Two possible conducting states of the influenza A virus M2 ion channel

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Abstract Molecular dynamics simulations have been performed on protonated four-helix bundles based on the 25-residue Duff–Ashley transmembrane sequence of the M2 channel of the influenza A virus. Well-equilibrated tetrameric channels, with one, two and four of the H37 residues protonated, were investigated. The protonated peptide bundles were immersed in the octane portion of a phase-separated water/octane system, which provided a membrane-mimetic environment. The simulations suggest that there could be two conducting states of the M2 channel corresponding to tetramers containing one or two protonated histidines. The more open structure of the doubly protonated state suggests it would have the higher conductance.

Key words: M2 proton channel; Four-helix bundle; Molecular dynamics; M2 protein; pH gating; Influenza virus

The native M2 protein of the Influenza A virus plays an essential role in the life cycle of the virus. Early research established that the M2 protein forms a homotetramer [1]. The recent cysteine-scanning mutagenesis experiments of Pinto et al. [2,3] provide a molecular model composed of four α-helices with four fold symmetry. Solid-state nuclear magnetic resonance experiments have shown that the membrane-bound M2 peptides tilt 33° with respect to the bilayer normal [4]. Activation of the channel on lowering pH has been attributed to the protonation of the H37 residue inside the channel [5], since the measured pK_a value is 5.77, very close to the pK_a of the imidazole. The Hill coefficient is 0.96, suggesting that the protonation of the channel is a non-cooperative process. Recent work of Gandhi et al. [6] also shows that transition metals, such as Cu^{2+} and Zn^{2+}, can bind to various parts of the protein, thereby providing a useful probe for future studies. The drug amantadine is believed to act via the disfunctioning of the M2 channel [7,8].

There is considerable interest in determining the origin of the observed pH-dependence of M2's conductance. Experimentally, M2 currents increase as the pH is lowered from around 7 to 5, and then begin to level off as the pH is further lowered to ∼4.0, the practical limit for experiments in oocytes [5,9]. Over this limited range, data have been fit [9] to a simple model which assumes conductance depends only on the concentration of a protonated form of M2, and that the concentration of this state depends on the external medium proton concentration. Because the pH (∼5.8) at the apparent inflection point of the fitted pH-dependent conductance occurs near the pK_a of histidine (6.2), it has been suggested [2,5,9] that protonation of His-37 in M2 is the 'activation' step in conductance.

Although the importance of this histidine in M2's proton conductance has been firmly established by mutagenesis studies (e.g. [2,5,6]), the mechanistic details remain unknown. For example, Sansom et al., using molecular dynamics (MD) simulation [10,11], proposed that when all four histidines in the homotetrameric bundle are protonated, the channel lumen becomes sufficiently hydrated to conduct. Subsequently [13,14], we showed using un-restrained MD simulations that the tetra-protonated state was unstable. This finding suggests that not all of the histidines can and/or need to be protonated in order for the channel to conduct. Additionally, the experimental data showing the dependence of M2 conductance on pH show that saturating levels of conductance were not achieved at pH 4. There is, therefore, a possibility that the M2 channel could have other conductance states, possibly less proton selective [12], at lower pH. Because the histidine residues of the M2 transmembrane segment are likely to be in close proximity in the tetramer, significant negative cooperativity (decreasing pK_a values) is expected in their successive protonations. It is of interest, therefore, to examine models of the M2 tetramer, which have different levels of protonation of the transmembrane histidines.

In this work, we have performed a MD simulation of the M2 proton channel without applied constraints in the presence of a membrane-mimetic environment. The simulation strategies and procedures have been used in a series of simulations of similar systems, including LS2, LS3, Vpu and M2, and have proved to be successful [13,15–17].

The paper is organized as follows. First, we present a brief outline of the simulation procedure and the preparation of the system. Further details of the simulation may be found in earlier publications. This section is followed by the presentation of our simulation on the protonated system and its stability. Next, we will discuss and characterize the observed two states. Finally, we propose a molecular model involving two possible conducting states; one state with a single protonated H37, and the other with two protonated H37's.

