Energetics of ion permeation through membrane channels
Solvation of Na^+ by gramicidin A

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ABSTRACT Calculations of the solvation energetics for a Na^+ ion inside the Gramicidin A channel and in water are presented. The protein dipoles Langevin dipoles (PDLD) method is used to obtain an electrostatic free energy profile for ion permeation through the channel. To gauge the quality of the PDLD results the solvation free energy of a Na^+ ion in water and in the center of the channel is also calculated using free energy perturbation (FEP) simulations. The effect of the polarisability of the surrounding lipid membrane is taken into account by representing the membrane by a large grid of polarisable point dipoles. The two methods give similar solvation energies in the interior of the channel and these are <5 kcal/mol above the solvation free energy for Na^+ in water, in good agreement with experimental data on the activation barriers for ion permeation. It appears that the problems associated with previous calculations of energy profiles in membrane channels can be overcome by a consistent treatment of all the relevant electrostatic contributions. In particular, we find that the induced dipoles of the membrane and the protein contributes with ~10 kcal/mol to the solvation energy inside the channel and can therefore not be discarded in a realistic description of ion solvation in the Gramicidin channel.

INTRODUCTION

Biological ion channels provide an extremely efficient way of allowing the transport of various ions through otherwise energetically prohibitive media, viz nonpolar lipid membrane environments. Due to the fact that the aqueous phase is such a good solvent for ions compared with lipid media of low dielectric constant, the energy required to move a small ion across a membrane is very high and consequently the spontaneous flux of ions through the membrane, without the aid of ion channels or pumps, is practically negligable. The predominant feature common to all biological ion channels is that they are capable of providing a local environment of rather polar nature within a non-polar medium, thereby allowing for relatively high rates of ion permeation. In addition to this general common property, the specific molecular structure of different channels results in particular selectivity and specificity properties, whereby some ions can be accepted while others are rejected. The most important factor governing the rate with which a given ion migrates through the channel is the difference in solvation free energy between the outside bulk solvent and the interior of the channel. The actual rate (in the transition state theory approximation) is determined by the highest free energy barrier that has to be surmounted.

Although the basic physics of ion permeation through membrane channels may appear simple, at least on a qualitative level, a detailed microscopic understanding of these processes poses a much more difficult problem. This is, of course, partly due to the complexity of system on a molecular level. For instance, the ion channel itself may be a very complicated protein molecule or an assembly of them (Noda et al., 1982; Brisson and Unwin, 1985; Young et al., 1985; Greenblatt et al., 1985), the structure of which is usually difficult to determine by ordinary crystallographic methods because of their highly hydrophobic surfaces. Furthermore, the membrane interacts directly with both the channel protein(s) as well as with the aqueous phase. In particular, many membranes have highly polar head groups in contact with the solvent, which may affect the energetics of an ion at the channel entrance as well the interfacial water structure. The orientation of solvent molecules inside and at the ends of the channel clearly plays a significant role for solvating the ion and consequently also for the permeation rates.

The most widely studied ion channel is the Gramicidin A (GA) system (for recent reviews, see Eisenman and Horn, 1983; Andersen, 1984; Urry, 1985*a*; Polymeropoulos and Brickmann, 1985; Jordan, 1987). This is a relatively simple molecular assembly made up from two identical 15 amino acid residue peptides which are blocked at their ends by a formyl group and an ethanolamine group. The predominant electroactive form is that of a head-to-head left handed (single stranded) helix with 6.3 residues per turn (Urry, 1971). The Nh_2-terminal formyl groups face each other in the central region of the membrane while the ethanolamine (COOh-terminal)

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ends are assumedly in contact with the solvent at either side of the membrane. The amino acid sequence of GA is very nonpolar, the only residues possessing a significant permanent dipole moment being eight tryptophan residues (four per monomer). Although there is no direct crystallographic evidence for this structure, the model proposed by Urry (1971) is supported by several experimental findings (see e.g., Bamberg et al., 1977; Urry, 1985*b*). Two other crystallographic structures of GA have recently been reported (Wallace and Ravikumar, 1988; Langs, 1988) in which the two peptide chains adopt antiparallel double stranded helical conformations. These structures, apparently, do not correspond to the major conducting conformation of membrane spanning GA channels.

Despite its relatively simple molecular structure, as compared with e.g., the acetylcholine receptor channel (Noda et al., 1982), GA possesses the basic properties of biological transmembrane channels in general. It is highly valence selective, allowing for the passage of small monovalent cations while rejecting anions and divalent cations (Myers and Haydon, 1972; Bamberg and Läuger, 1977). It also displays fairly high ion conductances when inserted into various membranes (Andersen, 1983; Eisenman and Sandblom, 1983). Data from electrokinetic experiments clearly indicate that the overall activation barrier for Na^+ permeation is at most on the order of 5–7 kcal/mol (Bamberg and Läuger, 1974; Eisenman and Sandblom, 1983; Urry, 1985*a*). Bamberg and Läuger (1974) have calculated an overall activation barrier for Na^+ in single ion channels induced by GA in dioleoyllethitin membranes of $E^a = 7.3$ kcal/mol and a corresponding value of $E^a = 4.5$ kcal/mol from experiments in glycerol monooleate (GMO) membranes (Hladky and Haydon, 1972). Eisenman and Sandblom (1983) used a single file kinetic model having four ion binding sites separated by three barriers to interpret conductance measurements on GA in GMO bilayers for a number of monovalent cations. Their model also gives free energy barriers of ~5 kcal/mol and well depths of about the same magnitude. In view of these experimental facts, it is evident that the free energy of a Na^+ ion inside the GA channel cannot differ by more than 5–7 kcal/mol from its solvation energy in water at the highest point of the free energy profile.

A large number of theoretical studies of ion permeation through transmembrane channels have been reported. However, none of these have yet been successful in reproducing the experimentally observed migration rates in a quantitative way. The different theoretical models used range from continuum electrostatic treatments (Parsegian, 1969; Levitt, 1978; Jordan, 1981) to microscopic calculations such as energy minimisation (Etchebest et al., 1984; Pullman and Etchebest, 1987; Sung and Jordan 1987), Monte Carlo (Kim and Clementi, 1985) and molecular dynamics (MD) simulations (Mackay et al., 1984; Lee and Jordan, 1984). The macroscopic calculations by Parsegian (1969), Levitt (1978), and Jordan (1981) have provided solutions to several interesting electrostatic problems and have also given qualitative insight on the dependence of the activation energy of ion migration on the geometric dimensions of the channel, dielectric constants, and ion radius. These treatments, however, did not take into account the detailed structure of the channel and its specific interactions with the ion and they generally tend to overestimate the free energy barrier associated with ion passage. More important, these models assumed rather than calculated the dielectric constant of the channel, thus preventing any specific predictions related to the function and design of a particular channel. It thus seems clear that in order to approach a detailed molecular understanding of ion channels which can account for migration rates, selectivity etc., one must consider the interactions between various components of the system on a microscopic level.

