First Facile Synthesis of Biomimetic Poly-(*R*)-3-hydroxybutyrate via Regioselective Anionic Polymerization of (*S*)-*β*-Butyrolactone

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Poly-(*R*)-3-hydroxybutyrate (PHB), a natural biopolymer, is produced by a great variety of microorganisms as intracellular carbon and energy storage material.[1,2] The low molecular weight PHB (100−150 units) forms channels in membranes of prokaryotic and eukariotic cells and is present in human blood plasma; thus, it obviously plays an important role in life processes.[3−5] Therefore, significant attention has been paid to the studies on the subtle structure of the native natural PHB and its synthetic analogues. The natural PHB, isolated from bacterial cells consists entirely of *R* units, and exhibits a high degree of crystallinity although it is completely amorphous in native granules, e.g. those of *Bacillus megaterium*.[5,6] PHB granules in native bacterial cells contain 97.7% PHB together with 1.9% proteins and 0.4% lipids, the latter presumably forming coatings in granules around pure PHB core.[6] It is assumed that such coatings slow polymer crystallization because the tendency to nucleation within granules is too small and crystallization is retarded. In the course of granules isolation from bacterial cells by all, even the mildest techniques as extraction with organic solvents, centrifugation, etc., instant polymer crystallization has been observed due to the removal of coatings from granules.

We report on the facile synthesis of PHB exhibiting almost identical structure with natural PHB isolated from bacterial cells. This synthetic biomimetic polymer would be useful as a model for preparation of synthetic PHB granules, as well as membrane channels mimicking natural ones. Such experiments would enable a better understanding of the role of PHB in life processes and its potential utility for various applications.

A great deal of research has been performed on the synthesis of PHB analogues via polycondensation procedure with various titanium and tin catalysts. However, low molecular weight polymers showing unsaturated end groups and rather broad molecular weight distribution were produced.[7] Seebach and his group[8,9] developed an elegant multistep condensation strategy starting from (*R*)-3-hydroxybutyric acid using protecting groups at each individual condensation step. This method yielded linear (up to a molecular weight of 11 000) and cyclic (*R*)-3-hydroxybutyric acid oligomers and was very laborious and time-consuming.

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Alternatively to the polycondensation procedure, PHB oligomers and polymers have been synthesized via ring-opening polymerization of *β*-butyrolactone initiated by various coordinative catalysts.[10−13] However, the polymers obtained exhibited polymodal molecular weight distribution, and their molecular architectures, as well as end groups, were different from those present in the natural poly-(*R*)-3-hydroxybutyrate.

Looking for suitable catalysts, we have used common anionic initiators such as alkali metal alkoxides and alkali metal carboxylate salts. However, *β*-butyrolactone is not polymerized by these initiators in contrast to other unsubstituted four-membered *β*-lactones. It is known that in some nucleophilic substitution reactions activation of an anion is necessary in order to enhance the rate of a given reaction.[14] Thus we have activated the above-mentioned anionic initiators by addition of a macrocyclic ligand, e.g., crown ether. Under such conditions the polymerization of *β*-butyrolactone could be accomplished. Due to the inversion of configuration from (*S*)-*β*-butyrolactone, which was used as the monomer, poly(*R*)-3-hydroxybutyrate was obtained. The structure of this polymer was similar to that of microbial PHB except for the presence of crotonate end groups.[15] The polymerization of *β*-butyrolactone via electron transfer from the catalyst consisting of alkali metal supramolecular complexes; e.g., K^+/18-crown-6, K^- yields polymers with similar architecture, however bearing acetoxy end groups.[16,17]

Considering the fact that even small structural defects, such as unsaturated crotonate or acetoxy end groups, can significantly change the biochemical behavior of a biopolymer, we have been looking for another regioselective initiator able to produce poly-(*R*)-3-hydroxybutyrate bearing only −OH and −COOH end groups which are typical for natural PHB. For this reason, the ability of the sodium salt of (*R*)-3-hydroxybutyric acid activated by a crown ether to function as an initiator has been examined. The experimental results have shown that the polymerization of (*S*)-*β*-butyrolactone with this initiator, performed in bulk phase or in an organic solvent, proceeds regioselectively with inversion of configuration, yielding poly-(*R*)-3-hydroxybutyrate with the almost identical structure and end groups as present in natural PHB isolated from bacterial cells.

