Ion Channels Formed by Biomimetic Oligo-(*R*)-3-hydroxybutyrates and Inorganic Polyphosphates in Planar Lipid Bilayers

Sudipto Das,[†] Piotr Kurcok,[‡] Zbigniew Jedlinski,[‡] and Rosetta N. Reusch*[,†]

Department of Microbiology, Michigan State University, East Lansing, Michigan 48824, and Center for Polymer Chemistry, Polish Academy of Sciences, 41-800 Zabrze, Poland

Received July 8, 1999; Revised Manuscript Received October 18, 1999

ABSTRACT: Biomimetic monodisperse oligomers of (*R*)-3-hydroxybutyrate (M_n 1670, M_w/M_n 1.2) (referred to as $OHB_{19/23}$) were complexed with inorganic polyphosphates (polyPs) to create large conductance, voltage-activated ion channels in planar lipid bilayers of 1,2-dierucoylphosphatidylcholine. The $OHB_{19/23}$/polyP channels displayed higher conductance (275 pS vs 100 pS) and significantly poorer selectivity for Ca^{2+} over Na^+ than channels formed by polyPs with polymers of (*R*)-3-hydroxybutyrate (PHBs) of 120−140 monomer units (>90:1 vs 4:1), suggesting that the oligomer channels are less well organized. In the absence of polyPs, $OHB_{19/23}$ formed channels only at 100-fold higher concentrations, and the channels were essentially nonselective open pores with fluctuating conductance that closed only rarely. The results suggest that polyPs serve as voltage sensors and have a major role in cation selection, whereas OHBs serve principally as solvating agents. It may be concluded that monodisperse biomimetic OHBs and polyPs can be used effectively to form voltage-activated artificial ion channels of limited cation selectivity.

Introduction

Low molecular weight polymers (50−150 units) (PHBs) and oligomers (<50 units) (OHBs) of (*R*)-3-hydroxybutyrate (3-HB) are ubiquitous constituents of prokaryotic and eukaryotic cells.[1−3] These linear polyesters have the conventional molecular characteristics of salt-solvating polymers, i.e., flexible backbones bearing electron-donating oxygens at intervals suitable for the formation of multiple coordinate bonds with cations.[4,5] Accordingly, they transport ions across chloroform layers in U-tubes,[6] form ion-conducting complexes with lithium perchlorate,[7] and transport ions across phospholipid bilayers in planar bilayer systems[8] and liposomes.[9]

Nonetheless, PHBs or OHBs, themselves, are not well suited for use as artificial ion channels because they must be incorporated into bilayers at high concentrations (>0.1%), and the resulting channels lack selectivity and voltage sensitivity.[8] More effectual ion transport systems may be formed by complexes of PHBs with inorganic polyphosphates (polyPs). PolyPs are chains of phosphates joined by high-energy anhydride bonds. At neutral pH, each residue carries a negative charge, giving polyPs a high capacity for ion exchange[10] and making them responsive to voltage. Complexes of PHB and polyP occur naturally in bacterial plasma membranes[11−14] and have been prepared synthetically.[14] The biological complexes from *Escherichia coli* membranes are comprised of PHBs of 130−140 units[3,13] and polyPs of 60−65 units.[15] Synthetic PHB/polyP complexes have been formed using the 128-mer of PHB (M_n = 11 026; M_n/M_w = 1.0), prepared by Lengweiler et al.[16] from (*R*)-3-hydroxybutyrate through an elegant multistep condensation strategy, and inorganic polyPs (average chain length 65) acquired from commercial sources. Both natural and synthetic complexes form voltage-activated ion channels in planar lipid bilayers that display many of the characteristics of protein calcium channels, i.e., selectivity for divalent over monovalent cations, permeance to calcium, strontium, and barium, and block by transition metal cations.[13,14]

The synthetic PHB_{128}/polyP channels were invaluable in furnishing definitive proof of the channel composition; however, the arduous exponential method of synthesis is impractical for routine preparation of artificial ion channels. Recently, Jedlinsky et al.[18] developed a facile method of PHB synthesis by regioselective ring-opening polymerization of (*S*)-β-butyrolactone, catalyzed by a supramolecular complex of a crown ether and sodium (*R*)-3-hydroxybutyrate. With this process, one can form linear isotactic optically active polymers with relatively narrow M_w/M_n. The end groups of these synthesized polymers (OH and COOH groups) are identical to those present in natural PHB, as shown by NMR and ESI-MS spectroscopy;[19] thus, polymers prepared by this method are referred to as biomimetic. Here we examine the characteristics of channels formed from biomimetic OHBs, M_n 1670, M_w/M_n 1.2, and their complexes with polyPs.

