Non-Storage Poly-(R)-3-hydroxyalkanoates (Complexed PHAs) in Prokaryotes and Eukaryotes

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16:0,18:1 PC 1-palmitoyl, 2-oleyl phosphatidyl choline

BSA bovine serum albumin
cPHA complexed PHA
cPHB complexed PHB
di20:1 PC 1,2-dieicosenoyl phosphatidyl choline
di22:1 PC 1,2-dierucoyl phosphatidyl choline; LDL: low-density lipoprotein

HDL high-density lipoprotein

R(3HB) (R)-3-hydroxybutyrate

R(3HV) (R)-3-hydroxyvalerate

NPN N-phenyl-1-naphthylamine

OHB oligomers of (R)-3-hydroxybutyrate

PHA poly-(R)-3-hydroxyalkanoate

PHB poly-(R)-3-hydroxybutyrate

polyP inorganic polyphosphate
1 Introduction

Poly-\(R\)-3-hydroxyalkanoates (PHAs) are fundamental constituents of all biological cells. Although storage PHAs (> 1000 units) are only found in certain prokaryotes and are sequestered within cytoplasmic granules, short-chain PHAs of < 150 units are present in all organisms, and are widely distributed among all cellular compartments and intracellular fluids. These low-molecular-weight forms of the polyesters are complexed to other cellular macromolecules; hence they are referred to as complexed PHAs (cPHAs). cPHAs may be roughly divided into two groups according to whether their association with macromolecules is (1) non-covalent or (2) covalent. The former, which comprises <1% of cellular cPHAs, are distinguished by their solubility in chloroform. They include the channel-forming coordinate complexes of poly-\(R\)-3-hydroxybutyrates (PHB) with inorganic polyphosphate salts (polyP). The latter, which comprise the majority of cPHAs (>99%), are generally chloroform-insoluble and frequently water-soluble. They are predominantly cPHAs conjugated to proteins. For reviews, see Müller and Seebach (1994), Reusch (1992, 1996b, 2000a, b), and Seebach et al. (1996a).

2 Historical Outline

Despite their universal presence in biological cells, cPHAs went unnoticed until the early 1980s. This oversight may be attributed to the low concentrations and sparse distribution of cPHAs within cells, their unremarkable physical properties, and the lack of sensitive methods for their detection. cPHAs have no signal atoms or moieties and no UV absorption. It is the high intrinsic viscosity of PHAs that led to the discovery of cPHB in bacterial cell membranes (Reusch, 1983). cPHAs associated with proteins escaped detection because of the greater lability of ester bonds as compared to amide bonds and the molecular flexibility of PHA molecules. Accordingly, they hydrolyze during Edman sequencing which exposes them to extremes of pH at elevated temperatures, they tend to fragment during mass spectroscopy, and their disordered conformations make them ‘invisible’ to X-ray spectroscopy. The presence of cPHAs in proteins was revealed by Western blot analysis, using antibodies prepared to haemocyanin-conjugated PHAs (Reusch, 1992).

2.1 Complexes of cPHB with Inorganic Polyphosphates (cPHB/polyP)

In 1983, Reusch and Sadoff discovered an ‘ordered gel structure’ in the membranes of the Gram-negative soil bacterium, Azotobacter vinelandii, during an investigation of membrane organization in genetically competent cells (cells able to take up exogenous DNA). Since genetic competence is a transient and labile phenomenon, it was essential to use minimally intrusive procedures to observe short-term changes in composition or structure. Hence modifications in the membrane environment were examined directly in whole cell cultures by spectrofluorometry using the hydrophobic fluorescent probe, N-phenyl-1-naphthylamine (NPN). At low concentrations (< 2× 10^{-5} M), NPN does not influence the temperatures of the lipid phase transitions and does not interact significantly with protein. The probe partitions into the hydrocarbon region of the cell membranes where it responds to changes in the viscosity of its environment by a change in fluorescence...
intensity. This procedure, developed by Trauble and Overath (1973), is rapid, results in minimal disturbance of cell processes, and reports lipid transitions specifically and in reasonable agreement with transitions determined by light scattering and X-ray diffraction (Overath and Trauble, 1973).

