Spreading of poly(β-hydroxylalkanoate)s at the air–water interface: a model system for the nascent lyotropic state of bacterial polyesters

Geoffrey A. R. Nobes* and David A. Holden†
Department of Chemistry, University of Waterloo, Waterloo, Ontario, Canada N2L 3G1

and R. H. Marchessault‡
Department of Chemistry, McGill University, Montreal, Quebec, Canada H3A 2A7
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Bacterial poly(β-hydroxybutyrate) and bacterial copolyesters of β-hydroxybutyric acid with up to 29% of β-hydroxyvaleric acid were shown to spread reproducibly from chloroform to give monolayers at the air–water interface. Typical limiting areas are 2.4 $m^2\ mg^{-1}$, corresponding to 0.35 nm^2 per repeating unit. Surface pressures of up to 36 $mN\ m^{-1}$ were reached, corresponding to packing densities of approximately 0.12 nm^2 per repeating unit. The spreading of chiral, water-insoluble biopolyesters to stable monolayers, in which a majority of the repeating units are in contact with the water subphase, has important implications for the structure of polyalkanoate inclusions.

(Keywords: bacterial polyesters; polyalkanoates; spreading)

Introduction

Bacterial poly(β-hydroxybutyrate) (PHB) and poly(β-hydroxybutyrate-*co*-β-hydroxyvalerate) (P(HB-*co*-HV)) are biopolymers synthesized by many bacteria as carbon-storage materials[1–3]. Copolymers containing up to 30 mol% β-hydroxyvalerate (HV) are currently manufactured by ICI, Billingham, UK, in a fermentation process[4], and commercial applications for these biodegradable materials are developing[5,6]. The biosynthesis of many new polyalkanoate copolymers through the use of different bacteria and growth substrates is also being reported[7,8].

One important but poorly understood aspect of polyalkanoate research concerns the structural differences between the polymer as it exists in the bacterial inclusions and after it has been isolated by chemical or biochemical techniques[9–12]. N.m.r. spectroscopic studies on whole cells showed that the polyesters in native inclusions were disordered and had more mobility than in the isolated state[12,13]. CP–MAS n.m.r. studies showed smaller differences between freeze-dried cells and solution-cast films of P(HB-*co*-HV), and in both cases the polymers displayed crystallinity[6]. A model[9] has been proposed in which polyalkanoates are formed as lyophilic colloidal inclusions in intimate association with the cellular fluid, and subsequently undergo crystallization if the associated water is removed by freeze-drying or solvent extraction and reprecipitation[6]. Differences in enzyme degradability also exist between 'never-dried' granules and 'once-dried' granules, reflecting the irreversible crystallization that results from water removal[14]. Furthermore, isolated granules show evidence of topotactic crystallization, with a hard exterior shell made of lamellar crystals surrounding the mobile core[10].

It would be advantageous to have a method for reconstituting the nascent lyotropic state of PHB and its copolymers in order to obtain greater insight into that state. Unfortunately, once it has crystallized, PHB is a crystalline thermoplastic quite resistant to moisture uptake. In the present communication we describe the spreading of PHB and P(HB-*co*-HV) to form true monolayers on water. The formation of these surface films has important implications for the affinity of polyalkanoates for water, and suggests a means by which the lyotropic state of poly(β-hydroxyalkanoate)s can be generated and studied.

Experimental

For studies of the spreading behaviour of polyalkanoates, PHB and P(HB-*co*-HV) samples were obtained from Aldrich Chemical Co. Samples of P(HB-*co*-HV) containing 4% and 29% HV units were also obtained from ICI through Marlborough Biopolymers, Billingham, UK (lot numbers BX P7/51 and F18 118/9). The synthetic poly(β-hydroxyvalerate) (PHV) was prepared using an $Al(Et)_3/H_2O$ catalyst system[15]. The compositions of all polymers were measured by ^1H n.m.r. spectroscopy on a 250 MHz Bruker instrument. *Table 1* lists the polymers studied in this work along with[2,3] viscosity average molecular weight (M_v) and melting point (T_m). X-ray diffraction on the never-dried and dried cells shows that the inclusions remain non-crystalline as long as they remain inside the cells[12,14].

