Totally Synthetic Voltage Dependent Ion Channel

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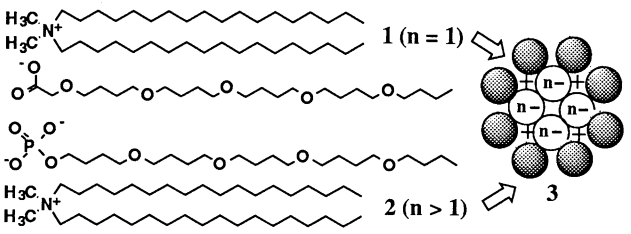
Phosphate monoester of tetra(butylene 1,4-glycol) monobutyl ether was combined with dioctadecyldimethylammonium cation to make an ion pair. When inserted into planar lipid bilayers, it afforded stable and constant single ion channel currents. The open probabilities varied depending on the applied voltage while keeping the conductance constant, giving rise to a mimic of the voltage dependence.

Recently, we have demonstrated that a simple, totally synthetic and nonpeptidic ion pair **1**, composed of glycolate ether of tetra(butylene glycol) monobutyl ether with hydrophobic ammonium cation, gave single ion channel characteristics closely resembled native ion channels.[1] The molecule was assumed to be self-assembled in lipid bilayers to make a supramolecular ion conducting pore, through which cations passed with a significant cation/anion selectivity.

Activities of biological ion channels are regulated by the gating mechanism equipped with sensors for membrane voltages, chemical ligands or mechanical tension. The molecular mechanism of channel gating is a subject attracting the top interest in the fields of biophysics and molecular biology. Particularly, the voltage gating has extensively been studied and a molecular domain for the voltage sensor which contains charges or dipoles is proposed to be primarily responsible.[2,3] As a target for the construction of totally synthetic ion channel function, here attempted was a voltage gating, to which only a few oligopeptides were hitherto accessible.[4-6]



We have followed the supramolecular approach to construct the voltage-gated ion channel by a simple modification of **1**. Instead of the carboxylate head group, phosphate was attached to tetra(butylene 1,4-glycol) monobutyl ether through an ester linkage and combined with dioctadecyldimethylammonium cation to afford the ion pair **2**. Since the phosphate group is dissociated statistically into ca. 1.5 minus at the operating pH 7.2, although perturbed to n (>1) minus at the membrane interphase, the ion pair formation with an equimolar ammonium cation leaves extra (n-1) anionic charges in the membrane phase as symbolically shown as **3** in the chart, while **1** is electrically neutral and its assembly is free from net charges.

When **2** was incorporated into planar lipid bilayers, single channel currents were observed as typical records at various voltages were illustrated in Figure 1. The striking observation is that the open probabilities were varied depending on the applied voltage. At 50 mV, the channel resides almost in the closed state with only occasional short openings in a short duration. At 70 to 85 mV, the opening frequency increases and their duration becomes longer significantly. Then at 100 mV, the channel stays almost completely in the open state with only instantaneous closings. The dwell time histogram analysis of this record shows a clear voltage dependence of the open and closed times, shifting to the open state at higher voltages (positive voltage dependence), while the conductance was kept constant at 126 pS for a series of the voltage variation. In other words, the open and closed times were varied in response to the voltage variation while

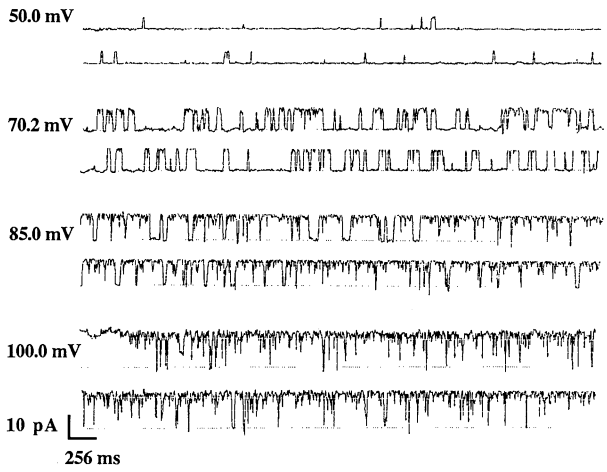


Figure 1. Typical records of ionic currents at several voltages under symmetric 0.5 M KCl solutions at pH 7.2. Currents increase upward from the closed level shown by dotted lines.

