FEBS 20750 FEBS Letters 434 (1998) 265–271

The M2 channel of influenza A virus: a molecular dynamics study

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Received 14 May 1998; revised version received 9 July 1998

Abstract Molecular dynamics simulations have been performed on a tetramer of the 25-residue (SSDPLVVAASIIGILHLIL-WILDRL) synthetic peptide [1] which contains the transmembrane domain of the influenza A virus M2 coat protein. The peptide bundle was initially assembled as a parallel α-helix bundle in the octane portion of a phase separated water/octane system, which provided a membrane-mimetic environment. A 4-ns dynamics trajectory identified a left-handed coiled coil state of the neutral bundle, with a water filled funnel-like structural motif at the N-terminus involving the long hydrophobic sequence. The neck of the funnel begins at V27 and terminates at H37, which blocks the channel. The C-terminus is held together by inter-helix hydrogen bonds and contains water below H37. Solvation of the S23 and D24 residues, located at the rim of the funnel, appears to be important for stability of the structure. The calculated average tilt of the helices in the neutral bundle is 27 ± 5°, which agrees well with recent NMR data.

Key words: Influenza A virus; M2 channel; Molecular dynamics; Four-helix bundle

1. Introduction

The native (97-residue) M2 coat protein plays an essential role in the life cycle of the influenza A virus. It consists of three domains: a 24-residue N-terminal extracellular sequence, a 19-residue transmembrane (TM) domain and a 54-residue cytoplasmic tail. Early research established that the M2 protein forms a homotetramer that exhibits ion-channel activity [2], which was found to be blocked by the anti-influenza drug amantadine hydrochloride [1,3]. The study of synthetic peptides incorporating TM domains has been shown to be a very successful strategy in characterizing ion-channel activity and thereby providing insight into membrane-protein function [4–6]. In this spirit, Duff and Ashley [1], utilizing a synthetic peptide with the 25 amino acid sequence SSDPLVVAASIIGILHLILWILDRL, hereafter referred to as M2-DA peptide, observed single proton-selective ion channels in planar lipid bilayers. The channels formed by M2-DA peptide were also effectively blocked by amantadine. Surprisingly, in the segment between residue P25 and residue H37, there is only one hydrophilic residue, i.e. S31. It remains a challenge to understand how such an unusually long hydrophobic segment, inside the TM domain, can form and function as an ion channel.

The recent cysteine-scanning mutagenesis experiments of Pinto et al. [7] have provided a molecular model of the M2-DA channel composed of a super-coiled peptide tetramer with four-fold symmetry. In this model, the H37 residues point into the lumen and effectively block the pore. Solid-state NMR experiments indicate that the membrane-bound M2-DA peptides tilt 33° with respect to the bilayer normal [8]. Activation of the M2 channel on lowering pH has been attributed to the protonation of the H37 residue inside the pore [9]. Experimental studies on M2-DA [3] show that the mutation of residue V27 to isoleucine does not change the pH gating of the channel but does affect amantadine blocking. More recent work of Gandhi et al. [10] shows that transition metal ions can bind to various parts of the protein, which may provide a useful probe for future research.

Molecular modeling, and the more CPU intensive molecular dynamics (MD) simulation technique, have both proven to be useful tools to understand the behavior of complex systems. Ion channel proteins, for which the hydrophobic environment is vital, have even been studied via MD simulation [11–16]. However, in previous studies on the M2 channel the membrane environment was omitted and structural constraints were applied to help stabilize the peptide bundle during the MD simulation [16]. The resulting M2 helix bundle was a left-handed supercoil surrounding a central pore with the (unprotonated) H37 residues blocking the channel. By contrast, a fully H37-protonated bundle was found to support a column of water. These calculations led to a model for pH activation of the M2 channel in which charging of the H37 residues causes their rotation to interfacial locations, thereby allowing water to permeate across the membrane. In the model suggested by Pinto et al. [7] no physical rotation of H37 is necessary in order to activate the channel.

