A Supramolecular Ion Channel Based on Amphiphilic Cholic Acid Derivatives

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Cholic acid was modified so as to represent the simplest expression of an artificial supramolecular ion channel by converting three hydroxyls to methyl ethers and a carboxyl to a methylene(trimethyl)ammonium grouping. It gave stable single channel currents showing several conductance values in planar bilayer lipid membrane.

Natural peptide ion channels have been known as excellent molecular system in the signal transduction of biological cells, and construction of artificial ion channels has been one of the most attractive research subjects connecting nature and chemistry, and various interesting approaches have been reported in this decade.[1,2] Mechanisms how high ion selectivities are maintained with allowing large ionic fluxes and how constant currents are kept with frequent open-closed transitions may give hints on designing future ionics devices. Recent advances of structural analysis of native ion channels have elucidated the mechanism even in part, and synthesis of artificial ion channel of simple and synthetically feasible structures may give us various informations to elucidate the mechanism, e.g. of ion selectivities and conductance values, and to establish the structure-function relationships.

We have succeeded in constructing artificial non-peptide ion channels by a combination of ion pairs of oligoether carboxylate and alkylammonium and its modifications.[3] The ion channels have been characterized well by the measurement of single ion channel currents, and the conductance values were found to be of the order of $10^1 - 10^3$ pS. The channels were active only when both hydrophilic oligoether and hydrophobic alkylammonium components were incorporated into bilayer lipid membrane. Their structures are considered to be of supramolecular aggregates: The central pore is constituted by the assembly of hydrophilic oligoether parts and stabilized by the surrounding hydrophobic alkyl ammoniums. Such a combination may be among the simplest expression of artificial ion channels. A further simplification may be allowed when a single amphiphilic molecule can afford both the required characteristics of hydrophilic and hydrophobic components. In this viewpoint, we have focused here on cholic acid derivatives, which have three hydrophilic hydroxyl groupings on only one side (alpha positions) extending from a rigid and hydrophobic steroidal skeleton. The molecular amphiphilic nature is expected to afford ion channels if proper modifications are made to stabilize the supramolecular assembly in bilayer lipid membrane. We report here the synthesis of an artificial ion channel based on a cholic acid derivative and its characterization of ion channel properties.

Cholic acid seems most appropriate as a candidate for supramolecular ion channel formation in view of following points as well as its plane amphiphilicity discussed above. The hydroxyl side chains themselves were too hydrophilic for the molecule to show channel activities and therefore tried to be converted to other moderately hydrophilic functionalities. The side chain having a carboxylic acid can easily be converted into other hydrophilic functional groups to anchor the substituent at the aqueous interphase. Further, the molecular length is estimated as approximately 16 Å, the molecule being adjusted to fit the lipid monolayer. Two molecules **3** and **6** were designed, where the terminal ionic groups were carboxylate and quarternary ammonium, respectively (Scheme 1). Treatment of cholic acid sodium salt **1** with sodium hydride and methyl iodide in dioxane gave methyl 3,7,12-trimethoxychlanoate **2**[4] in a 38% yield. Hydrolysis of the ester portion in **2** gave 3,7,12-trimethoxycholanoate **3** in a 87% yield. On the other hand, reduction of ester **2** with $LiAlH_4$,[5] followed by chlorination[6] by thionyl chloride and treatment of the product with methanolic trimethylamine solution afforded the quarternary ammonium derivative **6**[7] in a total yield of 60% starting from **2**.

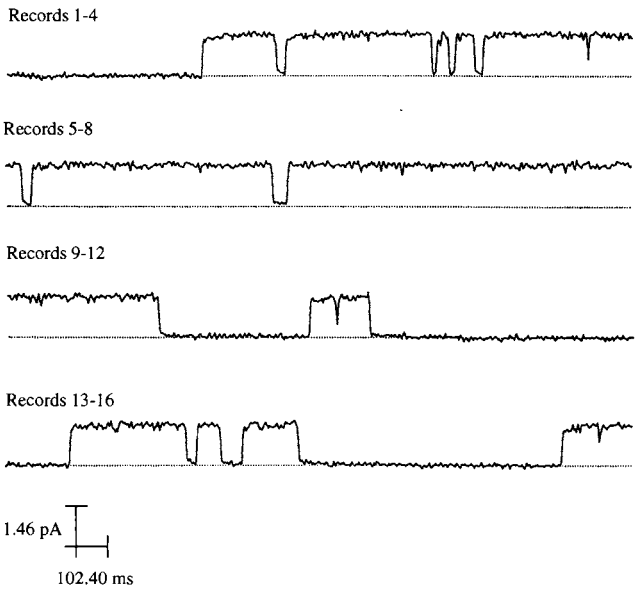
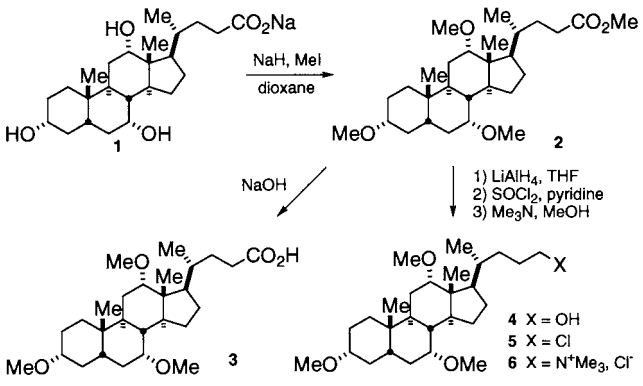


Figure 1. Record of single channel currents obtained by using ammonium channel component **6** at a bath condition of symmetric 500 mM KCl, pH 7.2 by tris-hepes buffer.