Results and Discussion

Channel Activity of Biomimetic $OHB_{19/23}$. The synthetic OHBs used in these studies (M_n 1670, M_w/M_n 1.2, isotacticity 94%) have an average of 19 residues by M_n measurements and 23 by M_w measurements. For simplicity, they will be referred to here as $OHB_{19/23}$.

No channel activity was observed with $OHB_{19/23}$ when it was incorporated into planar bilayers of synthetic 1-palmitoyl-2-oleoylphosphatidylcholine (16:0, 18:1 PC) at concentrations up to 2.5%. This result did not concur with earlier studies by Seebach et al.[8] in which nonselective channels were observed with monodisperse OHBs of 16 (or multiples of 16) residues in bilayers of 16:0, 18:1 PC at concentrations of 0.1−1.0%.

We considered that this discrepancy might be due to a mismatch between oligomer length and bilayer width. The structure of PHB in the solid state, as observed by X-ray scattering,[20,21] is a 2_1 helix with a pitch of 6 Å. In the bilayer, it is presumed that each OHB molecule crosses the hydrophobic region and is stabilized at each

[†] Michigan State University.
[‡] Polish Academy of Sciences.

10.1021/ma991105t CCC: $18.00
Published on Web 12/03/1999

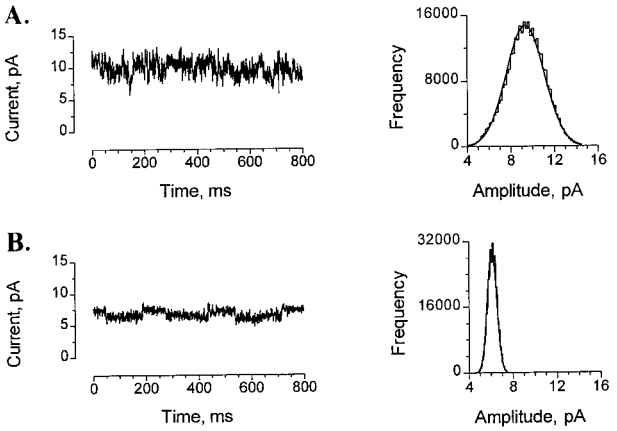

Figure 1. Profiles of single channel currents of $OHB_{19/23}$ in planar lipid bilayers: (A) 1,2-dieicosenoylphosphatidylcholine (di20:1 PC); (B) 1,2-dierucoylphosphatidylcholine (di22:1 PC). $OHB_{19/23}$ was incorporated into planar lipid bilayers, composed of synthetic phosphatidylcholines and cholesterol (5:1 w/w), between symmetric bathing solutions of 200 mM $CaCl_2$, 5 mM $MgCl_2$, 10 mM Tris-Hepes, pH 7.4, at 22 °C as described in Materials and Methods. Data were filtered at 1 kHz. All points amplitude histograms are shown to the right of each trace.

end by the formation of hydrogen bonds from the terminal hydroxyl and carboxyl groups to the ester groups of the phospholipids.[8] Accordingly, the length of the OHB molecule should ideally correspond to the width of the hydrophobic region of the bilayer. For planar bilayers of phosphatidylcholine lipids with 16:0, 18:1 fatty chains prepared from decane solutions, this value is estimated from electrical capacitance measurements as 48 Å.[22] This length corresponds well to the average 50 Å thickness of the lamellar crystals formed by OHBs (16−17 monomer units) as determined from fiber small angle X-ray scattering, transmission electron microscopy, and atomic force microscopy measurements.[23−27]

Solid-state measurements based on a left-handed 2_1 helix conformation indicate that the length of oligomers with19 units and 23 units are ca. 57 and 69 Å, respectively. Other solid-state structures have been observed, e.g., a right-handed 3_1 helix that would significantly reduce this distance.[26] However, it is reasonable to expect that OHBs assume more flexible structures when "dissolved" in a fluid bilayer. Geometric considerations indicate that at least six oligomers must assemble to form a pore large enough to conduct ions.[9] For $OHB_{19/23}$, the probability is high that most of these oligomers will be too short to fold and too long to remain fully extended in a bilayer of 48 Å.