Genetic competence develops in *A. vinelandii* when log-phase cells are transferred to an iron-deficient medium (Page and von Tigerstrom, 1979). Transformation becomes measurable after ca. 12 h, reaches a maximum at ca. 18 h, and then slowly diminishes. At stages during this process, the NPN fluorescence spectra were observed as temperatures were slowly increased from 15 to 65 °C. In addition to the broad and reversible gel to liquid-crystalline phase transition of the membrane phospholipids, which begins below 0 °C and ends at about 25 °C, the spectra of normal log-phase cells displayed a small and relatively sharp, irreversible fluorescence peak at ca. 56 °C (Figure 1A). After transfer of the cells to the iron-deficient competence-inducing medium, the intensity of the 56 °C peak increased dramatically with time, reaching it highest intensity at the time of maximal competence.

The sharp, intense 56 °C transition signifies a rapid increase in membrane viscosity, most likely due to changes in lipid composition. Extensive analysis of the phospholipid, glycolipid, and neutral lipid composition of the cell membranes throughout this period revealed no significant changes, but the plasma membranes showed a steady increase in PHB content (Reusch and Sadoff, 1983) (Figure 1B). There was a corresponding increase in storage PHAs, but the granules do not display appreciable fluorescence with NPN and could not be responsible for the 56 °C peak. The outer membranes were not analyzed because they cosedimented with PHA granules when the membranes were separated on sucrose density gradients. The molecular weight of the plasma membrane PHB was estimated at 13,000 ± 15% Da by viscosity measurements.

Similar studies of competence in the Gram-positive soil bacterium, *Bacillus subtilus*, showed a comparable correlation between competence, plasma membrane PHB, and the intensity of a fluorescence peak at ca. 56 °C (Reusch and Sadoff, 1983). PHB was also found in the membranes of competent cells, but not log-phase cells, of the Gram-negative pathogen, *Haemophilus influenzae*.

In 1986, PHB was detected in the plasma membranes of *Escherichia coli*, a bacterium that does not synthesize storage PHAs (Reusch et al., 1986). Indeed, *E. coli* was believed incapable of synthesizing PHAs. Normal log-phase *E. coli* DH1 cells did not show any notable transitions at temperatures above the gel to liquid-crystalline phospholipid transitions, but an irreversible fluorescence peak was observed at ca. 56 °C in stationary-phase cells and the intensity of this transition peak became very strong when *E. coli* cells were made genetically competent by suspending log-phase cells in cold Ca²⁺-containing buffers (Hanahan, 1983) (Figure 2A). Analysis of the membranes indicated that stationary-phase cells and competent cells, but not log-phase cells, contained low molecular weight PHB, and PHB concentrations strongly correlated with 56 °C peak intensities and transformation efficiencies. Since there were no interfering PHA granules, it was possible to establish that the outer membranes did not contain PHB.

Freeze-fracture electron microscopy studies of *A. vinelandii* and *E. coli* membranes supported the fluorescence data (Reusch et al., 1987). The micrographs of log-phase cells showed a typical mosaic of particles and pits on both concave and convex surfaces of
Fig. 1 (A) Fluorescence spectra of NPN in *A. vinelandii* UW1 cells during log-phase vegetative growth and at various stages of genetic transformability. Log-phase growth was in Burk's nitrogen-free buffer (Wilson and Knight, 1952), pH 7.2, plus ammonium acetate (1.1 mg/mL) and glucose (1%). Cells were cultured at 30°C with moderate aeration. For development of genetic transformability, cells were transferred to the same medium, except that Fe⁺⁺ was omitted from the buffer, and cultured as above (Page and von Tigerstrom, 1979). At the indicated times, NPN was added to 4 mL of cell culture to a final concentration of 10⁻⁵ M and the thermotropic fluorescence spectra were recorded. Fluorescence intensities are relative. Excitation, 360 nm; emission, 410 nm. Measurements were made at increasing temperature (ca. 2°C/min). From Reusch and Sadoff (1983).

(B) Relationship between the concentration of total PHB (•), plasma membrane PHB (○), and transformation efficiency (△). Membranes were separated on linear sucrose gradients (25–65%). PHB concentrations were determined by the method of Law and Slepecky (1961). Transformation efficiency was determined by the method of Page and von Tigerstrom (1979). From Reusch and Sadoff (1983).
the plasma membranes. As cells incorporated PHB into the plasma membranes, small semiregular plaques which possess shallow particles were formed (Figure 3). These plaques grew in size and frequency as the concentration of membrane PHB increased. No plaques were visible in the outer membranes of either organism.

