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# Biomimetic polyesters and their role in ion transport across cell membranes✪★

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**Syntheses of biomimetic low-molecular weight poly-(*R*)-3-hydroxybutanoate mediated by three types of supramolecular catalysts are presented. The utility of these synthetic polyesters for preparation of artificial channels in phospholipid bilayers capable of sodium and calcium ion transport across cell membranes, is discussed. Further studies on possible applications of these bio-polymers for manufacturing drugs of prolonged activity are under way.**

Two types of natural aliphatic polyesters having the structure of poly-(*R*)-3-hydroxybutanoate (PHB) are present in living systems:

- High-molecular-weight polymers (Mw up to hundred thousands) produced in prokaryotic cells as microbial storage material
- Low-molecular-weight polymers (20/120 mers) present in prokaryotic and eukaryotic cells, forming complexes with poly-Ca-phosphate as building blocks of channels in cell membranes, responsible for ion transport across a membrane. The low-molecular-weight polyesters are present also in human blood plasma [1].

The presence of low-molecular-weight PHB polymers in living cells and their obvious importance in life processes have attracted at-

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**Abbreviations:** PHB, poly-(*R*)-3-hydroxybutanoate; OHB, oligo-(*R*)-3-hydroxybutanoate; poly-P, calcium polyphosphate; Mw, weight average molecular weight; Mn, number average molecular weight; GPC, gel permeation chromatography; ESI-MS, electrospray ionization-mass spectroscopy; 18-Crown-6, 1,4,10,13,16-hexaoxacyclooctadecane; 15-Crown-5, 1,4,7,10,13-pentaoxacyclopentadecane.

tention of chemists and biologists, and a lot of attempts have been made to synthesize analogues of natural PHB using various synthetic methods.

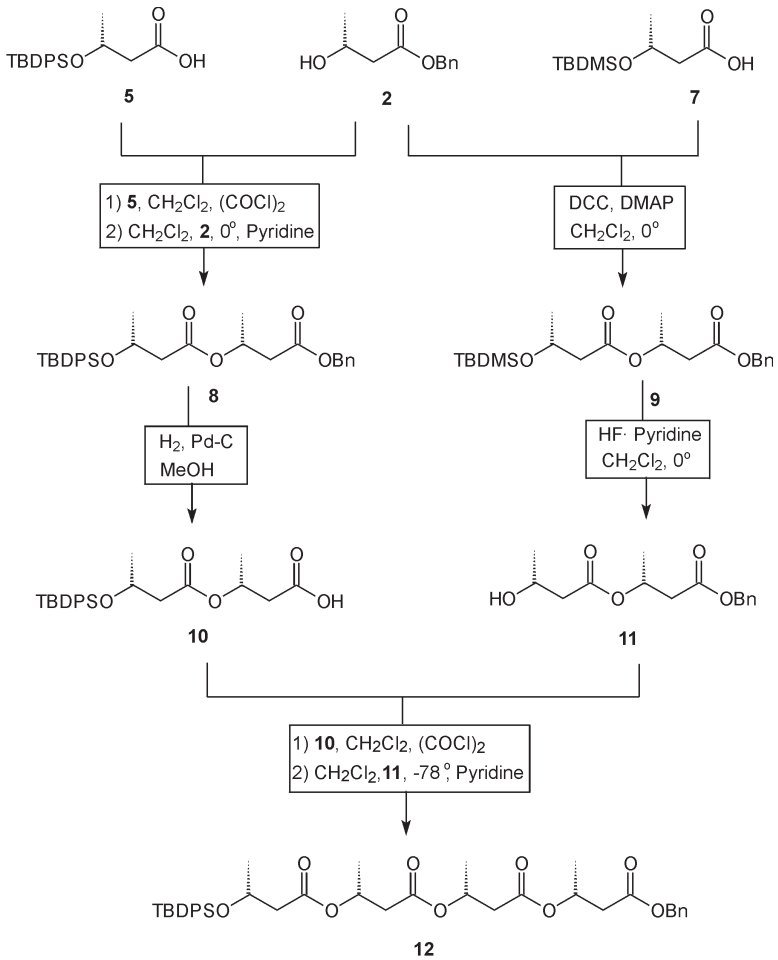
D. Seebach and his associates have developed an elegant method of PHB preparation using step-by-step polycondensation of (*R*)-3-hydroxybutanoic acid. However, this procedure is very laborious and time-consuming because protection and deprotection of end groups of the monomer and intermediate oligomers are necessary at each polycondensation step [2, 3] (Scheme 1).

