Molecular dynamics study of the LS3 voltage-gated ion channel

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Received 19 February 1998

Abstract Molecular dynamics calculations have been carried out on a model of the LS3 synthetic ion channel in a membrane mimetic environment. In the absence of an external electrostatic field, the LS3 channel, which consists of a bundle of six α-helices with sequence Ac-(LSSLLSL)$_3$-CONH$_2$, exhibits large structural fluctuations. However, in the presence of the field, the bundle adopts a well defined coiled-coil structure with an inner pore of water. The observed structural changes induced by the applied field are consistent with the proposed gating mechanism of the ion channel.

Key words: Ion channel; Self-assembly; Helical bundle α-helix; Electrostatic field; Molecular dynamics

Ion channels are ubiquitous. The typical ion channel is a transmembrane protein consisting of an α-helix bundle with axis normal to the lipid membrane. Under a channel-specific gating stimulus (voltage, pH, etc.), ions are transported across the membrane via the channel pore. The structure, gating mechanisms, and other properties of ion channels are poorly understood at the molecular level, presenting a major challenge.

The idea of using designed transmembrane peptide sequences in order to mimic an ion channel and thereby understand the principles governing its structure and function was pioneered by Montal [1–3] and DeGrado [4–8] in the late 1980s. In this article, we focus on the work of DeGrado et al. [4], in which the synthesized peptides contained simple repetitive sequences that retained only the basic structural elements thought to be important in natural channel forming proteins. This 'minimalist' approach, which depends on the ability of α-helical peptides to self-assemble into a functioning channel, provides model systems on which atomistic molecular dynamics (MD) calculation can be carried out to complement the laboratory experiments.

This letter reports MD results on one of these designed synthetic ion-channels. Specifically, we focus on the LS3 system, which is composed of the α-helical peptide Ac-(LSSLLSL)$_3$-CONH$_2$ (where L = leucine and S = serine). Conductivity data suggest that in a membrane this peptide functions as a channel and is probably a hexameric aggregate [4–8]. Based on fluorescence studies, it was proposed [5] that in the major non-conducting state the channel peptides are oriented parallel to the interface of the lipid bilayer, close to the lipid head group region. Reorientation of the peptides is thought to occur upon application of a voltage gradient to the membrane, and this is believed to constitute the voltage-gating mechanism.

MD simulation is an ideal tool to study ion channels [9–14]. Recent methodological developments have been aimed at larger systems and longer time scales. Efficient Ewald summation of the electrostatic interactions and multiple-time-step algorithms are two of the important developments that have enabled us to characterize the LS3 synthetic ion channel. Although experiments are carried out in the presence of an external electrostatic field, to date, MD simulations on biophysical systems have not considered this effect [11]. Anticipating our results we show that the external field is crucial for the formation of a stable channel and that LS3 indeed aggregates to a hexameric bundle (see Fig. 1).

The setup procedure and the simulation methods we employ have been described in detail elsewhere [15]. For completeness, we give only the salient facts. We used INSIGHT (BIOSYM, San Diego, CA) to set up our systems, starting with the six ideal α-helices arranged as parallel rods with hexagonal symmetry. The bundle was then minimized and equilibrated with backbone atoms fixed using an optimized version of the CHARMM program. The bundle was then immersed in a pre-equilibrated box of octane (C_8H_{18}), cut to the same height as the bundle. The octane slab provides the membrane mimetic hydrophobic environment. Layers of water (H_2O) were added to the two ends of the helices (see Fig. 1). The composite system was then equilibrated with fixed helices by carrying out a NVE MD run on the H_2O and C_8H_{18} to eliminate the possible tension between the different components. The whole system was equilibrated for about 200 ps with the velocities reassigned every 0.1 ps.

In order to get optimal performance in both system size and time scale, we use the technique of explicit reversible integrators combined with a multiple time step scheme [16]. The van der Waals and electrostatic interactions are each divided into short and long range parts. The short range cut-off is 7.0 Å for electrostatic interactions and 2.0 σ, where σ is the usual Lennard-Jones parameter, for van der Waals interactions. The bonding interactions, including stretching, bending and dihedral angles (both proper and improper), are calculated every 0.3 fs. The short range interactions have to be calculated every 1.5 fs, however they only contain a small portion of the non-bonding interactions. The major part of the non-bonding interaction is the long range part, which is calculated every 3 fs.

