

Physicochemical Properties of Multicomponent Poly(hydroxyalkanoates)

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Received April 27, 2006

Abstract—The properties of new five-component poly(hydroxyalkanoates) (PHA) formed by short- and medium-chain monomers synthesized by the bacterium *Wautersia eutropha* B5786 were studied by X-ray diffraction, IR spectroscopy, differential thermal analysis, and viscometry. The degree of crystallinity of PHA decreased from 72 to 57% as the molar fraction of hydroxyhexanoate increased from 2.5 to 18.0 mol%. The melting temperature (T_m) and decomposition temperature (T_d) of the multicomponent PHA are lower than those for poly(hydroxybutyrate), whose T_m and T_d are 168–170 and 260–265°C, respectively. Both parameters of the multicomponent PHA decrease to 156 and 252°C, respectively, as the hydroxyhexanoate mole fraction is raised. The effect of hydroxyhexanoate on the physicochemical properties of the PHA is similar to that of hydroxyvalerate observed previously.

DOI: 10.1134/S0006350907030062

Key words: poly(hydroxybutyrate), hydroxyhexanoate, hydroxyvalerate

INTRODUCTION

Analysis of literature data indicates fast development in the synthesis and study of polymers based on carboxylic acid derivatives. Poly(hydroxyalkanoates) (PHA) produced by microorganisms are of special interest as biodegradable polyesters, along with poly(lactides) and poly(glycolides). The known PHA include substances of various structure from highly crystalline thermoplastics to thermolabile rubber-like elastomers [1, 2]. There are three groups of PHA according to the number of carbons in the monomer: short-chain (C_3 – C_5 , PHA_{SC}), medium-chain (C_6 – C_{14} , PHA_{MC}), and long-chain ($>C_{17}$, PHA_{LC}) [3]. The most valuable property of PHA is the possibility to make polymers of different composition, i.e., to control their properties. However, this requires basic knowledge on the features of PHA synthesis and the effect

of chemical structure and composition on the polymer physicochemical properties.

Short-chain PHA, poly(hydroxybutyrate) (PHB) and hydroxybutyrate–hydroxyvalerate copolymers, are the best studied. Active studies of PHA_{MC} and PHA_{LC} have started recently, and very little is known about multicomponent PHA of a new type consisting of short- and medium-chain monomers (PHA_{SC+MC}) [1, 2].

It should be noted that the synthesis of multicomponent PHA is a complex biotechnological problem. To solve the problem, a number of factors should be taken into account: (1) high yields of PHA, except for *Alcaligenes latus*, are impossible in fast-growing cultures, therefore special conditions are necessary to provide the high yields of polymers at total high biproductivity of culture with respect to biomass; (2) the rates of synthesis differ for monomers of different chain length, this results in the instability of monomer ratio during the incubation of microorganisms; (3) carboxylic acids used as a co-substrate are toxic for

Abbreviations: PHA, poly(hydroxyalkanoates); SC, short-chain; MC, medium-chain; LC, long-chain; PHB, poly(hydroxybutyrate); DTA, differential thermal analysis; TG, thermogravimetry; DTG, derivative thermogravimetry.

