Analytica Chimica Acta 808 (2014) 104–114

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

Molecular level structure of novel synthetic analogues of aliphatic biopolyesters as revealed by multistage mass spectrometry

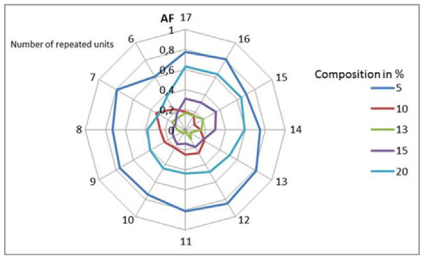
Grażyna Adamus[a], Iwona Kwiecień[a], Magdalena Maksymiak[a], Tomasz Bałakier[b], Janusz Jurczak[b], Marek Kowalczuk[a,*]

[a] Centre of Polymer and Carbon Materials, Polish Academy of Sciences, 41-819 Zabrze, Poland
[b] Institute of Organic Chemistry, Polish Academy of Sciences, 01-224 Warsaw, Poland

HIGHLIGHTS

- We examine the molecular structure of novel PHA analogues by ESI-MS^n.
- Sequence distribution of low molecular mass copolyesters has been determined.
- ESI-MS^n approach for structural studies of random copolyesters is described.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 16 May 2013
Received in revised form 28 August 2013
Accepted 3 September 2013
Available online 6 September 2013

Keywords:
Polymer mass spectrometry
Anionic copolymerisation of β-substituted β-lactones
Copolymer sequence distribution
Polyhydroxyalkanoate analogues

ABSTRACT

The present study focuses on electrospray ionisation (ESI) tandem mass spectrometry of novel copolyesters obtained by anionic ring-opening copolymerisation of β-substituted β-lactones. Detailed analysis of these copolyesters, including molecular chain architecture as well as the structures of the end groups, was performed using ESI-MS/MS collision-induced dissociation spectra. The random arrangement of comonomeric units along the copolyester chains was demonstrated by comparison of ESI-MS^n fragmentation spectra and fragmentation pathways. Sequence distribution analysis of comonomeric units confirmed the copolymer's random structure. ESI-MS^n proved to be a promising technique for structural analysis of copolyesters obtained *via* anionic ROP.

1. Introduction

Mass spectrometry has become a comprehensive analytical technique for copolymer characterisation. Possessing much more complex structure than homopolymers, copolymers can exhibit a variety of comonomer arrangements along the macromolecular chain, such as block, gradient, random or alternating copolymers. The molecular mass distribution (MMD) and comonomer composition distribution (CCD) of copolymers influence their physical properties for a given chemical composition. Soft ionisation techniques such as matrix-assisted laser desorption/ionisation (MALDI) and ESI are suitable for MS copolymer characterisation, in conjunction with nuclear magnetic resonance (NMR) spectrometry. The application of these MS techniques for characterisation of various copolymers has been reviewed recently [1].

* Corresponding author. Tel.: +48 32 271 60 77; fax: +48 32 271 29 69.
E-mail addresses: cchpmk@poczta.ck.gliwice.pl, marek.kowalczuk@cmpw-pan.edu.pl (M. Kowalczuk).

0003-2670/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.aca.2013.09.001

In our systematic studies of the molecular structures of aliphatic biopolyesters (polyhydroxyalkanoates, PHA) and their synthetic analogues, the ESI-MS fragmentation technique is preferred because with this technique each comonomer shows distinct fragmentation pathways along the chain. This method has been successfully applied to the characterisation of several copolymer systems, including microbial and synthetic aliphatic copolyesters [2–13]. It has been reported that random and diblock copolyesters derived from the same comonomeric units in the same molar ratios may be differentiated by comparing the fragmentation patterns of their molecular ions [14]. However, in some cases tandem MS (MS/MS) may be difficult due to peak overlap or low-intensity parent ions caused by large differences in comonomer composition. In such cases, the sequence distribution of the copolymer chains can be determined from the relative intensities of the detected molecular anions. The experimental values are compared to the theoretical ones (*e.g.*, calculated according to Bernoullian chain statistics) for copolymers of similar composition but with randomly distributed comonomers [2]. This approach was previously applied successfully to ESI-MS analyses of the composition and sequence distribution of various bacterial copolyesters and their mixtures [3,5,15]. Moreover, the sequence distributions of comonomer units in microbial PHA copolyesters inferred from ^{13}C NMR spectroscopy using dyad and triad analysis were comparable to those derived from ESI-MS [15].

An understanding of copolymer formation mechanisms, and a detailed description of the individual reactions taking place during initiation, propagation, and termination of chain growth, are essential to relate copolymer structure, properties, and function. This is of particular importance in the case of ring opening (co)polymerisation (ROP) of cyclic esters because (co)polymers prepared in this way are extremely useful, particularly for biomedical and pharmaceutical applications. For such requirements, MS offers new opportunities, enabling the elucidation of novel polymerisation mechanisms or the verification of proposed mechanistic pathways [16,17].