In order to get optimal performance in both system size and time scale, we have employed a MD program based on reversible integrators, combined with the multiple time step method (RESPA) [18]. The long range contribution of van der Waals and electrostatic interactions, which compose the interatomic forces, can be treated accurately and efficiently by RESPA using Ewald summation. The simulation was carried out at nominal room temperature, 300 K, with the temperature controlled by a Nosé–Hoover chain thermostat [19]. In the part

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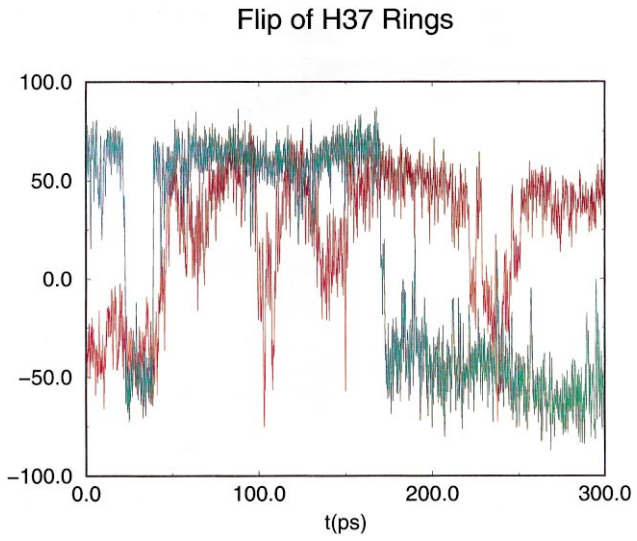

Fig. 1. Time evolution of the orientation of two of the four different H37 pyrrole rings, which block the M2 channel in neutral system. θ is defined as the angle between channel axis (z-direction) and the normal to the plane of the ring.
←

of the trajectory where the averaged properties are calculated, no constraints were applied to the system.

The approach based on synthesizing only the transmembrane domain of proteins has been shown to be very successful in characterizing ion channels and providing insight to understand protein function [20–22]. All of the present MD calculations were carried out on the sequence used by Duff and Ashley in their experiments [7]. It consists of a 25 amino acid sequence: SSDPLVVAASIIGILHLILWILDRL. The transmembrane domain is considered to begin from residue S22 and end with residue L43 [23].

The simulation was set up with ideal α-helices embedded in the octane portion of a octane/water phase separated box. A

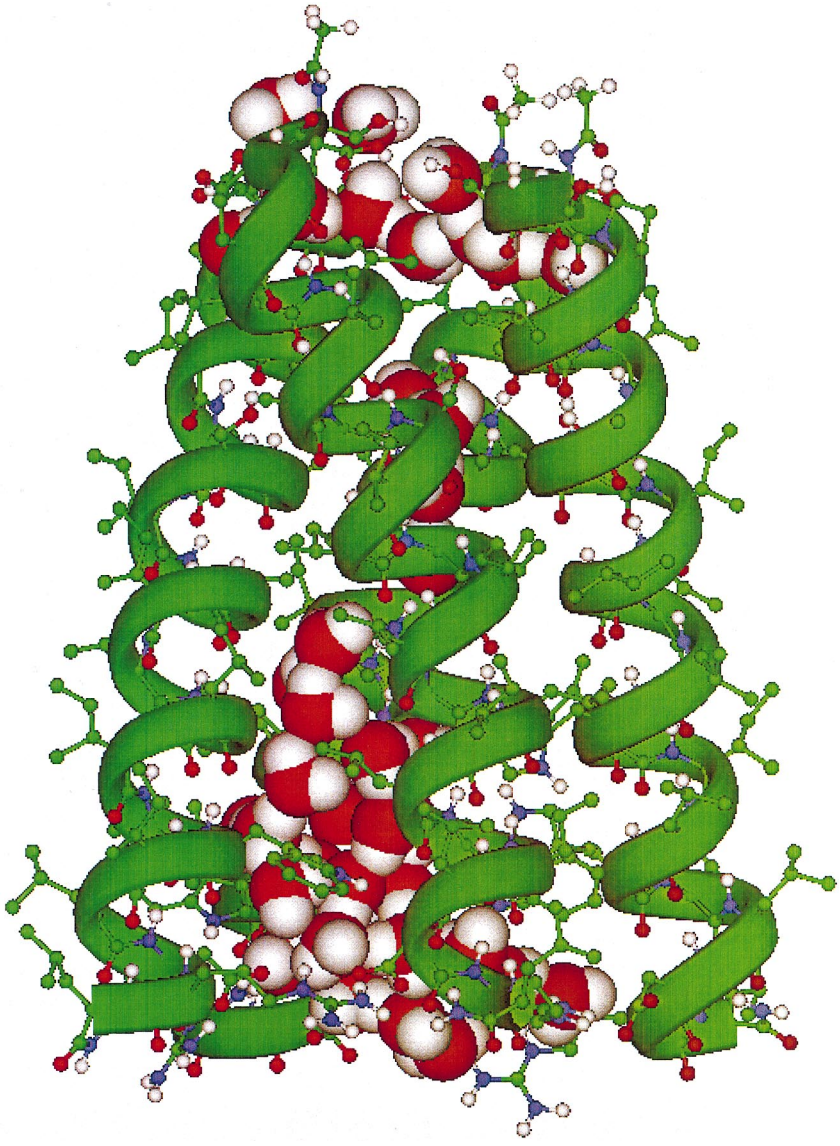

Fig. 2. Instantaneous configuration of the four-helix M2 peptide bundle, with singly protonated H37. The H_2O molecules are drawn with van der Waals radii. The carbonyl oxygens are drawn in red, the amide nitrogen and hydrogens in blue and gray, respectively. All other atoms are omitted for clarity.