Another synthetic procedure was based on ring-opening polymerization of $\beta$-butyrolactone (Scheme 2) using organometallic coordinative initiators. However, the resulting polymers exhibited very broad molecular weight distribution and end groups which were different from those found in natural polymers present in living systems [4–7].

In this article we present the synthesis of biomimetic analogues of natural PHB using $\beta$-butyrolactone as a monomer and supramolecular complexes with alkali metals as catalysts.

## MATERIALS AND METHODS

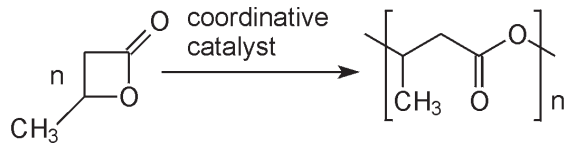
***Materials.*** (*S*)-$\beta$-Butyrolactone (97.5% (*S*) + 2.5% (*R*) (gift from Central Research Laboratory, Takasago International Corp., Hiratsuka, Japan) was purified as described




Bn = $PhCH_2$, TBDPS = (t-Bu)$Ph_2Si$, TBDMS = (t-Bu)$Me_2Si$,
DMAP = 4-(Dimethylamino)pyridine

**Scheme 1. Synthesis of (*R*)-3-hydroxybutanoic acid tetrameter by multistep condensation reaction [3].**

previously [8]. Potassium metal (Fluka) was purified by washing with boiling toluene and dried under vacuum. (*R*)-3-Hydroxybutanoic




**Scheme 2. Synthesis of poly-β-butyrolactone with a coordinative catalyst**

acid sodium salt (Aldrich) was dried under reduced pressure at 50°C for 48 h. Penicillin G potassium salt (Merck) was used as received. THF was purified as described in ref. [9] and then distilled over sodium-potassium alloy just before use. 18-Crown-6 (Fluka) was purified as described earlier [10]. 15-Crown-5 (Aldrich) was dried under vacuum at 40°C for 10 h. Inorganic poly-P (type 65) was obtained from Sigma. Buffers were ultrapure (> 99%) (Aldrich). The lipid used was 1,2-dierucoylphosphatidylcholine (di22:1 PC) (Avanti Polar lipids).

***Preparation of catalysts***. The solution of potassium supramolecular complex was obtained by the contact of the potassium mirror with THF solution of crown ether 18-crown-6 (0.1 mol/dm$^3$) at 0°C. After exactly 15 min the resulting blue solution was filtered through a coarse frit to the reactor (concentration of potassium anions: [$K^-$] = [18-crown-6] = 0.1 mol/dm$^3$). (*R*)-3-Hydroxybutanoic acid sodium salt/15-crown-5 complex and penicillin G potassium salt/18-crown-6 complex were prepared similarly as described previously [10, 11].

