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The interaction of Cl^- with a gramicidin-like channel

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Molecular dynamics simulations have been used to study the interaction of Cl^- with a gramicidin-like channel. The results suggest that there is a high-energy barrier at the entrance of the channel, which would correspond to a permeability 10^{-9}-times that of a cation of the same size. This could account for the cationic selectivity of the gramicidin channel and indicates that valence selectivity is kinetically controlled.

1. Introduction

Many experimental and theoretical studies have been designed to elucidate the molecular basis of the selectivity of biological channels. However, even for the simplest model channel, gramicidin, the molecular mechanism that accounts for its valence selectivity has not been unambiguously identified. The interactions of cations with a gramicidin channel have been investigated using several different theoretical approaches [1–9]. In this paper we describe a molecular dynamics study, of which a preliminary account has already been given [10], of the interaction of Cl^- with a gramicidin-like channel. As we have done before, we employ the polarizable electropole model proposed by Barnes et al. [11]. We have chosen this model because it accounts, in part, for the electronic reorganization that must take place when ionic sources interact with polarizable bonds.

2. The model and method

The membrane-bound gramicidin channel is the head-to-head dimer originally proposed by Urry [12–16]. In our simulations the coordinates of the atoms of the helical gramicidin backbone are obtained from the conformational analysis of Koeppe and Kimura [17]. A detailed description of our model and method has been provided in a previous publication [4]. Here we will only mention the main features of the method and some revisions.

In our simulations each polar group (CO and NH) of the peptide linkage is modeled by a polarizable dipole oscillating and tilting about its equilibrium position and orientation, respectively. The force constant and the torsional constant are chosen to be 0.5 mdyn/Å and 0.5×10^{-18} J, respectively, corresponding to vibrational frequencies in the range 200–500 cm^{-1}. The permanent dipole moments of the CO and NH groups [18] determined from their uncompensated partial charges are 2.262 and 0.864 Debye; the polarizabilities of CO and NH groups [19] are 1.82 and 1.40 $Å^3$, respectively.

Cl^- is a negatively charged, polarizable sphere; its polarizability, estimated by Gowda and Benson

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[20], is 2.156 Å^3. A water molecule is described as a sphere with a dipole and a quadrupole located at its center of mass. The permanent dipole moment is 1.855 Debye, determined experimentally for gaseous water [21]. The quadrupole moments are $Q_{xx} = -4.844$ and $Q_{yy} = 5.060$ Debye Å, obtained from quantum-mechanical calculation [22] and the polarizability [23] is 1.444 Å^3. The total dipole moment of the various polarizable moieties is the sum of the permanent dipole moment and an induced dipole moment, assumed to be the product of the electrical field and the isotropic polarizability. The resultant dipole moments are computed by an iterative method until self-consistency is achieved. Thus, electronic reorganization is taken into account in this approach. Then the energy, forces and torques are evaluated.

The total energy is the sum of five terms: electrostatic, polarization, Lennard-Jones, vibrational and torsional energy. The parameters for the 6-12 Lennard-Jones potential describing water-water interaction are 3.149 Å and 0.425 kcal/mol, determined by Gellatly et al. [24] by fitting the water dimer properties. For ion-water interaction the parameters were obtained by fitting the experimental hydration enthalpies [25] and ion-water distances [26]. The details have been published separately [27]. In our simulations we assume one set of Lennard-Jones parameters for water interacting with other water molecules, CO groups and NH groups and a different set of parameters for Cl^- interacting with water and NH. Because of the charge on Cl^- and the large size of both C and O atoms, new Lennard-Jones parameters describing the interaction of Cl^- with CO were calculated. To do this we constructed atom-based Lennard-Jones parameters for C and O atoms [5] interacting with Cl^- [28] using the combination rules of Kong [29]. These data then yielded a configurational potential dependent upon the CO-Cl^- orientation and separation. This potential was spherically averaged and fit to a new Lennard-Jones function of a single variable, the CO-Cl^- separation. The Lennard-Jones parameters used in the simulations are listed in table 1. The Lennard-Jones interactions among the CO and NH groups are not included in the simulations because these groups are constrained to the vicinity of their equilibrium positions.

Table 1

Lennard-Jones parameters

Interaction	σ (Å)	ϵ (kcal/mol)
Cl^--water	3.8	0.1
Cl^--NH	3.8	0.1
Cl^--CO	4.0	0.2
Water-water	3.149	0.425
Water-NH	3.149	0.425
Water-CO	3.149	0.425

The algorithm proposed by Gear [30] and the Cayley-Klein parameter method of Evans and Murad [31,32] were used in solving the equation of motion in the simulations. Each time step in the simulations is 1.5×10^{-15} s. At each energy minimum and saddle point the system is cooled down to 10^{-5} K to obtain the equilibrium geometry.

