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Energetics of Ion Transport in a Peptide Nanotube

François Dehez, Mounir Tarek, and Christophe Chipot*

Equipe de Dynamique des Assemblages Membranaires, Unité Mixte de Recherche CNRS/UHP 7565, Nancy Université, BP 239, 54506 Vandœuvre-lès-Nancy Cedex, France

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Ion channels constitute an important family of integral membrane proteins responsible for the regulation of ion transport across the cell membrane. Yet, the underlying energetics of the permeation events and how the latter are modulated by the environment, specifically near the mouth of the pore, remain only partially characterized. Here, a synthetic membrane channel formed by cyclic peptides of alternated D- and L-hydrophobic α-amino acids was considered. The free energy delineating the translocation of a sodium ion was measured along the conduction pathway by means of molecular dynamics simulations. The free-energy profiles that underly the permeation of the open-ended tubular structure are shown to not only depend on the characteristics of the latter but also inherently on the location of the mouth of the synthetic channel with respect to the membrane surface.

Nature has tailored a variety of ion channels that transport different ions at different rates across the cell membrane.[1] Although the three-dimensional structure of the internal pathway is central to the conduction properties of the channel, a number of other features are also at play. Among these features, the location of the mouth of the pore with respect to the membrane surface is also envisioned to modulate the conduction rates.[2] In essence, two scenarios can be considered, whereby the mouth of the ion channel lies in the water-membrane interfacial environment, for example, gramicidin A,[3] or sufficiently far from it, as instantiated in ligand-gated channels like the nicotinic acetylcholine receptor (nAChR).[4] Modulation of ion permeation by the local dipole of the lipids has been shown for prototypical channels based on Poisson−Nernst−Planck (PNP) theory.[5−7] Owing to their simple three-dimensional structure, peptide nanotubes provide a convenient framework for investigating permeation events. Synthetic channels resulting from the stacking of hydrophobic cyclic peptides of alternated D-L-α-amino acids,[8] which self-assemble through a network of intermolecular hydrogen bonds, exhibit a marked propensity to insert into the lipid bilayer as individual transmembrane nanotubes.[9,10] The latter have been shown to conduct ions;[11] albeit the underlying atomic-level mechanism and energetics of ion transport are still only partially characterized. Permeation events in these channels, however, can be captured at the theoretical level, employing free-energy methods that artificially accelerate ion translocation by means of externally applied biases.[3,12−14] Here, we quantify using all-atom molecular dynamics simulations the effect of the membrane environment on the free energy of ion translocation through a peptide nanotube and demonstrate that transport phenomena in membrane channels are strongly modulated by the local dielectric surroundings at the mouth of the pore.

The free energy delineating the translocation of a sodium ion was measured along the conduction pathway of a tubular structure formed by eight stacked cyclo-(LW)$_4$ peptide units, where underlined letters denote D-α-amino acids. The diameter of the pore is approximately 6 Å. Ion transport was examined both in a fully hydrated palmitoyloleylphosphatidylcholine (POPC) bilayer and in a bulk aqueous medium that reflects the situation where the mouth of the pore is located sufficiently far from the surface of the membrane, as instantiated in ligand-gated channels like the nicotinic acetylcholine receptor (nAChR).[4] To preserve the structure of the nanotube in bulk water, which can be viewed as a proof-of-concept model, the separation between adjacent cyclic peptides was restrained by means of soft harmonic potentials.[15] The reaction coordinate (RC), ξ, was chosen as the distance separating the ion from the center of mass of the hollow cylindrical structure, projected onto its longitudinal axis. Variation of the free energy, $\Delta G(\xi)$, along that direction was determined using the ABF method,[16] which relies upon the integration of the average force acting on the RC, obtained from unconstrained molecular dynamics simulations[17] carried out with the Namd program[18] and the Charmm27 force field.[19] Owing to the reduced size of the pore formed by the cyclic peptides, no axial−radial deconvolution of the free-energy change was performed;[13] the force was averaged radially in the plane defined at a given value of ξ. The pathway connecting the two ends of the synthetic channel, viz., approximately $-20 \leq \xi \leq +20$ Å, was divided into four nonoverlapping windows. Instantaneous values of the force were accrued in bins 0.1 Å wide.[17] The total simulation time in bulk water was purposely extended to 90 and 185 ns in the membrane model to guarantee that the free-energy profiles obtained in the two environments essentially coincide with those derived from the antisymmetrized average force across the reaction path. Quasi nonergodicity scenarios prone to occur in multistage approaches[20] were probed by running additional ABF simulations using a single, large window embracing the entire RC.

The energetics of ion transport in the synthetic channel immersed in a bulk water medium and in a hydrated POPC

* To whom correspondence should be addressed. E-mail: Christophe.Chipot@edam.uhp-nancy.fr.

