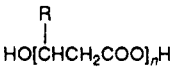
Syntheses of Poly(3-hydroxyalkanoate) (PHA) Conjugates: PHA–Carbohydrate and PHA–Synthetic Polymer Conjugates

Introduction. Poly(β-hydroxyalkanoates) (PHAs, 1) are highly crystalline, optically active materials that are elaborated by a wide variety of microorganisms.[1] The best



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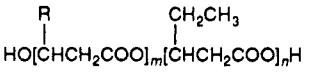

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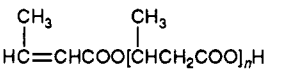
known PHA representatives are poly(3-hydroxybutyrate) (PHB, 1; R = CH_3) and poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBcoHV, 2).[2] PHAs with 4-hydroxybutyrate repeat units[3] {$[CH_2CH_2CH_2CO_2]_n$} and with longer alkyl side chains[4] are also known, as are terpolymers containing units with vinyl side chains (1, R = $CH{=}CH_2$) together with HB and HV repeat units.[5] A recent report also describes the natural occurrence of unique cellular materials consisting of a polysaccharide backbone which bears polyester substituents comprised of β-hydroxybutyrate monomer units.[6]

Considerable interest in PHAs is focused on their biodegradability, biocompatibility, and other physical properties that range from thermoplastic to elastomeric. A variety of applications have been suggested, such as for controlled drug release and biomedical devices. However, their actual use as plastics has so far been hampered by their thermal instability.[7] This prompted us to explore chemical methods for modifying PHAs to obtain materials with improved properties.

PHAs with termini other than the native hydroxyl and carboxyl groups are obtainable via various chemical and thermal modifications.[8] Thus, alkaline degradation[9] affords free carboxylic acid or salt end groups, while alcoholysis with methanol and *p*-toluenesulfonic acid[10] gives rise to ester terminals. Thermal degradation of PHA copolyesters yields[11] via β-elimination mono-, oligo-, or polymeric PHAs with olefinic end groups of type 3 (for PHB).



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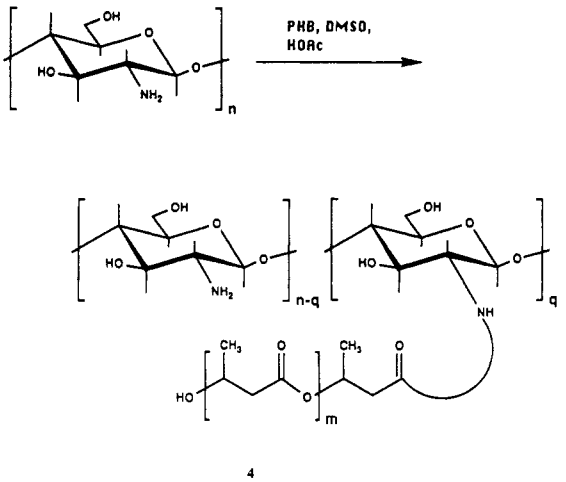
The available array of potential reactive PHA termini has not been exploited to any extent for chemical modifications. We report here on the synthesis of a series of new types of PHA polymer conjugates.

Results and Discussion. For the preparation of the PHA conjugates we employed the carboxyl terminal for amidation and esterification reactions. Commercial PHB samples (M_w 70 000) were partially depolymerized either prior to use or in situ by controlled acid hydrolysis, in order to reduce solution viscosities and to facilitate subsequent modifications. Thus, chitosan solutions in dilute acetic acid were treated with different molar ratios of reduced molecular weight PHB, to afford the corresponding amide conjugates 4 (Scheme I). The degree of substitution (DS) of the chitosan amine functions in 4 was very low (DS = 0.02–0.03), according to elemental analysis, varying insignificantly over the range of PHB–chitosan ratios we examined here. The molecular weight of the PHB branches in 4 was estimated[12] to be approximately 10 000. The formation of PHB conjugates was attested by the unique solubility features of 4. While neither of the parent polymers is water soluble, the PHA–chitosan

Scheme I



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derivatives formed opaque, viscous solutions in water. Upon drying of these solutions, strong, elastic films could be prepared. ^{13}C CPMAS NMR investigation of solid 4 showed a spectrum dominated by the characteristic PHB resonances,[13] with superimposed smaller carbohydrate contributions in the 60–110 ppm region (see Figure 1A). When the ^{13}C NMR spectrum of 4 in D_2O was examined, however, the chitosan resonances were more evident[14] (Figure 1B).