The relatively steep slope of the transition led to the inference that membrane PHB was part of an organized 'gel structure'. Presumably, PHB has little influence on the NPN environment when it is part of an ordered structure, but the release of the polyester chains into the bilayer when the structure dissociates effects a sharp increase in bilayer viscosity and a consequent abrupt increase in NPN fluorescence. The complex nature of the membrane gel structure was indicated by changes in the thermotropic fluorescence curves when the cells were washed with water or Ca\(^{2+}\)-chelating buffer; the sharp 56 °C transition diminished in intensity or disappeared and was replaced with broad fluorescence at lower temperatures (Reusch et al., 1986) (Figure 2B). Such broad fluorescence over a large temperature range below 45 °C was observed for uncomplexed PHB in liposomes. These data suggested that the structure responsible for the 56 °C transition was labile and that it had water-soluble as well as lipid-soluble components.

The gel structure responsible for the 56 °C fluorescent peak was extracted from *E. coli* competent cells and its components were identified. The cells were washed with methanol and acetone to remove water and most lipids, and then extracted with dry, cold chloroform. In addition to PHB, the chloroform solution contained short-chain polyP. Since polyP is highly insoluble in chloroform, it was suggested that polyP was surrounded and solvated by PHB. PolyP was extracted with 10 mM EDTA, and Ca\(^{2+}\) was determined to be the predominant neutralizing cation by graphite furnace
atomic absorption spectroscopy (Reusch and Sadoff, 1988). It was surmised that Ca\(^{2+}\) ions form bridges between the two polymers by forming strong ionic bonds to polyP and coordinate bonds to PHB. The complexed short-chain PHB was termed cPHB to distinguish it from the high-molecular-weight PHA granules and the complexes are referred to as cPHB/polyP.

2.2 Protein-associated cPHAs

Water-soluble cPHAs were first discovered in bovine serum albumin (BSA) in 1992 by Reusch et al. The presence of cPHAs in BSA was first noticed when BSA was being used as a blocking agent for the newly developed PHA antibody in a dot-blot ELISA immunoassay. The antibody had been raised in rabbits to PHAs from Alcaligenes spp. conjugated to keyhole limpet haemocyanin. After treatment with the second antibody, goat anti-rabbit IgG conjugated to alkaline phosphatase, and color development with nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate, the entire nitrocellulose membrane turned a deep purple. The presence of PHAs in BSA was confirmed by chemical assay (Karr et al., 1983) and later by \(^{1}H\)-NMR (Reusch, 1992; Seebach et al., 1994a) (Figure 4). The affinity of albumin for PHAs was established by measuring the transfer of \(^{14}C\)PHAs from chloroform solution into aqueous solutions of BSA. Surprisingly, \(^{14}C\)PHAs could not be extracted from aqueous solutions of BSA with warm chloroform or recovered from solid BSA by digestion with alkaline hypochlorite.

3 Occurrence and Distribution of cPHAs

cPHAs appear to be fundamental cell constituents, having been found in representative organisms of all phyla. Chloroform-soluble cPHAs have been isolated from bacterial and eukaryotic membranes (Reusch and Sadoff, 1983; Reusch et al., 1986; Reusch, 1989, 1999b), low-density serum lipoproteins of humans (Reusch et al., 1992), and extracts of spinach, bovine serum albumin, bovine heart, liver mitochondria, and human aorta (Reusch, 1992; Seebach et al. 1994a; Müller and Seebach, 1994). Chloroform-insoluble cPHAs have been found associated with proteins of
membrane, ribosomal, and cytosolic fractions of *E. coli* (Huang and Reusch, 1996), and proteins of diverse eukaryotic cells and tissues (Reusch et al., 1992; Seebach et al., 1994a; Reusch and Gruhn, 1997).

4 Synthesis and Degradation of cPHAs

It is apparent from genomic analysis that synthases and depolymerases of PHAs are not present in non-storage prokaryotes or in eukaryotes. Enzyme(s) responsible for cPHA synthesis have not yet been identified; however, cPHA synthase and depolymerase activities have been observed in *E. coli* lysates (unpublished result), and the monomer, (R)-3-hydroxybutyrate (R(3HB), is a well-known, universal metabolic intermediate in fatty acid biosynthesis. Depolymerases of storage PHAs show no significant activity toward protein-associated cPHAs (unpublished result), but they are degraded by some proteases, e.g. trypsin (unpublished results).