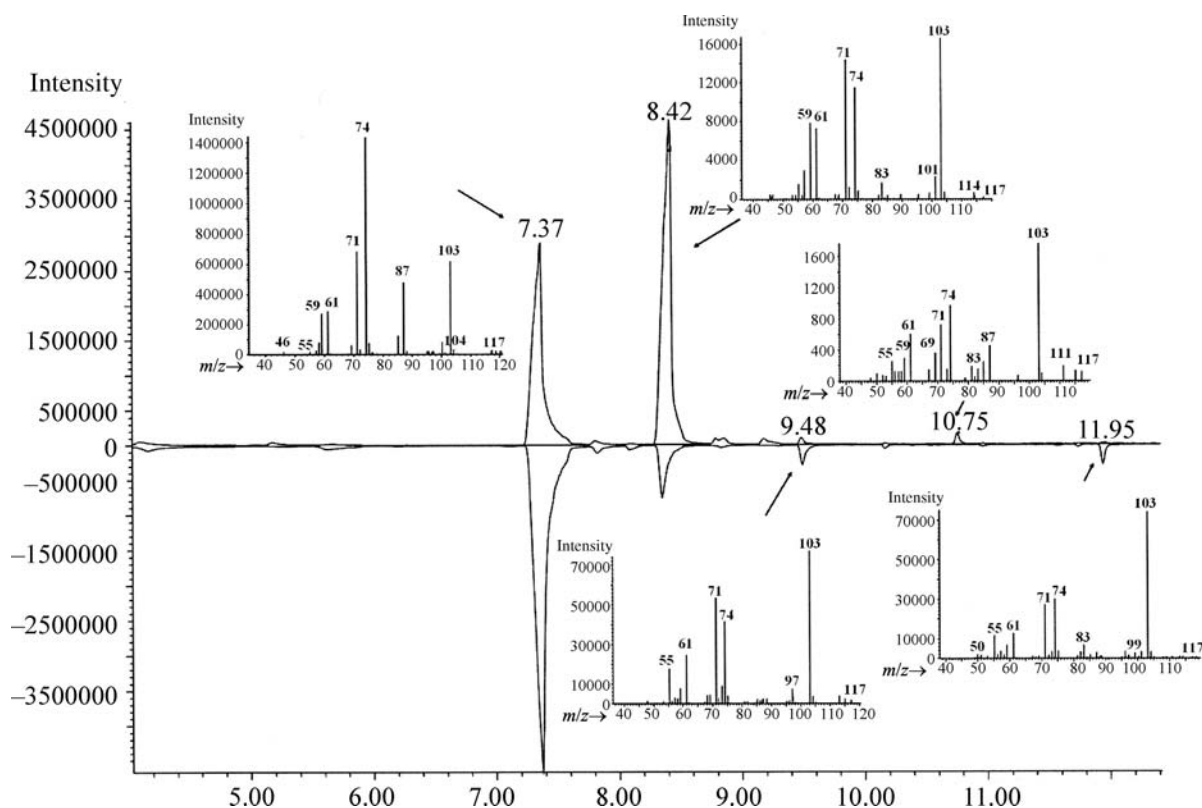


Fig. 1. Ion chromatograms of two samples of multicomponent PHA of different composition synthesized by *W. eutropha* in a medium with addition of heptanoic (up) and octanoic acid (mirror down) and the mass spectra of monomers: hydroxybutyrate, hydroxyvalerate, hydroxyhexanoate, hydroxyheptanoate, and hydroxyoctanoate with retention times 7.37, 8.42, 9.48, 10.75, and 11.95, respectively.

the majority of bacterial cultures, consequently, the admissible concentration must be determined for each co-substrate for each specific producer. Therefore it is very difficult to obtain multicomponent PHA in dense cultures at high PHA content in cells (above 50–60%) where medium- and long-chain monomers are included as the main monomers.

The bacterium *Wautersia (Ralstonia) eutropha* [4] is the most promising producer of PHA because it synthesizes polymers of different chemical structure (PHA_{SC} and PHA_{SC+MC}) in high yields (up to 80–90%) on different substrates (mixtures of hydrogen and carbon dioxide, sugars, organic acids, alcohols, factory and farm waste).

The aim of this work was to compare the physicochemical properties of new multicomponent PHA comprising short- and medium-chain monomers.

EXPERIMENTAL

We studied PHA samples produced by the bacterium *Wautersia eutropha* B5786 by the technology

developed in the Institute of Biophysics [5], adding valerate, hexanoate, heptanoate, and octanoate (all from Sigma) as co-substrates. PHA were extracted from bacterial biomass with chloroform and precipitated with hexane. The chemical structure of purified PHA samples was determined after methanolysis of samples to form methyl esters of fatty acids, using a Hewlett Packard GC–mass spectrometer.