***Polymerization of* (S)-β-*butyrolactone***. (*S*)-*β*-Butyrolactone was polymerized in bulk or in solution (THF or $CHCl_3$), respectively, under stirring in a previously flamed and argon-purged glass reactor. The monomer and solvent were added into the reactor containing the required amount of initiator (potassium ion pairs, (*R*)-3-hydroxybutanoic acid sodium salt/15-crown-5 complex or penicillin G potassium salt/18-crown-6 complex, respectively) under dry argon atmosphere. The progress of polymerization was measured by the FT-IR technique (based on the comparison of the band intensities at 1823 and 1740 cm$^{-1}$ corresponding to absorption of carbonyl carbons of monomer and polymer, respectively). When polymerization was complete, ethyl ether solution of HCl was added into the reactor and after 10 min the polymer formed was precipitated in hexane. Next the polymer was redissolved in $CHCl_3$, and the alkali metal chloride/18-crown-6 complex was extracted (five times) with water. Then the polymer was precipitated in hexane, dried under vacuum for 48 h, and analyzed by GPC, $^1$H NMR, ESI-MS and optical rotation measurements. The degree of isotacticity was determined by the method described previously [11].

***Preparation of PHB/poly-P complexes.*** A chloroform solution of low molecular PHB (Mn 1670, Mw/Mn 1.2 and a degree of isotacticity of 94% as determined by $^1$H NMR) was added to dry, pulverized poly-P 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 for 30 min at 4°C.

***Reconstitution of PHB/poly-P channels in lipid bilayer membranes*** was described in detail previously [12]. An aliquot of a chloroform solution of $OHB_{19/23}$/poly-P complexes was added to the phospholipid/cholesterol mixture (5:1; w/w) in decane (40 mg/cm$^3$). 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 about 200 $\mu$m diameter.

***Analyses***. $^1$H NMR spectra of obtained PHB oligomers and polymers were recorded by using a Varian VXR-300 spectrometer in $CDCl_3$ with tetramethyl silane as the internal stan-

dard. FT-IR spectra were recorded using a 40A Bio-Rad spectrometer at room temperature. GPC was performed at 30°C, using a Spectra Physics 8800 gel-permeation chromatograph with two PL-gel packed columns (103 Å and 500 Å). THF was used as the eluent at a flow rate of 1 mL/min. Polystyrene standards with low polydispersity (PL-Lab.) were used to generate a calibration curve. Number average molecular weight Mn of the obtained polymers was determined by GPC and confirmed by the vapour pressure osmometry technique in chloroform. Optical rotation measurements were conducted in $CHCl_3$ using a Perkin-Elmer 141 polarimeter.

## RESULTS AND DISCUSSION

Three initiators have been used for initiation of (*S*)-$\beta$-butyrolactone polymerization.

The preparation of the first one was based on discoveries of Dye [13] and Edwards [14] concerning the dissolution of alkali metals: potassium or sodium in an aprotic solvent, such as THF, containing a macrocyclic organic ligand e.g. 18-crown-6 or cryptand [2.2.2]. The specific procedure (see Materials and Methods) enabled the preparation of an unique alkali metal supramolecular complex forming in THF solution an alkali metal ion pair, e.g. $K^+$,L/$K^-$ (where L = 18-crown-6) (Fig. 1).

Such alkali metal ion pairs are capable of two electron transfer from the potassium anion towards a suitable substrate, e.g. $\beta$-butyrolactone with formation of a respective carbanion (Scheme 2). The strong tendency to two electron transfer is due to the unusual oxidation state of potassium anion bearing on its outer *s* orbital two labile electrons shielded from the positive potassium nucleus by inner orbitals. Using *S*-enantiomer of $\beta$-butyrolactone as a monomer and potassium supramolecular complex as catalyst, enolate carbanion is formed as the first reactive intermediate which induces polymerization, yielding poly-(*R*)-3-hydroxybutanoate [15,16]. Direct evidence for two electron transfer from the supramolecular complex to the monomer is provided by $^{39}$K NMR (Fig. 1). The resulting biomimetic polyester has the structure similar to native PHB produced in nature, except for acetoxy-end-groups (Scheme 3) which are formed instead of the hydroxyl ones typical for natural PHB.