Monolayer studies were conducted on a Langmuir balance which has been described previously[16]. For the present work the balance was controlled by an IBM PC-XT computer through an Intel 6522 interface. This allowed rapid sampling of the surface pressure (at a

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* Present address: Department of Chemistry, McGill University, Montreal, Quebec, Canada H3A 2A7
† Deceased
‡ To whom correspondence should be addressed

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Table 1 Properties of bacterial copolyester samples

Sample no.	HV (mol %)	Supplier, code	M_v ($\times 10^{-3}$)	T_m (°C)
1	0	Aldrich, 28,249-9	150	178
2	4	ICI, BX P7/51	113	169
3	13	Aldrich, 28,246-4	800	126
4	14	Aldrich, 28,247-2	150	138
5	20	Aldrich, 28,248-0	800	127
6	29	ICI, F18 118/9	–	86
7	100	Synthetic sample	190	112

compression rate of 0.05 nm^2 (repeating unit)$^{-1}$ min^{-1}), so that a typical surface pressure–area isotherm consisted of 1000 data points, each point representing an average of 100 surface pressure measurements recorded over about 1/3 of a second. The isotherms are plotted as continuous curves, as the separation between data points is less than the pen width of the plotter; the standard deviations of surface pressure and area are 1.0 mN m^{-1} and 0.01 nm^2 per repeat unit, respectively. Dilute (0.1 wt%) solutions of the polymers in chloroform (J. T. Baker Photrex Grade) were spread on the subphase (water purified to a resistivity of 18 MΩ cm^{-1} with a pair of Millipore filtration systems, connected in series) at 18°C by means of a gas-tight 50 μl syringe fitted with a Chaney adaptor.

Results and discussion

Figure 1 compares the spreading behaviour of PHV (A), a P(HB-*co*-HV) copolymer (B), and PHB (C) on pure water at 18°C. The isotherms for the three polymers are strikingly similar, increasing very gradually from surface areas of 0.35 nm^2 per repeat unit, corresponding to 2.4 m^2 mg^{-1}. The surface pressures increase without obvious collapse points. *Table 2* lists the average maximum surface pressures reached by the polymers studied. Typically, surface pressures of 20 mN m^{-1} are reached at areas of about 0.12 nm^2 per repeating unit, corresponding to 0.84 m^2 mg^{-1} for PHB. However, the 13 mol% HV sample reached a maximum surface pressure of 36 mN m^{-1}. By studying spreading on a slightly acidic or alkaline solution, there was no evidence that the polymers underwent ionization on the subphase.

The fact that these polyalkanoate samples form stable monolayers on a pure subphase indicates that they are able to overcome their inherent bulk cohesion and that they possess sufficient attraction for the subphase to spread. In order to form stable monolayers, every monomer segment is probably constrained to remain in the air–water interface for the duration of the experiment. From these results it is expected that the polymer exists on the surface in a random or partially ordered helical coil. Based on the average surface area of the *ac* and *bc* faces of the PHB unit cell, one can calculate a packing density of 0.19 nm^2 per repeating unit. The observed monolayer density is 0.12 nm^2 per repeating unit. These values indicate fairly close agreement between the calculated and observed packing densities. However, the molecular conformation then corresponds to a 2_1 helix. A similar calculation based on the nearly extended crystalline conformation[17] leads to a packing density of 0.22 nm^2 per repeating unit, hence the conclusion of dense packing with all repeat units in contact with the water surface appears reasonable. The nearly extended conformation would allow better hydrogen-bonding interaction with the water surface[11] and support the proposed water involvement in maintaining a non-crystalline state in the nascent morphology of PHB and P(HB-*co*-HV) granules[11–13].

A model[11] has recently been proposed wherein a 'pseudo-network' of hydrogen-bonded water molecules is formed between adjacent portions of growing polyalkanoate chains during biosynthesis. The hydrogen bonds could best form with the chains in the near-straight β conformation[17]. *Figure 2* shows a PHB polymer chain in a coiled state. Two successive 'zoom-ins' show the details of formation of the 'pseudo-network'. The results presented in this communication provide physical evidence for such a model.