According to our established premix methods, cholic acid derivatives **3** and **6** were incorporated into planar bilayer lipid membrane formed in a small aperture on a polypropylene cell.[3] In the case of using carboxylate **3**, no ion channel currents were observed. In a sharp contrast, incorporation of ammonium derivative **6** in 0.2 wt% relative to lipid gave stable single chan-

nel currents at various applied voltage values. A typical record at 152.6 mV was illustrated in Figure 1. In the whole experimental series using steroidal channel compound **6**, the open states stayed long compared to the closed ones, corresponding to 76 and 24%, respectively, in this specific run. Another characteristic to be noted is relatively slow open-closed transitions, mean open and closed times being 0.3 s and 0.07 s, respectively. Conductance values were obtained from the linear relationship between the voltages and the currents for a large number of experimental runs and gave a range of 3 – 10 pS. Compared to oligoether ion channels having relatively large conductance values and its wide range of 10^1 to 10^3 pS, steroidal channels gave significantly small values with variation only in a narrower range. Such conductance values find similarities in various native Na^+ and K^+ channels[8] and seem significant on considering the mechanism how the conductance value is determined. Therefore, the supramolecular channel structure directing ether functionalities inward to make a pore, anchored with ammonium groupings[3] may rationally be assumed (see Graphical Abstract).

The characteristic behaviors different from oligoether channels may have origin in the structural feature of the steroidal channel unit. The lipid membrane would resist to allow a supramolecular assembly formation of large numbers of steroidal components having a relatively large molecular section area and a rigid skeleton, because it almost corresponds to breakdown of the membrane structure. Instead, flexible oligoether of smaller molecular area may find methods somehow to stabilize the pore by using large assembling units. When a hydrophilic pore of the same section is constructed either by steroid or oligoether, a larger number of oligoether units are certainly required and it means a wider distribution of conductance values depending on the difference of the assembling number. At the same time, a higher density of ether functions may lead to larger conductance values. In addition, linear array of methylene and ether oxygen functions in the oligoether case is expected to allow fast conformational movement to induce rapid open-closed transitions of the ion channel. On the other hand, steroidal ion channels have restricted conformational freedom because of their rigid polycyclic structure and therefore the open state is expected to be stable and long-lasting once it appears.



Scheme 1. Synthetic scheme of cholic acid derivatives leading to new artificial ion channels.

In conclusion, we have successfully introduced a new artificial ion channel based on self-aggregation of a cholic acid derivative. Measurements of single channel currents clearly demonstrated a series of conductance values to support the supramolecular assembly formation of the structural unit and characterized a stable open state. These features are considered to originate from the rigid and amphiphilic structural nature of the steroidal component. These results are good indications to design novel artificial ion channels by using cholic acid derivatives as a simple and synthetically feasible construction unit and we are currently under investigation to design a new expression of artificial ion channels.

References and Notes

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4 **2**; Yellow oil after column chromatographic separation. ^{1}H NMR (270 MHz, $CDCl_3$): δ 3.65 (s, 3H), 3.34 (m, 1H), 3.31 (s, 3H), 3.23 (s, 3H), 3.19 (s, 3H), 3.12 (m, 1H), 2.98 (m, 1H), 2.40-0.60 (m, 33H).

5 **4**; Colorless oil in a 90% yield from **2**. ^{1}H NMR (270 MHz, $CDCl_3$) δ 3.72 (t, 1H), 3.58 (m, 2H), 3.31 (m, 1H), 3.31 (s, 3H), 3.24 (s, 3H), 3.18 (s, 3H), 3.12 (m, 1H), 2.97 (m, 1H), 2.4-0.6 (m, 33H). IR (NaCl) 3414 cm^{-1} (OH).

6 **5**; Yellow oil in a 79% yield from **4**. ^{1}H NMR (270 MHz, $CDCl_3$) δ 3.49 (m, 2H), 3.36 (m, 1H), 3.32 (s, 3H), 3.24 (s, 3H), 3.20 (s, 3H), 3.17 (m, 1H), 2.97 (m, 1H), 2.4-0.6 (m, 33H).

7 **6**; Colorless solid in an 84% yield from **5**. ^{1}H NMR (270 MHz, $CDCl_3$): δ 3.35 (m, 1H), 3.29 (s, 3H), 3.26 (m, 9H), 3.22 (s, 3H), 3.17 (s, 3H), 3.10(m, 1H), 2.99 (m, 1H), 2.4-0.6 (m, 33H). MS (Ion Spray) *m/z* 478.426 ([M-Cl] $^+$).

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