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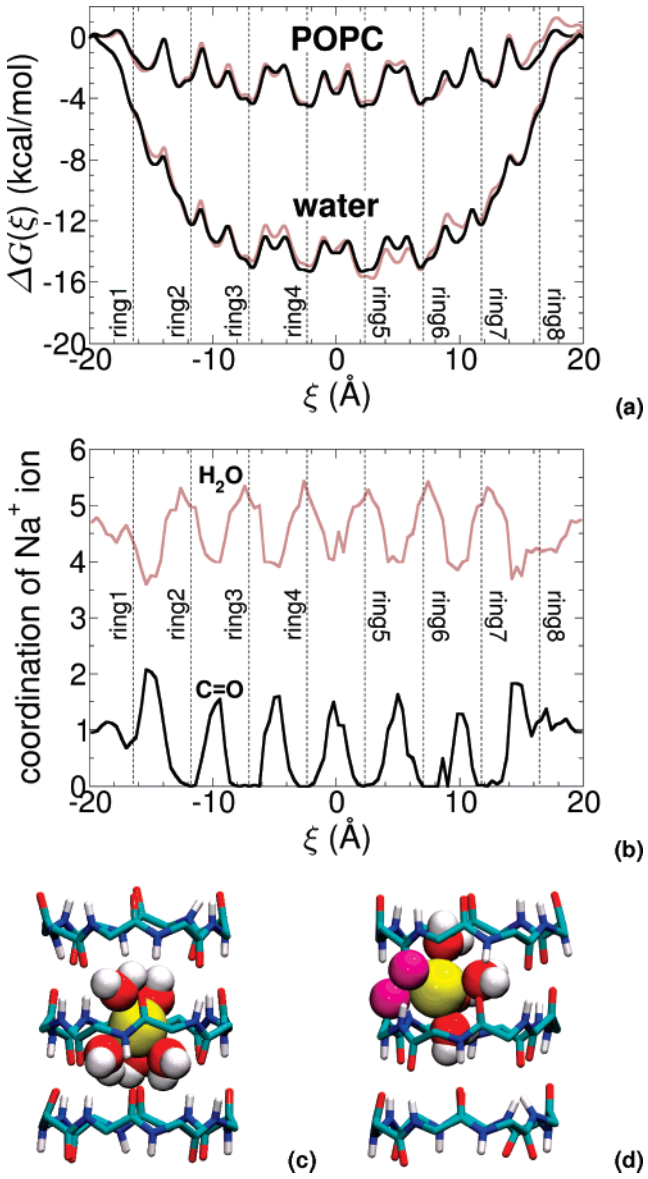

Figure 1. Free-energy profile delineating the transport of a sodium ion across a synthetic channel formed by eight cyclo-(*L*W)$_4$ units, in bulk water and in a hydrated POPC bilayer (a). Comparison of the profiles obtained from the antisymmetrized average force (dark lines) and from the average force measured across the entire conduction pathway (light lines). Number of oxygen atoms pertaining to water molecules and carbonyl moieties chelating the cation (b). Essentially identical results are obtained in the two environments. Coordination patterns of the sodium ion in the in- or α-plane (c) and in the mid-plane (d) of the cyclic peptides. The oxygen atoms of the carbonyl moieties are depicted as purple spheres. Image rendering was done with *VMD*.[21]

bilayer is depicted in Figure 1. Irrespective of the surroundings, the free-energy landscape within the tubular structure, that is, between its second and its seventh ring (i.e., approximately $-12 \leq \xi \leq +12$ Å) exhibits a pronounced roughness, reflecting the geometry of the cavity. The sharp local minima of the free-energy surface separated by ca. 4.7 Å, namely, the experimentally determined distance between the consecutive rings that form the synthetic channel,[9] correspond to in- or α-plane water chelation of the sodium ion. Ion conduction proceeds through hopping over adjacent, ca. 2 kcal/mol-high free-energy barriers representing the penalty for the transient dehydration of the cation. As may be noted in Figure 1, markedly weaker minima of the free-energy surface indicate that binding can also occur when the sodium ion is located equidistantly from two contiguous cyclic peptides. The roughness of the free-energy profiles is consistent with the coordination of the ion. As the latter diffuses along the RC, the number of chelating oxygen atoms pertaining to either carbonyl moieties or water molecules averages to ca. five, both in the in-plane and in the mid-plane of the cyclic peptides, the water molecules being replaced intermittently by carbonyl groups—analogous in spirit to what evolution has devised for overcoming dehydration barriers in more-complex ion channels, like potassium channels.[22−24]

In a similar synthetic channel immersed in water, Asthagiri and Bashford observed that the hydration number of the sodium ion was equal to four,[15] which may be the consequence of imposing harmonic restraints to their tubular structure. In both instances, however, lower hydration of the sodium ion, compared to that measured with the present force field in a bulk aqueous environment and found to be equal to ca. six, is due to the confinement in the synthetic channel. One of the consequences of this spatial restriction is the reduced mobility of the cation, which is rate-limited by the surrounding water molecules, thereby baring some analogy with the gramicidin A channel.[3] Removal of the confinement in wider peptide nanotubes is expected to yield somewhat smoother free-energy landscapes. A similar effect can be witnessed in carbon nanotubes on account of the uniform chemical nature of the tubular structure.[25]