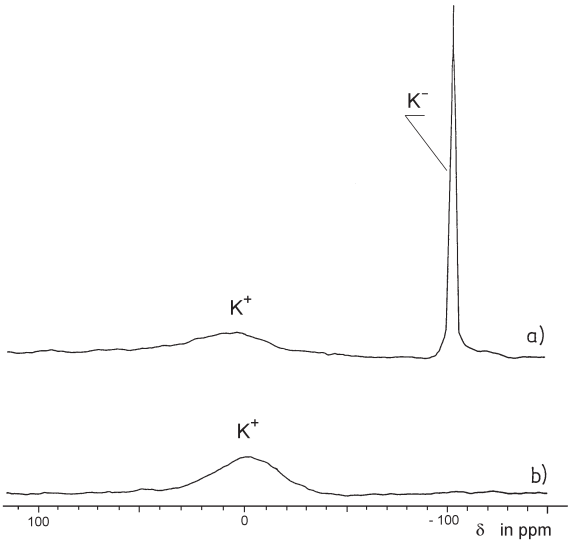
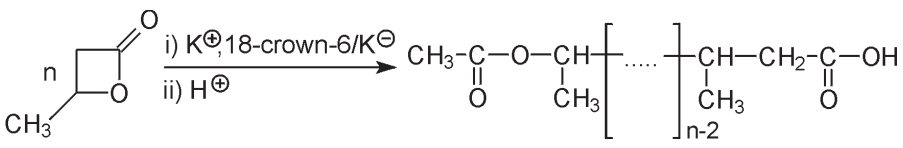


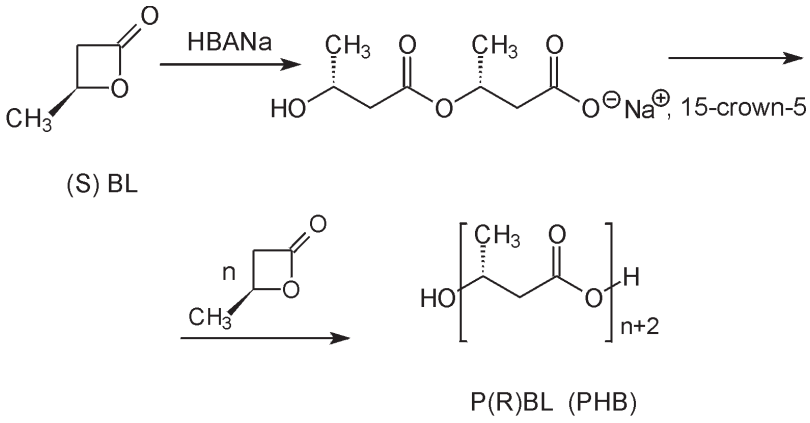

**Figure 1. a) $^{39}$K NMR of the potassium ion pair with 18-crown-6 in THF solution before a reaction; b) $^{39}$K NMR of this solution after a reaction.**

Considering the fact that even small structural defects can change the bioactivity of a biopolymer we have been looking for another regioselective initiator which would be able to produce poly-(*R*)-3-hydroxybutyrate bearing




**Scheme 3. Synthesis of biomimetic PHB using potassium supramolecular complex as initiator.**

only –OH and –COOH end groups typical for natural PHB. It turned out that sodium salt of (*R*)-3-hydroxybutanoic acid activated by added crown ether can act as a very effective




**Scheme 4. Synthesis of biomimetic PHB using ((*R*)-3-hydroxybutanoic acid sodium salt/15-crown-5) complex as initiator.**

initiator inducing polymerization of (*S*)-$\beta$-butyrolactone. The hydroxybutanoate anion of the initiator attacks the chiral carbon atom of the monomer, as it is usual in ring-opening reactions of $\beta$-lactones induced by carboxylate anions [17] (Scheme 4). The structure of synthetic biomimetic PHB is identical as that of the natural polymer.

The artificial model of a cell membrane was prepared using biomimetic PHB with the calcium polyphosphate (poly-P) complex incorporated into lipid bilayers of 1,2-dierucoylphosphatidylcholine. It was found that PHB poly-P channels show high conductance for $Ca^{2+}$ and $Na^{+}$ cations [12].