Several calculations of energy profiles for ion migration along the GA channel, employing microscopic models, have been reported. These include the energy minimisation studies by Pullman and coworkers, some of which neither included water nor structural mobility of the peptide backbone (e.g., Etchebest and Pullman, 1984) as well as subsequent investigations, which incorporated a limited number of water molecules but still kept the helix rigid (Pullman and Etchebest, 1987). The latter calculation gave a potential energy difference for Na^+ of ~18 kcal/mol between the outside and the interior of the helix. However, because only very few of the waters that actually would solvate the ion outside the channel were included, the solvation energy in this region must be severely underestimated. Kim and Clementi (1984) have performed Monte Carlo simulations of Na^+ and K^+ ions interacting with the GA channel including a larger number of waters, but also keeping the helix rigid. They obtain an energy difference of 25–30 kcal/mol between the outside and the central region of the channel. Unpublished free energy calculations by Wilson and coworkers (quoted as reference 88 in Jordan, 1987) indicate a 35 kcal/mol higher free energy for Cs^+ in the interior of the channel relative to bulk water.

Clearly, these results must be considered somewhat disappointing as far as the magnitude of the reported energy variations is concerned. The most reasonable results obtained so far appear to be the calculations comparing energy profiles for Cs^+ and Cl^- by Sung and Jordan (1987). Their model gives an entrance barrier for Cs^+ that is ~8 kcal/mol higher than the solvation energy

just outside the entrance. However, as was recognised by Sung and Jordan (1987), the neglect of bulk water in this case (and also in the study by Pullman and Etchebest, 1987) should lead to a considerable overestimation of the relative stability of the ion inside the channel (a larger error would be obtained for smaller ions such as Na^+). It is also important to keep in mind (Jordan, 1987) that all microscopic studies of the GA system have neglected the influence of the lipid membrane on the ion solvation energetics. The fact that the lipid phase constitutes a nonpolar region of comparatively low dielectric constant does not imply that it can be safely discarded when evaluating ion solvation energies in a transmembrane channel. On the contrary, one would expect the induced polarisation of the lipid hydrocarbon chains (due to interactions with the permanent charge distribution) to result in additional stabilisation of the ion in the channel. This is so, simply because the electrostatic screening of the net charge by the permanent dipoles of the protein and the linear water array inside the channel is bound to be less effective than in bulk water.

Here we will examine these effects and demonstrate that by representing all the relevant elements of the ion-water-channel-membrane system one obtains reasonable values of the solvation energy for Na^+ in the GA channel. We will also stress the importance of considering complete thermodynamic cycles when assessing the validity of calculated energy profiles for this system (as well as others). That is, unless comparisons are made to ion solvation energies in bulk water large errors in calculated energy profiles may remain undetected. In dealing with the problem of calculating electrostatic free energies in the GA channel we present the results from both a simplified and a more rigorous treatment. The former is based on the PDLD (protein dipoles Langevin dipoles) model (Warshel and Russell, 1984; Russell and Warshel, 1985), with which a complete electrostatic free energy profile for Na^+ is calculated. This approach, which was originally introduced in a first attempt to obtain a quantitative microscopic estimate of electrostatic energies in proteins (Warshel and Levitt, 1976), is based on the following assumptions. (*a*) It is assumed that the electrostatic energy evaluated using the average structure (usually a crystallographic one) of the given macromolecule is a good approximation to the corresponding electrostatic free energy. (*b*) No macroscopic dielectric constant is used, but instead a microscopic model that includes the protein partial charges, induced dipole moments and surrounding solvent. (*c*) Instead of explicitly including a large number of water molecules (that requires considerably heavier computations for convergence) it is assumed that the average polarisation of the water molecules can be described by using a grid of polarisable dipoles whose response to the solute field is determined by a Langevin type of relationship. Although the Langevin polarisation formula was originally derived for dipoles in the gas phase (see e.g., Hill, 1962), it has been shown that this provides a reliable representation of the polarisation of solvent molecules also in the condensed phase. In fact, the Langevin polarisation law is simply scaled by comparing its results with those obtained by simulations of ions in solution (Warshel and Russell, 1984) and then used for representing the solvent in simulations of macromolecules. The resulting model for a solvated macromolecule is a simplified microscopic model that does not include phenomenological parameters such as a dielectric constant or cavity radius.

In addition to the PDLD calculations we also employ a more rigorous (and much more expensive) approach, evaluating the electrostatic free energy of the ion in the center of the channel by a free energy perturbation (FEP) method (e.g., Warshel et al., 1986). In these calculations the charge of the ion is gradually changed in its given environment, while sampling the configuration space by molecular dynamics (MD) simulations and using a thermodynamic perturbation method to evaluate the corresponding electrostatic free energy. This FEP/MD treatment is obviously more rigorous than the simplified PDLD method. Yet, as will be shown below, once the main physics of electrostatic energies is taken into account both methods give reasonable results for the energetics of ion solvation in a transmembrane channel, which has appeared to be a major problem in previous studies.

MODEL AND METHODS

The head-to-head dimer of GA (Urry, 1971) is represented by its polyglycine analogue, while retaining the formyl and ethanolamine end groups. Because the actual conformation of the side chains in the membrane are not known and their positions may be dependent on interactions with the lipid hydrocarbon chains, we have chosen to leave these out of the present study. The effect of neglecting the hydrophobic side chains is more or less compensated for by our representation of the lipid membrane, which will occupy the positions otherwise occupied by side chains. The only sizable permanent dipole moments not included in our simulations are thus those associated with the tryptophan $N_{\epsilon 1} - H_{\epsilon 1}$ groups (this will be discussed below). An all atom representation of the surrounding membrane in energy minimisation and MD calculations would be computationally very expensive. Furthermore, the detailed structure of the hydrocarbon chains is not likely to be relevant for the overall ion-membrane energetics. Therefore, we have chosen to represent the membrane by a cylindrical grid of radius 17.5 Å, height 30 Å, and spacing 2.5 Å embedding the GA helix (cf. Fig. 1). Each grid point is a polarisable dipole with a polarisability of $\alpha = 1.0$ Å^3, but with no permanent dipole moment. This choice of grid spacing and polarisability gives a dielectric constant of $\epsilon = 2$, using the Clausius-Mossotti equation, in accordance with the experimental value for hydrocarbon media.