The influence of bilayer width on channel formation was examined by incorporating $OHB_{19/23}$ into bilayers composed of phosphatidylcholine with fatty acyl chains of 1,2-dieicosenoylphosphatidylcholine (di20:1 PC) or 1,2-dierucoylphosphatidylcholine (di22:1 PC) and cholesterol (5:1 w/w) between symmetric bathing solutions of 200 mM $CaCl_2$, 5 mM $MgCl_2$, 10 mM Tris-Hepes, pH 7. Each additional methylene group adds about 1.27 Å to the bilayer;[28] hence, the widths of di20:1 PC and di22:1 PC are estimated as 53 and 58 Å, respectively.

Representative current records of the dominant channels observed in each bilayer are shown in Figure 1. $OHB_{19/23}$ displayed activity at concentrations ≥1% of phospholipids (w/w) in both bilayers; however, channel formation was observed much more frequently in di22:1 PC. The channels in both cases were essentially open pores of fluctuating conductance. Over long periods of recording (>10 min), full closures were brief (order of milliseconds) and extremely rare (<1 per min), indicating a very high open probability (>0.99). A high opening probability (0.85) was observed previously for the 16-unit oligomer in 16:0, 18:1 PC.[8] As shown by the all points amplitude histograms (Figure 1), channels in di22:1 PC had lower average conductance as well as a much narrower amplitude distribution than in di20:1 PC (61 ± 4 vs 109 ± 13 pS), suggesting that more organized channels were formed in the wider bilayer.

Channel Activity of $OHB_{19/23}$ Complexes with Synthetic PolyPs. Complexes were formed from $OHB_{19/23}$ with the calcium salt of polyPs (average 65 units). Details are provided in the Experimental Section. Essentially, a chloroform solution of $OHB_{19/23}$ was added to an excess of dry polyP. After evaporation of the chloroform, the dry mixture of polymers was heated briefly in a microwave oven, and then suspended in chloroform and gently mixed in a bath ultrasonicator. PolyPs are highly insoluble in chloroform; hence, only polyP complexed with $OHB_{19/23}$ is found in solution. Uncomplexed $OHB_{19/23}$ will also be present, but $OHB_{19/23}$ channels are not observed at the concentrations used to form $OHB_{19/23}$/polyP channels.

The complexes were incorporated into bilayers of di22:1 PC and cholesterol (5:1 w/w) at concentrations <0.01% of phospholipids (w/w) between symmetric bathing solutions of 200 mM $CaCl_2$, 5 mM $MgCl_2$, 10 mM Tris-Hepes, pH 7.4, at 22 °C. The current records of $OHB_{19/23}$/polyP, shown in Figure 2A, display high conductance and complex channel activity. All points amplitude histograms[29] for single channel records at different clamping potentials indicate a major fully open state with a conductance of 260 ± 8 pS (Figure 2B) and a minor open state with conductance of 153 ± 3 pS (Figure 3A,B). These conductances were substantially higher than the ∼100 pS conductance reported for the major open state of the biological or synthetic PHB/polyP complexes.[14]

The open probability of single channels was voltage-dependent. Most frequently, open probabilities were higher at more positive potentials (Figure 2C). About 10% of single channels showed the opposite pattern, i.e., higher open probabilities at more negative potentials. Completely symmetrical relationships were never observed. This indicates that $OHB_{19/23}$ /polyP channels, like *E. coli* PHB/polyp channels,[30] are asymmetric. Since polyPs are completely symmetrical, the structural asymmetry is likely established by the different end groups of OHBs that allow the oligomers to adopt one of two opposite orientations in the bilayer. The energetically most favored arrangements of OHBs in a channel may be those that yield unequal numbers of carboxyl and hydroxyl end groups on the two sides of the bilayer. These patterns may be more likely to have high open probabilities at positive potentials.