The molecular weight (M_r) of the polymers was determined using an Ubbelohde viscometer with capillary diameter of 0.34 mm at 30°C. PHA concentrations in chloroform solutions (C , g/l) were 0.25–1.0. Relative (η_{rel}), specific (η_{sp}) viscosity, and η_{sp}/C ratio were calculated from the experimental data of outflow time for the solvent and the studied polymer solutions of different concentrations. The dependence of η_{sp}/C ratio on polymer concentration (C) was plotted. The value of intrinsic viscosity $[\eta]$, which is proportional to M_r , was obtained by extrapolating the results to $C = 0$.

To study the thermal characteristics and the degree of crystallinity, we used PHA samples as films

obtained by casting from solutions. The thermal characteristics were measured on a MOM derivatograph (Hungary) with simultaneous recording of differential thermal analysis (DTA), thermogravimetry (TG), and derivative thermogravimetry (DTG) curves. The samples of 0.1-mm-thick films made of PHA of different composition were placed in platinum crucibles; the analysis was carried out in inert gas atmosphere in the temperature range from 20 to 300°C at a heating rate of 5°/min. The temperatures of melting and decomposition were determined as the temperatures of corresponding endothermic maxima. The error in the determination of endotherms from derivatograms was $\pm 1^\circ\text{C}$.

The x-ray diffraction study of films obtained from PHA samples of different composition was performed with a Bruker Avance D8 diffractometer (graphite monochromator on reflected beam). To determine the degree of crystallinity C_x , the measurements were made in a scan-step mode with a 0.04° step and 2-s exposure for measuring intensity at a point (instrument operating mode was 40 kV \times 40 μA).

The IR absorption spectra of PHA films were recorded with an Infralyum FT-02 Fourier-transform infrared spectrometer (Lyumeks, Russia).

RESULTS AND DISCUSSION

The chemical structure of a number of studied PHA samples and certain properties thereof are shown in the table. We have studied five-component PHA samples containing hydroxyhexanoate along with hydroxybutyrate and hydroxyvalerate as main monomers, minor components were hydroxyheptanoate and hydroxyoctanoate. The multicomponent PHA of such a type were obtained using a natural strain of *W. eutropha* for the first time. Figure 1 exemplifies chromatograms of five-component PHA of different composition containing short- and medium-chain monomers.

The determination of physicochemical properties of five-component polymers comprising mainly short-chain hydroxybutyrate (31–93 mol%) and hydroxyvalerate (40–68 mol%) with a small content of medium-chain hydroxyhexanoate (0.07–0.34 mol%), hydroxyheptanoate (0.09–0.33 mol%) and hydroxyoctanoate (0.06–0.10 mol%) (samples 1–5 and 11–12, table) confirms previous results indicating that

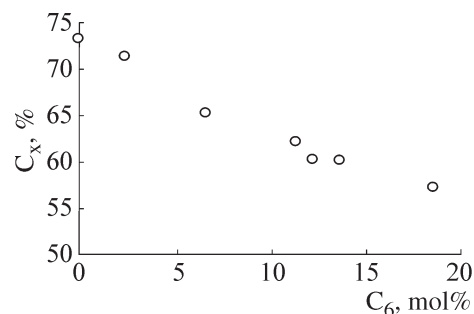


Fig. 2. The dependence of the degree of crystallinity of multicomponent PHA on the fraction of hydroxyhexanoate.

the inclusion of hydroxyvalerate brings about a considerable redistribution of amorphous and ordered phases in the polymer to decrease the degree of crystallinity. However, these changes are typical only of hydroxyvalerate content from 5 to 30 mol% because further growth of hydroxyvalerate fraction does not alter the C_x value. Indeed, PHA samples containing 47–68 mol% of hydroxyvalerate have C_x values about 50%. The presence of medium-chain monomers

Composition of multicomponent PHA synthesized by *W. eutropha* and containing hydroxybutyrate (C_4), hydroxyvalerate (C_5), hydroxyhexanoate (C_6), hydroxyheptanoate (C_7), and hydroxyoctanoate (C_8)

