solutions can provide useful insight because it has been observed that the binding of common monovalent cations to the GA channel is analogous to the thermodynamic equilibrium partition of those

ions between bulk water and DMF (Hinton et al., 1986). The experimental free energy needed to transfer one K^+ from bulk water to DMF is -2.3 kcal/mol (Cox et al., 1974). In striking contrast, the free energy needed to transfer one Cl^- from water to DMF is $+11.0$ kcal/mol. Of course, DMF does not have the ability to form hydrogen bonds, thus making the transfer more unfavorable for anions than for cations. However, the transfer of anions from water to liquid formamide is also unfavorable, despite the possibility of forming hydrogen bonds. The free energy needed to transfer one K^+ from water to formamide is -1.5 kcal/mol, whereas that needed to transfer Cl^- is $+3.3$ kcal/mol, implying a difference in solvation free energy of 4.8 kcal/mol. There are no experimental estimates for the transfer of ions from water to liquid N-methylacetamide, which would be a better solvent for modeling the backbone of the GA channel. In summary, free energy thermodynamic data indicate that the transfer of cations from water to liquid amides is favorable, whereas that of anions is not.

It ought to be possible, in principle, to understand how the GA channel can be valence selective based on a microscopic model. Yet because the selectivity involves a delicate balance between very large solvation free energies (in bulk water and in the channel), it is difficult to assess the various contributing factors. The contradictory views emerging from previous theoretical studies illustrate the difficulties in attempting to understand the molecular basis for the charge specificity of the GA channel. Urry and co-workers (1981) argued that cations are effectively well coordinated by the carbonyl oxygen ligands in the channel, whereas anions are less well accommodated by the amide hydrogen. The energetics of peptide librations was examined and indicated that a slight distortion of the channel backbone is sufficient to solvate a cation (Urry and Venkatachalem, 1984). In contrast, Sung and Jordan (1987) argued that the presence of an anion inside the channel is energetically favorable, but permeation is opposed by a large electrostatic barrier located at the entrance of the pore due to the asymmetrical charge distribution of the β-helical dimer; the carbonyl oxygens point outward, creating an effective quadrupolar moment at the entrance of the channel. More recently, Dorman, Partenskii, and Jordan (1996) addressed questions about the charge specificity using a mixed atomic/continuum semimicroscopic approach (SMC). Again, it was observed that the absolute ion-channel interaction is effectively more favorable in the case of anions than that of cations. However, it was concluded that the charge specificity of GA arises because an anion binds water more strongly than a cation of the same size and is thus unstable in the channel. Despite the previous studies, a central question remains: is the cation selectivity of the GA channel thermodynamically or kinetically controlled? Sung and Jordan (1987) argued that the presence of anions inside the channel is energetically favorable, but that the channel selectivity is a consequence of slow kinetics due to a large energy barrier opposing anion entry. Urry et al. (1981) argued that specificity of the GA channel is a consequence

of thermodynamic equilibrium: the anions do not partition spontaneously inside the channel, where they are not well coordinated by the backbone amide hydrogen. Dorman et al (1996) concluded that the valence selectivity of GA is thermodynamically controlled, but argued that the anions do not partition spontaneously inside the channel because they bind water more strongly and are more difficult to dehydrate than a cation of the same size.

The goal of this paper is to examine the molecular basis of the valence selectivity of the GA channel. In the present study, the relative affinity of K^+ and Cl^- for the interior of the channel is examined using molecular dynamics simulations based on atomic models and free energy perturbation calculations. These two ions were chosen because they both have a solvation free energy on the order of -80 kcal/mol in liquid water (for a summary of solvation data, see Dorman et al., 1996). The interior of the GA dimer is represented as a simple GA-like periodic poly-alanine β-helix similar to that used in previous studies, and the influence of the membrane lipids and entrance/exit effects are ignored. In addition, the ions are described in terms of simple charged Lennard-Jones particles, and induced polarization effects are not included in the potential function, in contrast with previous studies (Roux, 1995; Roux and Karplus, 1994). Such a simple model was used intentionally. Although it is generally desirable to construct atomic models that are as realistic as possible to make comparison with experiments, the increasing complexity of current simulations has left the impression that molecular dynamics calculations are computationally prohibitive. In recent years, increasingly sophisticated computations have been used to study ion permeation through the GA channel (Roux, 1995; Roux and Karplus, 1994). Extensive molecular dynamics simulations of the GA in a phospholipid membrane involving several thousands of atoms have been performed (Woolf and Roux, 1994, 1996; Woolf et al., 1994). The effect of ion-induced polarization to first and second order have been incorporated (Roux, 1993; Roux et al., 1995). Our purpose is to retain only the essential ingredients needed in a simple, and yet realistic, atomic model to reveal the microscopic origin of the valence selectivity of the GA channel. The simulation systems are intentionally kept as small and simple as possible; e.g., there are only around 360 atoms in the periodic β-helix simulation systems. It is hoped that the present investigation can illustrate how significant biophysical questions can be addressed, in a meaningful way, using a well-designed computational strategy applied to small and simple simulation systems. In the next sections the microscopic models are described briefly and the results of the free energy calculations are discussed.

METHODOLOGY

Microscopic model and potential function

The GA-like channel used in this work is similar to that described previously (Roux and Karplus, 1991a, b). The

channel is represented in terms of a periodic poly (L, D)-alanine β-helix; the side chains of GA are neglected to simplify the system and introduce periodicity. Alanine residues were chosen because the gramicidin monomer has only one glycine residue, and all the other residues yield ϕ, ψ energy maps that are similar to alanine. In contrast with the previous studies, which were based on an extended atom model, all of the atoms, including all hydrogens, are represented. The fundamental unit, treated with periodic boundary conditions to avoid end effects (images of the system are repeated along the helix axis), includes 34 alanine residues, a cation, and eight water molecules. The main system has a length of 26.35 Å, and the helix is oriented along the x axis. The total number of particles forming the simulation system is 365. Helical periodic boundary conditions were implemented using the IMAGE facility of CHARMM (Brooks et al., 1983). The initial system was constructed by putting the ion at the center of the channel and placing the eight water molecules in single file, four on each side of the ion in the helix. No constraints were applied on the helix to maintain the β-helical conformation.