Here, we report the comprehensive ESI-MS analysis of novel, potentially random synthetic PHA copolymers prepared *via* anionic ROP of β-butyrolactone with comonomeric β-substituted β-lactones containing aliphatic or aromatic moieties. The comonomeric β-lactones were synthesised by carbonylation of the respective epoxides under CO at ambient pressure [18]. Developed in our laboratories, anionic ROP of β-substituted β-lactones appears to be a perfect tool for preparation of PHA analogues of a desired structure, including the structure of the end groups [19,20]. However, the influence of the structure of the β-substituted monomer on the formation of these copolymers requires clarification. Mass spectrometry can be of particular help in such cases, as we will show in this study.

2. Experimental part

2.1. Materials

The (*R,S*)-β-butyrolactone (**1**), Sigma–Aldrich Chemie GmbH, Steinheim, Germany, was distilled over CaH_2, and the fraction boiling at 56 °C (9 mmHg) was collected. Racemic β-butoxymethylpropiolactone (**2a**), β-phenoxymethylpropiolactone (**2b**) and β-benzoxymethylpropiolactone (**2c**) were prepared by carbonylation of the respective oxiranes using the catalyst and conditions previously reported in Ref. [18] and were characterised by NMR and IR [18]. Tetrabutylammonium acetate ($AcNBu_4$), Sigma–Aldrich Chemie GmbH, Steinheim, Germany, was used as received.

Table 1
Copolyesters and their characteristics.

Sample	Composition[a] [mol%]	M_n[b] [g mol^{-1}]	M_w/M_n[c]
1	3-HB/BuOHB 82/18	1200	1.18
2	3-HB/PhOHB 84/16	1000	1.20
3	3-HB/BnOHB 86:14	1100	1.21

[a] Molar composition of copolymers estimated from ^1H NMR (see Section 2).
[b] Number-average molar mass estimated by GPC.
[c] Molar mass distribution estimated by GPC.

2.2. Synthesis of poly[(R,S)-3-hydroxybutyrate)-co-(R,S)-3-hydroxy-4-butoxybutyrate] (3-HB/BuOHB), poly[(R,S)-3-hydroxybutyrate)-co-(R,S)-3-hydroxy-4-phenoxybutyrate] (3-HB/PhOHB) and poly[((R,S)-3-hydroxybutyrate-co-(R,S)-3-hydroxy-4-benzoxybutyrate] (3-HB/BnOHB) random copolyesters

The (3-HB/BuOHB), (3-HB/PhOHB) and (3-HB/BnOHB) copolyesters were synthesised by anionic ring opening copolymerisation of racemic β-butyrolactone (**1**) with β-butoxymethylpropiolactone (**2a**), β-phenoxymethylpropiolactone (**2b**) or β-benzoxymethylpropiolactone (**2c**), respectively. The reactions were initiated with tetrabutylammonium acetate ($AcNBu_4$). The initial molar composition of the mixtures of (*R,S*)-β-butyrolactone **1** and β-substituted-β-lactones **2** was the same in each reaction (*ca.* 85:15 mol%).

Copolymerisation experiments were carried out in round-bottom flasks equipped with magnetic stir bars at 23 °C. The respective amount of initiator ($AcNBu_4$) was weighed into the flask, then THF solutions of monomers **1** and **2** were added under argon. The mixture was stirred until the reaction was nearly complete. The progress of copolymerisation was followed by FTIR spectroscopy, based on the relative intensities of carbonyl group bands of the lactones (1830 cm^{-1} (**1**), 1825 cm^{-1} (**2a**), 1830 cm^{-1} (**2b**) and 1828 cm^{-1} (**2c**)) and the copolyesters (*ca.* 1740 cm^{-1}). After the completion of polymerisation, the resulting copolymers were precipitated from cold hexane and dried under vacuum.

2.3. Protonation of copolyester carboxylate end groups

Chloroform solutions of copolymers (10%, w w^{-1}) were acidified with dilute HCl(aq) and the mixtures were stirred vigorously for 10 min. After phase separation the organic layer was removed and acidified again (as described above). Then, the polymer solution was washed 10 times with 10 mL of distilled water. Next, the solvent was evaporated and the copolymer was dried under vacuum at room temperature. Final products were characterised by ^1H NMR, gel permeation chromatography (GPC) and ESI-MS techniques.

2.4. Measurements

2.4.1. FTIR spectroscopy

FTIR spectra were recorded using a Bio-Rad FTS 40A spectrometer at room temperature.

2.4.2. Nuclear magnetic resonance (NMR) spectroscopy

^1H NMR spectra were recorded at 600 MHz with a Bruker Avance II at room temperature in $CDCl_3$ with tetramethylsilane (TMS) as internal standard. The spectra were recorded with 64 scans, 2.65 s acquisition time and 11 μs pulse width.

The chemical compositions of the copolyesters obtained are listed in Table 1. These were inferred from ^1H NMR, based on the areas of the signals corresponding to —C**H**(CH_3)— at 5.25 ppm in the 3-hydroxybutyrate (HB) repeating units