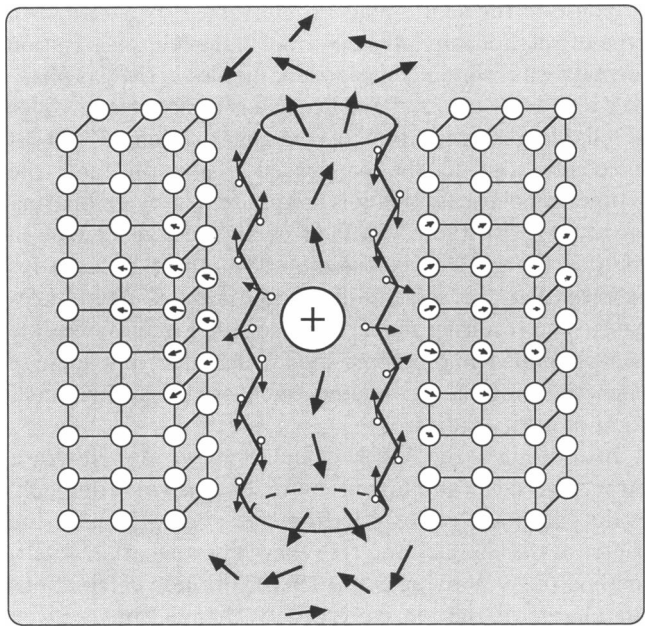

FIGURE 1 Schematic representation of the model system consisting of the GA helix dimer, a NA^+ ion, solvent and the cylindrical membrane grid. The solvent molecules are either modeled by Langevin dipoles or explicit water molecules (see text for details).

PDLD calculations

The PDLD treatment has the major advantage that it can take into account the effects of bulk solvent (at a low computational cost), which is particularly important in the region near the channel entrance. In these calculations, the solvent molecules are modeled by a 16 Å × 16 Å × 16 Å grid with 3 Å spacing truncated to a sphere and centered on the ion. All grid points in the membrane are deleted together with those that are closer than 2.6 Å to a heavy backbone atom or closer than 2.0 Å to a hydrogen atom. For the Na^+ ion a corresponding Langevin dipole exclusion radius of 2.4 Å is used (this radius was obtained from PDLD studies of solvated Na^+ in water). Each solvent grid point is a point dipole whose average polarisation obeys the Langevin formula

$$\mu_i^L = e_i\mu_0\left(\coth \chi_i - \frac{1}{\chi_i}\right)$$

$$\chi_i = C'\mu_0(\xi_i - \xi_i^C)\frac{\xi_i^0}{\xi_i^0 k_b T}, \qquad (1)$$

where e_i is a unit vector in the direction of ξ_i^0, the field from the permanent charge distribution on the ith dipole. μ_0 is taken as 1.8D, C' is a parameter, ξ_i is the local field on the ith dipole and ξ_i^C the field from its nearest neighbors. One should not get the impression that the parameters in this model can be adjusted at will. The value used for the parameter C' was 1.0 in all calculations (this number had previously been determined empirically by using the Langevin dipole model to evaluate solvation energies of ions in water [Russell and Warshel, 1985]). If one were to adjust such a parameter to the specific system under study the resulting model would, of course, have little predictive value. The equation for the effective Langevin dipoles, μ_i^L, is solved iteratively as described by Russell and Warshel (1985). When evaluating the interaction energy between the Langevin dipoles and the rest of the system it is essential to take an average over several grid configurations. In the present calculations we have used 50 different grids with their origins randomly distributed within a 1 $Å^3$ cube centered on the ion, from each ion position. The Langevin dipole energy was then calculated as the Boltzmann average over these grid configurations.

In order to obtain an optimised structure of the solvated ion-channel system (which will approximate the relevant average structure), the GA dimer with the Na^+ ion and 20 explicit all atom water molecules (inside and at the ends of the channel) were subjected to potential energy minimisation (EM). The optimisation was carried out for each of the different ion positions of the calculated profile with the ion constrained (by a harmonic potential) to lie in a particular z-plane (the z-axis in our system coincides with the helix axis and the origin corresponds to the dimer symmetry center). The Lennard-Jones parameters for Na^+ were calibrated by FEP/MD calculations on Na^+ in water to reproduce the experimental solvation energy as well as nearest neighbor distances ($V_{ij}^{LJ} = A_iA_j/r_{ij}^{12} - B_iB_j/r_{ij}^6$; $A_{Na} = 150$, $B_{Na} = 5$). Other parameters of the potential are taken from Warshel and Levitt (1976). Another EM cycle was then performed with the ion replaced by an additional water molecule to obtain an optimised structure of the water filled channel in absence of the ion. All water molecules except those two constituting the first solvation shell were subsequently removed and the Langevin dipole and membrane grids built around the resulting EM structures. The reason for explicitly representing the first solvation shell is that the Langevin dipole model is parametrised to represent the average position dependent polarisation of a water molecule surrounded by other water molecules (Warshel and Russell, 1984). The resulting model underestimates the interaction between an ion and an individual water molecule, which is not surrounded by other water molecules (this deficiency can be corrected by relating the Langevin polarisation to the actual number of dipoles around the given dipole).

The electrostatic free energy of the Na^+ ion at a given position is calculated from the differences in the various energy components between the ion containing system and the one in which the ion position is occupied by a water molecule. First we evaluate the interaction between the ion and the permanent dipoles (i.e., those of the protein and the two explicitly treated water molecules), $V_{Q\mu} = 332\Sigma_j Q_{Ion}q_j/r_{Ij}$, and the energy of the interaction between the permanent dipoles themselves, $V_{\mu\mu} = 332\Sigma_{i<j}q_iq_j/r_{ij}$. The energy of the induced dipoles of the protein and the membrane grid in the field of the permanent charge distribution is then calculated iteratively as described by Russell and Warshel (1985); $V_{ind} = -166\Sigma_k\xi_k^0 \cdot \mu_k$, where ξ_k^0 is the field on the kth dipole from the permanent charge distribution and $\mu_k = \alpha\xi_k$ is the induced dipole moment of the kth dipole and ξ_k the total field on the dipole. Finally, the interaction between the solvent Langevin dipoles and the rest of the system, $V_{Lgvn} = -166\Sigma_i\xi_i^0 \cdot \mu_i^L$, is calculated. The electrostatic free energy of the ion in the channel is thus obtained as

$$\Delta G_{tot}^{GA} = \Delta V_{Q\mu} + \Delta V_{\mu\mu} + \Delta V_{ind} + \Delta V_{Lgvn} + \Delta G_{bulk}, \qquad (2)$$

where ΔG_{bulk}, the contribution from the bulk solvent/membrane (not explicitly represented by Langevin or induced dipoles), is evaluated using the approximate Born type of formula

$$\Delta G_{bulk}(z_{Na}) \approx -\frac{166q^2}{a}\left\{\left(1 - \frac{1}{\epsilon_w}\right) - \frac{1}{4}\right.$$

$$\left.\cdot\left[\arctan\left(\frac{z_{Na} + h/2}{a}\right) - \arctan\left(\frac{z_{Na} - h/2}{a}\right)\right]\left(\frac{1}{\epsilon_m} - \frac{1}{\epsilon_w}\right)\right\}. \qquad (3)$$

Here, h is the height of the membrane cylinder and a is the Born radius (17.5 Å in our case). This expression was derived by simply integrating the electrostatic energy stored in an infinite slab of height h (outside the Born radius) in a homogeneous medium. The energy associated with changing the dielectric constant of the slab from ϵ_w to ϵ_m was then

approximated by the difference in the slab energy between the two homogeneous media with $\epsilon = \epsilon_w$ and $\epsilon = \epsilon_m$. The dielectric constants for bulk water and membrane, ϵ_w and ϵ_m, are taken as 80 and 2, respectively. All parameters not quoted above that were used in the PDLD calculations are given in Russell and Warshel (1985). A dipole–dipole cutoff radius of 15 Å was used consistently in both the PDLD and FEP/MD calculations.

