JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY
COMMUNICATION
pubs.acs.org/JACS

How Far Can a Sodium Ion Travel within a Lipid Bilayer?

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S *Supporting Information*

ABSTRACT: Analogues of a synthetic ion channel made from a helical peptide were used to study the mechanism of cation translocation within bilayer membranes. Derivatives bearing two, three, four, and six crown ethers used as ion relays were synthesized, and their transport abilities across lipid bilayers were measured. The results showed that the maximum distance a sodium ion is permitted to travel between two binding sites within a lipid bilayer environment is 11 Å.

Ion-channel proteins are of fundamental importance in various biological processes and represent a key target in pharmaceutical research.[1,2] Although intensive work has been done over the past 20 years, many aspects of their mechanism are still not completely understood. For instance, how do ions migrate through the channel? What is the maximum distance an ion can travel between two relay sites in a lipid membrane? What is the hydration level of cations during their transmembrane journey? These are questions for which answers are still elusive. Here we report that Na^+ ions can translocate efficiently between two polar sites separated by as much as 11 Å in the low-polarity environment of a phospholipid bilayer.

For a better understanding of natural ion membrane transport, we and other groups opted for a strategy using simpler models of ion channel proteins.[3−12] Our approach was inspired by natural protein features, in particular those of the KcsA channel,[13] and we incorporated them into a minimalist molecular system consisting of a single helical peptide framework of 21 amino acids containing six phenylalanine residues substituted with 21-crown-7 macrocyclic ligands.[14] The sequence was designed in such a way that the macrocycle side chains are positioned on top of each other when the peptide framework adopts an α-helix conformation. Previously, with the help of several techniques, we showed that such molecular devices facilitate membrane ion transport using a monomolecular channel mechanism.[15] Our design strategy has the advantages of being simple and allowing for rapid molecular engineering by solid-phase peptide synthesis, which permits efficient engineering of channel end groups, length, and diameter.[16,17]

In order to establish the minimal distance between two relay sites for efficient ion translocation, we prepared a series of analogues bearing different numbers of 21-crown-7 ligands (Figure 1). Peptide **1** bears six crown ether-modified phenylalanines at positions 2, 6, 9, 13, 16, and 20. The remainder of the sequence is composed of leucines and alanines. These amino acids were chosen for their hydrophobicity, as the peptide should be lipophilic enough to be incorporated readily into a lipid bilayer membrane.[18,19] These amino acids also have a strong propensity to favor the α-helix conformation. Peptides **2**−**4** are based on the same framework as model **1** but have two or four crown ether residues replaced by phenylalanines, yielding peptide devices with four (**2**, **3**) or two (**4**) binding sites. For peptide **5**, the crown ether residues were incorporated at positions 4, 11, and 18 to obtain a nanostructure with three binding sites; **6** is similar to peptide **5** but has a phenylalanine at position 11 to generate a peptide device with two crown ethers. All of the peptides were designed to orient their crown ethers on the same side when in a helical conformation. Under such a conformation, the distances between two crown ethers were estimated and vary from 6 to 28 Å (Figure 1).[20]

Peptide devices **1**−**6** were prepared by solid-phase synthesis using Wang resin as a solid support and *N*-Fmoc-protected amino acids.[21,22] The protected crown ether-modified L-phenylalanines were synthesized from L-dopa and poly(ethylene glycol).[15,23] The target compounds were purified by high-performance liquid chromatography (HPLC) and characterized by mass spectrometry. Circular dichroism measurements on each peptide confirmed that they adopt a strong α-helical conformation in trifluoroethanol and lipid bilayers, as predicted. Thus, all of the peptide devices orient the crown ethers appropriately in stacking arrangements when in a low-polarity environment.

The transport abilities of the different analogues were evaluated by fluorescence assays using pyranine as a fluorescent probe.[24,25] This pH-dependent probe was encapsulated in egg yolk phosphatidylcholine vesicles at pH 6.2 and placed in the tested cation solution at pH 7.2. Efficient channels allow cations to enter the vesicles while protons escape to preserve the ionic equilibrium, leading to an increase in the pH of the intravesicular milieu. Therefore, the membrane transport efficiency can be characterized by the increase of fluorescence over time.[16] Each peptide solution was added to a solution of vesicles, and transport was measured until 400 s, where 100% transport was established by lysing all of the vesicles with Triton X-100. It is important to note that although compounds **1**−**9** have very similar solubilities, slight differences in solubility may also account in part for the observed differences in transport rates. The results of a typical experiment are presented in Figure 2.