The channel structure is unknown, but it is proposed that polyP stretches across the bilayer, encircled and solvated by an indeterminate number of OHBs. The space between the two polymers, lined with ester carbonyl oxygens on one side and phosphoryl oxygens on the other, would accommodate multiple conductive pathways for cations. This arrangement could generate higher conductance than single lane central pores such as those presumably created by $OHB_{19/23}$ alone. The polyP polyanion, with its high negative charge and

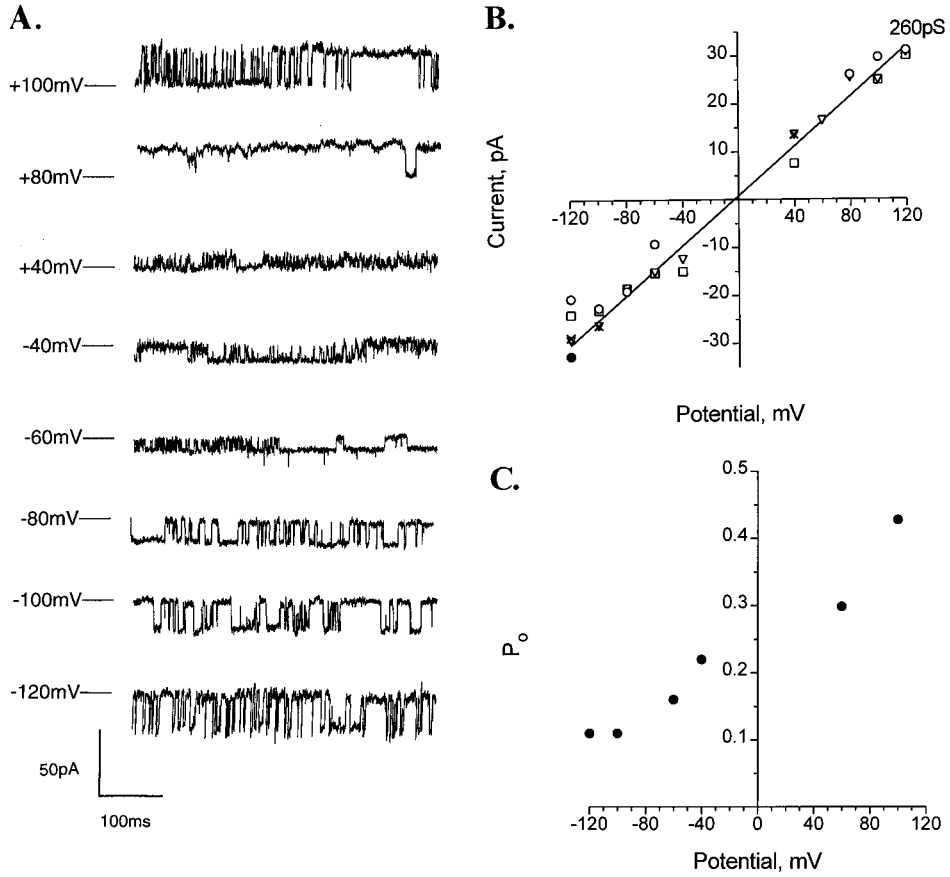

Figure 2. Characteristics of $OHB_{19/23}$/polyP complexes in di22:1 PC/cholesterol bilayers. (A) Profiles of single channel currents. $OHB_{19/23}$/polyP complexes were incorporated into planar lipid bilayers, composed of synthetic di22:1 PC/cholesterol (5:1 w/w), between symmetric bathing solutions of 200 mM $CaCl_2$, 5 mM $MgCl_2$, 10 mM Tris-Hepes, pH 7.4, at 22 °C as described in Materials and Methods. Data were filtered at 1 kHz. **(**B) Current−voltage relations for the major conductance state of $OHB_{19/23}$/polyP channel complexes. All points amplitude histograms were constructed for single channel records at each indicated potential. Data were filtered at 2 kHz. The points show the mean peak position of Gaussian distributions, fit by a simplex least-squares procedure at respective clamping potentials. The best fit in each case was obtained using two distributions, yielding one major fully open state and one minor open state (see Figure 3) at the indicated potentials. The data shown here are mean amplitudes of the major open state from several experiments. Best fit obtained by linear regression yields a single channel conductance of 260 ± 8 pS. Each symbol represents an independent experiment. (C) Open probability of $OHB_{19/23}$/polyP complexes. The complexes were incorporated into planar lipid bilayers as in (A). The data show the open probability at the indicated clamping potentials.

conformational polymorphism, would reasonably be responsive to voltage change.