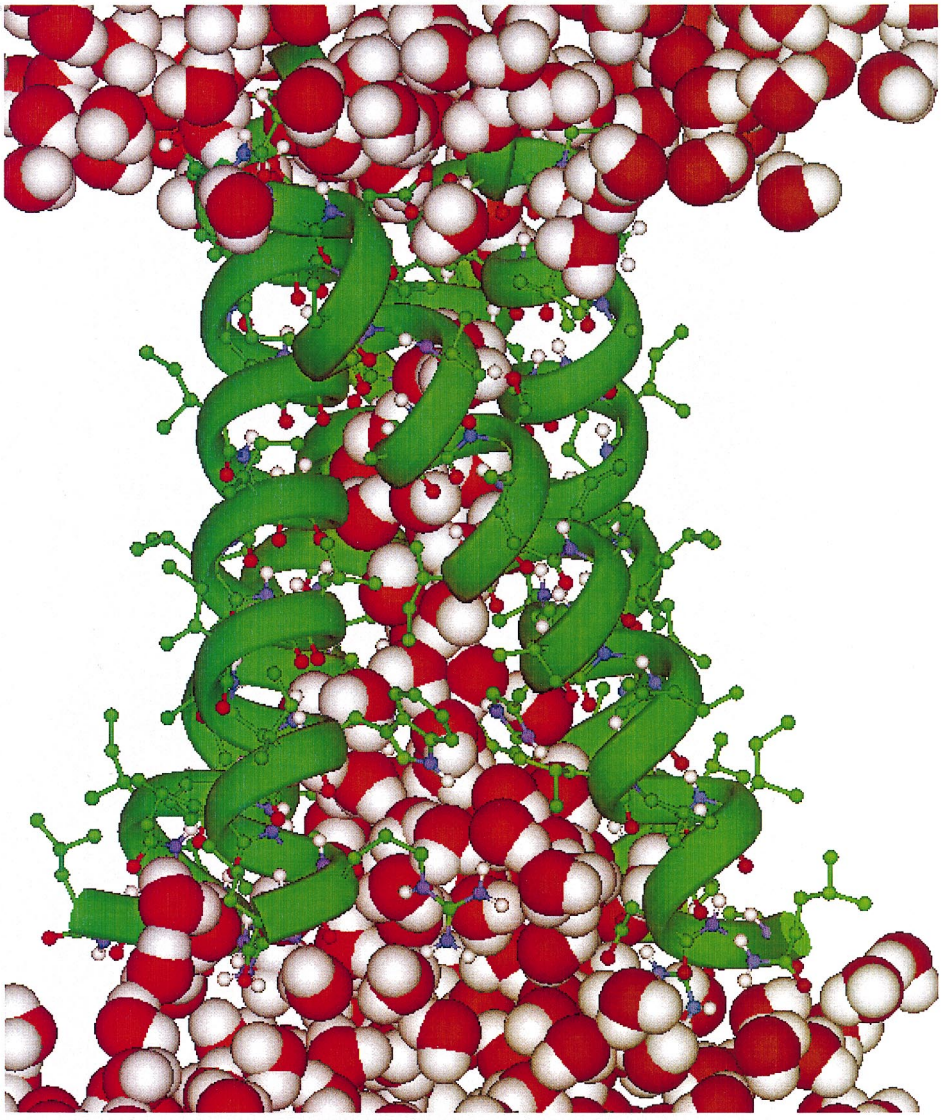

Fig. 3. Instantaneous configuration of the M2 protein with a pair of opposite H37 residues protonated. Notation as Fig. 2.

nanosecond simulation has been performed in order to reach the equilibrium structure, which was reported in our earlier paper [13].

For the channel having no charged histidine resides, simulation [13] shows that the H37 residues play a channel-blocking role. Two H37 residues from diagonally opposite peptide chains are positioned with their rings facing each other and block the channel completely. The other two H37 residues are stacked above these two. Because this structure has no discernible diffusion pathway for ionic conduction, it would conventionally be considered a 'closed' ion channel state. An important observation is that even though all the H37 residues have their imidazole amide N–H groups pointed predominantly toward the C-termini of the channel, and the H37 residues are closely compacted, their rings can still flip (see Fig. 1). The motion and flip of the H37 rings do not appear to be highly correlated, i.e. each of them moves independently.