The fluorescence underwent a rapid increase until it reached a plateau. For comparison purposes, we considered the percentage of transport observed at 400 s and the initial rate for each compound; the values are reported in Table 1. Peptide **1** was

Received: November 17, 2010
Published: March 08, 2011

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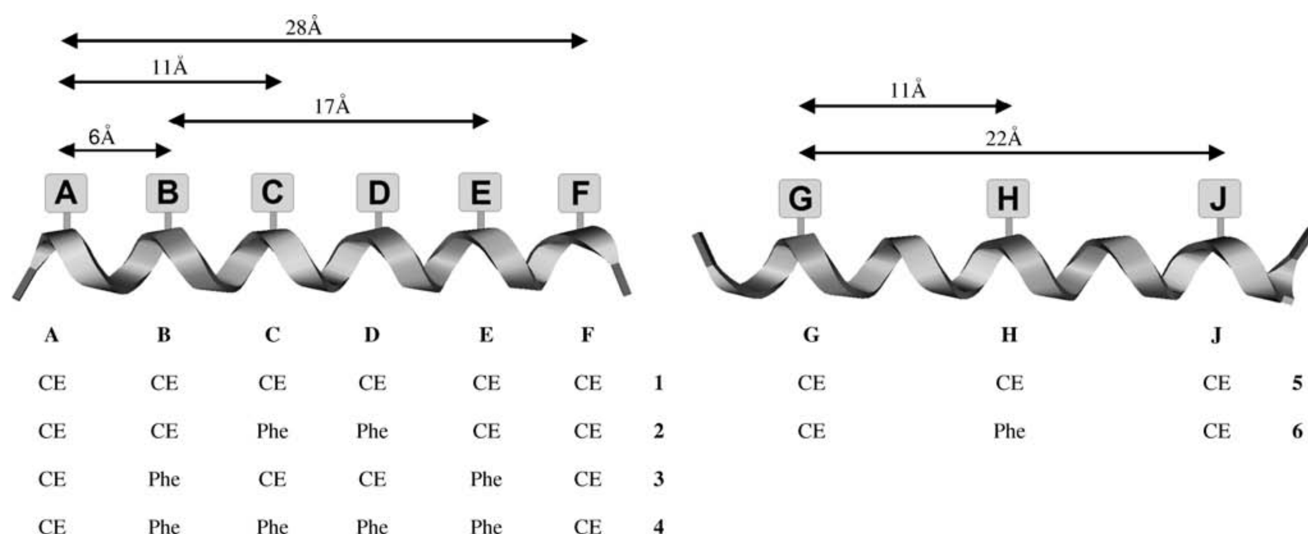

Figure 1. Structures of the analogues used in this study. Peptides **1**−**4** have the sequence H-Leu-**A**-Leu-Ala-Leu-**B**-Leu-Leu-**C**-Leu-Ala-Leu-**D**-Leu-Leu-**E**-Leu-Ala-Leu-**F**-Leu-OH, while peptides **5** and **6** have the sequence H-Leu-Ala-Leu-**G**-Leu-Leu-Phe-Leu-Ala-Leu-**H**-Leu-Ala-Leu-Phe-Leu-Leu-**J**-Leu-Ala-Leu-OH. CE = 21-crown-7−L-phenylalanine.

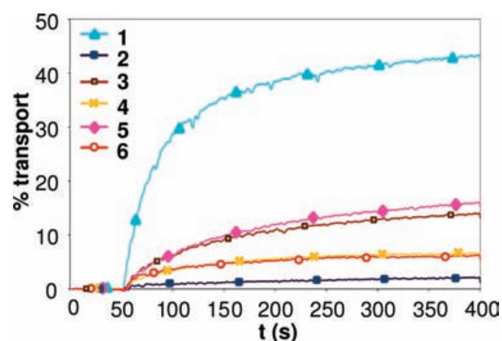

Figure 2. Transport of sodium ion as a function of time. Peptides **1**−**6** were added at 50 s. Experiments were stopped at 400 s by adding Triton X-100.

Table 1. Percentages of Sodium Cation Transport at 400 s and Relative Initial Rates for Peptides Bearing Six, Four, Three, or Two Crown Ethers

compound	% transport	relative initial rate
1	43	1
2	2	0.21
3	14	0.54
4	7	0.26
5	16	0.46
6	6	0.24