The all-hydrogen PARM22 potential function of CHARMM (Mackerell et al., 1992) was used with the TIP3P water model (Jorgensen et al., 1983). In contrast with previous studies, the K^+ and Cl^- were represented by a simple charged Lennard-Jones (LJ) particle with no induced polarization. Although many-body effects due to ion-induced polarization effects are quite significant in the case of small ions such as Li^+ and Na^+ (Roux, 1993), a simple LJ particle with a point charge can provide a reasonable model for larger ions such as K^+ and Cl^-. In addition, the ion-induced polarization was ignored to show that simple atomic models can be used to address questions about the valence specificity of the GA channel. The Lennard-Jones parameters of the ions were developed to reproduce the experimental solvation free energy in liquid water, following the approach of Åqvist (1990). To compute the free energy of K^+ and Cl^- in water, a spherical system of 120 TIP3P water molecules was constructed. The ions were placed in the center of the sphere. A statistical mechanical spherical solvent boundary potential (SSBP) was applied to reproduce the behavior of an infinite system (Beglov and Roux, 1994). The SSBP includes a reaction field contribution, calculated from the Kirkwood multipolar expansion (Kirkwood, 1934), to approximate the dielectric response of the surrounding bulk water. A dielectric constant of 78.4 was assumed for the surrounding continuum, and the electrostatic multipoles from $l = 0$ to 15 were included in the reaction field sum (the $l = 0$ term corresponds to the familiar Born correction). The LJ parameters were adjusted iteratively to yield a solvation free energy of -80 kcal/mol for both the cation and the anion. This is very close to the best estimates of the chemical potential for K^+ and Cl^- in bulk water reported by Dorman et al. (1996). The LJ parameters of the ion are reported in Table 1. The free energy calculations are described in the next section.

TABLE 1   Nonbonded Lennard-Jones ion parameters

Ions	$R_{min}/2$ (Å)	E_{min}
K^+	1.76375	-0.0870
Cl^-	2.27000	-0.1500

Ab initio calculations were performed to compare with the potential energy function. The ab initio interaction energy of a chloride anion with formamide and N-methylacetmamide were calculated at the Hartree-Fock level using the 6-311G** basis set as implemented in the GAUSSIAN 90 program (Frisch et al., 1990). The ab initio interaction energy of a potassium cation with formamide was calculated at the Hartree-Fock level using the 6-31G basis set of Wachters (1970) with the contraction of Clementi (Gianolio et al., 1978). All ab initio calculations were performed using the GAUSSIAN 90 program (Frisch et al., 1990). In the ab initio calculations, the experimental internal gas phase geometries of formamide (Hirota et al., 1974) and N-methylacetamide (Kitano et al., 1973) molecules were used. To account for the systematic errors due to basis set superposition and overestimated dipole moments, all of the interaction energies were corrected, as described previously (Roux and Karplus, 1994), according to the equation $E_{correc} = (E_{scf} - E_{bsse})\mu_{exp}/\mu_{scf}$, where μ_{exp} and μ_{scf} are the experimental and the computed ab initio electric dipole of the molecule in the absence of the ion, and E_{bsse} is the correction for the basis set superposition error obtained using the counterpoise technique of Boys and Bernardi (1970).

To analyze the average solvation structure around the K^+ and the Cl^-, production trajectories of 200 ps were generated for the water sphere and the periodic helix systems. In all of the simulations the nonhydrogen atoms were submitted to dissipative and stochastic Langevin forces corresponding to a temperature T of 300K and a velocity relaxation rate of 25 ps^{-1}. Such coupling to a heat bath was introduced to obtain better convergence of the Boltzmann statistics characteristic of a canonical ensemble. No cut-off was used for the simulations in the water, NMA, and formamide spheres, and all interactions were included (Beglov and Roux, 1994). A group-based cut-off was used for the simulations in the periodic helix. The nonbonded interactions were smoothly switched to zero over a distance of 3 Å and truncated at 13 Å. The lengths of all bonds involving hydrogen atoms were kept fixed using the SHAKE algorithm (Ryckaert et al., 1977). The integration time step was 0.002 ps for the dynamics. The simulation systems were equilibrated for at least 50 ps.

In addition to the water sphere and the periodic helix systems, spheres of 50 formamide and 50 N-methylacetamide (NMA) molecules were constructed to calculate the relative solvation free energy of K^+ and Cl^- in these two liquid amides. The total number of particles forming the all-hydrogen formamide and NMA systems is 301 and 601, respectively. Dielectric constants of 107.5 and 178.9, consistent with the experimental value for formamide and

NMA (Bass et al., 1964), were used to represent the surrounding continuum in SSBP. Lastly, a periodic chain of eight water molecules with no channel was constructed to calculate the relative free energy of the ions solvated by a bare linear single-file arrangement of waters. To maintain the linear configuration in this simplified system, the single-file waters were confined to a cylinder of 0.5-Å radius using a flat-bottomed harmonic potential with a force constant of 100 kcal/mol/Å.