In this work, we have combined some of the recently introduced novel simulation methodologies [17,18] with state-of-the-art parallel computing to probe the structural and dynamical properties of the M2-DA peptide bundle in the presence of a membrane-mimetic environment. Specifically, the latter has been taken into account by placing a four-helix Duff-Ashley peptide bundle [1] in the octane region of a water/octane simulation box. The evolution of the four-helix bundle was then followed during extensive MD simulations, which spanned several nanoseconds. The present calculations are far from routine and required several months on a dedicated machine with parallel processors. Anticipating our results we will see that our MD simulation for the unprotonated H37 bundle indeed yields a supercoil structure that has similarities to that proposed earlier [16]. However, the present results suggest that residues outside of the TM domain may play a role in determining the structure of bundle. Our simulation of a fully H37-protonated bundle yielded a rather unstable asymmetric aggregate, which therefore seems to be an unlikely candidate for the pH activated state of the channel.

The paper is organized as follows: First, we present a brief

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PII: S0014-5793(98)00988-0

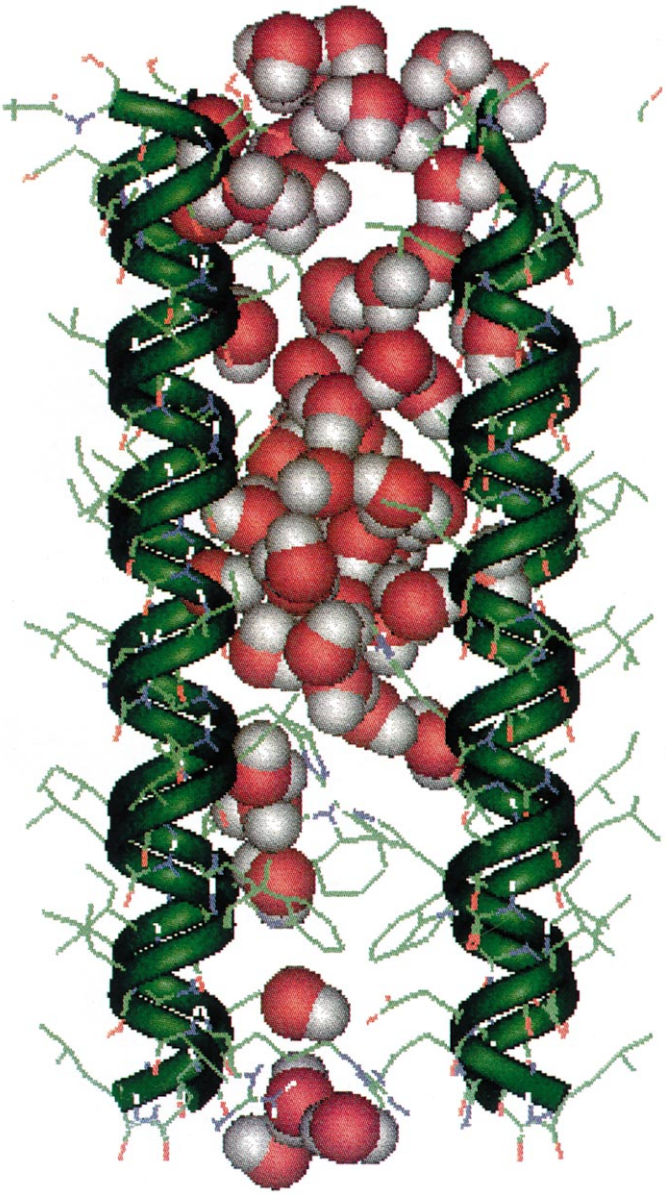

Fig. 1. The initial configuration used to launch the MD simulation of the 4-helix M2 peptide bundle. The membrane mimetic octane/water system has been omitted for visual clarity. The channel water molecules are rendered with van der Waals radii and the peptides as ideal α-helices oriented normal to the octane/water interface.

outline of the simulation procedures. Then, we discuss the system set-up and the assembly of the peptide bundle. This is followed by the presentation of our simulation on the neutral system and a discussion characterizing the observed structures. Next, the fully H37-protonated bundle is discussed. Finally, we make comparison with the available experimental data.

2. Multiple-time-step molecular dynamics

We have employed an MD scheme based on reversible integrators, combined with the multiple-time-step method [19]. The van der Waals and electrostatic interactions, which compose the interatomic forces, were each divided into short- and long-range parts. The short-range interactions were truncated at 7.0 Å for the electrostatic terms and at 2.0 σ, where σ is the usual Lennard-Jones parameter, for van der Waals interactions, respectively. The short-range interactions were calculated every 1.5 fs. However, with the chosen cutoffs, they only contain about half of all the non-bonding interactions. The major part of the non-bonding interaction, the long-range part, fluctuates slowly and was therefore calculated only every 3 fs. The intramolecular interactions, including stretching, bending, and dihedral angles, are calculated every 0.3 fs.