clearly the most efficient channel, although this was somewhat expected as it contains the largest number of crown ethers (six), distanced from one to the other by ~6 Å. It is noteworthy that this distance matches well with the one found for the KcsA channel. Indeed, crystallographic data from the KcsA protein established that K^+ ions within the selectivity filter of the channel are spaced by 7.5 Å, in good agreement with the distance between crown ethers in **1** (6 Å).[13,26−28] For peptides **3** and **5**, sodium ion transport was effective, but they allowed only one-third of the transport found for **1**. With these two peptides, the longest distance between two crown ether relays is 11 Å. By comparison, peptides **2**, **4**, and **6** gave roughly 10% of the transport allowed by **1**, with maximum distances between crown ether binding sites of 17, 28, and 22 Å, respectively. According to these results, a distance of 17 Å is too long to allow efficient ion translocation and good transport. Looking at the initial rates of transport confirmed the above conclusion. Indeed, peptides having crown ether relays separated by more than 11 Å (i.e., **2**, **4**, and **6**) had significantly lower initial rates than **1**, whereas crown peptides **3** and **5** showed 54 and 46% of the activity of **1**. Overall, results show that even if a 6 Å distance between two relays is ideal for sodium cation transport, a distance of 11 Å can be tolerated for relatively efficient transport. The measured distances compare well with the ones determined for other natural and artificial channels. Indeed, a maximum distance of 14 Å was found for Na^+ with tris(aza)crown hydraphiles.[29,30] Likewise, it has been shown that the open form of gramicidin A possesses two ion-relay sites separated by 11.6 Å.[31,32] However, for the same peptide, a different channel form with a much longer distance between the two relay sites, 19 Å, was proposed.[33,34] These observations illustrate the difficulty of identifying salient information on functional natural ion channels and the utility of model systems. Also, although unlikely, it is possible that the inability of compound **2** to perform ion translocation is partially due to channel blockage by intrusion of lipid chains between the more distant crown ethers.

To confirm that the increase in fluorescence observed during pyranine assays was due to transport instead of membrane perturbation, we performed calcein leakage experiments.[16,35] In all cases, less than 5% calcein release was measured, showing that very little membrane perturbation, if any, occurred in any of the peptide devices. Furthermore, to prove that ions traveled through the channel formed by the crown ether stacks and not through undefined pores created by peptide aggregates, we prepared and tested analogues of the most efficient peptide device **1** bearing crown ethers with different diameters. Peptides with six 18-crown-6 (**7**), 15-crown-5 (**8**), and 13-crown-4 (**9**) were studied for their ion transport ability using the pyranine fluorescent assay with different ions. The results are shown in Table 2.

Table 2. Percentages of Cation Transport Observed with Hexacrown Peptide Channels Having Crown Ethers of Different Diameters[a]

compound	crown ether	% transport Na$^+$	K$^+$	Cs$^+$
1	**21C7**	33	53	62
7	**18C6**	28	38	13
8	**15C5**	12	7	8
9	**13C4**	2	2	2

[a] Measured at 400 s.

For Cs$^+$, we observed that the excellent transport capacity of peptide **1** bearing 21-crown-7 decreased dramatically with the 18-crown-6 analogue **7** (62 vs 13%) and was almost negligible for the 15-crown-5 and 13-crown-4 analogues (**8** and **9**). The large radius of Cs$^+$ (169 pm) can be invoked to explain the difficulty of its translocation with crown ethers smaller than 21-crown-7, which has a pore radius of ~170 pm.[36,37] Transport of K$^+$ was performed efficiently with 21-crown-7 (**1**) and 18-crown-6 (**7**) channels (53 and 38%, respectively) but was not significant for the analogues bearing 15-crown-5 (**8**) or 13-crown-4 (**9**) macrocycles.

The size of K$^+$ (133 pm) matches that of 18-crown-6 (134−143 pm) but is larger than the 86−92 pm cavity of 15-crown-5 and smaller crown ethers. Finally, for Na$^+$, which has a radius of 95 pm, transport was obvious for channels with larger crowns, difficult but feasible in **8** bearing 15-crown-5, and inefficient in **9** holding 13-crown-4. These results strongly support a channel mechanism in which ions pass through the crown ether stacks. Indeed, ion transport via an aggregated pore mechanism would not lead to the significant ion selectivity observed with **1**, **7**, **8**, and **9**. Furthermore, the results also point to the possibility of modulating the ion selectivity of our peptide channels, an important feature for future practical applications of such nanoscale devices.

In summary, by using simple models of ion channel proteins, we have demonstrated that ions travel through membranes by a hopping mechanism in which ions jump from one relay point to another. We have also established that the maximum distance between two relays for which significant transport activity can be observed is 11 Å. The capacity to modulate ion selectivity by modifying the crown diameter has also been demonstrated. We are currently exploring the use of functionalized channels for the development of efficient detection and ion-separation devices.

ASSOCIATED CONTENT

Supporting Information. Characterization data for all new compounds, experimental procedures, and fluorescence assays. This material is available free of charge via the Internet at http://pubs.acs.org.

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ACKNOWLEDGMENT

This work was supported by the NSERC of Canada, the FQRNT of Quebec, and Université Laval. C.R.-B. thanks PROTEO for an undergraduate research scholarship. We are grateful to Ms. R. Théôrêt for the artwork and illustrations.

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