X-ray investigation of 4 displayed either powder patterns of PHB or powder patterns with superposition of reflections of crystalline chitosan and crystalline PHB. This is a manifestation of the greater crystallizability of PHB and does not allow conclusions as to whether or not there is a chemical linkage between the two biopolymer moieties.

Solid-state ^{13}C NMR inversion–recovery experiments provided evidence for large domains of PHB and chitosan for conjugate 4, similar to the results from the X-ray studies. The ^1H T_1 values for the chitosan and PHB were found to be significantly distinct from each other, a situation encountered only when the two fractions are not well mixed on a scale of >100 Å.

Further insight into the nature of the PHA conjugates was derived from differential scanning calorimetry (DSC) investigations. We obtained DSC thermograms of solid samples of each PHB, chitosan, and physical mixtures of chitosan and PHB and compared them against that of the PHB–chitosan conjugate 4. In the DSC thermograms the melt transition, T_m, of PHB shifted from 173 to about 150 °C for conjugate 4 with a concomitant decrease in the sharpness of melting. At the same time, the endotherm of chitosan also decreased from 116 to 105 °C for 4. On the other hand, the T_m value of PHB remained essentially unaltered for a physical mixture of PHB and chitosan. DSC thermograms were also obtained for samples containing water (~1 μL/mg): a single melt transition at 178 °C was observed for conjugate 4, compared to T_m values of 121 and 173 °C for the mixture. Thus, the DSC profiles of conjugate 4 suggest the formation of a covalent material.[15]

In another approach, PHB was linked to a cellulose backbone via a transesterification reaction. Using cellulose acetate, a film-forming PHB conjugate 5 (Chart I) was obtained, whose solid-state ^{13}C CPMAS NMR spectrum again displayed only narrow PHB signals (Figure 2A). The DSC thermogram of conjugate 5 displayed a melt transition at 181 °C (ΔH_m = 96 J/g), compared to a T_m at 173 °C (ΔH_m = 70 J/g) for a physical mixture of PHB and cellulose

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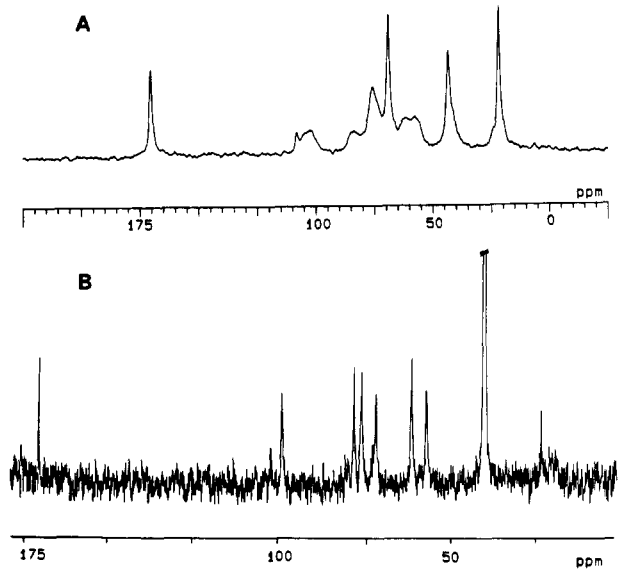
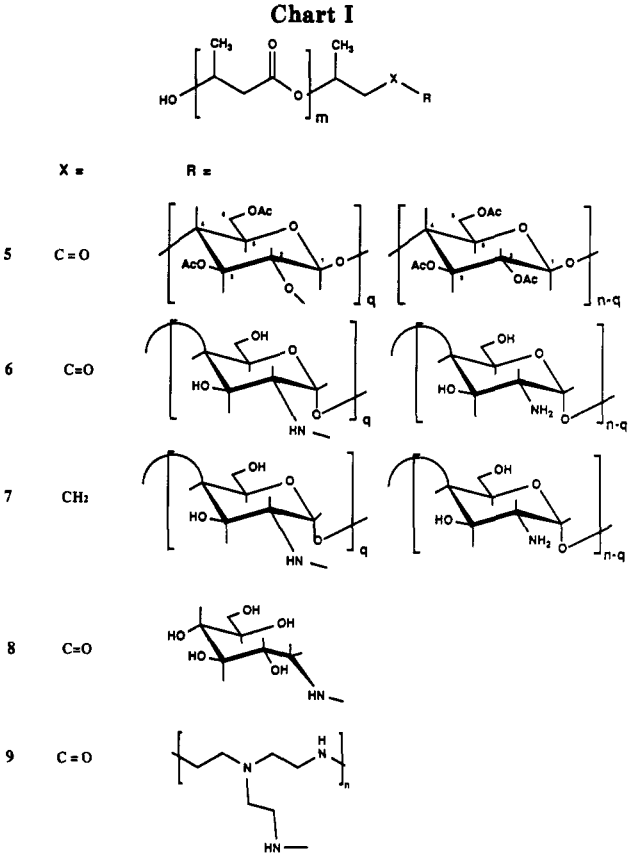