Free energy calculations

It is assumed that questions about selectivity can be addressed on the basis of the equilibrium association of ion i and ion j with the channel. For ion i, the association constant K_i is given by

$$K_i \sim e^{-[\mathscr{A}_i^c - \mathscr{A}_i^w]/k_B T}, \tag{1}$$

where $\mathscr{A}_i^c$ and $\mathscr{A}_i^w$ are, respectively, the free energy in the interior of the channel and in bulk water. A similar expression can be written for ion j. The ratio of the association constant of two ions can be expressed as

$$-k_B T \ln\left[\frac{K_i}{K_j}\right] = (\mathscr{A}_i^c - \mathscr{A}_j^w) - (\mathscr{A}_j^c - \mathscr{A}_j^w)$$
$$= \Delta\mathscr{A}_{ij}^c - \Delta\mathscr{A}_{ij}^w, \tag{2}$$

where $\Delta\mathscr{A}_{ij}^c = (\mathscr{A}_i^c - \mathscr{A}_j^c)$ and $\Delta\mathscr{A}_{ij}^w = (\mathscr{A}_i^w - \mathscr{A}_j^w)$ represent the relative solvation free energy of the two ions in bulk water and in the interior of the channel. Those free energy differences correspond to the "alchemical" transformation from an ion of type i to an ion of type j in the interior of the channel (c) and in bulk water (w), respectively, which can be calculated using the well-documented computational methodologies of molecular dynamics free energy perturbation or thermodynamic integration (for a recent review, see Kollman, 1993). In the present calculations, the perturbation corresponds to the transformation of a K^+ into a Cl^-, thus involving simultaneous changes in both the ionic charge and the nonbonded LJ parameters. The perturbations were calculated using the intermediate potential function, $U[\lambda] = (1 - \lambda)U[K^+] + \lambda U[Cl^-]$, using the free energy perturbation technique. The free energy differences were calculated using the windowing method, with 20 trajectories generated for λ values given by 0.025, 0.075, 0.125, ..., 0.925, and 0.975; the perturbations were calculated with $\Delta\lambda$ of ± 0.025 for all configurations during the simulation. For

TABLE 2   Solvation free energy in liquid water (kcal/mol)

	CHARMM	Experiments*
K^+	−80	−78.9
Cl^-	−80	−78.0

*The experimental solvation free energies, taken from table 2 of Dorman et al. (1996), correspond to a best estimate obtained from an average over several independent reported values.

TABLE 3   Interaction with a water molecule

	CHARMM-TIP3P		Ab initio*		Experiments
Ions	E (kcal/mol)	d (Å)	E (kcal/mol)	d (Å)	E (kcal/mol)
K^+	−18.9	2.62 (O)	−16	2.60 (O)	−17.9
Cl^-	−14.7	2.24 (H)	−12		−13.1
		3.11 (O)		3.32 (O)	

*Ab initio for Cl^- from Foresman and Brooks (1987); K^+ from Roux and Karplus (1995). The gas phase geometry of water was used for all calculations.

each of the 20 windows, the system was equilibrated for 5 ps, and a production trajectory of 15 ps was generated; the complete free energy calculation for one alchemical transformation of K^+ into Cl^- is equivalent to a single trajectory of 400 ps. Similar procedures were used to compute the charging free energy of K^+ and Cl^- in the water sphere. Only 10 windows were used to turn off the charge of the ion from its full value to zero. This is justified because the process does not involve the full change of sign of the ion charge. Forward (K^+ to Cl^-) and backward (Cl^- to K^+) calculations were performed for the periodic helix; the spheres of water, NMA, and formamide; the periodic helix in the absence of single-file waters; and the linear single file of waters without any channel. All of the free energy calculations required a total of approximately 10 days on one CPU of a SGI Super Challenge.

RESULTS AND DISCUSSION

Table 1 shows the LJ parameters corresponding to the model cation and anion used in the present calculations. The parameters were adjusted empirically to reproduce the experimental solvation free energy of K^+ and Cl^- in bulk water, following the procedure of Åqvist (1990). The experimental solvation free energies of K^+ and Cl^- are roughly similar, although there remains a significant uncertainty as to what the exact values should be (Dorman et al., 1996) (Table 2). To simplify the analysis of the results, the LJ parameters were adjusted so that both the cation and anion models have exactly a free energy of solvation of −80 kcal/mol. Because this is an effective pair model that was

TABLE 4   Interaction with a formamide molecule

	CHARMM*		Ab initio#	
Ions	E (kcal/mol)	d (Å)	E (kcal/mol)	d (Å)
K^+	−23.8	2.54 (O)	−26.1	2.49 (O)
Cl^-	−19.0	2.17 (H)	−15.8	2.31 (H)
		3.17 (N)		

*The geometry of formamide was optimized in the calculations with the CHARMM potential.
#The gas phase geometry of formamide was used (Hirota et al., 1974). The ab initio calculations were performed at the HF/6-31G* for K^+ and at the HF/6-311G** level for Cl^-, including corrections for systematic errors according to Roux and Karplus (1995).

TABLE 5 Interaction with a single NMA molecule

Ions	CHARMM*		Ab initio#	
	E (kcal/mol)	d (Å)	E (kcal/mol)	d (Å)
K⁺	−24.2	2.54 (O)	−24.8	2.53 (O)
Cl⁻	−17.3	2.23 (H)	−15.5	2.39 (H)
		3.22 (N)		

*The geometry of NMA was optimized in the calculations with the CHARMM potential.
#The gas phase geometry of NMA was used (Kitano et al., 1973). The HF/6-31G* ab initio value for K⁺ was taken from Roux and Karplus (1995); the ab initio calculations for Cl⁻ were performed at the HF/6-311G** level, including corrections for systematic errors (see Roux and Karplus, 1995).

optimized for the condensed phase, it is possible that the interaction of isolated fragments is not well reproduced. To assess the present parametrization, the interaction of the ions with a single water molecule are compared with ab initio calculations. The results of those calculations are reported in Table 3. The empirically derived LJ model reproduces the essential features of the interaction with a water, indicating that the parameterization is reasonable. Interestingly, although the interaction energy of Cl⁻ with a single water is significantly smaller than that of K⁺, the resulting free energies are similar. In bulk water, the smaller interaction energy of the anion relative to that of the cation is compensated by a more favorable hydration structure: the Cl⁻ and the K⁺ ion are surrounded by 8.3 and 7.4 water molecules, respectively, in the first shell. The interactions of the ions with formamide and NMA were also compared with the ab initio calculations. The results of those calculations are reported in Tables 4 and 5. The empirically derived LJ model reproduces the essential features of the interaction with formamide and with NMA, indicating that the parameterization is reasonable.