Cation Selectivity of $OHB_{19/23}$ and $OHB_{19/23}$/PolyP Complexes. There was no significant difference between Ca^{2+} and Ba^{2+} conductance measured under the same conditions (not shown), indicating that $OHB_{19/23}$ channels, like synthetic OHB channels,[8] do not differentiate between cations by size. The selectivity of $OHB_{19/23}$ channels for divalent over monovalent ions was examined in planar bilayer membranes composed of synthetic di22:1 PC and cholesterol (5:1 w/w), between asymmetric bathing solutions of 65 mM $CaCl_2$, 10 mM NaCl, 5 mM $MgCl_2$, 10 mM Tris-Hepes, pH 7.4 (cis side), and 200 mM NaCl, 0.1 mM $CaCl_2$, 5 mM $MgCl_2$, 10 mM Tris-Hepes, pH 7.4 (trans side), at 22 °C. The reversal potential, determined graphically from current−voltage relationships, was ∼0 mV, indicating that $OHB_{19/23}$ channels do not discriminate between cations by charge.

The selectivity of $OHB_{19/23}$/polyP complexes for divalent over monovalent ions was examined in the same manner; i.e., the complexes were incorporated in planar bilayer membranes composed of synthetic di22:1 PC and cholesterol (5:1 w/w), between asymmetric bathing solutions of 65 mM $CaCl_2$, 10 mM NaCl, 5 mM $MgCl_2$, 10 mM Tris-Hepes, pH 7.4 (cis side), and 200 mM NaCl, 0.1 mM $CaCl_2$, 5 mM $MgCl_2$, 10 mM Tris-Hepes, pH 7.4 (trans side), at 22 °C. The reversal potential was −20 mV as determined graphically from current−voltage relationships. The Nernst equilibrium potentials (calculated from concentrations) were $E_{Ca} = -82$ mV, $E_{Cl} = +9$ mV, and $E_{Na} = +76$ mV. These data indicate selectivity for Ca^{2+} over Na^+ of about 4:1. This degree of selectivity shows significant improvement over $OHB_{19/23}$ channels but much poorer discrimination than that demonstrated by natural and synthetic PHB/polyP complexes (>90:1).[14]

Conclusions

We find that biomimetic oligomers of 3-HB aggregate to form ion-permeant pores when incorporated into bilayers at high concentrations (>1% of phospholipids by weight), provided that oligomer lengths approximately match bilayer width. $OHB_{19/23}$ channels in bilayers of di22:1 PC display fluctuating conductance, no cation selectivity, and no voltage sensitivity. Of greater import are complexes of biomimetic $OHB_{19/23}$ with polyPs that form high-conductance, voltage-dependent ion channels when incorporated into suitable bilayers at low concentrations (<0.01% of phospholipids by weight). Apparently, polyP provides a framework for

