J|A|C|S
COMMUNICATIONS

Published on Web 02/06/2002

SCMTR: A Chloride-Selective, Membrane-Anchored Peptide Channel that Exhibits Voltage Gating

Paul H. Schlesinger,[‡] Riccardo Ferdani,[†] Jun Liu,[†] Jolanta Pajewska,[†] Robert Pajewski,[†] Mitsuyoshi Saito,[‡] Hossein Shabany,[†] and George W. Gokel*[,†]

Program in Bioorganic Chemistry, Division of Bioorganic Chemistry, and Departments of Molecular Biology and Pharmacology and Cell Biology and Physiology, Washington University School of Medicine, 660 South Euclid Avenue, St. Louis, Missouri 63110

Received August 7, 2001; Revised Manuscript Received November 1, 2001

Modern cation and anion channels are complex proteins that perform marvelous functions. They are highly selective for either a particular cation or an anion, which they conduct across a phospholipid bilayer membrane at a high rate. In addition to selectivity of cation over anion or one cation over another, these proteins conduct ions in only one direction (rectification). Further, their open−closed behavior may depend on the membrane potential (voltage gating). Notwithstanding the remarkable features found in modern protein channels, these functions must have been performed by much simpler structures at an earlier stage of evolution. It seems unlikely that "primordial channels" were as efficient or selective as are modern examples.

Our belief that simple but functional channel compounds must have operated in early biological development has led us to prepare what we consider to be "working models" of cation channels (hydraphiles).[1] We now report our successful strategy leading to a chloride-selective channel that exhibits voltage-gating properties when it is present in a phospholipid bilayer membrane.

Anion, particularly chloride, permeability is essential for volume, pH, and membrane potential regulation in all cells.[2,3] Currently, four major families of chloride channels are known[4,5] and we have used these proteins as a guide and inspiration for the design of a synthetic, chloride-selective transporter, **6**, that is active in phospholipid bilayers.[6]

Our design incorporates structural patterns from naturally occurring chloride transporters. Several lines of evidence suggest a critical role for proline in the putative chloride entry portal. (1) All members of the ClC family of chloride protein channels contain the conserved motif GKxGPxxH in the anion pathway.[7,8] (2) Nicotinic acetylcholine receptors are converted to anion selectivity by the substitution of a proline into the intrinsic channel-selectivity filter.[9,10] (3) Proline in channel-forming peptides may form a "hinge-bend" regime,[11] GxxP, to extend the peptide across the membrane bilayer.[12] (4) Proline may induce a surface "kink" in membrane transport proteins.[13] (5) The helix−loop−helix motif of C-peptide has proline at the loop's apex that is required for ion channel activity.[14]

In **6**, $(C_{18}H_{37})_2NCOCH_2OCH_2CO$-GGGPGGG-$OCH_2Ph$, the sequence places proline at the pinnacle of an "arch" flanked with glycine residues. We propose that the heptapeptide resides at the top of the mid-polar regime to form an uncharged, chloride-selective portal, which is held in place by the hydrophobic anchor.[15]

The membrane anchor was designed to mimic a phospholipid monomer in size, polarity, and functional group position. The anchor is a dialkylamino derivative of diglycolic acid: $(C_{18}H_{37})_2NCOCH_2OCH_2COOH$.[16] Related anchors have been described in the past,[17,18] but no characterization data appear in those reports or in the Beilstein database.[19]

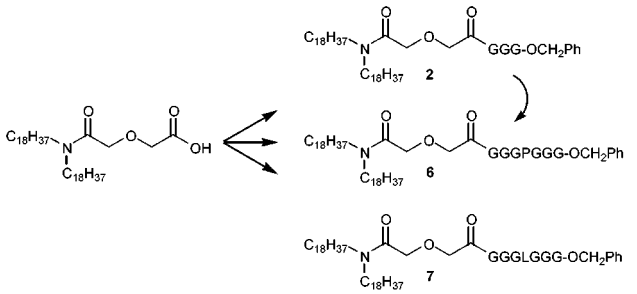

Figure 1. Preparation of the synthetic Cl^- channel. Synthetic approaches to **2**, **6**, and **7**.