Periodic boundary conditions are introduced with a cut-off 10 Å for the electrostatic interactions and 2.5 σ for the van der Waals interactions. Beyond that, the van der Waals interaction is taken into account, by a standard long range correction, on an averaged basis [17,18]. The Ewald method is used to take into account the long range contribution from the electrostatic interaction [17,18]. We use 10 Å^{-1} as the cut-

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PACS Classification codes: 31.15.Qg; 87.15.By; 87.15.He

PII S0014-5793(98)00304-4

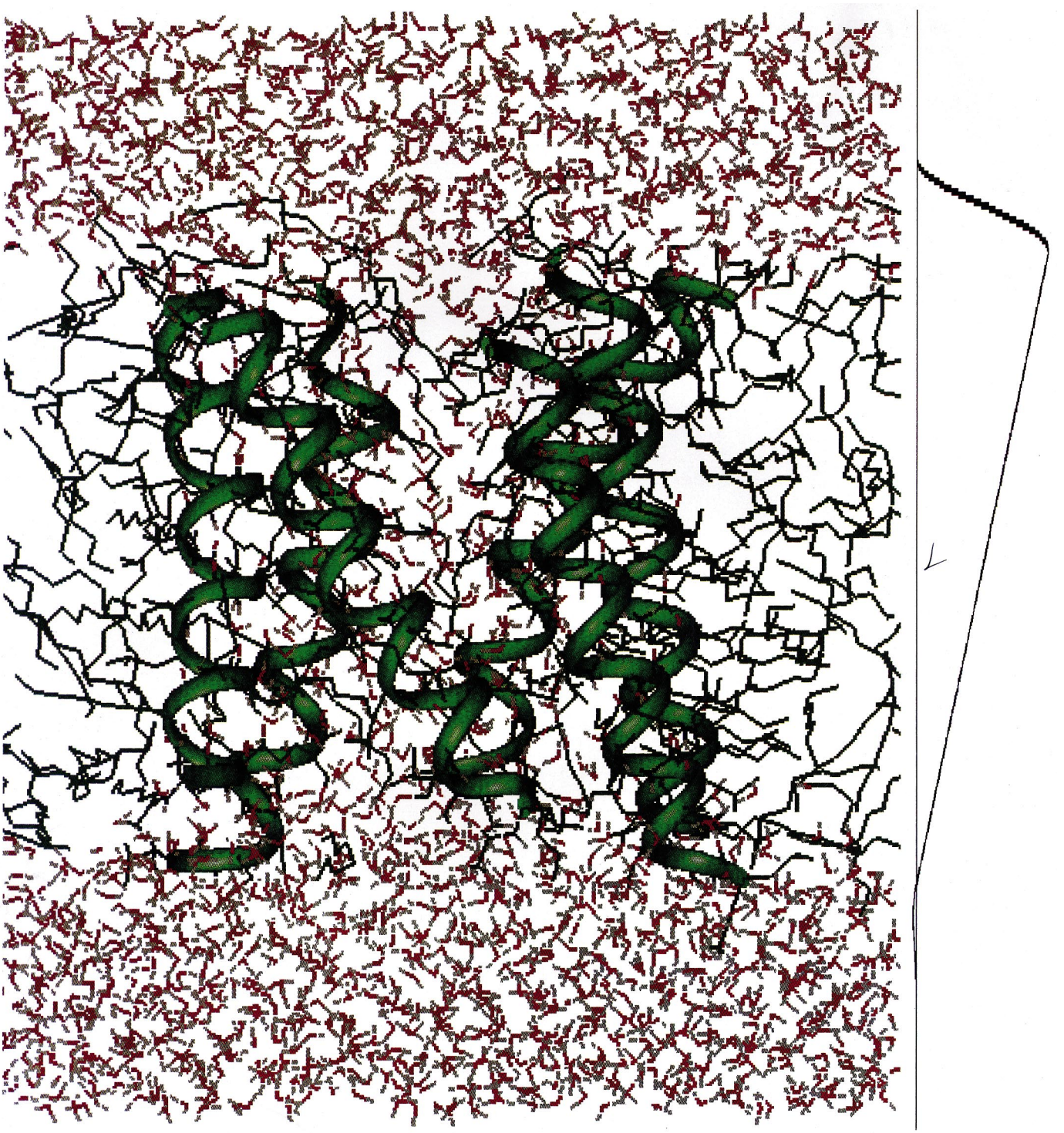

Fig. 1. An instantaneous configuration of the octane–water–peptide simulation system after 500 ps. The six helices, shown in a ribbon representation, have the coiled-coil hexamer arrangement, which has roughly three-fold symmetry (see text). The N-terminus is at the bottom. The trace at the right hand side is the field applied over the membrane region (32 Å) during the simulation. The maximum amplitude of the field is 200 mV.

off in *k*-space and the calculation is converged at $\alpha = 0.3$ Å^{-1}, where α is the weight of the Gaussian damping factor. We use a Nosé-Hoover chain of length 3 to control the temperature and the frequency of the chain is chosen as 2 ps^{-1}. The resulting equations of motion give continuous dynamics that generates a canonical distribution [19,20].