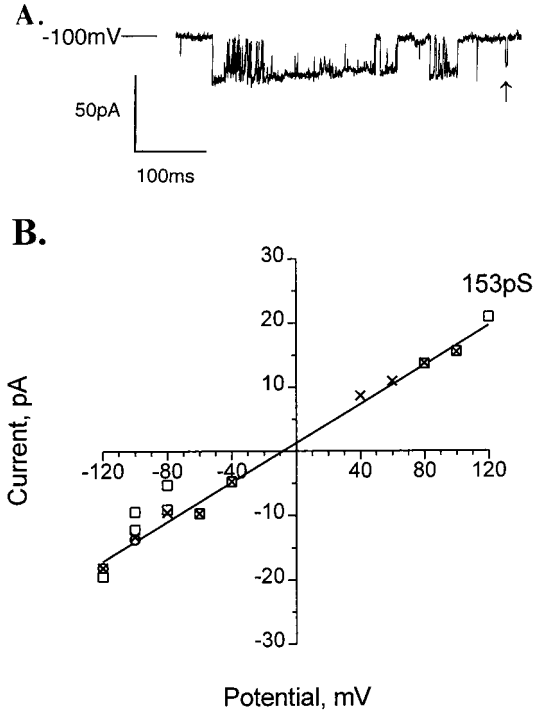

Figure 3. Characteristics of the minor conductance state of $OHB_{19/23}$/polyP complexes in di22:1 PC/cholesterol bilayers. (A) $OHB_{19/23}$/polyP complexes exhibit an open state with a minor conductance. Trace showing a minor independent open state in the PHB/polyP channel in a bilayer at −100 mV clamping potential. Channels were incorporated into planar lipid bilayers as described in Figure 2. Data were filtered at 1 kHz. The bar at the side of the profile indicates the position of the fully closed state of the channel. There was no evidence of a second channel in the current records even at potentials with very high opening probabilities, thus confirming that the transitions originated from the minor open state. (B) Current−voltage relations for the minor conductance state. All points amplitude histograms were constructed for single channel records at each indicated potential. The points show the mean peak position of Gaussian distributions fit by a simplex least-squares method at respective clamping potentials. The best fit in each case was obtained using two distributions, yielding one major fully open state and one minor open state at the indicated potentials. The data shown here are mean amplitudes of the minor open state from several experiments. Each symbol represents an independent experiment. Best fit to the $I-V$ curve, obtained by linear regression, indicates a single channel conductance of 153 ± 3 pS. Data were filtered at 2 kHz. Other experimental conditions were same as described in Figure 2.

assembly of $OHB_{19/23}$ and imposes a measure of order that results in more uniform conductance. PolyP is known to be an efficient ion transporter,[10] and as a polyanion it may reasonably be expected to be responsive to voltage change. $OHB_{19/23}$ acts primarily by forming a sheath around polyP thus solvating it in the bilayer. Though superior to uncomplexed $OHB_{19/23}$ in many respects, $OHB_{19/23}$/polyP channels apparently do not have the degree of structural organization required for high cation selectivity. This demands precise ligand geometries, more easily contrived by long PHB molecules than multiple short OHBs. In summary, we find that complexes of monodisperse biomimetic OHBs and polyPs can be used to create serviceable artificial cation channels of limited divalent cation selectivity in planar bilayers.

Experimental Section

Materials and Reagents. Inorganic polyP glass (type 65) was obtained from Sigma Corp. Buffers and salts were ultrapure (>99%) (Aldrich Corp.). Lipids were 1-palmitoyl-2-oleoylphosphatidylcholine (16:0 18:1 PC), 1,2-dieicosenoylphosphatidylcholine (di20:1 PC), or 1,2-dierucoylphosphatidylcholine (di22:1 PC) (Avanti Polar lipids).