The hydroxybutyrate anion of the initiator attacks, as is usual in ring-opening reactions of *β*-lactones induced by carboxylate anions, the chiral carbon atom of the monomer implying alkyl-oxygen bond scission with inversion of configuration at the chiral carbon atom (Scheme 1). The polymer chain growth proceeds entirely via carboxylate anions, and polymers formed bear hydroxy and carboxy end groups (Figure 1). A very small amount of crotonate unsaturated end groups, evidenced by ESI−MS spectroscopy, is negligible.

Consequently linear monodisperse optically active poly-(*R*)-3-hydroxybutyrate oligomers and polymers (Table 1) are formed with the use of this supramolecular complex of hydroxybutyric acid salt as the initiator.[18]

The molecular weight of resulting linear polymers depends on the monomer-to-initiator molar ratio, so that oligomers and polymers of desired molecular weights (up to 20 000) can be synthesized. The molecular weight

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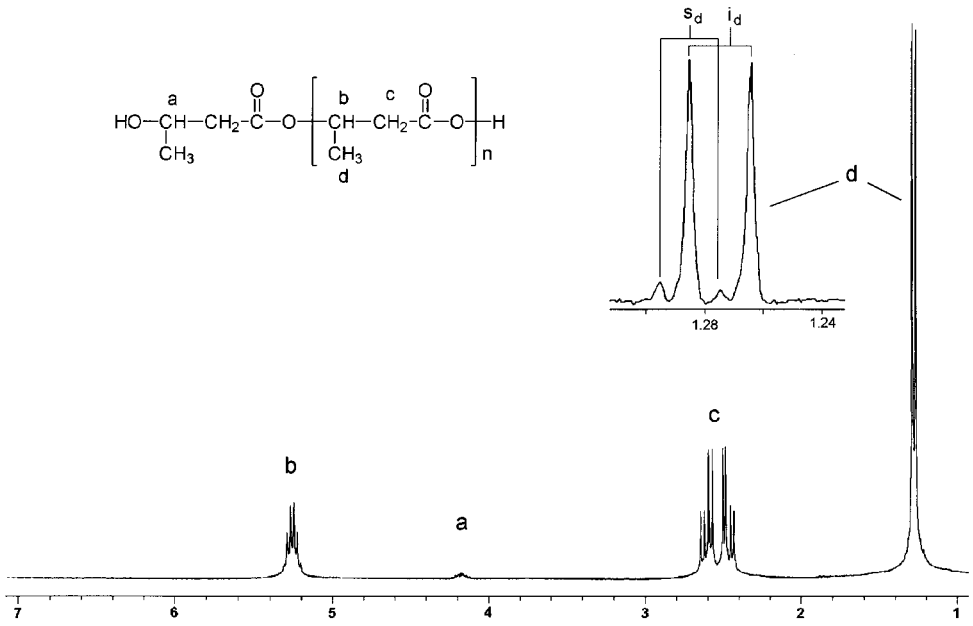
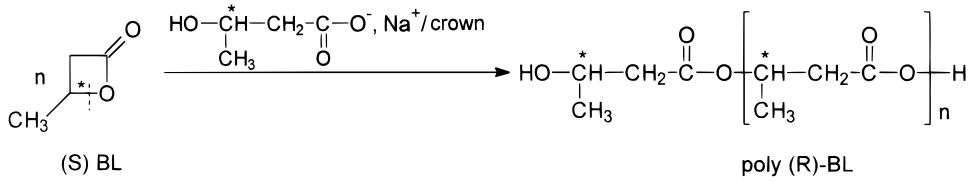


Figure 1. ^{1}H NMR spectrum of poly-(*R*)-3-hydroxybutyric acid obtained with 3-hydroxybutyric acid sodium salt/18-crown-6 as initiator; $M_n = 2000$. In the expanded methyl group region, the signals corresponding to *iso* (i_d) and *syndio* (s_d) diads are visible.