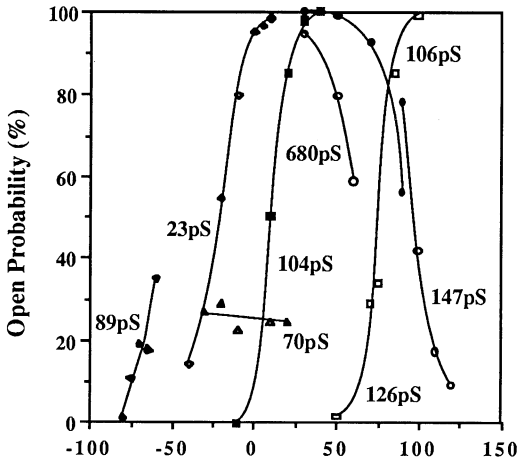


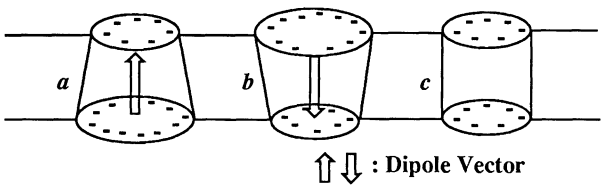
Figure 2. Plots of open probabilities against voltages applied across the bilayer membrane. Values in the Figure show the conductance of each single ion channel.

keeping the conductance constant, exhibiting exactly the same controlling mode as the biological ion channels do.

We recorded numbers of ionic currents at various voltages by using the same molecule. For all runs, single channel characteristics, e.g. stable current, open-closed transition, constant conductance, ion selectivity, were maintained satisfactorily.[7] The data after the amplitude histogram analysis in terms of voltage-dependence were shown in Figure 2, where the open probability was plotted against the applied voltage. The line having the conductance of 126 pS represents the case described above.

Interestingly, positive and negative voltage dependencies were observed with an almost equal probability along with less frequent appearance of almost zero voltage dependence, irrespective of the magnitude of single channel conductance. For any supramolecular channels, the observation of several conductance levels is not unusual, but rather common[4-6,8,9] and it should be reserved to comment that the linear dependence of the current on the applied voltage was maintained for each run to give rise to a specific conductance. This indicates that we are observing a single molecule of supramolecular ion channel persisting stably in the course of voltage variation experiments with continuous variation of open-closed probabilities, and a different single molecule in the next run.

It should be noted that such a voltage dependence has never been observed when an electrically neutral ion pair **1** was employed. The net negative charges of the ion pair are therefore responsible for the voltage dependence of **2**. Based on the supramolecular ion channel model of **1**,[1] the combined observations suggest the following mechanism for the voltage dependence as schematically shown in the Scheme: Amphiphilic molecules are assembled to construct a half channel in each membrane layer. This process occurring in one of the membrane layers should be independent of the one occurring in the other layer. When two supramolecular half channels thus formed are interconnected, the assembly numbers in two layers are generally different from each other unless strong interactions are operating and the resulting transmembrane channel may possess a net dipole vector across the membrane. When the case *a* channel is stabilized by e.g., a positive external voltage and destabilized by a negative one, a positive voltage dependence may result. The case *b* channel having the opposite dipole vector should then give a negative voltage dependence. Since no asymmetry exists across the membrane and we are observing a single molecule of ion channel formed in the membrane in each run, the formation of



Scheme. A plausible mechanism for the formation of voltage dependent ion channels. Trapezoidal cylinders symbolize the transmembrane ion channel composed of assembles of uneven (cases *a* and *b*) or even (case *c*) aggregation number of the net negative ion pair **2**, which is symbolized by -, at both surfaces of the membrane.

case *a* and *b* channels must be of statistically equal probability and we observe positive and negative voltage dependences in an equal frequency. When the assembly numbers in both layers happens to be identical (case *c*), no voltage dependence should be observed. These are what we observed exactly in the above experiment.[10]

Although these explanations still wait further proofing experiments, it was clearly demonstrated that such the simplest combination as phosphate monoester and hydrophobic tetraalkylammonium cation can mimic the fundamental biological function of signal transduction in excitable cells. A cummurative success for artificial ion channel functions by such simple molecules may disclose a wide gate for the design of ion channels and possible applications to ionics devices.

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References and Notes

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10 The midpoint voltage, i.e. the voltage at 50% open probability, varied also irrespective of the magnitude of conductance or of the positive/negative voltage dependence. The shift may correspond to changes in the net voltage across the membrane, which is produced by the asymmetric change of the surface potential on each side of the membrane. Asymmetric distribution of the molecular aggregates with net negative charges may be responsible for producing such a situation.