The result of all of the free energy calculations is given in Table 6. Except in the case of liquid NMA, the forward and backward free energy perturbations yield consistent results, indicating that the calculations are well converged. In all cases, it appears that the cation is favored over the anion in the liquid amides and in the interior of the channel. The result of the free energy perturbation for transforming K⁺ into Cl⁻ in liquid formamide is 12.6, which is almost 2 to 3 times larger than the experimental estimate of 4.8 kcal/mol. Although there are no direct experimental estimates of

TABLE 6 Transformation of K⁺ into Cl⁻

Environment	Free energy (kcal/mol)		
	Forward	Backward	Average
Liquid water	0.1	0.4	−0.2
Liquid formamide	12.9	−12.3	12.6
Liquid NMA	38.6	−9.0	23.8
β-Helix with single-file waters	58.6	−59.0	58.8
β-Helix with no single-file waters	61.7	−62.6	62.1
Single-file water (no β-helix)	1.3	−0.2	0.7

the free energy of Cl⁻ in liquid NMA, the present result of +23 kcal/mol is probably too large. Similarly, the free energy difference between K⁺ and Cl⁻ in the interior of the channel appears to be overestimated. The overestimated K⁺ to Cl⁻ free energy differences in liquid amides and in the channel are probably due to the use of a potential function with a permanent charge distribution and neglect of induced polarization. In the case of a cation such as K⁺, the free energy is dominated by the interaction with the permanent dipole of the peptide moiety, particularly with the negative carbonyl oxygen, which is readily accessible because of the libration mechanism. Thus a simple nonpolarizable model can represent the interactions effectively. In contrast, an anion does not interact favorably with the permanent charge distribution of the backbone, and induced polarization may have a strong influence on the stabilization energy in the interior of the channel. In addition, a Cl⁻ is more polarizable than a K⁺ because of the supplementary electron. The polarizability of K⁺ is around 0.8 Å³ (Öpik, 1967; Fowler and Madden, 1984), whereas that of Cl⁻ is 5.5 Å³ (Fowler and Madden, 1984). Therefore it is not surprising that induced polarization is necessary for a quantitative account of the energetics of anion association to the GA channel. In a nonpolarizable molecular mechanical model such as the one used here, the charge specificity of the channel is significantly exaggerated and somewhat caricatured. Nevertheless, the present model, in which all atoms including the hydrogens were represented, is sufficiently realistic to indicate that anions are poorly solvated by amides compared to cations. The calculated relative free energy of transfer from bulk water to the channel environment is consistent with the measured free energy of transfer of cations and anions from water to liquid amides.

The K⁺ and Cl⁻ ions have nearly the same free energy in bulk water. In contrast, there is a free energy difference of almost 60 kcal/mol in favor of K⁺ relative to Cl⁻ in the periodic helix. Thus the cation has a much higher affinity for the interior of the channel than the anion, even though they have, by design, exactly the same solvation free energy in bulk water. Although the calculated free energies are certainly overestimated, the results show that the channel is even more unfavorable than the liquid amides (formamide and NMA). This suggests that the channel backbone is a particularly unfavorable environment for the Cl⁻. The large free energy difference can be understood in terms of the solvation structure in the environment provided by bulk water and by the channel interior. The free energy and interaction of a charged ion with its surroundings are dominated by the electrostatic contribution. The latter can be expressed in terms of $\langle \rho_{elec}(r) \rangle$, the average radial charge distribution around the ion arising from the surrounding atoms,

$$\langle U_{elec} \rangle = 4\pi \int_0^\infty r^2 dr \frac{q}{r} \langle \rho_{elec}(r) \rangle, \tag{3}$$

where $\langle \rho_{elec}(r) \rangle$ is given by

$$\langle \rho_{elec}(r) \rangle = \left\langle \sum_j q_j \delta(r - r_j) \right\rangle. \tag{4}$$

To gain more insight into the structural origin of the free energy contribution, the average charge distribution function around the ions was calculated from a simulation of 200 ps in water and in the periodic helix. The distribution functions are shown in Fig. 1. It is observed that the average charge distributions surrounding a K^+ in bulk water and in the β-helix are very similar. In bulk water, the K^+ ion is surrounded by approximately 7.4 water molecules in the first solvation shell; the oxygen of the water molecules

FIGURE 1   Average radial charge distribution around the K^+ and Cl^- in bulk water (*solid line*) and in the periodic β-helix (*dashed line*) calculated from 200-ps molecular dynamics simulations. For the K^+, the first peak in bulk water caused by the electronegative water oxygen is replaced by an equivalent peak from the backbone carbonyl oxygen. For the Cl^-, the first peak in bulk water caused by the electropositive hydrogens is only poorly substituted by the amide backbone hydrogens in the channel interior. In the bulk liquid, the integral of the radial charge density surrounding an ion of charge Q is equal to Q $(1/\epsilon - 1)$.

points toward the ion, causing a negative peak at a contact distance of 2.6 Å, which provides a favorable electrostatic interaction energy. In the channel, the solvation is provided primarily by the oxygen of the backbone carbonyl group. Although the two environments are significantly different, the resulting radial charge distributions around a cation such as K^+ are remarkably similar. In contrast, the radial charge distribution is markedly different in bulk water and in the β-helix in the case of a Cl^- ion. In bulk solution, the water molecules provide a favorable electrostatic interaction energy by forming hydrogen bonds with the anion. The Cl^- ion is surrounded by approximately 8.3 water molecules in the first shell, and a significant positive peak at a hydrogen bonding distance of 2.0 Å provides a favorable electrostatic interaction energy. In the channel, similar hydrogen bonds with the NH amide backbone group are not easily formed. In the bulk liquid, the integral of the radial charge density surrounding an ion of charge Q is equal to $Q(1/\epsilon - 1)$, based on an exact sum rule (Roux et al., 1990). No similar sum rules have been derived for an ion inside a periodic helix.