Sample no.	PHA composition, mol%				
	C_4	C_5	C_6	C_7	C_8
1	31.22	68.46	0.13	0.16	0.04
2	36.01	63.64	0.12	0.21	0.02
3	49.50	50.01	0.34	0.09	0.06
4	52.25	47.33	0.09	0.33	none
5	59.36	40.48	0.07	0.09	0.1
6	77.41	4.3	18.0	none	0.29
7	81.81	3.69	13.8	none	0.74
8	88.38	4.47	6.63	none	0.52
9	90.63	2.29	6.52	none	0.56
10	91.7	4.6	2.5	none	1.2
11	41.94	56.30	0.71	0.98	0.06
12	93.31	3.34	2.38	none	0.97
13	100	none	none	none	none

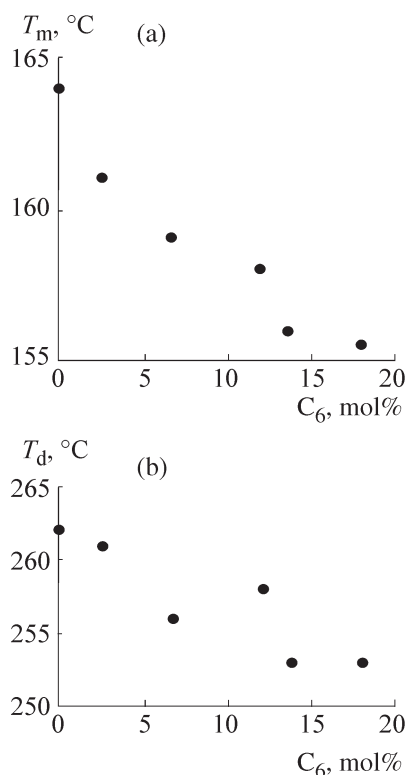


Fig. 3. The dependence of the melting temperature (a) and decomposition temperature (b) of multicomponent PHA on the fraction of hydroxyhexanoate.

(C_6 – C_8) at fractions of a percent (table) did not influence the degree of PHA crystallinity. Our previous study of three-component samples of PHA also showed that the inclusion of hydroxyhexanoate in concentration up to 2.4 mol% has no effect on the ratio of amorphous and crystalline phases [6].

It was most interesting to study the samples of five-component PHA containing appreciable amounts of hydroxyhexanoate in addition to hydroxybutyrate and hydroxyvalerate. It should be noted that the studies of PHA formed by short- and medium-chain monomers began only recently, after several research groups prepared such polymers. The studies of hydroxybutyrate–hydroxyhexanoate copolymers synthesized by natural and genetically-modified producers were reported recently [7–9], while a family of multicomponent PHA containing hydroxyhexanoate and hydroxyoctanoate units in addition to hydroxybutyrate fragments was obtained [10] using biosynthesis and chemical synthesis. The authors of this work showed that an increase of the portion of medium-chain monomers in PHA decreases the melting temperature and the degree of crystallinity.

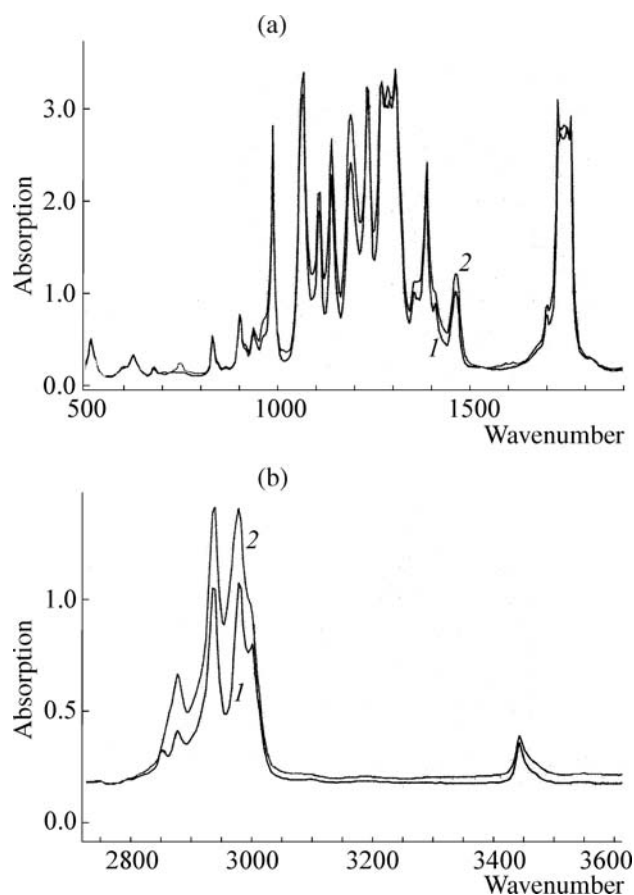


Fig. 4. The IR spectra of PHB (1) and multicomponent PHA with 18 mol% of hydroxyhexanoate (2) in the ranges 500–1800 cm^{-1} (a) and 2700–3600 cm^{-1} (b).