FEP/MD calculations

With the recent availability of supercomputers it has become possible to start exploiting free energy perturbation methods (e.g., Zwanzig, 1954) in combination with MD or Monte Carlo simulations (Valleau and Torrie, 1977). In this approach one determines the free energy difference between two states of a given system, A and B, defined by their hamiltonians $\mathcal{H}_A$ and $\mathcal{H}_B$ and potential energy surfaces ϵ_A and ϵ_B. The basic idea is then to slowly vary the effective potential in an MD simulation from ϵ_A to ϵ_B in order to drive the system from state A to B. This is usually done by introducing a linear combination of the potential surfaces (Warshel, 1982)

$$\epsilon_m = (1 - \lambda_m)\epsilon_A + \lambda_m\epsilon_B, \tag{4}$$

where λ_m is a dimensionless parameter that will be successively changed from 0 to 1. For small steps in λ, the free energy associated with changing λ_m to $\lambda_{m'}$, can be treated as a perturbation on the ensemble belonging to λ_m

$$\delta G(\lambda_m \rightarrow \lambda_{m'}) = -RT \ln < \exp - (\epsilon_{m'} - \epsilon_m)/RT >_m. \tag{5}$$

The total free energy difference between the states A and B is obtained by summing up the contributions from each λ step.

$$\Delta G_{\text{FEP}}(\lambda_0 \rightarrow \lambda_n) = \sum_{m=0}^{m=n-1} \delta G(\lambda_m \rightarrow \lambda_{m'}). \tag{6}$$

The FEP/MD approach has previously been employed in calculations of (solvation) free energies for a number of different systems (see e.g., Warshel, 1982; Warshel, 1984; Warshel et al., 1986; Straatsma et al., 1986; Wong and McCammon, 1986; Bash et al., 1987; Warshel et al., 1988) and has been shown to give reasonable results when compared with experimental data.

The FEP/MD calculations for a Na^+ ion in the GA channel were done as follows. We let the states A and B above denote the uncharged and charged Na^+, respectively. With the ion initially at $(x, y, z) = (0, 0, 0)$ a series of MD simulations were carried out on the ion-GA system, including 20 water molecules, where the charge of the sodium ion was successively changed from 0 to +1. It was also necessary to repeat the procedure decreasing the charge from +1 to zero in order to assess the accuracy of the calculated free energies (for too short trajectory length at each λ_m or too large steps, $\Delta\lambda = \lambda_{m'} - \lambda_m$, one will get some hysteresis between the forward and backward charging direction). In the present calculations we used $\Delta\lambda = 0.02$ and a total simulation length of 20 ps for data collection in each direction. It is evident that this calculation would be significantly more time consuming at the channel entrance, because one would then have to include a much larger number of water molecules. In the center of the channel, however, the limited number of waters used here is sufficient to take into account most of the long-range ion-water interactions. The remaining bulk contribution is again estimated using (3). The potential function parameters used in the MD calculations are the same as in the energy optimisations (see Warshel and Levitt, 1976; Warshel et al., 1988). In order to determine the contribution from the induced membrane and protein dipoles to the total free energy, the polarisable membrane grid was built around the charged and uncharged systems and ΔV_{ind} calculated from the resulting MD structures. The total electrostatic free energy is thus given by

$$\Delta G^{\text{GA}}_{\text{tot}} = \Delta G_{\text{FEP}} + \Delta V_{\text{ind}} + \Delta G_{\text{bulk}}. \tag{7}$$

The solvation energy of Na^+ in water was also computed with free energy perturbation simulations where the solvent was treated with the SCAAS (surface constrained all atom solvent) model (Warshel and King, 1985) including 100 explicit water molecules. This model incorporates dynamical polarisation constraints on the surface of the water drop so that it behaves as if it were part of a bulk sample.

RESULTS AND DISCUSSION

Let us first examine the relevant thermodynamic cycles underlying the PDLD and FEP/MD calculations. In the former model we are evaluating the electrostatic free energy of the Na^+ ion in the channel solvated by the permanent dipoles of the GA helix and the two explicit water molecules, the induced dipoles of both the helix itself and the polarisable membrane grid and by the Langevin dipoles in the interior of the channel and outside the entrance(s). This is done by calculating the corresponding interaction energies for a system where the ion is replaced with an additional (explicit) water molecule and evaluating the energy differences according to Eq. 2. Thus having obtained the free energy $\Delta G^{\text{GA}}_{\text{tot}}$ associated with changing (or substituting) a water molecule to a Na^+ ion at a given position in the channel, one must perform the same calculation in (bulk) water in order to obtain the actual free energy of transferring the ion from the solvent to its position in the channel. Hence, we are considering the free energy difference between the two systems depicted in Fig. 2. Our reference free energy is the solvation energy of $Na^+ + 2H_2O$ in water minus the solvation energy of $3H_2O$ in water. The electrostatic free energy of moving the ion from water to the channel (Fig. 2) is thus given by

$$\Delta\Delta G[Na^+(\text{aq}) \rightarrow Na^+(GA)] = \Delta G^{GA}_{\text{tot}} - \Delta G^{w}_{\text{sol}}[w \rightarrow Na^+]. \tag{8}$$

The calculated value for $\Delta G^{w}_{\text{sol}}[w \rightarrow Na^+]$ is −101.5 kcal/mol, using the 16 Å Langevin grid described above.