Thus, although the channel is electrically neutral, the strong asymmetry in the permanent charge distribution of the channel backbone results in a significant reduction of favorable interaction energy in the channel for negatively charged ions. The electronegative carbonyl oxygen can become easily accessible to a cation with a small distortion of the channel. Favorable contacts of an anion with the electropositive amide hydrogen appear to be more difficult to establish. The marked difference in the average charge "solvating" distribution for a cation and an anion inside the β-helix is responsible for the valence specificity of the GA channel. The structure of the backbone channel is such that the amide HN group is far less effective than water molecules at solvating an anion, whereas the carbonyl CO can effectively replace the hydration shell of a cation.

The present free energy calculations demonstrate that the interior of the GA channel provides an unfavorable environment for Cl^- relative to K^+ (much worse in fact than liquid amides). The valence selectivity of the GA channel is seen to be a direct consequence of the structural charge asymmetry of the backbone relative to that of water. The analysis is entirely consistent with Urry's, who was the first to point out that the structural features of the backbone make the GA channel an energetically favorable interaction site for cations but not for anions (Urry et al., 1981; Urry and Venkatachalem, 1984). Peptide libration, i.e., torsional motions of the peptide moiety that arise from a simultaneous anticorrelated rotation of the backbone dihedral angles, $\pm\Delta\psi_i$ and $\mp\Delta\phi_{i+1}$, is the dominant mechanism of the small structural fluctuations that are necessary to better solvate an ion inside the channel. A small inward displacement of the backbone carbonyl of the peptide group is able to stabilize a cation inside the channel. This is an effective mechanism because of the length of the carbonyl CO bond and the significant electronegative charge of the carbonyl oxygen $(-0.51e)$. In contrast, an equivalent libration of the carbonyl oxygen outward and of the amide hydrogen inward
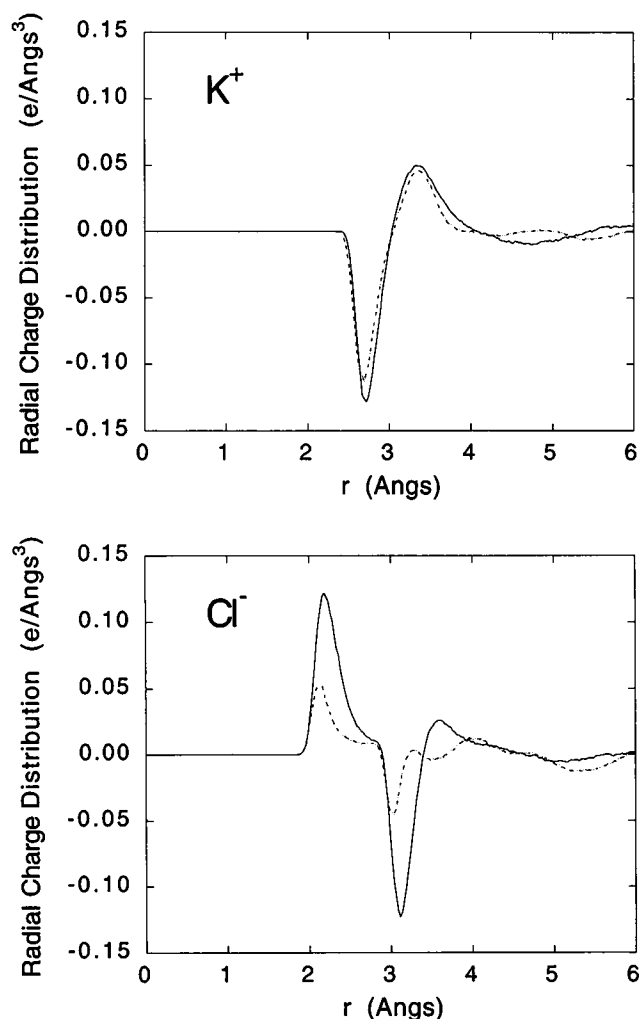
is much less effective in coordinating an anion because of the shorter NH bond length and the smaller effective positive charge on the amide hydrogen ($+0.31e$). The average radial charge distribution around the K^+ is very similar in bulk water and in the periodic helix (see above). In contrast, the average radial charge distribution around Cl^- is much reduced in the periodic helix relative to the bulk liquid. Thus, the conclusion of the present study is essentially in complete agreement with Urry's picture. As a consequence of the structural asymmetry of the backbone charge distribution, cations can partition spontaneously from bulk water to the interior of the GA channel whereas anions cannot.