The key anchor portion of the molecule, $(C_{18}H_{37})_2NCOCH_2$-OCH_2COOH, **1**, is formed in a single step by heating equivalent amounts of diglycolic anhydride and bis(octadecyl)amine in refluxing toluene for 48 h. Evaporation of the solvent afforded crude **1** (∼100%), which was crystallized from $CHCl_3$ to afford a colorless solid (87%, mp 81−82 °C). Compound **1** and $TsOH{\cdot}H_2N$-GGG-OCH_2Ph were coupled using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) and Et_3N in CH_2Cl_2 (0 °C → RT, 30 h) to afford $(C_{18}H_{37})_2NCOCH_2OCH_2CO$-GGG-$OCH_2Ph$, **2** (89%). The benzyl group was removed by hydrogenolysis (H_2, Pd/C, abs. EtOH, 65 psi, 3 h, 96%) to give **3** (mp 163−164 °C). The tetrapeptide fragment was added in three steps: (1) coupling $TsOH{\cdot}H_2N$-GGG-OCH_2Ph with Boc-proline to give Boc-PGGG-OCH_2Ph, **4**, (71%, mp 176−177 °C); (2) Boc group removal (4 N HCl/dioxane, 1 h, 25 °C, 100%) to give $ClH_3NPGGGOCH_2Ph$, **5** (mp 145−146 °C); and (3) coupling (EDCI, Et_3N, CH_2Cl_2, 0 °C → rt, 30 h) of **3** and **5** to give $(C_{18}H_{37})_2NCOCH_2OCH_2CO$-GGGPGGG-$OCH_2Ph$, **6** (82%, mp 116−118 °C). Proton and ^{13}C NMR were in concert with all expected structures. The leucine analogue, $(C_{18}H_{37})_2NCOCH_2OCH_2CO$-GGGLGGG-$OCH_2Ph$, **7**, was prepared in an analogous fashion (final coupling step, 83%, mp 164−165 °C) (Figure 1).

Chloride release mediated by **6** was rapid, concentration-dependent, and went to completion in defined unilamellar liposomes (150 ± 16 nm, Figure 2). Chloride will exit these vesicles rapidly; pore activation kinetics are shown in Figure 2. Replacement of the proline in **6** by leucine (→ GGGLGGGOCH_2Ph, **7**) dramatically reduced chloride efflux. The truncated analogue of **6** [$(C_{18}H_{37})_2$-$NCOCH_2OCH_2CO$-GGG-OCH_2Ph], **2**, exhibited 6-fold lower Cl^- release (data not shown). Neither **7** nor **2** contains proline; therefore, no obvious source of a bend is available as in **6**. On the basis of its

[†] Department of Molecular Biology and Pharmacology.
[‡] Department of Cell Biology and Physiology.

10.1021/ja016784d CCC: $22.00

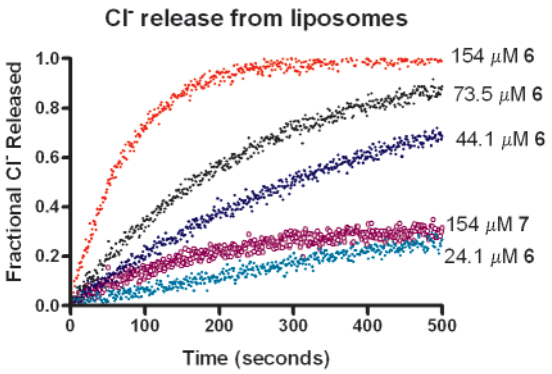

Figure 2. Chloride release from liposomes. Liposomes were prepared by reversed-phase evaporation[21] as previously reported.[22] Cl^- release was determined with a resin, chloride-specific electrode.[23] The kinetics of Cl^- release at 24−154 μM **6** (●) and with 154 μM **7** (○).