Periodic boundary conditions were used with an overall cut-off of 2.5 σ for the van der Waals interactions. A standard correction [20] was utilized for the neglected long-range contributions of van der Waals interactions. The Ewald method was employed to take into account the long-range electrostatic interactions [20,21], with a 10-Å real-space truncation, 10 $Å^{-1}$ as the cut-off in reciprocal space, and a value $\alpha = 0.3$, for the weight of the Gaussian damping factor.

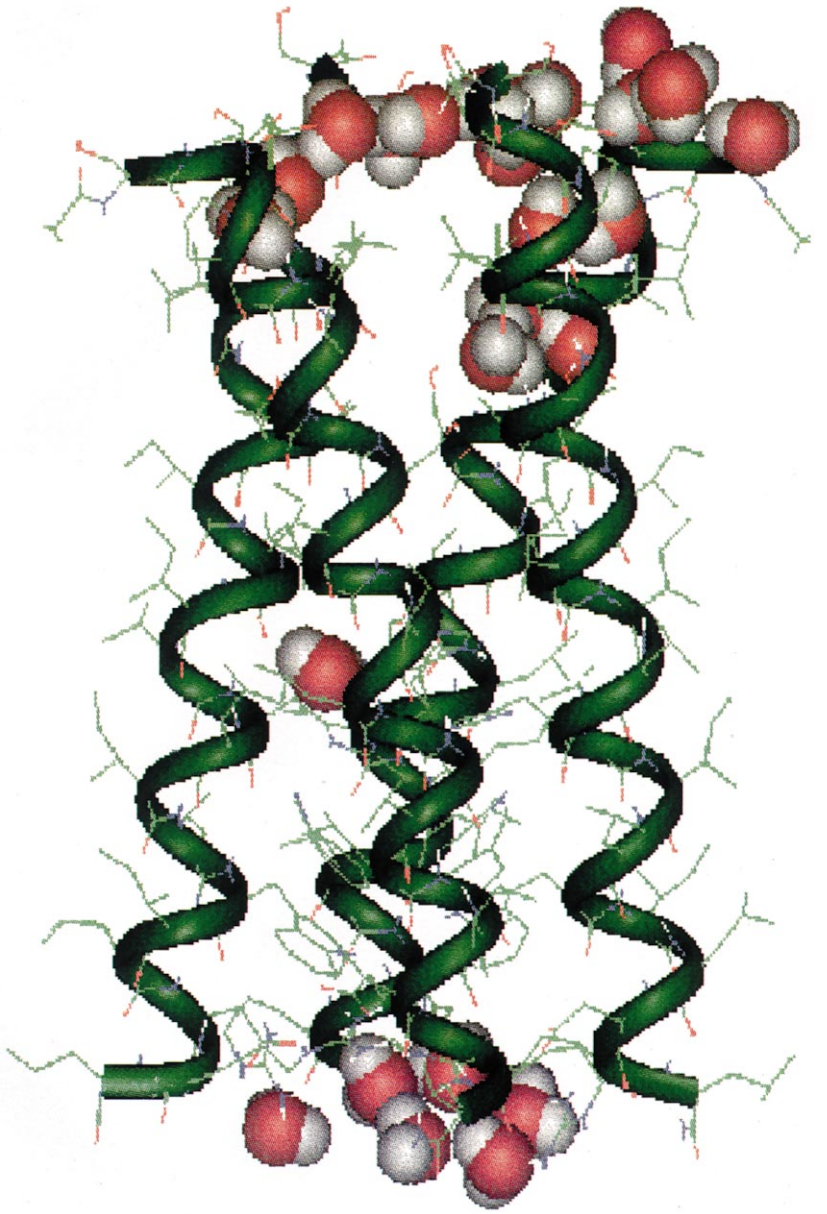

Fig. 2. The intermediate compact state of the peptide bundle at about 1 ns. The structure is a left-handed supercoil with roughly four-fold symmetry about the long axis. The bundle is held together via helix-helix hydrogen bonds between the S23 and D24 residues at the N-terminus and between D44 and R45 at the C-terminus (see text).

The simulation was carried out at nominal room temperature, 300 K, with the temperature controlled by a Nosé-Hoover chain thermostat [17]. We used a single Nosé-Hoover chain of length 3 with the frequency factor of the chain chosen as 2 ps^{-1}. The thermostated equations of motion yield continuous dynamics that generate a canonical distribution [18]. In the part of the trajectory where the averaged properties are calculated, no constraints were applied to the system.