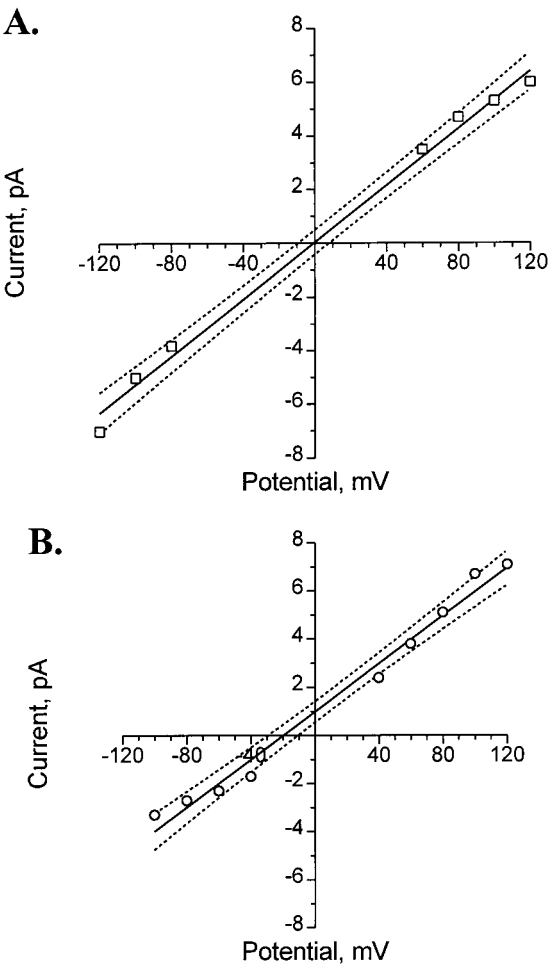

Figure 4. Ca^{2+} selectivity of (A) $OHB_{19/23}$ and (B) $OHB_{19/23}$/polyP channels. (A) Single channel current−voltage relationships of $OHB_{19/23}$ incorporated in planar bilayer membranes composed of synthetic di22:1 PC/cholesterol (5:1 w/w), between asymmetric bathing solutions of 65 mM $CaCl_2$, 10 mM NaCl, 5 mM $MgCl_2$, 10 mM Tris-Hepes, pH 7.4 (cis side), and 200 mM NaCl, 0.1 mM $CaCl_2$, 5 mM $MgCl_2$, 10 mM Tris-Hepes, pH 7.4 (trans side), at 22 °C as described in Materials and Methods. The Nernst equilibrium potentials (calculated from concentrations) were $E_{Ca} = -82$ mV, $E_{Cl} = +9$ mV, and $E_{Na} = +76$ mV. Error bars were smaller than the symbol sizes in most cases. The dotted lines indicate 95% confidence limits. (B) Single channel current−voltage relationships of $OHB_{19/23}$/polyP complexes incorporated in planar bilayer membranes composed of synthetic di22:1 PC/cholesterol (5:1 w/w), between asymmetric bathing solutions as above. The straight line indicates the best fit obtained by linear regression, yielding a permeability ratio (Ca^{2+}:Na^+) of ∼4:1. The dotted lines indicate 95% confidence limits.

Synthesis of $OHB_{19/23}$. Biomimetic oligomers with M_n 1670, M_w/M_n 1.2, and a degree of isotacticity of 94% as determined by ^1H NMR[19] ($OHB_{19/23}$) were prepared by polymerization of (*S*)-*β*-butyrolactone initiated by a supramolecular complex of crown ether 15-crown-5 and the sodium salt of (*R*)-3-hydroxybutyric acid.[18] The percentage of unsaturated end groups, as determined by ESI-MS spectroscopy, was negligible.

Preparation of $OHB_{19/23}$/PolyP Complexes. A chloroform solution of $OHB_{19/23}$ was added to dry, pulverized polyP, and chloroform was removed with a stream of purified nitrogen gas. The mixture was heated in a microwave oven (2 × 30 s). Chloroform was added, and the mixture was sonicated in a Branson ultrasonication bath (model 2210) for 30 min at 4 °C.

Reconstitution of $OHB_{19/23}$/PolyP Channels in Lipid Bilayer Membranes. An aliquot of a chloroform solution of $OHB_{19/23}$/CapolyP complexes was added to the phospholipid/cholesterol mixture (5:1; w/w) in decane (40 mg/mL). The ratio of PHB to phospholipid was <1:10 000. After removal of the chloroform by evaporation with a stream of dry nitrogen, the solution was used to form a bilayer across an aperture of ~200 μm diameter in a Delrin cup (Warner Instruments) between symmetric bathing solutions of 200 mM $CaCl_2$, 5 mM $MgCl_2$, 10 mM Tris-Hepes, pH 7.4.

Single-Channel Recording and Data Analyses. Single channel currents were recorded using an Axopatch 200A integrating patch clamp amplifier (Axon Instruments). The cis solution (voltage command side) was connected to the CV 201A headstage input, and the trans solution was held at virtual ground via a matched pair of Ag−AgCl electrodes. The output of the amplifier was stored in unfiltered form (10 kHz bandwidth) on videocassettes after digitization through an A−D converter (VR 10B, Instrutech Corp.). The data were analyzed offline using pClamp software (version 6.0.3, Axon Instruments) after filtration through an eight-pole Bessel filter (902LPF, Frequency Devices) at 500−2000 Hz. Sampling was done using a TL-1 interface (Axon Instruments) at 10−15 kHz.

Acknowledgment. This work was funded by Grant MCB-9816053 from the National Science Foundation to R. N. Reusch and Grant PAN/NSF-98-330 from the NSF-M. Sklodowska-Curie Fund II to Z. Jedlinsky.