3. Results and discussion

In our simulation the calculated potential energy of an empty gramicidin-like channel is −147.04 kcal/mol at its equilibrium conformation. This energy reflects the electrostatic interactions, polarizations, relocations and reorientations of the CO and NH groups. The potential energy of Cl^- in a gramicidin-like channel relative to an empty channel is shown in table 2 and fig. 1. The striking features of fig. 1 are the positive energy when the ion is about 14 Å from the dimer junc-

Table 2

Potential energies of Cl^- in channel

z (Å)	x (Å)	y (Å)	E (kcal/mol)
14.0	0.47	−0.42	0.41
12.0	−0.12	−0.04	−10.45
10.0	−0.01	−0.05	−18.47
8.0	−0.01	0.04	−20.88
6.0	−0.06	−0.04	−21.65
4.0	0.04	−0.02	−22.41
2.0	−0.06	0.02	−22.95
0.0	−0.06	−0.09	−23.48

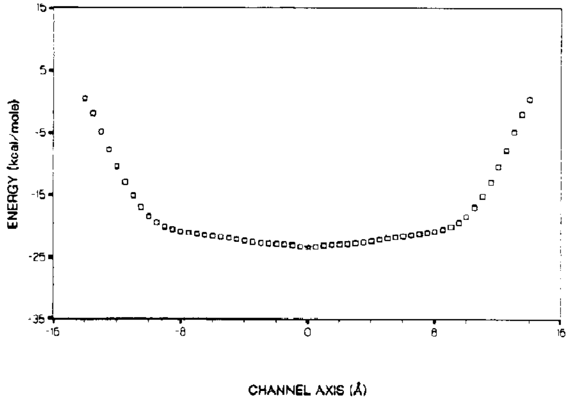

Fig. 1. The potential energies of Cl^- in a gramicidin-like channel without water relative to that of an empty channel. The only minimum (☆) is in the center of the channel.

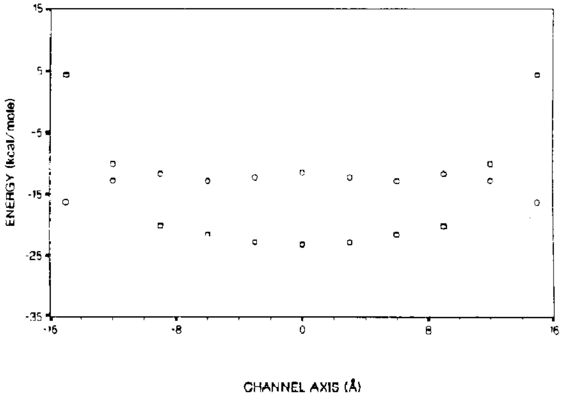

Fig. 2. The energy profiles of Cl^- (□) and a positively charged ion with the same parameters (○) at the axis of a gramicidin-like channel without water.

tion and the deep well in the center of the channel. In contrast, cations are most stable near the channel mouth [1–4]. These calculations, which do not consider the influence of water, indicate that Cl^- is significantly less stable near the channel mouth than an alkali cation. If this energy difference is also a property of the hydrated ions, it suggests that there is a substantial energy barrier for Cl^- entry into the channel, an observation that would account for the channel's impermeability to anions.

Could this be an artifact of our parameters? To answer this question we performed simulations for a positively charged ion otherwise identical to Cl^-, i.e., the same mass, polarizability and Lennard-Jones parameters. The energy profiles are contrasted in fig. 2 where both the positive and negative ions are constrained to the channel axis. This method of comparison is chosen, since otherwise the cation and anion prefer different radial positions for the same axial coordinates. Two differences are apparent: at the channel center the anion is more stable than the cation; at the mouth the reverse is true.

In order to find the origin of the qualitative differences between these energy profiles we examined the orientations of the dipole moments of the groups in the channel. Our convention is that the dipole points toward its positively charged end; a radial dipole is positive if it points toward the outside of the channel. The channel axis is the z-axis. The CO and NH groups of a gramicidin monomer are numbered from the N end, for example, the formyl CO group is the CO0 and the ethanolamine COH group is denoted as CO16. Table 3 lists the z and r components of the dipole moments for the monomer in the upper half (positive z) of the empty channel at its equilibrium conformation. The dipole moments of groups in the lower half of the channel are in one to one correspondence with those of groups in the upper half channel; their z components have opposite sign. The dipoles of the CO and NH groups of the peptide linkage are nearly parallel to the channel axis and antiparallel to one another [12–17]. Before polarization and reorientation are treated the sum of the z components of the dipole moments in the upper half of the channel is about -0.5 Debye and that of the r components less than 0.1 Debye. Because of the electrostatic interaction, the dipoles polarize each other and reorientate, the sum of the z components become much larger, about -1.7 Debye, as shown in table 3. The lower half of the channel has an equivalent opposing dipole pointing toward the center of the channel. This effect gives the whole channel a linear axial quadrupole moment which is positive at the channel center and negative at the two entrances of the