3. Simulation system

The M2-DA peptide was first set up in an ideal right-handed α-helical form using INSIGHT (BIOSYM Technologies, San Diego, CA, USA). Then, the peptide with caps added was minimized via an Adapted Basis Newton-Raphson algorithm (ABNR) using an optimized version of the CHARMM program, and the CHARMM 19 united atom parameter set [22], with all backbone atoms fixed, so that the atoms on the side chain could relax. Next, the minimized helix was duplicated, and assembled as a parallel tetramer, with four-fold symmetry. The inter-helical separation (about 10 Å) was chosen to avoid any unphysical repulsions. The energy of the four-helix bundle was then minimized by ABNR, with all backbone atoms fixed, to eliminate any possible bad contacts between the helices. The resulting bundle provided the starting configuration for the MD simulations (see Fig. 1).

In order to reduce the overhead in simulation time, the helical bundle was solvated in the octane part of a phase-separated octane/water box. The mimicking of a planar lipid bilayer as an octane slab has proven successful in our earlier simulations [25,26]. One of the most important properties of

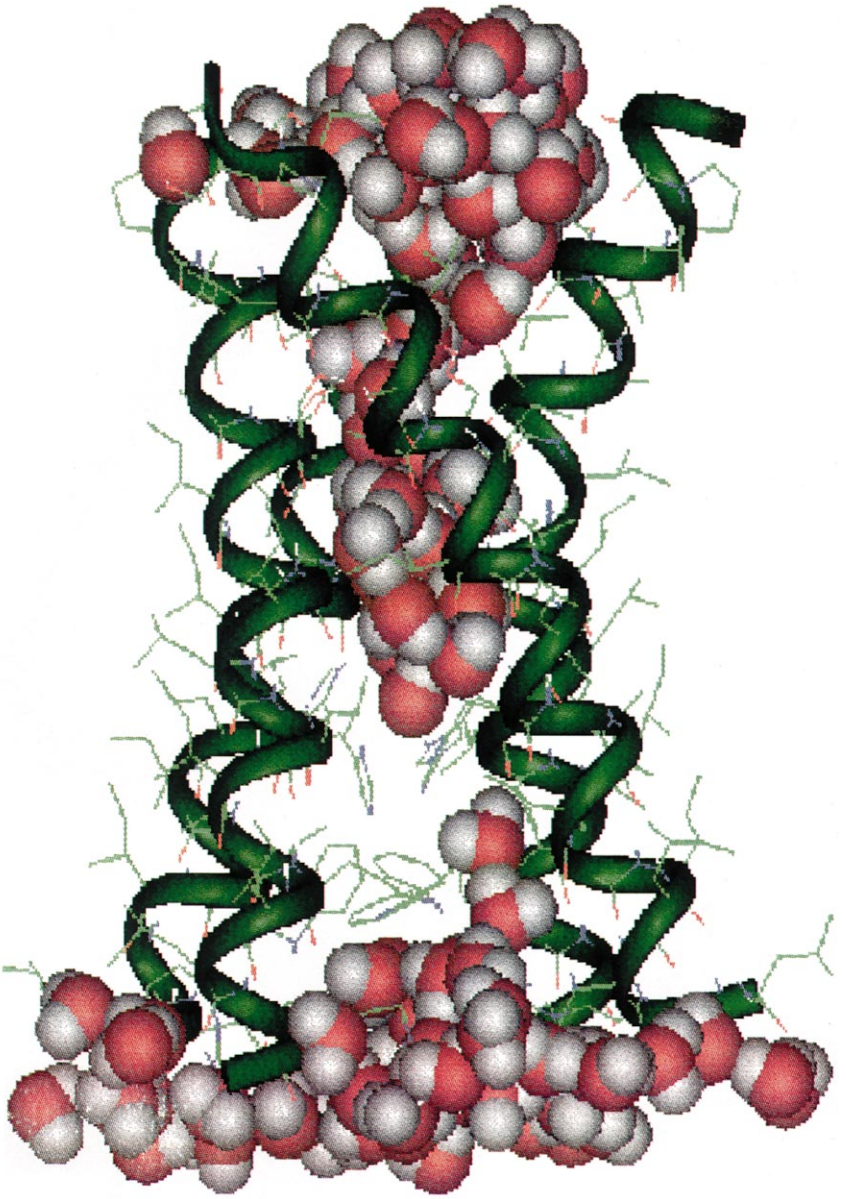

Fig. 3. An instantaneous configuration of the four-helix peptide bundle at 3 ns. The left-handed supercoil is maintained but the interior of the bundle now contains water with an overall hour-glass structural motif. The channel waters are blocked by the four H37 residues.

the lipid bilayer, among others, is a well-defined hydrophilic/hydrophobic interface whose essential properties have been shown to be preserved in this simple mimetic system.