The analysis of x-ray diffractograms of the five-component PHA shows a clear C_x vs. C_6 dependence (Fig. 2): as the hydroxyhexanoate content increases from 2.5 to 18.0 mol%, the degree of crystallinity of PHA decreases monotonically from 74 to 57%. Thus, incorporation of hydroxyhexanoate equalizes the ratio of amorphous and crystalline phases in PHA, similarly to hydroxyvalerate. This makes the material less crystalline and more suitable for processing.

The derivatograms reveal two distinct endothermic effects within the positive temperature range studied, with transition intervals about 20°C. The first endotherm causes no change in molecular weight and is identified as melting of the crystalline fraction of the polymer. The second endotherm is detected within the temperature range of complete thermal decomposition of the polymer. DTA, TG, and DTG curves show no appreciable signs of inhomogeneity of the samples or the presence of fractions with diverse

melting and decomposition temperatures or different rates of thermal decomposition, suggesting good compatibility of components in the formulation. Both the melting and the decomposition temperature decrease from 163 and 263 to 156 and 252°C, respectively, as the hydroxyhexanoate fraction increases (Figs. 3a, 3b). It should be noted that the change in T_m and T_d is virtually the same; i.e., the temperature difference between polymer melting and thermal degradation is retained, which is very important for the thermal processing of PHA.

As in the previous works [6, 11], the spread of the M_r values for the studied series of multicomponent PHA was found very significant, from 150–200 to 450–800. No relation between the chemical structure of PHA, the presence of any monomer, and M_r was revealed. The molecular weight of PHA is known to be a very variable parameter affected by many factors including the source of carbon for bacteria, duration of cultivation, and polymer extraction procedure.

We used IR spectroscopy to reveal the possible structural differences in the PHA samples of different composition. The IR spectra of the series of samples (table) were recorded in the range of 500–4500 cm^{-1} . The IR absorption spectra of multicomponent PHA with different ratio of SC and MC monomers showed identity even in small detail, with absorption bands corresponding to the vibrations of the main structural units of PHA, except for those of terminal C–OH and COOH groups. All the spectra showed absorption from the asymmetric stretching vibrations of CH_3 and CH_2 groups (2994, 2974, 2936 cm^{-1}), symmetric stretching vibrations of CH and CH_2 groups (2874, 2878 cm^{-1}), and stretching vibrations of carbonyl groups C=O (1700–1760 cm^{-1}). Some difference was revealed upon comparison of the spectra of the PHB homopolymer with that of a five-component PHA richest in hydroxyhexanoate (18 mol%). Figure 4 shows detailed spectra as fragments expanded along the wavenumber axis in the ranges 500–1800 cm^{-1} (Fig. 4a) and 2800–3000 cm^{-1} (Fig. 4b). The increase in the intensity of absorption bands in the short-wavelength region (2800–3000 cm^{-1}) is the most typical distinction of the IR spectra of multicomponent PHA with 18 mol% of hydroxyhexanoate from the spectra

of 100% PHB. This feature is most probably due to the increased share of stretching vibrations of C–H and CH_2 groups in PHA samples with a large content of hydroxyhexanoate.

ACKNOWLEDGMENTS

This work was supported by the United States Civilian Research and Development Foundation (CRDF) and by the Ministry of Education of the Russian Federation (grant no. PG05-002-1), the Russian Foundation for Basic Research (project no. 05-04-08024ofi-a), and the Program of Interdisciplinary Integration Projects of the Siberian Division of the RAS (projects nos. 24 and 54).

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