In the FEP/MD simulations we are gradually charging a sphere surrounded by explicit waters in the center of the channel. To complete the relevant thermodynamic cycle and obtain the proper reference free energy the same process must also be carried out in solution. This was also necessary in order to calibrate the Na^+ potential parameters since the same parameters were used in the EM calculations. The resulting Na^+ parameters give nearest neighbor distances to the water oxygens of 2.3–2.4 Å and a solvation free energy of $\Delta G^{w}_{\text{sol}}(Na^+) = -103.2$ kcal/mol, in good agreement with experimental data (see e.g.,

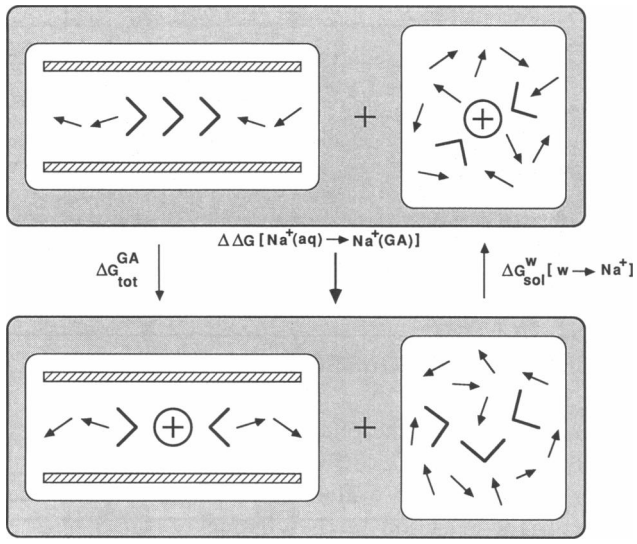

FIGURE 2 The two systems between which the free energy difference $\Delta\Delta G(Na^+[aq] \rightarrow Na^+[GA])$ is calculated. The channel is energy minimised both with and without the ion, in presence of explicit waters. All waters outside the first hydration shell are subsequently replaced by the Langevin dipole grid. $\Delta G^{w}_{sol}(w \rightarrow Na^+)$ is calculated using the 16 Å spherical grid described in the text.

Burgess, 1978). The free energy of transferring the ion from water to the interior of the GA channel is thus given by

$$\Delta\Delta G[Na^+(aq) \rightarrow Na^+(GA)] = \Delta G^{GA}_{tot} - \Delta G^{w}_{sol}(Na^+), \quad (9)$$

where ΔG^{GA}_{tot} is evaluated according to Eq. 7. In principle, one should also include the energies of creating the uncharged sodium sphere in the two environments. However, this contribution to $\Delta\Delta G$ is found to be negligible (and within the errors of the calculations) in our case.

Comparison of PDLD and FEP/MD free energies

In view of the fact that the PDLD/EM scheme used here is clearly a simplified model compared with the more rigorous FEP/MD calculations, it is interesting to compare the results from the two methods. This is also necessary in order to gauge the overall accuracy of the free energy profile calculated with the PDLD model. Table 1 lists the values of $\Delta\Delta G$, calculated according to Eq. 8, together with its decomposition into different energy contributions, for a number of ion positions. It can be seen that the calculated ion solvation energies in the channel are within just 5 kcal/mol from the reference free energy in solution. The largest contribution naturally comes from $\Delta V_{Q\mu}$, the ion-permanent dipole interactions, of which ~ -50 kcal/mol is due to the two water molecules in the first solvation shell. However, favorable interactions between the ion and the permanent dipoles (notably in the central region of the helix) are opposed by less favorable interactions between the permanent dipoles themselves ($\Delta V_{\mu\mu}$). This reflects both local distortions of the peptide structure as well as the different orientation of the explicit water molecules compared with the case where no ion is present. It is also noteworthy that the induced dipole energy, ΔV_{ind}, can amount to as much as 10–12 kcal/mol.

In the central region of the channel the difference in solvation energy with respect to water, $\Delta\Delta G$, is ~ -2 to $+3$ kcal/mol. This value can be directly compared with the FEP/MD result of $\Delta\Delta G = 2.3$ kcal/mol given in Table 2. The latter value represents an (ensemble) average in the neighborhood of $z \approx 0.9$ Å (corresponding to the average position of the ion during the MD simulations), because in this case no constraints were applied. In fact, the average ion position in the MD simulation corresponds to a minimum on the free energy profile calculated with the PDLD/EM method (cf. Fig. 3 *a*). This demonstrates that the two methods give similar values of the total free energy associated with transferring the ion from bulk solvent to the center of the channel. However, since the MD trajectory samples a large number of configurations while the PDLD structures are optimised, it seems reasonable that the former calculation gives a slightly higher free energy in the neighborhood of a given minimum. Nevertheless, the fact that the two methods give similar differential free energies in the center of the channel is encouraging, because it supports the validity of the assumptions underlying the PDLD scheme. It is noteworthy that calculations of a complete free energy profile using the FEP/MD method would be very expensive, in particular at the channel entrances where a large number of waters would have to be included to account for bulk effects.

The most important observation to be made, however, is that the stabilising contribution to the ion solvation free energy in the channel from the induced dipoles of the membrane and the protein can amount to as much as 10–12 kcal/mol (the membrane alone is responsible for up to 70% of ΔV_{ind}). This suggests that as long as this energy component is neglected in calculations of energy profiles, these will inevitably give too high energies inside the channel (if compared with solvation energies in water) and be offset by roughly the same number. With the requirement that $\epsilon = 2$ for the membrane the Clausius-Mossotti only defines the relation between the polarisability and spacing of the grid. We have examined the sensitivity of ΔV_{ind} to the actual geometry of the grid by performing additional calculations using a grid with 2 Å spacing and $\alpha = 0.5$ Å^3 ($\epsilon = 2$ also for this choice) and approximately the same van der Waals contact distances to the protein. This grid gives ΔV_{ind} values that are 0.5

TABLE 1 PDLD/EM calculations of Na^+ solvation in the GA channel

z_{Na} (Å)	$\Delta V_{Q\mu}$	$\Delta V_{\mu\mu}$	ΔV_{ind}	ΔV_{Lgvn}	$\Delta G_{no\ bulk}$	ΔG_{bulk}	ΔG_{tot}^{GA}	$\Delta\Delta G$
0.4	−119.3	+38.4	−7.7	−3.7	−92.3	−7.7	−100.0	+1.5
0.9	−111.4	+27.0	−8.3	−3.4	−96.1	−7.7	−103.8	−2.3
2.7	−106.9	+34.2	−10.2	−7.8	−90.7	−7.7	−98.4	+3.1
5.8	−101.3	+31.8	−12.0	−7.4	−88.9	−7.8	−96.7	+4.8
6.9	−103.1	+32.4	−12.3	−9.7	−92.7	−7.8	−100.5	+1.0
8.6	−101.7	+29.7	−10.0	−11.0	−93.0	−7.9	−100.9	+0.6
10.9	−102.9	+39.3	−9.9	−16.2	−89.7	−8.0	−97.7	+3.8
11.5	−93.1	+34.8	−11.5	−19.7	−89.5	−8.0	−97.5	+4.0
12.9	−81.4	+23.0	−8.4	−24.9	−91.3	−8.1	−99.4	+2.1
14.0	−79.0	+16.4	−8.4	−27.1	−98.1	−8.1	−106.2	−4.7
14.8	−74.1	+18.0	−7.1	−31.3	−94.5	−8.2	−102.7	−1.2

Free energy differences (units in kilocalorie/mole) between the energy minimised system with the charged Na^+ ion and the energy minimised water filled channel. The reference free energy in water is $\Delta G_{sol}^{w}[w \rightarrow Na^+] = -101.5$ kcal/mol. The highest and lowest points of the profile (cf. Fig. 3 *a*) occur at $z = 5.8$ Å and $z = 14.0$ Å, respectively.

kcal/mol higher than those reported in Table 1, a number which can be considered to define the error range for ΔV_{ind}.