Figure 1. (a) 75-MHz solid-state ^{13}C CPMAS NMR spectrum of chitosan–PHB conjugate **4** (the PHB signals are the sharp resonances and the chitosan signals are the broader resonances between 60 and 110 ppm); (b) 100-MHz ^{13}C NMR spectrum of **4** in D_2O (114 000 scans).



acetate, while cellulose acetate did not exhibit an endotherm.

PHB substitution of the chitosan analogue poly(α-1,4-galactosamine) in the absence or presence of reducing agents afforded the amide conjugate **6** and the corresponding amine conjugate **7**, respectively. The DS values of these derivatives were somewhat higher (DS = ~0.05–0.07) than those of **4**. Interestingly, the solid-state NMR spectra of **6** (Figure 2B) revealed a slightly smaller contribution of the carbohydrate resonances than in the case of **4** (compare Figure 1A).

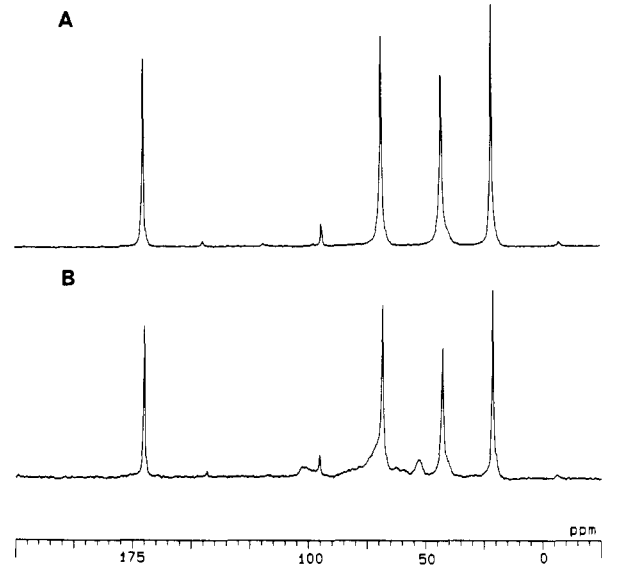


Figure 2. 75-MHz solid-state ^{13}C CPMAS NMR spectra of (a) cellulose acetate–PHB conjugate **5** (only the PHB resonances are visible) and (b) poly(α-galactosamine)–PHB conjugate **6** (the broad carbohydrate resonances appear between 50 and 110 ppm). The small peaks are spinning sidebands.