The present analysis differs essentially from that of Sung and Jordan (1987), who concluded that Cl^- can be thermodynamically stable inside the GA channel but does not permeate easily through the channel, because of a large entrance barrier arising from the "effective quadrupolar moment" of the dimer channel. The basis of valence selectivity was attributed to a kinetic factor (i.e., a slow rate to hop across a large energy barrier). In a more recent study, Dorman et al. (1996) revised that conclusion. They found that the free energy needed to transfer an anion from bulk water to the channel interior is unfavorable and concluded that the valence selectivity of the GA is thermodynamically controlled. However, the valence selectivity of GA was not attributed to the unfavorable interaction of anions with the channel backbone. Based on their analysis, an anion has a significant affinity for the channel; the free energy needed to transfer an ion of a given size from a vacuum to the interior of the channel is more favorable for an anion than for a cation, but the tendency is reversed because of the stronger hydration energy of anions. It is possible that the disagreement is due in large part to the significant differences in the microscopic representation of the channel backbone. The early study of Sung and Jordan (1987) was based on a simplified gramicidin-like system in which the backbone C_α were suppressed and the CO and NH groups were modeled as point dipoles; the CO and NH, modeled as a single LJ-6-12 particle, were mobile, reorientable, and polarizable. The channel system and its content were even further simplified in the study of Dorman et al. (1996). Only the 32 CO carbonyl dipoles of the channel were included explicitly; the C_α and amide NH groups were suppressed and the remainder of the system was represented in terms of continuum dielectrics. The study was based on a semimicroscopic continuum theory (SMC). In this approach, the ion and the single-file water molecules inside the channel are represented explicitly in terms of a hard sphere with an embedded dipole, and the surrounding bulk water is represented in terms of a dielectric continuum. In both studies, the carbonyl groups were disposed according to an ideal helical arrangement, and their equilibrium orientation was chosen to lie alternatively parallel and antiparallel (0° or 180°) with respect to the channel axis. Fluctuations of the peptide units around the ideal helical arrangements were permitted. In contrast, a detailed microscopic model with all atoms based on realistic interactions and allowing full flexibility of the backbone structure was used in the present study. Thus the models differ in terms of both the atomic representation of the backbone (i.e., all backbone atoms are included) and its flexibility (all fluctuations are allowed). Even though the carbonyl group provides the dominant contribution to the electrostatic charge distribution of the backbone, the other molecular groups are also important. For example, in the CHARMM potential energy function, the partial charges of the CO are $\pm0.51e$, whereas the charges of the HN, N, C_α, and H_α are $+0.31$, -0.47, $+0.07$, and $+0.09$, respectively. In particular, the charge distribution of such an all-atom model of the channel does not possess the "effective quadrupolar moment" that is observed in previous studies of Jordan and co-workers (Sung and Jordan, 1987; Dorman et al., 1996). The direct consequences of a simplified representation of the backbone and atom suppression for the energetics are difficult to assess, because the individual interaction of one ion with one model peptide unit (such as a NMA molecule) was not reported. Differences with the present results may have arisen from the assumed ideal helical geometry and the limited flexibility included in the model. In Jordan's models the peptide groups were allowed to fluctuate around an ideal helical geometry. However, the carbonyl oxygens are not perfectly oriented parallel and antiparallel to the channel axis in an all-atom model of the β-helical structure (Roux, 1995). In fact, the carbonyl oxygens are tilted slightly inward, causing a negative electrostatic potential that spontaneously makes the interior of the channel more favorable for cations than for anions, even in the absence of structural distortions. Such an inward tilting is observed both in the gramidicin-like periodic β-helix as well as in the head-to-head channel dimer.

CONCLUSION

The structural and thermodynamic factors responsible for the valence selectivity of the gramicidin channel were investigated with molecular dynamics simulations and free energy perturbation methods. The free energy of solvation was calculated and compared for a K^+ cation and a Cl^- anion. The results of the present calculations demonstrate that the interior of the GA channel provides an energetically favorable interaction site for a cation but not for an anion. The trend is a consequence of the structural asymmetry of the backbone charge distribution.

In the present study, the interior of the channel was modeled as a periodic poly-alanine β-helix, and the influence of the entrance/exit, the channel side chains, and the surrounding lipids was neglected. However, because the analysis demonstrates that the valence specificity originates in the detailed interactions in the first solvation shell of the ions, it can be reasonably expected that the influence of other factors does not depend on the sign of the ion and that those contributions would balance each other. Using a simplified helical model, Sung and Jordan (1987) found that

anions can be solvated favorably in the channel and that a large energy barrier prevented their entry near the channel's end. In contrast, we attribute the molecular origin of the valence selectivity of the GA channel to the nature and the asymmetry of the permanent charge distribution of the backbone structure relative to that of water. The anions do not permeate into the channel, because their presence anywhere in the channel is energetically unfavorable relative to bulk water.

The present analysis of solvation in bulk water and in the interior of a periodic β-helix clearly shows that continuum and semicontinuum models must be used with caution in complex molecular systems. For example, the molecular dipole moments and the dielectric constant of the liquid amides have little to do with the magnitude of the relative solvation free energy of the cations and anions. The dipole moments of formamide, NMA, and DMF are 3.71, 3.73, and 3.81 Debyes, respectively (Kurland and Wilson, 1957; Meighan and Cole, 1964). However, the dielectric constants of formamide, DMF, and NMA, 107.5, 36.3, and 178.9, respectively (Bass et al., 1964), can be smaller or larger than that of water (80). The origin of the free energy difference lies in the detailed molecular interactions in those liquid systems. A major difficulty with a continuum or semicontinuum dielectric description is to define unambiguously the ionic radii in liquid and channel environments. Ionic crystal radii do not provide appropriate estimates for cations and anions because of the strong asymmetry of the charge distribution in a water molecule. Statistical mechanical analysis of the molecular basis of the Born solvation model has shown that the appropriate radius for obtaining the solvation free energy is related to the first peak in the ion-solvent radial distribution function (Roux et al., 1990). Because the details of the radial distribution function result from the interactions between the ion and the surrounding solvent molecules, the so-called Born radius must be considered an empirical parameter that is dependent on the molecular nature of the solvent and on the sign of the ion charge. As a consequence, the ionic Born radius is not a microscopic parameter and a unique value does not exist. In effect, the optimal Born radius for representing an ion in liquid water does not necessarily correspond to the best choice for the same ion in liquid amides. Furthermore, the solvation free energy of a charged Lennard-Jones particle differs, depending on the sign of the charge. This is due to the internal structure and the charge asymmetry of the water molecule. The electrospositive hydrogen of the water can get much closer to a negatively charged particle than the oxygen. In the present study, we have chosen to compare models of K^+ and Cl^- designed to have the same solvation free energy in water to resolve the ambiguity with ionic radii. It is clear that the radii of a cation and an anion must differ significantly to yield similar free energies in liquid water. The LJ parameters of K^+ and Cl^-, adjusted to yield the same solvation free energy, differ by 0.5 Å. The LJ parameters of the Cl^- correspond roughly to those used to model a Cs^+.