5 Physical Properties of cPHAs

cPHAs are linear, amphiphilic molecules with solvent properties. Hydrophobic methyl groups alternate with hydrophilic ester carbonyl oxygens along the polyester chain. At physiological temperatures, cPHAs are well above the glass temperature, estimated for storage PHAs at –0.5–4 °C (Lauzier et al., 1992; Abe et al., 1994; Holmes, 1988); hence, at physiological temperatures cPHAs are flexible, amorphous molecules in

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**Fig. 4** 1H-NMR spectra of the chloroform:methanol (9:1) extracts of BSA. The spectra show the characteristic methylene and methine protons of cPHB; the methyl protons are hidden under resonances of impurities. Assignments: methylene protons split into an octet at 2.42 – 2.62 p.p.m. JAX 5.7, JBX 15.5; methine protons form a multiplet centered at 5.23 p.p.m. The assignments were confirmed by selective decoupling of the methine resonances. From Reusch (1992).
‘solution’ in the bilayer or in hydrophobic pockets of proteins. The shape of in the molecules can be described by the four backbone dihedral angles of the monomer unit. Computer modeling studies (Insight II, Biosyms) indicate that cPHB may assume a large number of exolipophilic–endopolarophilic or exopolarophilic–endolipophilic forms, when the ester groups are maintained in the preferred antiperiplanar orientation and slight changes are made in one or more of the three less constrained backbone dihedrals (Reusch et al., 1995) (Figure 5). The argument is not that cPHB retains such conformations; rather that it has the flexibility to wrap around other macromolecules or conform to the contours of peptide segments (Figure 6). Thus, cPHAs can act as intermediaries between polar molecules and the bilayer or they can adjust or reverse the polarity of peptide segments in a protein. When the methyl groups of cPHAs engage in hydrophobic interactions with nonpolar residues, the free ester carbonyl oxygens form a hydrophilic solvating surface; conversely, when the carbonyl oxygens form hydrogen or coordinate bonds to polar amino acids or their salts, the exposed methyl groups create a hydrophobic surface.

An equally important property of cPHAs is their ability to ‘dissolve’ salts. Indeed, cPHAs may be unique among biological polymers in having the structural features that are common to a small group of synthetic polymers known as polymer electrolytes, which are distinguished by their ability to form ion-conducting salt complexes (Armand, 1987; Watanabe and Ogatu, 1987; MacCallum and Vincent, 1987; Gray, 1992).

Fig. 5. Some exolipophilic–endopolarophilic helical conformations of cPHB. Computer-generated models (Insight II, Biosyms) of the amphiphilic cPHB molecule in helical conformations with the methyl groups facing outward and the ester groups facing inward. All three conformers are 140 residues in length and have the ester groups in the preferred antiperiplanar arrangement. (A) Ester groups are aligned at each turn of the helix, (B) ester groups alternate, and (C) ester groups are helically skewed. From Reusch et al. (1995).
Important characteristics of this class of polymers are (1) flexible backbones with low barriers to bond rotation to ease segmental motions of the polymer chain, (2) heteroatoms that have sufficient electron donor power to form coordinate bonds with cations, and (3) a suitable distance between heteroatoms to permit the formation of multiple intrapolymer coordinate bonds to cations. The stability of these polymer salt complexes is enhanced, as compared to complexes formed by small molecules, by the entropic advantage known as the ‘polymer effect’, attributable to the cooperative effect of neighboring ligands attached to a common backbone.

The most well-known member of this class is the polyether, polyethylene oxide, whose complexes with lithium perchlorate have been used commercially in lithium batteries. The good solvating power of polyethylene oxide is attributed to an optimal spacing of the electron-donating ether oxygens along a flexible backbone that allows multiple contacts between the polymer backbone and cations. When this distance is decreased, as in polymethylene oxide, chain flexibility is greatly reduced; when it is increased, as in 1,3-polypropylene oxide, the distance between oxygens is too great to allow the polymer to assume the low energy conformations that maximize polymer cation coordinations. In both cases, the ability to solvate cations is lost or greatly diminished. However, when substituents are pendant to the backbone, as in 1,2-polypropylene oxide, the polymers still form conducting complexes, albeit the solvating ability may be attenuated by the increasing steric hindrance.