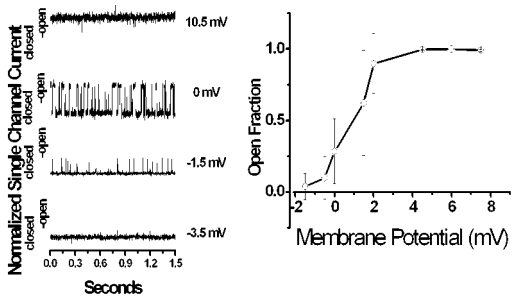

Figure 3. Planar lipid bilayers were formed as previously described.[26] SCMTR was applied to the cis chamber at 60 μM with a 450/150 KCl gradient present across the membrane. Seconds later Cl^- currents appeared, and voltage was varied from +20 to −2 mV. Fractional open time was computed using Fetchan and pStat, Axon Instruments, CA.

activity, we have called this compound ***s***ynthetic ***c***hloride ***m***embrane ***tr***ansporter or SCMTR.[20]

Substituting an impermeant external ion ($SO_4^=$) for a permeant one (NO_3^-) inhibited Cl^- release. The 5-fold reduction in chloride efflux (data not shown) reflects the diffusion potential resulting when a SCMTR pore is present in an external solution of impermeant ($SO_4^=$). Addition of K^+-selective valinomycin reduced the potential and permitted the remaining chloride to exit the liposomes.

The chloride selectivity of **6** was assessed by voltage clamp methods in planar phospholipid bilayers. Addition of **6** produced large currents within seconds in a 450:150 mM KCl ion gradient. The sign and the magnitude of this current indicated Cl^- transport by a nanoSiemen-sized channel. A linear current−voltage relationship (−20 to 60 mV) gave a conductance of 1.3 ± 0.01 nS. The reversal potential, E_{rev}, was 28 ± 0.45 mV, corresponding to the calculated chloride Nernst potential, E_{Cl}, of 28.2 mV. This confirms the >10:1 Cl^-/K^+ selectivity observed in liposomes. The ion selectivity and conductivity indicate that **6** forms a large, stable, anion-selective aqueous pore in bilayer membranes.[24,25]

Extensive biophysical studies have characterized the gating phenomenon in numerous proteins. Although a great deal is known about what occurs, the mechanism of gating is simply not understood. The activity of SCMTR is similar to that of numerous proteins in the sense that the membrane potential modulates the behavior. SCMTR also exhibits voltage-dependent gating between −3 and 10 mV, as illustrated in Figure 3. The open time and current dependence upon membrane potential clearly demonstrate characteristic voltage gating by the scmtr anion current.[24] The left panel shows characteristic channel transitions that progress from rapid and frequent at potentials <10 mV to stable activity at potentials >10 mV. Using the data at the left (in the Figure), we calculated the dependence of channel open time as a function of positive membrane potential that is shown in the right panel of Figure 3. We note that in this case there is a strong dependence of open time on transmembrane voltage.

On the basis of the data obtained, we believe that two molecules of SCMTR associate in the external leaflet of the bilayer. This assembly creates a pore of approximate diameter 6−7 Å and the anchor group's tails penetrate the other bilayer sufficiently for a transmembrane pore to form. Synthetic modifications and a structure−activity relationship will be required to confirm this hypothesis.

The design, preparation, and characterization of the first synthetic chloride membrane transporter are presented. The novel compound imparts anion permeability to phospholipid bilayers. The successful structure, **6**, inserts rapidly into liposomes and planar lipid bilayers. SCMTR has a 1.3 ± 0.01 nS chloride diffusion pathway (>10:1 Cl/K selectivity). SCMTR shows classic channel kinetics and (open−close) behavior with clear evidence for voltage-dependent gating. Preliminary data, not presented here, show that SCMTR also modulates cellular volume in mammalian cells. The high throughput, ion channel behavior of this simple molecule demonstrates that even modest synthetic structures can afford selective membrane permeability equivalent to that seen in protein channels.