Individual simulation systems of both octane and water were first prepared using constant volume (NVT) and constant pressure (NPT) MD simulations, at room temperature and atmospheric pressure, each of which ran for over 500 ps. Then, the octane box was cut into a slab 45.45×47.45 Å^2, with the same height (40.00 Å) as the ideal α-helical Duff-Ashley sequence. The lateral dimensions of the simulation box were chosen in such a way that the four-helix bundle is reasonably far away from any of its images. Next, a hole was cut in the middle of the octane box and the bundle was inserted. Then, two slabs of water molecules, each 10 Å thick, were taken from the H_2O simulation and added to both ends of the peptide bundle. The layer thickness was chosen based on the known characteristics of the octane/water interface. Then, the interior of the laterally expanded bundle was filled with nominal pore water molecules. The resulting system with 162 octane molecules, 1986 water molecules, and 4 α-helices with dimension $45.45 \times 47.45 \times 60.00$ Å^3 constituted the starting configuration for this simulation study.

Initially, the bundle was constrained and an NVE-MD run carried out on the octane and water subsystem for 100 ps (see Fig. 1). The well-known TIP3P model [27] was used for both the bulk and pore H_2O. However, because of the multiple time-step integrator used for the equations of motion, the O–H bond length was not constrained. The parameters used for octane were those recommended by Siepmann et al. [28] for a fully flexible united atom model of methyl and methylene groups. The topology and parameters for the peptide were taken from CHARMM 19 [22]. This version of the parameter set is based on a united atom model for the peptide residues and their interactions and hence is consistent with the molec-