Lastly, the stability of cations and anions in gramicidin may be very sensitive to the channel structure and the environment. In fact, the presence of chloride ions inside a gramicidin pore structure is observed in the x-ray crystallography study of Wallace and Ravikumar (1988), in which the GA molecule crystallized in a double-stranded helix conformation from a 0.1 M cesium chloride methanol solution. The situation appears quite puzzling, because the pore incorporates five ions disposed in single file in the pore: $Cl^- - Cs^+ - Cl^- - Cs^+ - Cl^-$ (a last Cs^+ needed for electroneutrality is located outside the pore between adjacent dimers). However, two of the Cl^- are located close to the channel entrance and the influence of the solvent is unknown. Further investigations will be needed to understand the factors contributing to the relative stability of Cl^- and Cs^+ in the crystal structure. Molecular dynamics simulations and free energy calculations will be performed when the coordinates of the refined x-ray structure will be made available from the Brookhaven Protein Data base. In particular, it will be interesting to calculate the free energy difference resulting from swapping the position of the cations and anions in the crystal structure.

REFERENCES

Andersen, O. S., and R. E. Koeppe II. 1992. Molecular determinants of channel function. *Physiol. Rev.* 72:S89.

Åqvist, J. 1990. Ion water interaction potential derived from free energy perturbation simulations. *J. Phys. Chem.* 94:8021–8024.

Bass, S. J., W. I. Nathan, R. M. Meighan, and R. H. Cole. 1964. Dielectric properties of alkyl amides. II. Liquid dielectric constant and loss. *J. Phys. Chem.* 68:509–515.

Beglov, D., and B. Roux. 1994. Finite representation of an infinite bulk system. Solvent boundary potential for computer simulations. *J. Chem. Phys.* 100:9050–9063.

Boys, S. F., and F. Bernardi. 1970. The calculation of small molecular interactions by the differences of separate total energies. Some procedures with reduced errors. *Mol. Phys.* 19:553–566.

Brooks, B. R., R. E. Bruccoleri, B. D. Olafson, D. J. States, S. Swaminathan, and M. Karplus. 1983. CHARMM: a program for macromolecular energy minimization and dynamics calculations. *J. Comput. Chem.* 4:187–217.

Catterall, W. A. 1988. Structure and function of voltage-sensitive ion channels. *Science.* 242:50–61.

Cox, B. G., G. R. Hedwig, A. J. Parker, and D. W. Watts. 1974. Solvation of ions. XIX. Thermodynamic properties for transfer of single ions between protic and dipolar aprotic solvents. *Aust. J. Chem.* 27:477–501.

Dorman, V., M. B. Partenskii, and P. C. Jordan. 1996. A semi-microscopic Monte Carlo study of permeation energetics in a gramicidin-like channel: the origin of cation selectivity. *Biophys. J.* 70:121–134.

Eisenman, G. 1962. Cation selective electrodes and their mode of operation. *Biophys. J.* Supp. 2: 259–323.

Foresman, J. B., and C. L. Brooks, III. 1987. Ab initio study of hydrated chloride ion complexes: evidence for polarization effect and nonadditivity. *J. Chem. Phys.* 87:5892–5894.

Fowler, P. W., and P. A. Madden. 1984. In-crystals polarizabilities of alkali and halide ions. *Phys. Rev. B.* 29:1035–1042.

Frisch, M. J., M. Head-Gordon, G. W. Trucks, J. B. Foresman, H. B. Schlegel, K. Raghavachari, M. A. Robb, J. S. Binkley, C. Gonzalez, D.

J. Defrees, D. J. Fox, R. A. Whitesides, R. Seeger, C. F. Melius, J. Baker, R. L. Martin, L. R. Khan, J. J. P. Steward, S. Topiol, and J. A. Pople. 1990. Gaussian 90. Gaussian, Inc., Pittsburgh, PA.

Galzi, J. L., A. Devillers-Thiery, N. Hussy, S. Bertrand, J. P. Changeux, and D. Bertrand. 1992. Mutations in the channel domain of a neuronal nicotinic receptor convert ion selectivity from cationic to anionic. *Nature.* 359:500–505.

Gianolio, L., R. Pavani, and E. Clementi. 1978. A new algorithm for obtaining contracted basis set from gaussian type functions. *Gaz. Chim. It.* 108:181–205.

Heineman, S. H., H. Terlau, W. Stühmer, K. Imoto, and S. Numa. 1992. Calcium channel characteristics conferred on the sodium channel by single mutations. *Nature.* 356:441–443.

Hille, B. 1992. Ionic Channels of Excitable Membranes, 2nd Ed. Sinauer Associates, Sunderland, MA.

Hinton, J. F., W. L. Whaley, D. C. Shungu, R. E. Koeppe, II, and F. S. Millett. 1986. Equilibrium binding constant for the group I metal cations with gramicidin-A determined by competition studies and Tl^+-205 nuclear magnetic resonance spectroscopy. *Biophys. J.* 50:539–544.

Hirota, E., R. Sugisaki, C. J. Nielsen, and G. O. Sorensen. 1974. Molecular force field and structure of water: recent microwave results. *J. Mol. Spectrosc.* 53:62–76.

