Plasticization of Bacterial Poly(3-hydroxybutyrate)

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Introduction

Many bacteria accumulate poly(3-hydroxybutyrate) [P(3HB)] as an intracellular carbon and energy-reserve material[1] in the form of granules. The fact that P(3HB) macromolecules are enzymically accessible in the granules in vivo but do not serve as a substrate to intracellular depolymerase after granule drying has been the subject of much debate.[2-5] As a matter of fact, the isolated polymer—whatever the extraction procedure—is a highly crystalline solid which melts at about 180 °C.[6] Studies of P(3HB) crystallization[7,8] have shown that the isolated polymer easily crystallizes from the melt over a wide range of temperatures, including room temperature.

X-ray evidence of the absence of crystallinity in P(3HB) native granules and of the occurrence of crystallization upon removal of the lipid component has been taken by Kawaguchi and Doi[4] as an indication of the importance of lipids in keeping P(3HB) molecules in a mobile state in vivo. On the other hand, owing to the very small amount of protein and lipid associated with the granules, Barnard and Sanders[3] have suggested that the substance present in the cell that is more likely to play a "plasticizing" role is water. Moreover, a recent ^{13}C NMR investigation on whole cells of *Alcaligenes eutrophus* has shown[5] that P(3HB) in native granules behaves like an amorphous elastomer, with an estimated T_g at about −40 °C.

The aim of this work is to give a contribution to the intriguing subject of P(3HB) mobility, through investigation of the plasticization phenomenon. The effect of increasing amounts of a biodegradable[9] plasticizer, di-*n*-butyl phthalate (DBP), on the glass transition of P(3HB) has been examined over the whole range of concentrations, from pure polymer to pure diluent. The influence of DBP on crystallization kinetics and melting of P(3HB) will be discussed elsewhere.

Experimental Section

Poly(3-hydroxybutyrate) was an ICI commercial sample (BX GV9; M_w = 1 140 000; M_n = 350 000), and di-*n*-butyl phthalate (purity > 99%) was synthesized as previously reported.[10]

Mixtures with different compositions were prepared by weighing appropriate amounts of P(3HB) and DBP directly in the DSC pans; the pans were sealed and heated above P(3HB) melting temperature, to promote mixing through diffusion of the plasticizer into the molten polymer. It can be safely assumed that an equilibrium mixing situation is achieved by this procedure since, on quenching to −150 °C after the heat treatment, excellent reproducibility was obtained in all subsequent DSC scans performed at 20 °C/min in the range −150 to +215 °C.

A Du Pont 9900 thermal analyzer calibrated with high-purity standards (*n*-heptane, *n*-octane, benzene, lauric acid, and indium) was used. The glass transition temperature (T_g) was taken both as the inflection point of the specific heat increment (T_g^i) and as the base-line deviation (T_g^b).

Results and Discussion

Figure 1 shows the DSC curves in the glass transition region for P(3HB), DBP, and their mixtures, obtained after quenching from +215 to −150 °C. The endothermal base-line shift corresponding to the glass transition is seen to move to lower temperatures with an increase in the DBP content, as a consequence of the plasticizing effect displayed by the diluent.

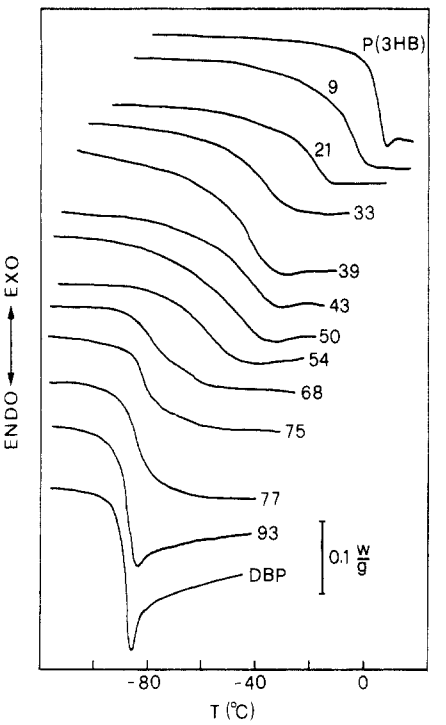

Figure 1. Calorimetric curves in the glass transition region for P(3HB)/DBP mixtures with different DBP content (numbers on the curves are the percent weight fractions).