The most-striking difference between the free-energy curves of Figure 1 is found at the thresholds of the peptide nanotube. The profile determined in the aqueous medium can be viewed as the superimposition of the rugged landscape characterizing the interior of the synthetic channel, and a ca. 12 kcal/mol deep, quasi-harmonic well that prevents the sodium ion from leaving the open-ended tubular structure. A similar trend has been observed recently in the case of a nanotube formed by only four stacked cyclo-(AEAQ)$_2$ peptide units, immersed in water.[13] Yet, the noticeably smaller well depth can be ascribed to edge effects, which evidently play a significant role on the overall shape of the free-energy profile. In the present calculations, use of a longer channel allows us to observe a plateau in Figure 1 revealing the spatial extent of such edge effects, viz., approximately 10 Å.

To rationalize the marked difference observed when the mouth of the pore is located either near or far from the membrane surface, the electrostatic potential has been mapped in the three dimensions of Cartesian space.[26] Figure 2 illuminates how this quantity varies dramatically as a function of the environment. In liquid water, the concentric isopotential surfaces are oblate and span the entire nanotube; the difference in electrostatic potential between the midst of the hollow structure and the bulk medium amounting to ca. 500 mV. The electric field has been derived from the three-dimensional maps of the electrostatic potential. It is oriented collinear to the longitudinal axis of the synthetic channel, pointing toward the center of the latter; that is, $\xi = 0$. As a result, the sodium ion is thrusted inside the cavity, where it must then overcome an appreciable free-energy penalty to exit the confined environment and return to the bulk.[27] In sharp contrast, the isopotential surfaces of the peptide nanotube immersed in a membrane model form a funnel-like passage between the two planes of the lipid bilayer so that the cation experiences no measurable electrostatic barrier and, hence, no net electric force.

The effect of the electrostatic potential on the permeation of sodium ions has been quantified further by estimating the reversible work due to the electric field acting at the thresholds of the hydrated synthetic channel. For instance, over a distance

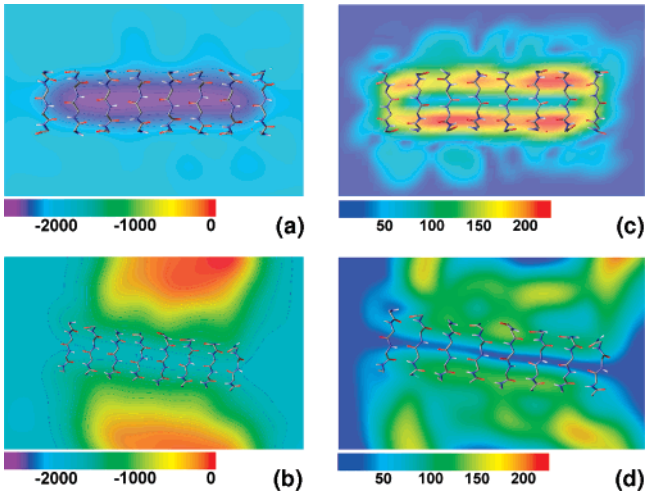

Figure 2. Electrostatic potential contour maps[26] generated in the aqueous medium (a) and in the model membrane (b). Units given in mV. Cross-sectional view of the three-dimensional map of the electric field determined when the synthetic channel is immersed in an aqueous medium (c) and in a model membrane (d). Units are given in mV/Å.

of 2.5 Å above and below the first or the last cyclic peptide, the electric field is equal to ca. 70 mV/Å. It follows that the extra energetic cost incurred by the cation in this region amounts to ca. 8 kcal/mol, which essentially coincides with the free-energy difference measured by the ABF method (see Figure 1). Over a distance spanning the two first cyclic peptides, this energetic penalty increases to ca. 12 kcal/mol, which is in line with the electrostatic component of the transfer free energy of a sodium ion from water to the interior of the peptide nanotube examined by Asthagiri and Bashford.[15]

The results reported here support at the atomic level the contentions inferred from computations relying on the PNP theory.[7] Our free-energy calculations demonstrate that permeation of open-ended tubular structures does not only depend on the characteristics of the latter but also inherently on the nature of the surroundings, more specifically near the mouth of the pore. In particular, a cation entering sufficiently far from the surface of the membrane a synthetic channel is rapidly shoved inside the hollow tubular cavity, where a net electric force creates an overwhelming energetic penalty too large to allow the ion to return to the bulk aqueous medium.[27] In sharp contrast, when the mouth of the peptide nanotube emerges in the water-membrane interfacial region, ion permeation is thermodynamically favored by a local reshaping of the electrostatic landscape. The present simulations suggest that the conduction properties of more-complex ion channels might follow a similar trend imposed by the location of their mouth with respect to the surface of the lipid bilayer.

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Supporting Information Available: Methodological details and further analyses of the simulations. This material is available free of charge via the Internet at http://pubs.acs.org.

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