Free energy profile from PDLD calculations

In view of the previous discussion, we feel that it is now warranted to examine the complete (PDLD/EM) free energy profile for Na^+ ion permeation through the GA channel. It should, of course, be kept in mind that we are still leaning on at least two simplifications that may have some effect on the results. These are, firstly, the neglect of the amino acid side chains and, secondly, the neglect of polar membrane head groups. The latter approximation should mainly affect the results near the channel entrance. In the case of e.g., dioleoyllethitin membranes the head group net charges may particularily influence the energetics at the channel entrance (see e.g., discussion in Eisenman et al., 1983). The observation (Bamberg and Läuger, 1974) that these membrane channels display slightly higher (~3 kcal/mol) barriers for Na^+ than in GMO bilayers probably reflects a repulsion between the ion and the $N(CH_3)_3^+$ charges. It has also been shown (Apell et al., 1979) that GA channels in negatively charged membranes display considerably higher cation conductances. If one considers GMO membranes, on the other hand, the neglect of head groups should not be as severe an assumption because they are much less polar. For these membranes, the overall effect of the head groups is expected to be only a few kilocalories (at the entrance) and can be neglected at present, because this error is small compared with those in previous studies.

TABLE 2 FEP/MD calculations of Na^+ solvation in the GA channel and in water

	ΔG_{FEP}	ΔV_{ind}	$\Delta G_{no\ bulk}$	ΔG_{bulk}	ΔG_{tot}
Na^+ in H_2O	−89.5		−89.5	−13.7	−103.2
Na^+ in GA	−83.0	−10.2	−93.2	−7.7	−100.9

Free energy of charging a Na^+ ion in water and in the center ($z_{Na} \approx 0$ Å) of the water filled GA channel, obtained from FEP/MD simulations (units in kilocalorie/mole). The errors in ΔG_{FEP} are $\leq \pm 2$ kcal/mol.

As for the first simplification above, the effect of the induced polarisation of the hydrophobic side chains can be assumed to be reasonably well modeled by our membrane grid. The neglect of the tryptophan $N_{\epsilon 1} - H_{\epsilon 1}$ dipole moments is, in our view, the most questionable approximation. As it is not within the scope of this study to actually energy minimise the side chain conformations in the presence of real hydrocarbon chains, we will address this problem in a preliminary manner. Taking the positions of the relevant $N_{\epsilon 1} - H_{\epsilon 1}$ groups from Urry's GA structure (Urry, 1971), we can evaluate the electrostatic influence of these dipoles on the profile without further ado. This was done by simply inserting the $N_{\epsilon 1} - H_{\epsilon 1}$ groups into the membrane grid and deleting those grid points within 3.0 Å of the $N_{\epsilon 1}$ atoms. The additional interaction energy will thus affect only $\Delta V_{Q\mu}$ and ΔV_{ind} (and marginally also ΔV_{Lgvn}). It then turns out that this contribution gives at most only a small stabilisation (0–2 kcal/mol) of the ion, which does not appear to be significant without full optimisation of the side chain conformations. Therefore the polyglycine representation of the GA helix does not seem to be a severe simplification given the present level of detail and the tryptophan residues, if included, are not expected to change the calculated profile drastically.

The calculated free energy profile is depicted in Fig. 3 *a* and its decomposition into different components in Figs. 3, *b–g*. As mentioned earlier, the interaction energy between the Na^+ ion and the two explicit water molecules (~−50 kcal/mol) is included in $\Delta V_{Q\mu}$ rather than in the

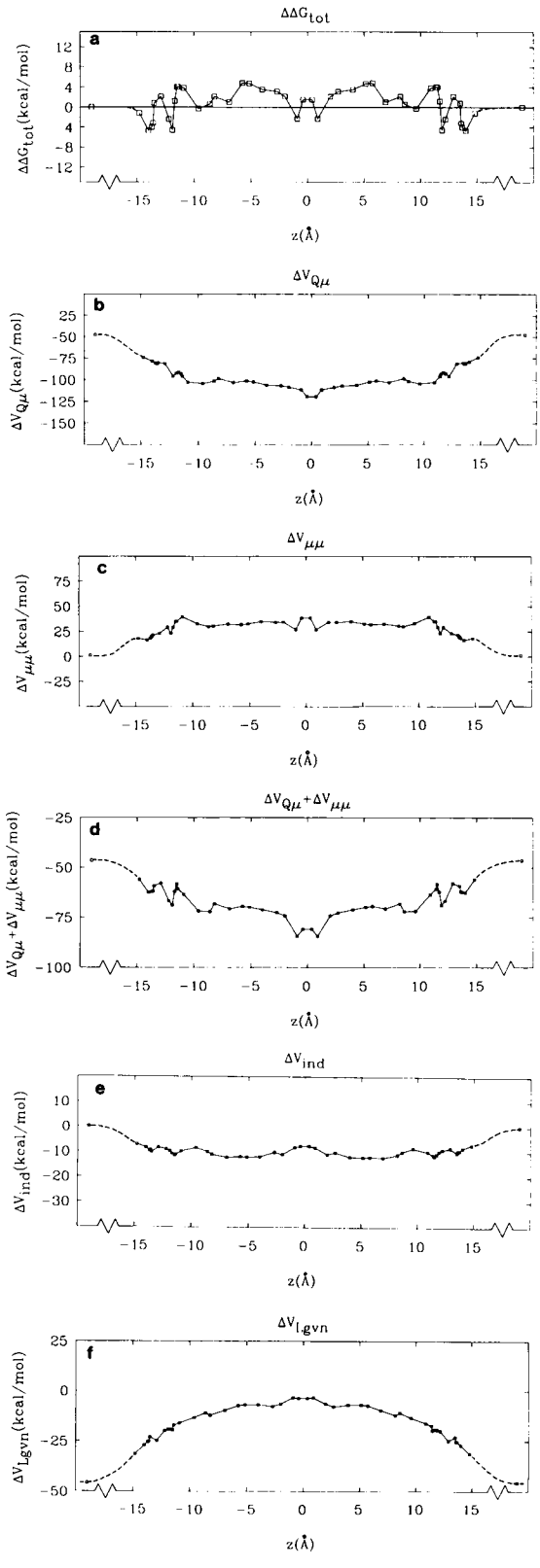

FIGURE 3 (*a*) Total electrostatic free energy of Na^+ with respect to bulk water as a function of the ion position. (*b–g*) Decomposition of the total free energy into different contributions. See text for details.