In order to probe possible open states and the gating mechanism of the channel, various charged systems were generated from the compact structure of the four-helix bundle at about 1.0 ns. Three different charged states were considered, namely, with either one, two or all four of the H37 residues carrying a unit positive charge. Starting from the equilibrated neutral structure, the charges were introduced adiabatically. Each charge was introduced in four steps with increments of a quarter charge. During each charging step, the system was equilibrated with about 300 ps of MD simulation. Thus, the whole charging process took more than 1 ns. Then, a trajectory was followed for more than 3.0 ns for each of the charged systems.

Like the neutral system, both the singly (Fig. 2) and doubly charged (Fig. 3) systems maintain a stable structure, although the previously studied, fully protonated system [13] does not. The secondary structure of the α-helices is also maintained.

In the singly protonated system, the bundle shows a left-handed coiled-coil with a tilt angle of about 28° between each helix and the central pore, which is close to the experimental data of Kovacs et al. [4] and, more recently, Kukol et al. [24].

In our simulation of the singly protonated system, the four H37 residues remain close-packed so that there is no continuous H_2O network (Fig. 2), although the water molecules can

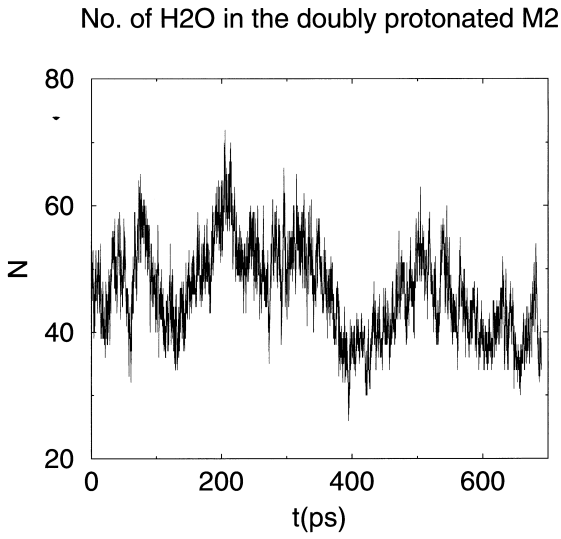

Fig. 4. Time evolution of the number of water molecules in the pore region of the doubly protonated M2 system. The time origin begins after 3 ns of simulation.

get into the channel as in the neutral structure [13]. Although without quantum simulation we cannot conclude that the singly protonated channel is a conducting channel, we find that the proton-relay mechanism, proposed by Pinto et al. [2], is consistent with our classical simulation. First of all, with continuous H_2O liquid penetrating far inside the channel, a proton can easily find its way up to the H37 blocker. Although the amide hydrogen of imidazole predominantly points to C-termini, the fact that the H37 ring can flip (Fig. 1) suggests that the energy difference and barrier between the two hydrogen orientations is small. When the pH drops at the N-terminus, the difference in proton density will drive proton transport via the flip process [2] so that the proton can be transferred from N-terminus to C-terminus. For pH values where the histidine is always protonated (proton access to the histidines is not rate-limiting), the transport process would be limited by the maximum flip rate of the H37 rings (Fig. 1).

The secondary structure and the helical bundle of the doubly charged system were also found to be stable. We have calculated the inertia tensor of the peptide bundle, which shows that the bundle fluctuates around the equilibrium position without any sign of dissociation or collapse. The number of water molecules present in the channel pore fluctuates stably around an average value of $\sim$46 (Fig. 4), which shows that the whole structure is stable without large fluctuations. However, due to the repulsion of the charged H37 groups, one of the charged imidazole H37 rings rotates towards the N-terminus, which effectively opens the channel and sets up a complete H_2O network (see Fig. 3). This 'open' structure contains significantly more water than the singly protonated structure and, since the water forms continuous channel through the lumen, it is conceivable that water and possibly even small ions could permeate and diffuse through the structure in a conventional manner.

In summary, we have performed several MD simulations, each spanning more than 3 ns, on a M2 proton channel consisting of four α-helix peptide bundles. The necessary hydrophobic/hydrophilic environment has been taken into account so that the channel can be followed dynamically during the simulation. We have shown that the four α-helices assembled in the presence of this membrane-like environment form a close-packed structure. Both single and double protonation maintained the compact bundle structure, though full protonation did not. The simulation suggests that both singly and doubly protonated states should be included in considerations of M2 proton conduction mechanisms. Proposed schemes [3] can be tested both theoretically, via quantum mechanical simulations [25], and experimentally by current–voltage measurements over a wide pH range, preferably in bilayer-reconstituted systems where pH gradients can be closely controlled and extended beyond normal physiological ranges.

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