The DSC thermogram of conjugate **6** revealed broad melt transitions at about 80 and 175 °C (ΔH_m = 20 J/g), while a mixture of PHB and poly(α-1,4-galactosamine) displayed a single T_m at 173 °C, and poly(α-1,4-galactosamine) displayed no distinguishable endotherm (only a shallow inflection centered at 100 °C). PHB conjugates were also prepared with D-glucamine (**8**) and poly(ethylenimines) of varying molecular weights (1800, 10 000, and 70 000 for **9a–c**, respectively).

Conclusions. The syntheses reported in this paper provide access to a new range of PHA derivatives. The observed physical and thermal characteristics, combined with the spectroscopic data, provide strong testimony for the formation of such PHA conjugates. Further evidence will be derived from studies currently in progress aimed at examining the physical and thermal properties of these materials and their films.

The commonly known noncarbohydrate substituents of glycans include acetate, fatty acyl, glycerate, pyruvate, succinate, and amino acid residues.[16] Hollingsworth and co-workers[17,18] and others[19] provided the first evidence for the occurrence of D-3-hydroxybutyrate residues covalently attached to the acidic capsular polysaccharides of *Rhizobium leguminosarum* and *Rhizobium trifolii*. The recent, still to be confirmed findings[6] of actual polyester–polysaccharide conjugates for gellan, welan, and rhamsan, as well as for the structurally related NW11 polymer (elaborated by a *Xanthobacter* sp. bacterium), suggest the possibility that such assemblies of PHB–microbial exopolysaccharides may also be found outside the "gellan series" of water-soluble polysaccharides. While the biological function of such conjugates, if indeed in existence, remains to be elucidated, the syntheses described here allow facile preparations of model materials for structure/activity investigations.

The combination of PHAs with synthetic polymers has recently been explored in a compatibility study involving physical blends of PHBcoHV and several commercial polymers.[20] Reeve et al.[21] have also reported on the preparation of polyurethane-type block copolymers of PHB and poly(ethylene glycol). The poly(ethylenimine) derivative **9** and the various carbohydrate polymer conjugates described here substantially extend the repertoire

of useful PHA derivatives. Our observations on the partial water solubility of the PHB–chitosan conjugate **4** and the film-forming capacity of other derivatives demonstrate the scope of new PHA-derived materials, whose properties can be designed to be intermediate between those of the microbial polyesters and various other polymers.

Experimental Section. Methods and Materials. ^{13}C CPMAS solid-state NMR spectra were observed at 75 MHz using a Chemagnetics CMX-300. The following parameters were employed: contact time 1 ms; recycle delay 5 s; and spin rate 5.5 kHz. ^{13}C NMR spectra were observed at 100 MHz using a Bruker WH 400. DSC thermograms were obtained using a Du Pont 9900 instrument. PHB samples (M_w 70 000) and sodium cyanoborohydride were obtained from Aldrich Chemicals, chitosan powder from Katakura Chikkarin Co., Tokyo, poly(α-1,4-D-galactosamine) from ICN Biochemicals, cellulose triacetate from Eastman (90% acetyl content, ASTM viscosity 25), and poly(ethylenimine) from Nippon Shokubai Kagaku Co., Ltd. 1-Amino-1-deoxy-D-glucitol (D-glucamine) was kindly supplied by Huels. Dialysis membranes (molecular weight cutoff 6000–8000) were obtained from Spectrapore. All solvents were reagent or HPLC grade.

Preparation of Low-M_w PHB. The partially depolymerized PHB samples were prepared either in situ or prior to use by dissolving PHB in a mixture (1:50) of acetic acid–DMSO and stirring for 16 h at ambient temperature.