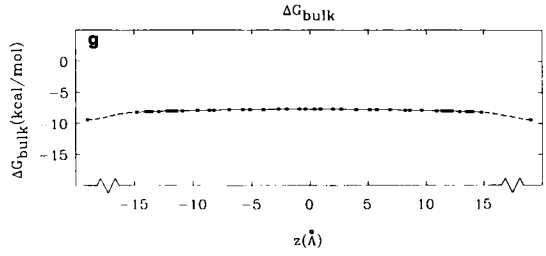

FIGURE 3 (*continued*)

solvent term ΔV_{Lgvn}. However, the variation in this interaction term is < 5 kcal/mol (its absolute value generally decreases as the ion is moved out of the channel), wherefore the shape of the $\Delta V_{Q\mu}$ profile (Fig. 3 *b*) is mainly determined by the change in ion-protein permanent dipole energy.

The main feature governing the overall free energy of the ion can be rationalised as a balance between the net gain in the interaction energy due to the permanent dipoles as the ion enters the channel (Fig. 3 *d*) and the loss in water solvation energy (Fig. 3 *f*). The latter term, ΔV_{Lgvn}, varies rather smoothly with the ion position as would be expected, while the term $\Delta V_{Q\mu} + \Delta V_{\mu\mu}$ (Fig. 3 *d*) which reflects the actual structure of the GA helix is the major source of barriers and minima in $\Delta\Delta G_{tot}$ (Fig. 3 *a*). It should also be mentioned in this context that the reference energy (corresponding to the upper system in Fig. 2) varies very little (< 3 kcal/mol) along the channel. The calculated value for $\Delta V_{Q\mu} + \Delta V_{\mu\mu}$ in the center of the channel is ~ -80 kcal/mol. As a check, this number can be directly compared with that obtained by Mackay et al. (1984) for sodium. They use similar Lennard-Jones parameters for the Na^+ interactions and also obtain a corresponding energy difference of -80 kcal/mol between the water filled channel and the case with the ion in the center (see Table 1 of Mackay et al., 1984). The study by Mackay et al. (1984) did not however, take into account bulk water or the membrane dielectric and would have given an unrealistic profile for ion permeation. On the other hand, the study by Lee and Jordan (1984) reported a decrease in the potential energy for Na^+ of only ~7 kcal/mol as the ion (with its flanking waters) was moved from z = 11–12 Å to the channel center. The corresponding change in our case for $\Delta V_{Q\mu} + \Delta V_{\mu\mu}$ (between z = 11.5 Å and z = 0.4 Å) is -23 kcal/mol. This discrepancy appears mainly to be due to the fact that Lee and Jordan (1984) used substantially larger repulsive Lennard-Jones parameters for Na^+, but could also reflect their simplified helix model. They report nearest neighbor Na^+-water oxygen distances of ≈2.6 Å, which disagrees with experimentally observed radial distribution functions in solution (Licheri et al., 1973; Pálinkás et al.,

1980). This indicates that the observed hydration energy of Na^+ would not be reproduced with their parameters.

The energy term $\Delta V_{Q\mu} + \Delta V_{\mu\mu}$ associated with the permanent change distribution of the helix and the explicit water molecules can also be viewed as the result of two competing trends as the ion enters the channel. While the favorable Na^+-helix interaction becomes more negative, this also results in local perturbations of the peptide structure that is reflected by a loss in the energy between helix (and water) dipoles themselves (Fig. 3 *c*). However, the former effect dominates wherefore the net energy due to the permanent dipoles decreases (becomes more negative). The contribution from the induced dipoles, ΔV_{ind} (Fig. 3 *e*), is generally on the order of 10 kcal/mol. It can be noted, though, that in the center of the channel, where there is a dip in $\Delta V_{Q\mu} + \Delta V_{\mu\mu}$ mainly due to interaction between the Na^+ ion and the formyl groups ΔV_{ind} becomes less favorable as the ionic charge is more effectively screened by the permanent dipoles. Finally, the bulk contribution (Fig. 3 *g*) increases slowly as the ion is moved out of the channel and acts mainly by adding an (almost) constant term to the total free energy.

The total (differential) free energy profile $\Delta\Delta G_{tot}$ (Fig. 3 *a*) shows barriers of ≤ 5 kcal/mol and two minima near the channel entrance of ~4–5 kcal/mol. These values are in good agreement with activation barriers derived from experimentally observed permeation rates (Bamberg and Läuger, 1974; Eisenman and Sandblom, 1983). Another interesting comparison that can be made is to the Cs^+ profile obtained by Sung and Jordan (Fig. 5 *B* in Sung and Jordan, 1987). Although we are considering two different ions, the two profiles are qualitatively very similar. Both show a relatively deep minimum near $z = 12$ Å followed by a fairly high barrier. This barrier appears to be slightly shifted towards the channel entrance for Na^+. Furthermore, a minimum in the interior of the channel is present in both cases at $z \approx 8.5$ Å and there also appears to be a local maximum in the channel center for both Na^+ and Cs^+. This is particularly interesting because it indicates that even a fairly simple model for GA (Sung and Jordan, 1987) in which only the dipolar CO and NH groups of the helix backbone are taken into account (together with the ends groups and a small number of waters) is capable of producing results similar to those obtained with more expensive all atom models, as far as the general shape of the profile is concerned. This should partly be due to the fact that ΔV_{ind} (as well as ΔG_{bulk}) to a first approximation can be considered as an additive constant to the profile in the channel. It also probably illustrates the importance of allowing the helix dipoles to reorient themselves, which is the case in Sung and Jordan's model, too (see also Schröder, 1983). That is, in a rigid helix model (e.g., Kim and Clementi, 1985; Pullman and Etchebest, 1987) the simultaneous optimisation of $V_{Q\mu}$ and $V_{\mu\mu}$ is completely suppressed and a major factor determining the structure of the energy profile is thus neglected.

Let us now examine the free energy profile in more detail and try to correlate the calculated energetics with the actual structure of the GA channel. As the Na^+ ion approaches the channel entrance it first encounters the carbonyl groups of (amino acid) residues 13 and 15 while still remaining more than half of its bulk solvation energy. This interaction gives rise to the observed minimum at $z \approx 14$ Å (just outside the entrance). At this point the ion still has three water molecules in the first solvation shell (together with CO-13 and CO-15) as judged from the EM structure containing all the explicit waters. As the ion is moved further into the channel (to $z \approx 12$ Å) it must give up one of these water ligands, but regains this energy loss by interacting with the ethanolamine tail. The carbonyl ligands are then also switched from CO-13 and CO-15 to CO-11 and CO-13 (Fig. 4 *a*). The barrier separating these two minima could thus be interpreted as an intermediate situation in which the third water ligand is loosening while the ethanolamine interaction is not yet optimal. Note, that although we only treat two water molecules explicitly when evaluating the PDLD energies, the effect of losing the third water ligand seems to be reasonably well modeled by the Langevin dipole grid. The optimised structure corresponding to binding site at $z \approx 12$ Å is depicted in Fig. 4 *a*.