Acknowledgment. We warmly thank the National Institutes of Health (GM 36262), the National Science Foundation (CHE-9805840), and the Kilo Foundation for grants that supported this work.

References

(1) Gokel, G. W. *Chem. Commun.* **2000**, 1−9.
(2) Halm, D. R.; Frizzell, R. A. *Intestinal Chloride Secretion*, Raven: New York, 1990; pp 47−58.
(3) Steinmeyer, K.; Ortland, C.; Jentsch, T. J. *Nature* **1991**, *354*, 301.
(4) Heiss, N. S.; Poustka, A. *Genomics* 1997, *45*, 224.
(5) Jentsch, T.; Gunther, W. *Bioessays* **1997**, *19*, 117.
(6) A lysine-modified peptide has recently been reported: Gao, L.; Broughman, J. R, Iwamoto, T.; Tomich, J. M.; Venglarik, C. J.; Forman, H. J. *Am. J. Physiol.* **2001**, *281*, L24−L30.
(7) Fahlke, C.; Yu, H.; Beck, C.; Rhodes, T.; George, A. *Nature* **1997**, *390*, 529.
(8) Fahlke, C.; Desai, R. R.; Gillani, N.; George, A. L. *J. Biol. Chem.* **2001**, *276*, 1759.
(9) Corringer, P. J. et al. *Neuron* **1999**, *22*, 831.
(10) Galzi, J. L. et al. *Nature* **1992**, *359*, 500.
(11) Gibbs, N.; Sessions, R. B.; Williams, P. B.; Dempsey, C. E. *Biophys. J.* **1997**, *72*, 2490.
(12) Yang, L. T. A. H.; Weiss, T. M.; Ding, L.; Huang, H. W. *Biophys. J.* **2001**, *81*, 1475−1485.
(13) Brandl, C. J.; Deber, C. M. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 917.
(14) Ido, Y. et al., *Science* **1997**, *277*, 563.
(15) Tieleman, D. P.; Sansom, M. S. P.; Berendsen, H. J. C. *Biophys. J.* **1999**, *76*, 40.
(16) Ihara, H.; Hashiguchi, Y.; Kunitake, T. *Chem. Lett.* **1983**, 733.
(17) Gildea, B. D. et al. *Tetrahedron Lett.* **1998**, 7255.
(18) McCrindle, R.; McAlees, A. J. *J. Chem. Soc., Perkin Trans. 1* **1981**, 741.
(19) Ebato, H. et al. *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 1087.
(20) We pronounce SCMTR "scimitar,"a name also suggested by its calculated shape.
(21) Szoka, F.; Papahadjopoulos, D. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 4194.
(22) Saito, M.; Korsmeyer, S. J.; Schlesinger, P. H. *Nat. Cell Biol.* **2000**, 553.
(23) Schlesinger, P. H. et al. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 11357.
(24) Hille, B. *Ionic Channels of Excitable Membranes 3/e*; Sinauer Press: Sunderland, MA, 2001.
(25) Miller, C. *Rev. Physiol.* **1984**, *46*, 549.
(26) (a) Edwards, J.; Tulk, B.; Schlesinger, P. H. *J. Membr. Biol.* **1998**, *163*, 119−127. (b) Schlesinger, P.; Gross, A.; Xin, X.; Yamamoto, K.; Saito, M.; Waksman, G.; Korsmeyer, S. J. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 11357−11362. (c) Schlesinger, P. H.; Blair, H. C.; Teitelbaum, S. L.; Edwards, J. C. *J. Biol. Chem.* **1997**, *272*, 18636−18643.

JA016784D