Thus the low molecular weight PHB-polymer (19–20 monomer units) can be used effectively for preparation of artificial ion channels mimicking natural ones. The model of channels proposed by Reusch [18] and depicted in Fig. 2, contains two helices: the outer one containing poly-(*R*)-3-hydroxybutanoate, complexed by hydrogen bonding with the inner helix of poly-P.

PHB polymers containing bioactive groups, e.g. $\beta$-lactame moieties characteristic for $\beta$-lactame antibiotics have also been prepared [19] (Scheme 5).

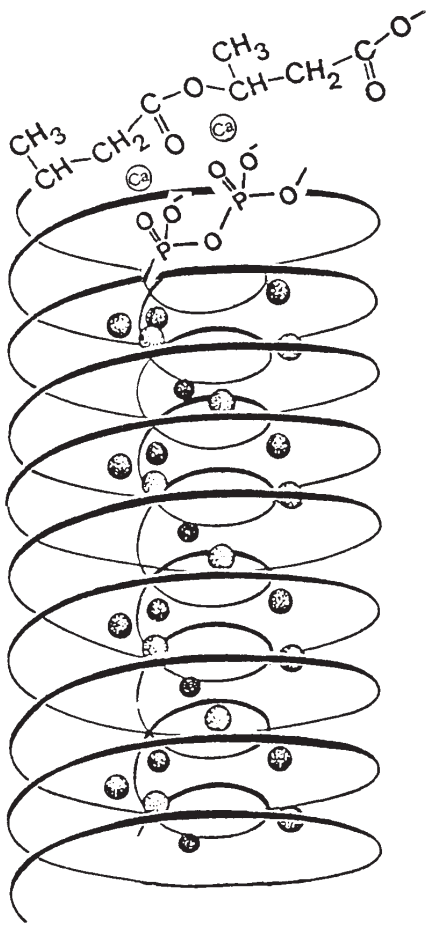
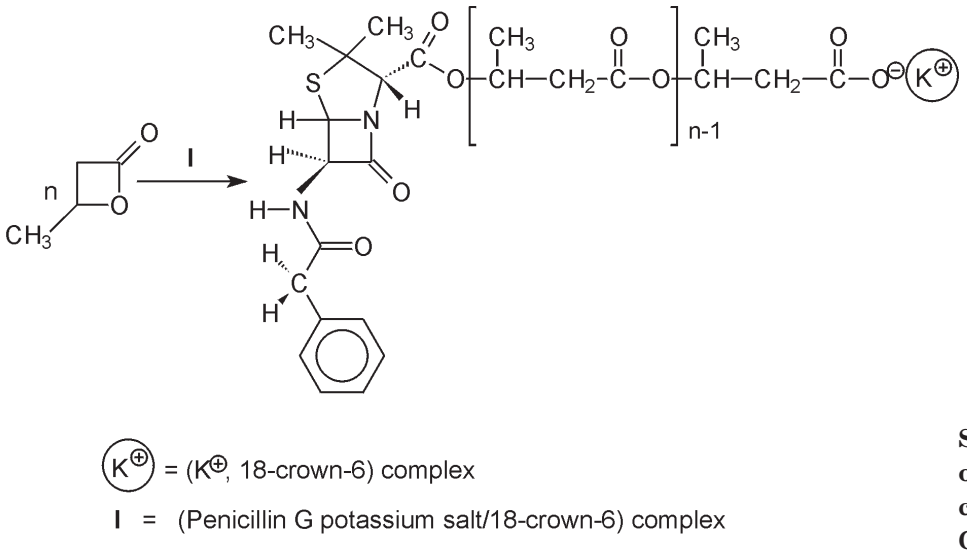


**Figure 2. Model of channels formed by the complex of biomimetic low molecular PHB with calcium polyphosphate.**




**Scheme 5. Synthesis of biomimetic PHB containing penicillin G units.**

Further studies on possible usefullness of such polymers for preparation of drugs showing prolonged activity are under way.

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