When the Na^+ ion is moved further into channel it will eventually (at the minimum around $z \approx 9$ Å) be solvated by four carbonyl groups in addition to the two water molecules, thus giving a total of six ligands. The barrier at $z \approx 11.5$ Å appears mainly to reflect strain (cf. Fig. 3 *c*) in the helical structure before this happens (notably involving the ligands from the previous minimum at $z \approx 12$ Å). This loss in interaction energy between the helix dipoles can be viewed as a consequence of the dipolar groups reorienting themselves in order to compensate for the overall loss in solvation energy from the water. At $z \approx 9$Å the Na^+ ion interacts with the carbonyl groups from residues 7, 9, 12, and 14 (Fig. 4 *b*) and this solvation pattern prevails as it is moved towards the center of the channel; e.g., at $z \approx 5$ Å the carbonyl groups have been switched to 3, 5, 8, and 10. The overall increase in $\Delta\Delta G_{tot}$ as the ion is moved towards the center from the point where it has first received the four carbonyl ligands appears to reflect mainly a loss of (long range) water solvation energy, i.e., a decrease (in the absolute value) of ΔV_{Lgvn}. Finally, the distinct minimum at $z \approx 1$ Å is caused primarily by favorable interaction with one of the formyl groups. The formyl group is more flexible than the carbonyl groups because of its location at the end of the peptide chain. This flexibility can apparently allow for a better optimisation of $V_{Q\mu}$ without significantly perturb-

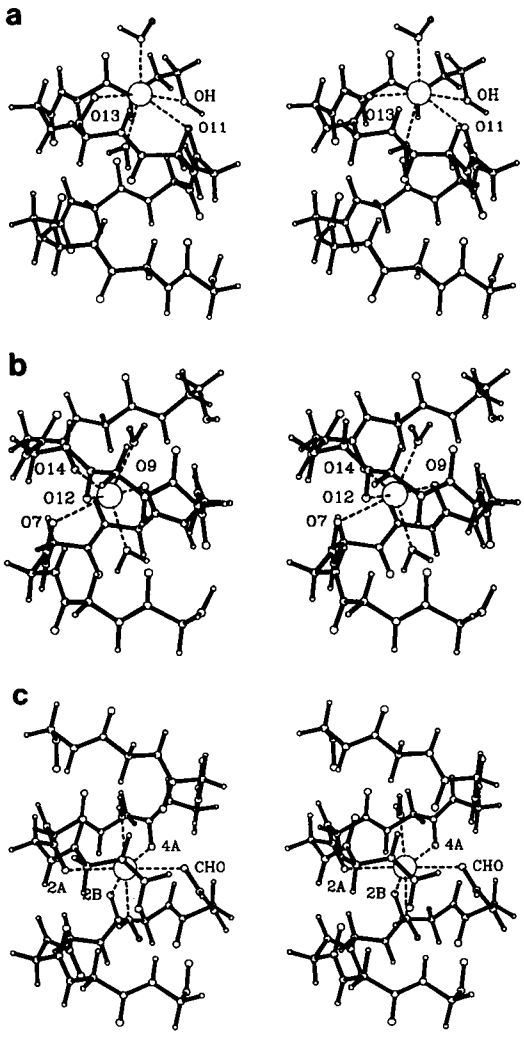

FIGURE 4 Stereo views of the energy minimised Na^+-GA-water structures at (*a*) z = 11.9 Å, (*b*) z = 8.6 Å, and (*c*) z = 0.9 Å. Numbers denote carbonyl groups, OH denotes the ethanolamine end and CHO is a formyl group.

ing the helix backbone. At this minimum (Fig. 4 *c*) the Na^+ ion interacts with CO-$2_{(1)}$, CO-$4_{(1)}$, $CHO_{(2)}$ and CO-$2_{(2)}$ (where the subindices enumerate the GA monomers and CHO is a formyl group). It can be noted that a local minimum at roughly the same position was observed for Cs^+ in the study by Sung and Jordan (1987) and that the ion ligands appear to be exactly the same in our case.

CONCLUDING REMARKS

We have presented calculations of the solvation energetics of a Na^+ ion in the interior of the Gramicidin channel using both the computationally inexpensive PDLD method, with which a complete energy profile has been obtained, as well as free energy perturbation simulations. The membrane surrounding the ion channel was represented by a large cylindrical grid where each grid point is a polarisable point dipole with no permanent dipole moment. Both the PDLD and the FEP/MD methods give solvation energies for Na^+ inside the channel that are <5 kcal/mol above the solvation free energy for the ion in water (Fig. 3 *a*). These values agree well with experimentally estimated activation barriers for ion migration (Bamberg and Läuger, 1974; Eisenman and Sandblom, 1983) that are at most of the order of 5–7 kcal/mol. It should be noted that we did not perform actual calculations of the rate constant and this type of calculation is now in progress in our laboratory. However, we would like to point out that the dynamical effects, which are clearly of interest, are merely second order effects compared with the energetics of ion solvation by the channel. That is, any model that predicts a translocation barrier of, say, 15 kcal/mol will be incorrect by six to seven orders of magnitude with or without consideration of dynamical effects. On the other hand, a model which gives free energy barriers of 5 kcal/mol cannot be wrong by more than two orders of magnitude due to neglect of dynamical effects (because we are concerned with processes in condensed phases where the transmission factor is close to unity [Warshel et al., 1988]). Thus, we consider our results to be quite encouraging and interpret them in support of the argument that electrostatic free energies in macromolecules can be evaluated in a semiquantitative (or even quantitative) way, provided all of their relevant components are taken into account in an explicit microscopic representation. On the other hand, it appears that using rigorous treatments for some parts of the system, while neglecting others, is less effective than an approximate but complete representation of the entire system (as illustrated by e.g., the effect of membrane polarisation in the present case).

An important conclusion emerging from our study is that the induced polarisation of the lipid membrane (and to a lesser extent) of the GA helix can play a significant role in determining the free energy of the ion inside the channel. Because the stabilising effect of the membrane has been neglected in all previous microscopic simulations of the GA system it is not surprising that the computed energy profiles generally give too high energies inside the channel. However, the effect of the induced membrane polarisation on the shape of the energy profile does not appear to be very large (at least not compared with the interaction between the ion and the permanent dipoles). Therefore, models incorporating essential features like flexibility of the GA helix and waters should be able to provide relevant information on the location (and maybe also relative energies) of barriers and ion binding sites.

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