Polyesters with suitable spacing between ester groups also form ion-conducting salt complexes. Accordingly, Watanabe et al. (1984) have demonstrated that poly-β-propiolactones forms ion-conducting salts with lithium perchlorate. PHB bears the same relationship to poly-β-propiolactone as does 1,3-polypropylene oxide to polyethylene oxide (Figure 7).
The salts ‘dissolved’ by aprotic polymers, such as cPHAs, are composed of hard cations and large anions with diffused charge. Ester carbonyl oxygens are weak Lewis bases of low polarity, thus they form coordinate bonds with cations that have large solvation energies, such as the four major physiological cations, Na$\text{^+}$, K$\text{^+}$, Mg$\text{^{2+}}$, and Ca$\text{^{2+}}$. cPHAs do not have the hydrogen-bond-donating groups needed to solvate anions, hence they ‘dissolve’ salts of large anions with diffused charge that require little solvation (MacCallum and Vincent, 1987; Gray, 1992), e.g. perchlorates or polyphosphates, in which a single negative charge is dispersed over several oxygens.

Cations are conducted in polymer electrolyte complexes by segmental motions of the polymer backbone which carry the cation from one complexation site to the next (Gray, 1992). As a result, the polymers are conductive only in their amorphous state, i.e. above the glass temperature. As stated above, this temperature for bulk PHA polymers is in the range of 0.5–4 °C. One would expect that single short-chain cPHA molecules undergo considerable segmental motions at physiological temperatures, when incorporated into fluid lipid bilayers or hydrophobic pockets of proteins.

There has been substantial experimental confirmation of the capacity of PHAs to solvate salts. Seebach et al. (1993, 1996a) prepared crown ester complexes from cyclic trimers (triolides) of R(3HB) and sodium thiocyanate, Bürger and Seebach (1993) demonstrated that cyclic oligolides and open-chain oligomers of R(3HB, known as OHB, transport alkali and alkaline earth picrate salts across methylene chloride layers in U-tubes, and Reusch and Reusch (1993) prepared ion-conducting complexes of PHAs with lithium perchlorate. Moreover, Fritz et al. (1999) showed that OHB transport Ca$\text{^{2+}}$ across vesicle bilayers, and, as discussed below, OHB form nonselective ion channels in planar lipid bilayers (Seebach et al., 1996b; Das et al., 2000).

### 6 Transbilayer Ion Transport by OHB

The ability of OHB to conduct salts across bilayers was examined by Seebach et al. (1996b) in a planar bilayer voltage-clamp setup (Miller, 1983; Alvarez et al., 1985). In this system, a bilayer is formed between two aqueous solutions by ‘painting’ a decane solution of phospholipids across a small aperture (about 0.2 mm in diameter) in a partition separating two chambers containing aqueous salt solutions. The hydrocarbon drains away and the phospholipids spontaneously arrange themselves into a planar bilayer (black lipid membrane). The aqueous solution on one side (cis side) of the bilayer represents the cell cytoplasm and the solution on the other side (trans) represents the aqueous environment outside the cell. The trans side is maintained as ground and external voltage steps are applied to the cis side (Figure 8).

Since bilayers are compartments of defined size, the effect of OHB length on channel formation was examined. Monodisperse oligomers of R(3HB) containing 8, 16, 32, 64, and 96 monomer units (Plattner et al., 1993; Seebach et al., 1994b), were incorporated into bilayers of synthetic 1-palmitoyl, 2-oleoyl-phosphatidyl choline (16:0,18:1 PC) by premixing the OHB and phospholipids before painting the bilayer. Bilayers formed from pure phospholipids or phospholipids with low concentrations of the oligomers were not conductive. However, when the concentrations of the oligomers were 0.1–5% of the phospholipid concentration, discrete current fluctuations were observed for all chain lengths of 16
monomer units or greater (Figure 9). For a given preparation, the conductance at a given potential was reasonably constant, but it varied considerably for different preparations of the same oligomer, so that current–voltage relationships could not be established. Furthermore, as expected, OHB did not discriminate among ions.

Oligomer size and end group structure proved to be critical factors in channel formation. High-molecular-weight PHAs from natural sources (>3000 units) and small synthetic oligomers of 8 units or less did not form channels, even at concentrations of 5% of phospholipids (w/w). Oligomers of 16, 32, 64, and 96 units in which the end groups had been derivatized also displayed no single-channel activity.