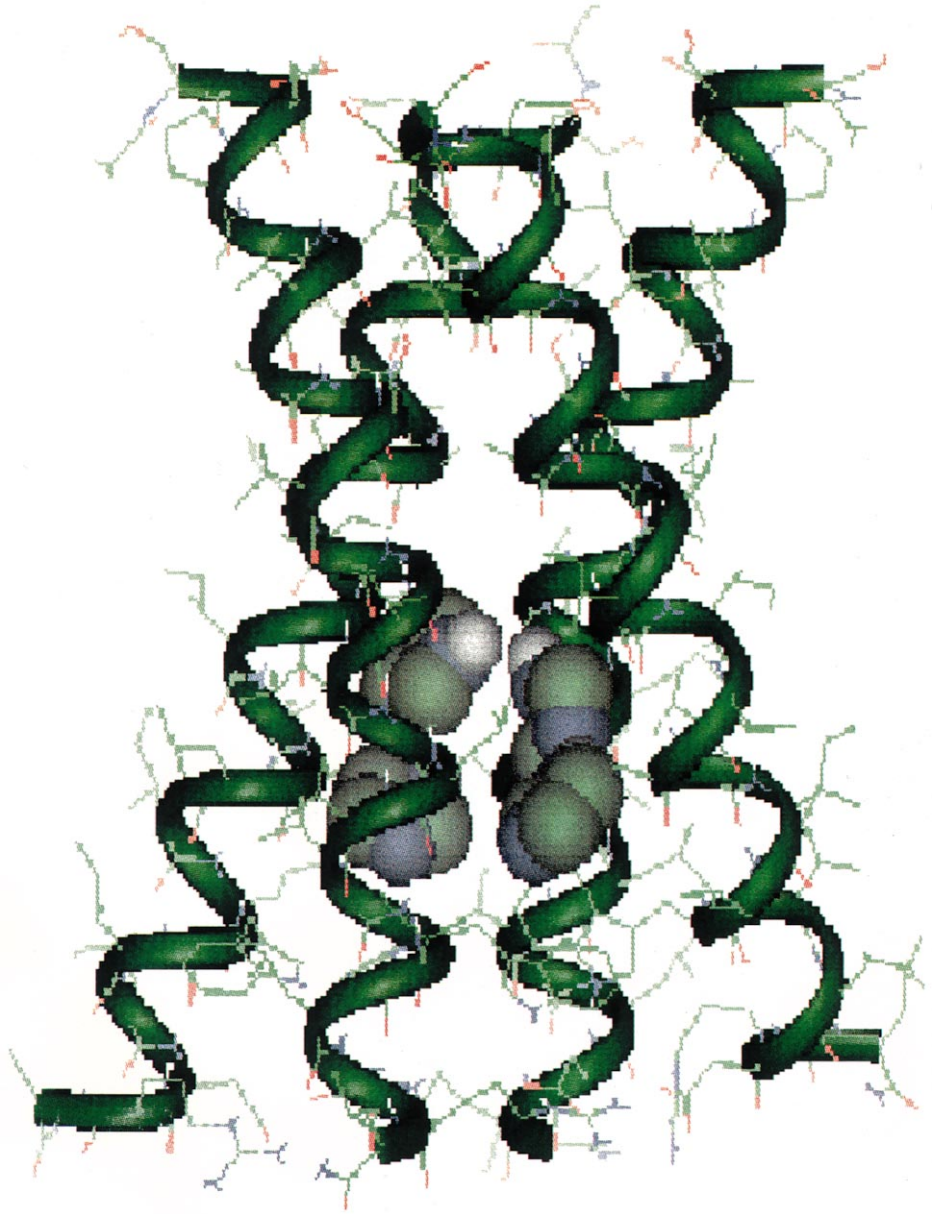

Fig. 4. The same configuration as in Fig. 3 showing the H37 channel blocking residues.

ular model used for the hydrophobic octane. All hydrogens, except the polar hydrogens, the amide hydrogens, and water hydrogens, were absorbed into heavy atoms, which leads to a significant reduction in the number of interaction sites. The resulting simulation system contains, in total, 8194 united atoms.

A 200-ps NVE-MD run was then started from the set-up described above. This was followed by a 400-ps run under NVT conditions. During both runs, the velocities were reassigned every 100 steps in order to hold the temperature at 300 K. After 600 ps of temperature scaling, a NVT-MD run was carried out, which lasted more than 3 ns. However, already within 500 ps, the helical bundle evolved into a coiled-coil structure with roughly four-fold symmetry. The peptides comprising the supercoil tilted on average $27 \pm 5°$ with respect to the interface normal, which is close to the value reported in the recent NMR measurements of Cross et al. [8]. At the same time, the water molecules inside the lumen were expelled and the bundle adopted a compact, essentially dehydrated, structure (see Fig. 2).

Detailed inspection of the compact structure reveals that the helices are linked by hydrogen-bonds at both the N- and C-termini. At the N-terminus the S23 side chains hydrogen-bond to the D24 side chains of adjacent helices, while at the C-terminus the D44 side chains hydrogen-bond to guanidino of R45 on neighboring helices. In this compact structure the middle of the helical bundle is completely closed by the close-packed side chains. Near the N-terminus the lumen is collapsed around V27, where about two turns of the helix are hydrophobic. It is apparently the attractive interactions between these residues that closed off the N-terminus of the channel. In this configuration, the imidazoles of the residues H37 are also inside the bundle. However, since the imidazole is a relatively large group, it acts to block the channel rather than causing a collapse of the bundle.

This compact metastable dehydrated state of the channel

lasted for about 1.5 ns. Then, water molecules gradually returned to the pore and the interior became hydrated. During the fluctuations and conformation changes caused by the rehydration process the supercoil remained intact. The resulting hydrated structure is shown in Fig. 3. Careful examination of this structure reveals that in the hydrated state the H-bond between D44 and R45, near the C-terminus, was still maintained. The long side chains of D44 and R45, along with the fact that R45 has multiple proton donors, make it possible for this part of the channel to open without breaking the H-bonded structure. However, the N-terminus opens with the breaking of the inter-helical H-bond between residues D24 and S23. After the breaking of these H-bonds the bundle opens and water enters this largely hydrophobic region (Fig. 3). During the whole process residues W41 stay in the inter-helical region and do not change much. We conclude that the hydration of residues S23 and D24, which reside at the octane/water interface, is likely crucial for the formation of the observed funnel-like structure at the N-terminal mouth of the M2-DA peptide bundle. This funnel-like structure permits water to enter the hydrophobic region and thereby allows protons access to residue H37 (see Fig. 4). Moreover, the four solvated D24 residues may even serve to attract extracellular protons into the funnel via a mechanism very similar to that proposed recently for the potassium channel selector [23,24].