Scheme 1



crown = 15-crown-5 or 18-crown-6

Table 1. Results of Anionic Polymerization of (*S*)-*β*-Butyrolactone[a] at Room Temperature Initiated with (*R*)-3-Hydroxybutyric Acid Sodium Salt in the Presence of a Crown Ether[b]

entry no.	solvent	yield (%)	M_n calcd	M_n found	M_w/M_n	% isotactic diads[c]	$[\alpha]^{25}_{365}$[d] (deg)
1	THF	89	2 100	2 000	1.30	95	+7.0
2	DMF	91	9 800	7 900	1.28	94	+6.5
3		94	10 800	8 100	1.30	95	+7.1
4	$CHCl_3$	96	11 800	10 500	1.01	95	+6.9
5		2					

[a] (*S*)-enantiomer content 97.5%. [b] Entries 1−3 with 18-crown-6, entry 4 with 15-crown-5, and entry 5 without crown ether. [c] Estimated from the intensities of the methyl group signals by ^{1}H NMR; calculated content of isotactic diads is 95%. [d] Concentration of polymer $c = 0.028$ g/cm^3 in $CHCl_3$.

distribution is relatively narrow ($M_w/M_n \sim 1.10$), which is indicative of the uniformity of obtained polymers. The obtained synthetic biopolymers are entirely isotactic (Figure 1) and crystalline.

The observation that both synthetic PHB and PHB extracted from bacteria cells display a high degree of crystallinity whereas PHB existing in the form of granules in bacteria cells in vivo is amorphous provides indirect evidence for the hypothesis that lipids and proteins in living cells inhibit the crystallization of native PHB.[6]

In summary, a novel facile method of regioselective synthesis of biomimetic oligomers and polymers analogous to natural PHB polyester produced by enzymes in living organisms is presented. The method is based on the regioselective ring-opening polymerization of (*S*)-*β*-butyrolactone, which proceeds with the inversion of configuration [(*S*)→(*R*)] and can be accomplished using the specific supramolecular complex of (*R*)-3-hydroxybutyric acid sodium salt as initiator.

The described synthesis of biomimetic poly-(*R*)-3-hydroxybutyrate yields polymer analogues to natural PHB produced in eukariotic and prokaryotic living organisms. This synthetic analogue of natural PHB could be used for the preparation of the models of artificial channels in cell membranes, which may be used for studies of transport phenomena across biomimetic membranes. This synthetic biomimetic polymer could be also employed for preparation of drug delivery systems. The presented polymerization reaction and its application to PHB total synthesis is an attempt to emulate nature.

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(18) Initiator, (*R*)-3-hydroxybutyric acid sodium salt, was put into the dried reactor containing a glass-covered stirring bar in an atmosphere of dry argon. Next the mixture of (*S*)-*β*-butyrolactone and crown ether (15-crown-5 or 18-crown-6) or its solution in DMF or $CHCl_3$ were added. The monomer conversion followed by FTIR 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), was almost quantitative. Obtained polymers were characterized by GPC, ^1H NMR, ESI−MS, and optical rotation measurements. ESI−MS experiments were carried out using the Finnigan MAT TSQ 700 triple stage quadruple mass spectrometer equipped with an electrospray ion source. Samples were dissolved in methanol or in $CHCl_3$ at a concentration of 0.5 mg/mL and introduced into the electrospray interface at a flow rate of 2 μL/min. The potential difference between the needle and the electrospray chamber was 4.5 kV. The capillary temperature was 250 °C. Mass spectra were acquired over the range of $m/z = 50-2000$.

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