The well-known TIP3P model [21] is used for both the bulk and pore H_2O. However, since we are using the multiple time step integrator, we do not introduce constraints for the O–H bond length. The parameters used for C_8H_{18} are for a fully flexible molecule, which consists of methyl and methylene groups [22]. The topology and parameter set for the peptide

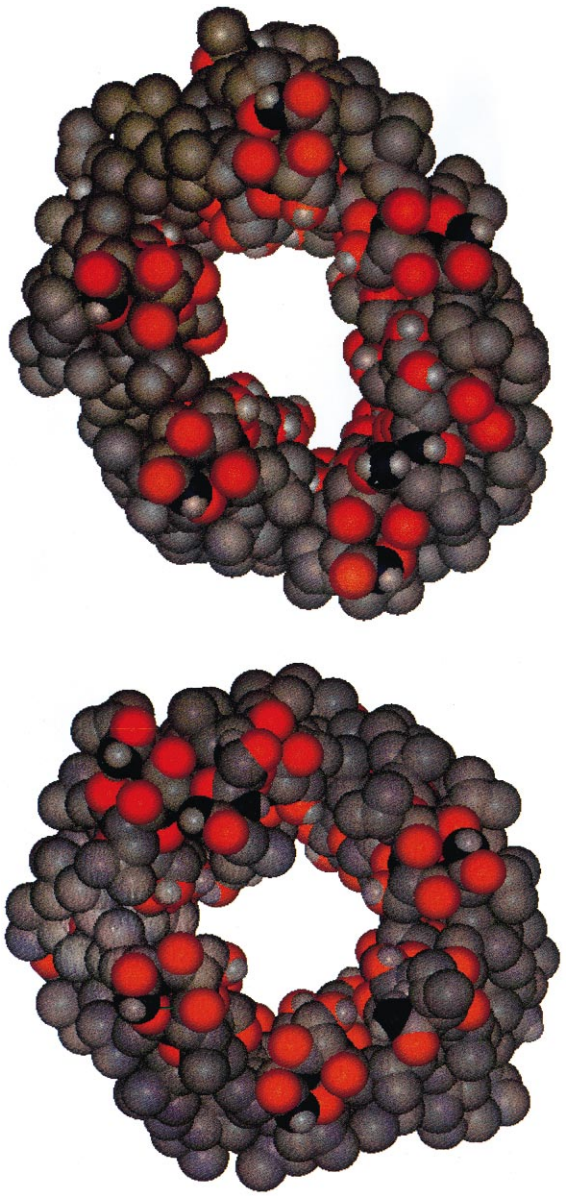

Fig. 2. Instantaneous configurations of the LS3 ion channel. Top: Under equilibrium conditions, the six α-helices form into three associated dimers. Although the dimers themselves are robust, they exhibit a tendency to translate along the pore direction. Bottom: The six α-helix bundle in the presence of an electrostatic field adopts a left-handed coiled-coil structure and the stability of the bundle has changed dramatically. The atoms of the pore structure are drawn with van der Waals radii and have the color code: red (oxygen), black (carbon), blue (nitrogen) and white (hydrogen). Water and octane are omitted for clarity.

are based on the CHARMM parameter set (CHARMM 19) [23], which is consistent with the molecular model used for C_8H_{18}. All hydrogens, except the polar hydrogen and the amide hydrogen, are absorbed in heavy atoms so that the simulation can be carried out more efficiently.

The simulations were all performed at room temperature, 300 K, and the equilibrium properties reported below were calculated from the MD trajectory with no constraints on the system. The MD code was developed in house [24] and parallelized using the Message Passing Interface (MPI).

One of the main issues is the possible geometry of the channel resulting from the stereochemistry of helix-helix interactions. Accordingly, three different initial configurations of LS3 were simulated, each for over 1 ns.

During the first 500 ps of MD simulation without the field applied, the bundle loses its initial hexagonal symmetry and the six α-helical peptides form three hydrogen-bonded dimers. In this configuration, there is a strong tendency towards dissociation; the dimers slide along each other, which contributes to the instability of the bundle. An instantaneous configuration after about 1 ns is shown in Fig. 2.

In order to eliminate the possibility that the apparent instability was caused by a poor starting configuration, an initial coiled-coil configuration, with a twist angle of 10°, was simulated in exactly the same way (we thank Dr. G. Dieckmann for providing this twisted initial configuration of the hexamer bundle). Essentially, the same behavior was observed after about 1 ns. The MD results strongly suggest that the hexamer is not stable under the normal simulation conditions.