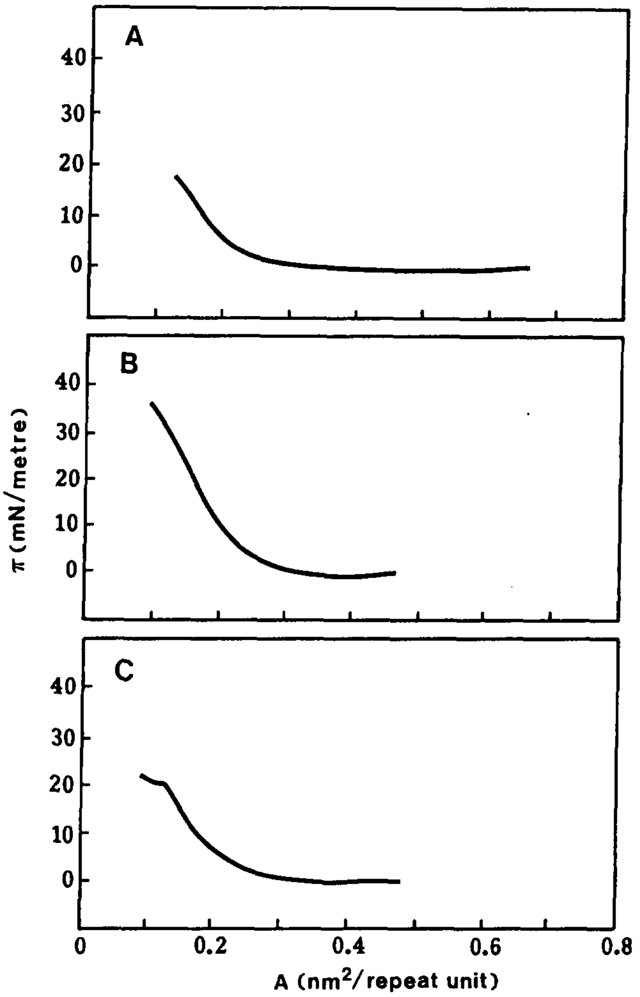

Figure 1 Surface pressure *versus* area isotherms of: (A) synthetic homopolymer of PHV; (B) bacterial copolymer of P(HB-*co*-13% HV); and (C) bacterial homopolymer of PHB

Table 2 Spreading behaviour of bacterial copolyester samples

Sample no.	HV (mol %)	Max. surface pressure (mN m^{-1})
1	0	22.6
2	4	18.3
3	13	35.9
4	14	23.3
5	20	20.9
6	29	21.0
7	100	17.8

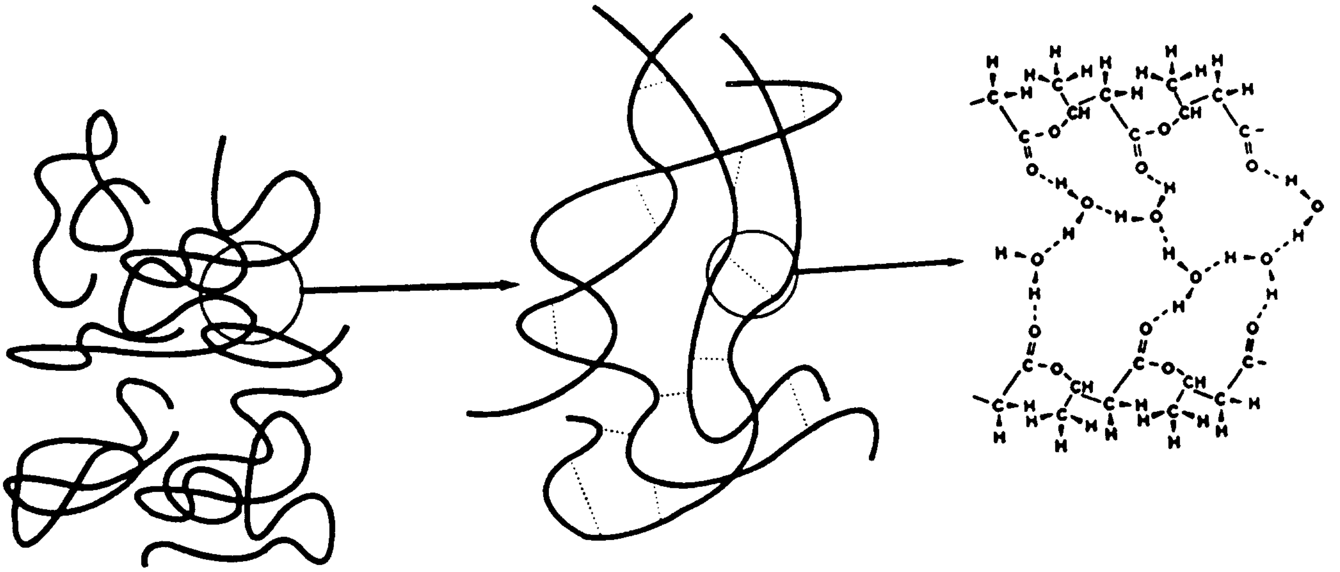

Figure 2 Schematic drawing of PHB chain in coiled state with zoom-ins showing formation of 'pseudo-network' of hydrogen-bonded water molecules formed between adjacent polymer chains

We expect that these results will provide new insight into the mobile nascent structure of the polymer inclusions. They also suggest an affinity for water by polyalkanoates. This is in keeping with contact angle measurements, which show a value of about 70° for water on solid PHB films[18], similar to polar polymers such as nylon 6,6.

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