The arrangement of the oligomers in the bilayer is uncertain, but if one assumes that OHB in the bilayer preserve the 2, helicity, 5.96 Å pitch, determined from X-ray diffraction of crystalline PHB (Cornibert and Marchessault, 1972; Yokouchi et al., 1973; Marchessault et al., 1981; Brückner et al., 1988), then the length of molecules containing 16 monomer units corresponds well to the 48 Å width of the 16:0,18:1 PC bilayer, as

![Diagram](image-url)

**Fig. 8** Planar bilayer setup. The system consists of two aqueous solutions, labeled cis and trans, separated by a planar bilayer. External voltage commands are applied to the cis side, with the trans side maintained at ground (defined as zero voltage). OSC, oscilloscope; VCR, recording tape system.

![Histogram](image-url)

**Fig. 9** Single-channel currents of synthetic OHB96. (Left) Representative current fluctuations obtained when the given voltage was applied at a planar bilayer made from 16:0,18:1 PC containing 0.1–1% of 96mer of PHA between symmetric solutions of 60 mM RbCl, 5 mM MgCl₂, 10 mM HEPES CsOH, pH 7.2. The solid horizontal bar in each record indicates the current level with the channel closed. pO is the probability that the channel is in the open state. (Right) Corresponding conductivity histograms. N indicates the total number of observations that have been analyzed. From Das and Reusch (2000).
estimated from electrical capacitance measurements (Fettiplace and Haydon, 1980). This bilayer width also corresponds to the average 50 Å thickness of lamellar crystallites formed by natural PHB and synthetic OHB of 16 units or greater (Seebach et al., 1994b; Barham et al., 1984; Sykes et al., 1995). These data imply that chains of more than 16 units fold back on themselves. It is presumed that each OHB molecule crosses the hydrophobic region and is stabilized at each end by the formation of hydrogen bonds from the terminal hydroxyl and carboxyl groups to the ester groups of the phospholipids (Figure 10). Considering the high concentrations of the polyesters required to form channels, it seems probable that the pores are formed by aggregates of several molecules. Seebach et al. (1996a) suggest that the oligomers form islands of lamellar crystallites in the bilayer, and that ion permeability results from areas of mismatch at the interfacial regions between phospholipids and the oligomers. Fritz et al. (1999) incorporated OHB of 8, 16, 32, and 64 units into the bilayers of Quin-2-encapsulating vesicles, formed by 16:0, 18:1 PC. Ca\(^{2+}\) transport into the vesicles was studied by following the Ca\(^{2+}\)-dependent Quin-2 absorption at 264 nm. In the presence of a concentration gradient, the 32mers and 64mers, but not the 8mers or 16mers, transported Ca\(^{2+}\) into the vesicles with turnover numbers in the range of 2–3 s\(^{-1}\), a rate that was comparable to that observed in the same system for the ionophore, calcimycin. The OHB-mediated transport was blocked by La\(^{3+}\). Other cations were not examined, hence specificity for Ca\(^{2+}\) was not demonstrated.

More insights into the relationship between polyester size and bilayer width were provided by Das et al. (2000), who examined the channel-forming ability of linear isotactic OHB. The oligomers were prepared synthetically by Jedlinsky et al. (1998) via regioselective ring-opening polymerization of \((S)-\beta\)-butyrolactone, catalyzed by a supramolecular complex of a crown ether and sodium R(3HB). This method is less arduous than the exponential fragment-coupling strategy and provides larger yields of the polyesters. The polymers or oligomers formed by this method do not have the advantage of identical size, but they have relatively narrow dispersity. The end groups of these synthesized polymers (OH and COOH groups), as well as the channel-forming oligomers prepared by the exponential fragment-coupling strategy, are identical to those present in natural PHAs, as shown by nuclear magnetic resonance and ESI-mass spectroscopy (Jedlinsky et al., 1998), thus they are referred to as biomimetic.

The channel activity of biomimetic oligomers of \(M_n\) 1670, \(M_w/M_n\) 1.2, isotacticity 94% was examined in planar lipid bilayers (Das and Reusch, 2000). Since these oligomers have an average of 19 residues by \(M_n\) measurements and 23 by \(M_w\) measurements, they are referred to as OHB\(_{19/23}\). OHB\(_{19/23}\) did not form channels in bilayers of 16:0 18:1 PC and cholesterol (5:1 w/w) at concentrations up to 2.5%. This failure was attributed to a mismatch between oligomer length and bilayer width. Solid state measurements based on the above 2 helix conformation indicate that the length of oligomers with 19 units and 23 units are around 57 and 69 Å, respectively. Geometric considerations indicate that at least six oligomers must assemble to form a pore.

**Fig. 10** Schematic representation of OHB of 32 units incorporated into planar phospholipid bilayer. From Seebach et al. (1996).