Jorgensen, W. L., R. W. Impey, J. Chandrasekhar, J. D. Madura, and M. L. Klein. 1983. Comparison of simple potential functions for simulating liquid water. *J. Chem. Phys.* 79:926–935.

Kirkwood, J. G. 1934. Theory of solution of molecules containing widely separated charges with application to zwiterions. *J. Chem. Phys.* 2:351.

Kitano, M., T. Fukuyama, and K. Kuchitsu. 1973. Molecular structure of *n*-methylacetamide as studied by gas electron diffraction. *Bull. Chem. Soc. Jpn.* 46:384–387.

Kollman, P. A. 1993. Free energy calculations: applications to chemical and biochemical phenomena. *Chem. Rev.* 93:2395–2417.

Kurland, R. J., and E. B. Wilson. 1957. Microwave spectrum, structure, dipole moment and quadrupole coupling constants of formamide. *J. Chem. Phys.* 27:585–590.

Lipkind, G. M., and H. A. Fozzard. 1995. A structural motif for the voltage-gated potassium channel pore. *Proc. Natl. Acad. Sci. USA.* 92:9215–9219.

Mackerell, A. D., Jr., D. Bashford, M. Bellot, R. L. Dunbrack, M. J. Field, S. Fischer, J. Gao, H. Guo, D. Joseph, S. Ha, L. Kuchnir, K. Kuczera, F. T. K. Lau, C. Mattos, S. Michnick, D. T. Nguyen, T. Ngo, B. Prodhom, B. Roux, M. Schlenkrich, J. Smith, R. Stote, J. Straub, J. Wiorkiewicz-Kuczera, and M. Karplus. 1992. Self-consistent parametrization of biomolecules for molecular modeling and condensed phase simulations. *Biophys. J.* 61:A143.

Meighan, R. M., and R. H. Cole. 1964. Dielectric properties of alkyl amides. I. Moments and polarization in benzene solution. *J. Phys. Chem.* 68:503–508.

Öpik, U. 1967. The polarizability of a closed-shell core of an atomic system by an outer electron. II. Evaluation of the polarizabilities from observed spectra. *Proc. Phys. Soc. Lond.* 92:566–572.

Roux, B. 1993. Nonadditivity in cation-peptide interactions: a molecular dynamics and ab initio study of Na^+ in the gramicidin channel. *Chem. Phys. Lett.* 212:231–240.

Roux, B. 1995. Theory of transport in ion channels: from molecular dynamics simulations to experiments. *In* Computer Simulation in Molecular Biology. J. Goodfellow, editor. VCH, Weinheim. 132–169.

Roux, B., and M. Karplus. 1991a. Ion transport in a gramicidin-like channel: structure and thermodynamics. *Biophys. J.* 59:961–981.

Roux, B., and M. Karplus. 1991b. Ion transport in a gramicidin-like channel: dynamics and mobility. *J. Phys. Chem.* 95:4856–4868.

Roux, B., and M. Karplus. 1994. Molecular dynamics simulations of the gramicidin channel. *Annu. Rev. Biomol. Struct. Dyn.* 23:731–761.

Roux, B., and M. Karplus. 1995. Potential energy function for cations-peptides interactions: an ab initio study. *J. Comp. Chem.* 16:690–704.

Roux, B., and M. Karplus. 1991a. Ion transport in the gramicidin channel: molecular dynamics study of single and double occupancy. *Biophys. J.* 68:876–892.

Roux, B., H. A. Yu, and M. Karplus. 1990. Molecular basis for the Born model of ion solvation. *J. Phys. Chem.* 94:4683–4688.

Ryckaert, J. P., G. Ciccotti, and H. J. C. Berendsen. 1977. Numerical Integration of the cartesian equation of motions of a system with constraints: molecular dynamics of *n*-alkanes. *J. Comp. Chem.* 23:327–341.

Sather, W. A., J. Yang, and R. W. Tsien. 1994. Structural basis of ion channel permeation and selectivity. *Curr. Opin. Neurobiol.* 4:313–323.

Sung, S. S., and P. C. Jordan. 1987. Why is gramicidin valence selective? A theoretical study. *Biophys. J.* 51:661–672.

Urry, D. W., and C. M. Venkatachalam. 1984. Theoretical conformation analysis of the gramicidin A transmembrane channel. II. Energetics of helical states of the channel. *J. Comput. Chem.* 5:64–71.

Urry, D. W., C. M. Venkatachalam, K. U. Prasad, R. J. Bradley, G. Parenti-Castelli, and G. Lenaz. 1981. Conduction process of the gramicidin channel. *Int. J. Quant. Chem. Quant. Biol. Symp.* 8:385–399.

Wachters, A. J. H. 1970. Gaussian basis set for molecular wavefunctions containing third-row atoms. *J. Chem. Phys.* 52:1033–1036.

Wallace, B. A., and K. Ravikumar. 1988. The gramicidin pore: crystal structure of a cesium complex. *Science.* 241:182–187.

Woolf, T. B., and B. Roux. 1994. Molecular dynamics simulation of the gramicidin channel in a phospholipid bilayer. *Proc. Natl. Acad. Sci. USA.* 91:11631–11635.

Woolf, T. B., and B. Roux. 1996. Structure, energetics and dynamics of lipid-protein interactions: a molecular dynamics study of the gramicidin A channel in a DMPC bilayer. *Protein Struct. Funct. Genet.* 24:92–114.

Woolf, T. B., J. Desharnais, and B. Roux. 1994. Structure and dynamics of the sidechains of gramicidin in a DMPC bilayer. *In* NATO ASI Series: Computational Approaches in Supramolecular Chemistry, Vol. 426. G. Wipff, editor. Kluwer Academic Publishers, Dordrecht. 519–531.