Table 3

The dipole components of the upper monomer

Group	μ_z (Debye)	μ_r (Debye)
CO0	2.42	0.05
CO1	−2.62	0.00
CO2	2.53	0.03
CO3	−2.65	0.00
CO4	2.54	0.02
CO5	−2.64	0.02
CO6	2.54	0.05
CO7	−2.64	0.01
CO8	2.48	0.07
CO9	−2.63	0.05
CO10	2.45	0.05
CO11	−2.44	0.14
CO12	2.43	0.03
CO13	−2.43	0.13
CO14	2.44	0.03
CO15	−2.40	0.06
CO16	1.09	−0.03
NH1	1.09	0.01
NH2	−1.32	0.05
NH3	1.06	−0.02
NH4	−1.32	0.05
NH5	1.06	−0.02
NH6	−1.31	0.04
NH7	1.09	−0.03
NH8	−1.33	0.05
NH9	1.07	−0.01
NH10	−1.35	0.07
NH11	1.00	−0.03
NH12	−1.32	0.08
NH13	0.94	−0.04
NH14	−1.31	0.09
NH15	0.96	−0.04
NH16	−1.19	0.05
Total	−1.73	1.00

channel. This stabilizes Cl^- in the center and creates the difference between anion and cation energy profiles. Here the polarization plays an important role, but the basis is the original orientation of the polar groups, i.e., the structure of the channel.

From table 3, we see that the sum of the radial components is positive. The total radial moment is smaller than the total axial moment. It reduces the influence of the axial quadrupole near the channel center and reinforces it at the channel entrance. Furthermore, the radial components affect the distance of the ions from the channel wall. Cations generally bind closer to the channel wall than anions and, consequently, have stronger local interactions. Especially at the entrance this effect lowers the cation energy and creates a binding site. Unlike the cation, Cl^- always stays close to the axis and, consequently, the energy profile exhibits much less structure. For example, at 12 Å from the center, Cl^- is most stable about 0.1 Å from the channel axis while a positively charged ion of the same size and polarizability is most stable about 0.7 Å from the channel axis.

So far we have only discussed the interaction of Cl^- with an otherwise empty channel. What happens when water molecules are present? Figs. 3 and 4 show the energy changes for the following process with two or four molecules present ($n = 2$ and 4).

$$Cl^-(H_2O)_n + \mathrm{Gram}(H_2O)_n = (H_2O)_n + \mathrm{Gram}[Cl^-(H_2O)_n]$$

In contrast to the case without water, there are now many energy minima. These binding positions in the main reflect the interactions of water with the channel. Nevertheless, the qualitative behavior is the same as in fig. 1. The high potential energy near the channel entrance and the deep well at the center of the energy profile are the features common to all three of figs. 1, 3 and 4. As more water molecules are included in the calcu-

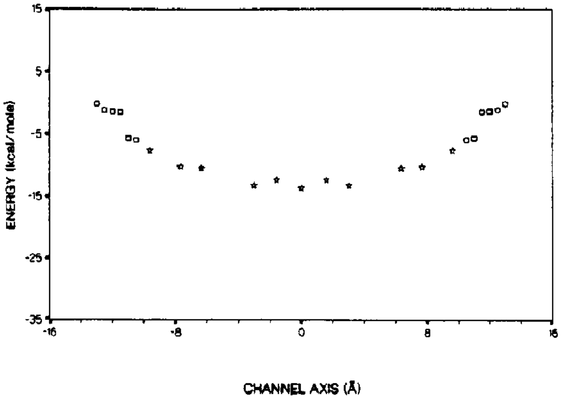

Fig. 3. The energy profile of Cl^- in a gramicidin-like channel with two water molecules present. (☆) Energy minima, (□) unstable states. The saddle points are not shown.

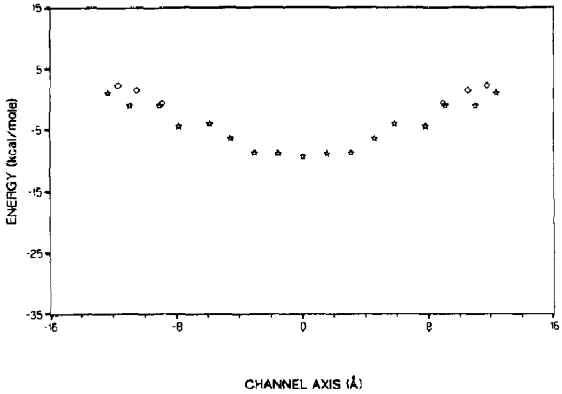

Fig. 4. The energy profile of Cl^- in a gramicidin-like channel with four water molecules present. (☆) Energy minima, (◇) saddle points (only six are shown).

lation, the whole energy curve moves up, because the dehydration energy increases as more water molecules are included. The dehydration energy in bulk water could further raise the energy at the entrance and make the Cl^- entry even more difficult. Again, it is useful to compare Cl^- to an otherwise identical, positively charged ion. With four water molecules present, at 12 Å from the center the energy for Cl^- is about 12 kcal/mol greater, corresponding to a permeability factor of 10^{-9}.

4. Conclusion

The impermeability of the gramicidin channel to Cl^- appears to be a consequence of a high-energy barrier to ion entry. Once inside the channel the ion could be quite stable. This suggests that the selectivity is kinetically controlled.

This energy barrier is due to the orientation of the polar CO and NH groups which form the helix. Including water in the simulations does not substantially alter the energy profile for either cation or anion. It appears that water does not significantly influence valence selectivity.

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