The present MD simulations suggest that the most stable state of the unprotonated system is the one with water occupying the largely hydrophobic region. However, the free energy difference between the dehydrated (Fig. 2) and hydrated state shown in Fig. 3 is sufficiently small that a seemingly minor change involving solvation of residues D24 and S23 can significantly perturb the system. That a change in H-bonding structure from helix-helix to helix-water can lead to a global conformation change of a peptide bundle has already been noted for LS2 and LS3 [25,26]. Thus, the choice of final structure possibly involves a delicate balance between hydration of the polar residues and inter-helix hydrogen bonding [29]. Given the apparently delicate stability of the structure, in future calculations we plan to explore the effect of using different interaction schemes.

4. Charged system

In order to investigate the previously proposed open state of the M2 channel [16] a fully H37-protonated system was generated from the compact 4-helix bundle shown in Fig. 2. The additional proton charges were introduced onto H37 in four steps with increments of a quarter charge. During each charging step the system was equilibrated for about 300 ps of MD simulation. Thus, the whole charging process took more than 1 ns. Then, an MD trajectory was generated spanning more than 3.0 ns.

The charging of the H37 residues obviously introduces repulsions between the helices. Indeed, the repulsion was sufficiently strong that the helix-helix H-bonds between residues D44 and R45 at the C-terminus become broken, and water enters the bundle. At the same time some of the H-bonds along the peptide backbone also break. However, these backbone H-bonds reformed when the system was equilibrated. With broken helix-helix H-bonds the bundle appears to be unstable. Snapshots from the MD simulation show that the helices now attempt to maximize not only their in-plane but also out-of plane separations, which leads to the helices sliding along each other. Although there was water in the interior, there was no obvious overall structure to the bundle. Thus, it appears that the C-terminus is a key element in the stability of the bundle. This idea is reinforced by our observation that MD simulation on a truncated peptide tetramer, lacking D44 and R45, did not result in a bundle with interior waters.

5. Conclusions

In summary, we have performed MD simulations, each spanning several nanoseconds, on the M2 proton channel consisting of four α-helix DA peptides. We have shown that the four α-helices assemble in the presence of a membrane-like environment to form a left-handed supercoil structure with helix-helix hydrogen bonds at the C-terminus. There is a funnel-like structure at the N-terminus with water occupying the hydrophobic region near the mouth.

Based on our simulation, it is tempting to speculate that there may possibly be two active centers in the bundle; one is the H37 channel blocker proposed previously [16] while the other might be the residues S23 and D24 on the rim of the funnel. These two centers could work independently. H37 is a blocker, which can be activated by excess protons. Residue V27 is at the neck of the funnel. A collapse at the neck will dehydrate the channel, and effectively close it. A possible trigger of this later process could be desolvation of the residues S23 and D24, which are located outside of the TM domain. This possible scenario is also consistent with experimental studies of the inhibition of M2 channel by transition metals, which suggest two independent binding sites: one inside the channel and the other outside [10].

Although the helical bundle with four H37-protonated residues yields a continuous column of water, it does so by breaking the inter-helix hydrogen bonds and translating the helical peptides, a process which results in a highly fluctuational bundle. Given this finding it seems worthwhile to explore other charged states, which may have a less drastic effect on the bundle and still allow a conventional proton conduction mechanism via an open channel defined by a continuous water network [30]. The present MD simulations cannot exclude the proton relay mechanism, proposed by Pinto et al. [7] as one of the likely schemes to explain the proton selectivity and pH-gating of the M2 channel.

Acknowledgements: This work was supported by National Institutes of Health under Grant GM 40712 and by International Business Machines Corporation through a joint study agreement No. 41680055. Q.Z. and T.H. would like to thank the Thomas J. Watson Research Center, IBM Corporation for support and hospitality. The simulation code we employed was developed at the University of Pennsylvania [31] and parallelized using the Message Passing Interface (MPI). The simulations were carried out on SP supercomputers at the Cornell Supercomputing Center, the Laboratory for Research on the Structure of Matter, IBM T.J. Watson Laboratory, and the Maui High Performance Computing Center. We thank Drs. J.D. Lear, L.H. Pinto, W.F. DeGrado, K.A. Sharp, D. Gregg, D. Scharf, K. Tu, and A. Woska for many illuminating discussions.

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