Preparation of PHB Conjugates. PHB–Chitosan Amide Conjugates 4. To a viscous solution of chitosan [**4a** (4.2 g, 26 mmol), **4b** (5.1 g, 32 mmol), **4c** (4.0 g, 25 mmol)] in acetic acid–DMSO (1:14, 150 mL) was added a solution of PHB (**4a** (2.65 g, 31 mmol), **4b** (4.5 g, 52 mmol), **4c** (3.4 g, 40 mmol)) in DMSO or in a mixture of methylene chloride–DMSO (1:1.5, 150 mL). The reaction mixture was stirred for 1, 2, or 5 days (**4b**, **c**, and **a**, respectively) at ambient temperature and then extensively dialyzed against water for several days. The resulting opaque, viscous solutions were then either precipitated with acetone or lyophilized. Yields were, for **4a**, 4.9 g, for **4b**, 9.6 g, and, for **4c**, 7.0 g. Microanalysis gave the following. For **4a**: C, 46.45; H, 6.85; N, 3.41. For **4b**: C, 41.31; H, 6.32; N, 2.93. For **4c**: C, 38.49; H, 6.14; N, 3.14.

PHB–Cellulose Acetate Ester Conjugate 5. To a solution of cellulose acetate (3.07 g, 11 mmol) in DMSO (150 mL) was added a solution of hydrolyzed PHB (3.9 g, 45 mmol) in acetic acid–DMSO (45 mL). The reaction mixture was stirred for 4 days, dialyzed, and precipitated with acetone, affording 3.21 g of the product **5** as a white material.

PHB–Poly(α-D-galactosamine) Amide 6 and Amine 7 Conjugates. To a solution of PHB (2.0 g, 23 mmol) in DMSO (120 mL) was added a solution of poly(α-D-galactosamine) (1.08 g, 6.7 mmol) in acetic acid–DMSO (1:63, 260 mL). The reaction mixture was stirred at ambient temperature for 2 days, extensively dialyzed against water, and then lyophilized to give **6** (1.69 g). Alternatively, the above reaction mixture was neutralized after 1 day with 1 N NaOH and then treated with sodium cyanoborohydride (1.2 g, 19.1 mmol) to afford **7** (1.43 g). Elemental analysis gave the following. For **6**: C, 49.74; H, 6.64; N, 1.47. For **7**: C, 48.23; H, 6.92; N, 2.10.

PHB–Glucamide Conjugate 8. A solution of PHB (4.36 g, 51 mmol) in acetic acid–DMSO (1:25, 80 mL) was treated with a D-glucamine (4.38 g, 25 mmol) solution in DMSO (60 mL). After reaction for 3 days, the mixture was dialyzed and lyophilized, yielding 5.74 g of product **8** (C, 43.33; H, 6.18; N, 0.46).

PHB–Poly(ethylenimine) Amide Conjugates 9. To solutions of poly(ethylenimine) (**9a** [PEI 1800], 3.94 g, 41 mmol; **9b** [PEI 10 000], 5.65 g, 60 mmol; **9c** [PEI 70 000], 4.65 g, 50 mmol) in DMSO (150 mL) was added hydrolyzed PHB (3.9 g, 45 mmol) in acetic acid–DMSO (45 mL). The resulting white suspensions were stirred at ambient temperature for 3 days, then dialyzed, and lyophilized, yielding 4.51, 3.40, and 4.57 g of **9a–c**, respectively. Elemental analysis gave the following. For **9a**: C, 49.07; H, 7.50; N, 6.92. For **9b**: C, 44.23; H, 8.77; N, 13.87. For **9c**: C, 50.28; H, 7.28; N, 4.90. The ^{13}C CPMAS NMR spectra of these conjugates (not shown) were dominated by the PHB resonances.

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(13) The fact that the PHB resonances are observable in the case of the microbial glycans has been ascribed[6] to solubilization of the polyester via bonding onto the water-soluble glycan backbone. This association is believed to provide the requisite rotational freedom for the appearance of the narrow PHB resonances. A somewhat similar mechanism may be involved for the spectrum of **4**. The chitosan resonances of **4** are broader than those of the native glycan, reflecting a less crystalline morphology. The PHB methyl and methylene resonances of **4** at 21 and 43 ppm, respectively, also reveal shoulders. For additional information on NMR features of PHB, see ref 6 and: Preusting, H.; Nijenhuis, A.; Witholt, B. *Macromolecules* **1990**, *23*, 4220–4224.

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