References and Notes

(1) Reusch, R. N. *Proc. Soc. Exp. Biol. Med.* **1989**, *191*, 377.
(2) Reusch, R. N. *FEMS Rev.* **1992**, *103*, 119.
(3) Seebach, D.; Brunner, A.; Burger, H. M.; Schneider, J.; Reusch, R. N. *Eur. J. Biochem.* **1994**, *224*, 317.
(4) Armand, M. B. In *Polymer Electrolyte Reviews-1*; MacCallum, J. R., Vincent, C. A., Eds.; Elsevier Applied Science: New York, 1987; pp 1−37.
(5) Gray, F. M. *Solid Polymer Electrolytes*; VCH: New York, 1992; pp 1−4.
(6) Bürger, H. M.; Seebach, D. *Helv. Chim. Acta* **1993**, *76*, 2570.
(7) Reusch, R. N.; Reusch, W. H. U.S. Patent No. 5,266,422, 1993.
(8) Seebach, D.; Brunner, A.; Bürger, H. M.; Reusch, R. N.; Bramble, L. L. *Helv. Chim. Acta* **1996**, *79*, 507.
(9) Fritz, M. G.; Walde, P.; Seebach, D. *Macromolecules* **1999,** *32*, 574.
(10) Corbridge, D. E. C. *Stud. Inorg. Chem.* **1985**, *6*, 170.
(11) Reusch, R. N.; Sadoff, H. L. *J. Bacteriol.* **1983**, *56*, 778.
(12) Reusch, R. N.; Sadoff, H. L. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 4176.
(13) Reusch, R. N.; Huang, R.; Bramble, L. L. *Biophys. J.* **1995**, *69*, 754.
(14) Das, S.; Lengweiler, U.; Seebach, D.; Reusch, R. N. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 9075.
(15) Castuma, C.; Huang, R.; Kornberg, A.; Reusch, R. N. *J. Biol. Chem.* **1995**, *270*, 12980.
(16) Lengweiler, U. D.; Fritz, M. G.; Seebach, D. *Helv. Chim. Acta* **1996**, *79*, 670.
(17) Hagiwara, S.; Byerly, L. *Annu. Rev. Neurosci.* **1981**, *4*, 69.
(18) Jedlinsky, Z.; Kurcok, P.; Lenz, R. W. *Macromol*ecules **1998**, *31*, 6718.
(19) Jedlinsky, Z.; Adamus, G.; Kowaiczuk, M.; Schubert, R.; Szewezuk, Z.; Stefanowicz, P. *Rapid Commun. Mass Spectrom.* **1998**, *12*, 357.
(20) Cornibert, J.; Marchessault, R. H. *J. Mol. Biol.* **1972**, *71*, 735.
(21) Yokouchi, M.; Chatani, Y.; Tadokoro, H.; Tani, H.; Teranishi, K. *Polymer* **1973**, *14*, 267.
(22) Fettiplace, R.; Haydon, D. A. *Physiol. Rev.* **1980,** *60*, 510.
(23) Marchessault, R. H.; Coulombe, S.; Morikawa, H.; Okamura, K.; Revol, J. F. *Can. J. Chem.* **1981**, *59*, 38.
(24) Seebach, D.; Bürger, H. M.; Müller, H.-M.; Lengweiler, U. D.; Beck, A. K.; Sykes, K. E.; Barker, P. J.; Barham, P. J. *Helv. Chim. Acta* **1993**, *76*, 2004.
(25) Seebach, D.; Bürger, M.; Müller, H.-M.; Lengweiler, U. D.; Beck, A. K. *Helv. Chim. Acta* **1994**, *77*, 1099.
(26) Seebach, D.; Hoffmann, R.; Kühnle, J. N. M.; Lengweiler, U. D. *Helv. Chim. Acta* **1994**, *77*, 2007.
(27) Sykes, K. E.; McMaster, T. J.; Miles, M. J.; Barker, P. A.; Barham, P. J.; Seebach, D.; Müller, H.-M.; Lengweiler, U. D. *J. Mater. Sci.* **1995**, *30*, 623.
(28) Small, D. M. *The Physical Chemistry of Lipids*; Plenum Press: New York, 1986.
(29) Colquhoun, D.; Sigworth, F. J. In *Single Channel Recording*; Sakmann, B., Neher, E.., Eds.; Plenum: New York, 1983; pp 91−263.
(30) Das, S.; Reusch, R. N. *J. Membr. Biol.* **1999**, *170*, 135.

MA991105T