Although the hexamer does not appear to form a stable bundle in the simulation, robust hydrogen-bonded peptide dimers are found and persist as a structural motif. The dimer consists of two α-helices shifted with respect to each other by about 0.75 of a turn, with a twist angle of about 7°. In this way, the leucine hydrophobic side chains of the neighboring helices pack into a 'knob-to-hole' pattern and the serine hydrophilic side chains form interhelix hydrogen bonds, which likely accounts for the stability of the dimers.

Since the MD simulations suggest that the equilibrium bundle is only marginally stable, an electrostatic field was introduced and a 1 ns simulation was carried out. In order to incorporate a linear electrostatic field across the model membrane into the periodic boundary conditions some care is needed. In particular, the field has to be smoothed so that the derivatives of the forces are continuous everywhere in the system (see Fig. 1).

In the presence of the field, the MD simulation shows that the helices adopt a coiled-coil structure [25]. The bundle, which consists of three dimers, has roughly three-fold symmetry. The tendency of dissociation along the channel axis is inhibited by the presence of the external field (see Fig. 2). Although we cannot simulate the actual process of gating

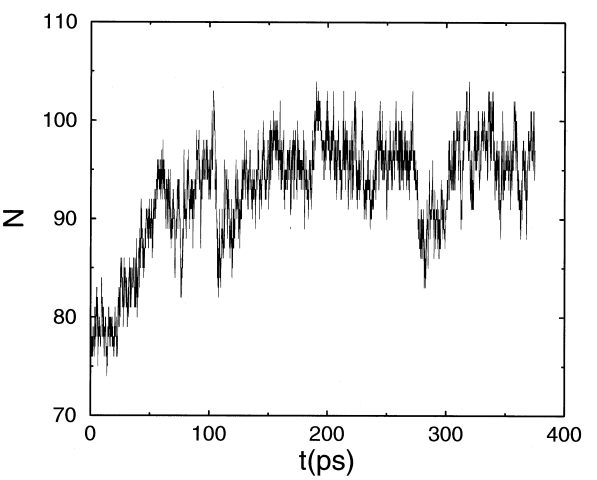

Fig. 3. The number of pore H_2O molecules, N, defined as those whose oxygen atoms lie within ±7.5 Å of the center of mass of the bundle as a function of time.

with the available techniques and resources, the observed change of stability caused by the field is consistent with the idea of a voltage-gated function. The helical bundle is coiled-coil with the an angle of 10° with respect to the H_2O–C_8H_{18} interface. The axis of each α-helix is inclined towards the pore by an angle of about 20°. Calculations of the dihedral angles φ and ψ show that the peptides remain predominantly α-helical, despite the thermal fluctuations.

Analysis of the structure shows that the helical bundle supports a pore with an average inner radius of about 5 Å, which is about 0.5 Å larger at the N-terminus (see Fig. 2). The number of H_2O in the pore is quite stable at 95 ± 10 (see Fig. 3). The H_2O molecules have an averaged diffusion constant of only about one-third of the bulk value. The relaxation time of the H_2O molecular dipole, which may be measured experimentally, has two components: one is about two-thirds of the bulk H_2O value, and the other one is about 10 times longer.

In conclusion, we have performed MD simulations, each spanning more than 1 ns, on the LS3 synthetic ion channel. The hydrophobic/hydrophilic environment has been taken into account and the channel is allowed to form spontaneously during the simulation without any artificial structural constraints. We have shown that the six α-helices self-assemble in the presence of a membrane-like environment to form a close packed pore structure, whose stability is changed dramatically only when an electrostatic field is introduced. In the presence of the field, the three α-helical dimers spontaneously adopt a left-handed coiled-coil structure. We have also investigated bundles of seven and five α-helices. The heptamer appears to be very unstable whereas the pentamer forms a very compact bundle with a very small pore. The enhanced stability in the field is consistent with the proposed voltage-gated mechanism. It remains to be determined whether or not the present detailed structural predictions are robust to changes in the force field used in the MD simulations. Nevertheless, the importance of the external field accords well with current experimental data [8].

Acknowledgements: This work was supported by the National Institutes of Health under Grant GM 40712 and by International Business Machines Corporation under Joint Study Agreement 41680055. Q.F.Z. would like to thank the Thomas J. Watson Research Center, IBM Corporation for support and hospitality. We thank the Maui High Performance Computing Center for generous use of their computational facilities. We thank Drs. D.F. DeGrado, T. Husslein, J.D. Lear, P.C. Pattnaik, D. Scharf, D.J. Tobias, K. Tu, and A. Woska for many illuminating discussions.

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