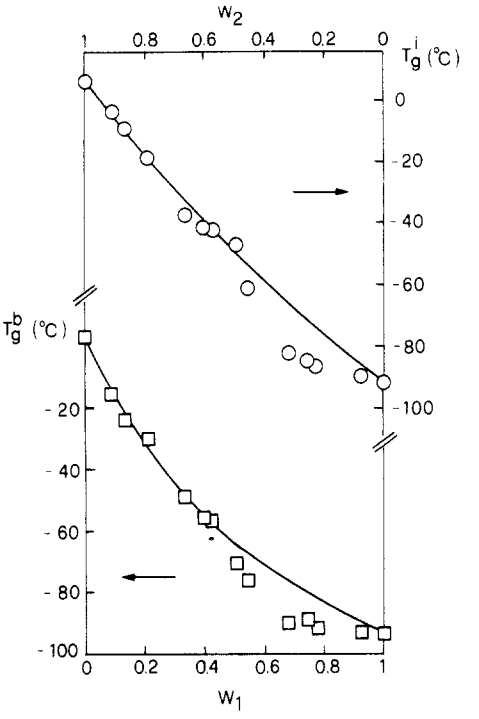

Figure 2. Glass transition temperature (T_g^i, inflection; T_g^b, base-line deviation) of P(3HB)/DBP mixtures as a function of composition.

The composition dependence of the glass transition temperature (T_g) is reported in Figure 2, where both "inflection" and "base-line deviation" values are plotted as a function of the DBP content. The curves drawn have

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been calculated according to the equation

$$T_g = (w_1 T_{g_1} + k w_2 T_{g_2})/(w_1 + k w_2) \quad (1)$$

where w_1 and w_2 are the weight fractions and T_{g_1} and T_{g_2} the glass transition temperatures of DBP and P(3HB), respectively. Equation 1 is a generalized version of some of the empirical equations commonly used to describe the T_g/composition dependence of plasticized polymers. The k parameter defines the curvature of the T_g/composition dependence, $k = 1$ corresponding to a straight line connecting the polymer and diluent glass transition temperatures. Depending on the particular significance attached to the parameter k and on the composition variable used, eq 1 corresponds to the well-known relationships given by Fox,[11] Kelley and Bueche,[12] and Gordon et al.[13] Here, k has been used as an adjustable parameter to give the best fit to the experimental T_g data in the range of polymer-rich mixtures and has been given the following values: 0.76 and 0.43 for T_g^i and T_g^b, respectively. The curves corresponding to eq 1, with the mentioned k values, have been drawn in Figure 2 to call attention to a feature that has been previously pointed out in a number of polymer–diluent systems:[14-17] a change of curvature in the T_g/composition dependence in the intermediate range of concentrations. This behavior is unaccounted for by most of the available empirical equations, which predict the T_g of a plasticized polymer to decrease regularly from that of the pure polymer to that of the pure diluent.

As first pointed out by Riande et al.,[17] results such as those of Figure 2 give evidence for the existence of two concomitant phenomena: a strong depression of the glass transition of the polymer due to the plasticizing effect of the diluent ($w_1 < 0.4$) and a slight increase of the glass transition of the diluent due to hindrance of diluent mobility caused by the dissolved polymer molecules ($w_2 < 0.4$). In the intermediate range of compositions the two mobilization processes merge into an apparently single, broad glass transition and cannot be individually identified by DSC unless T_{g_1} and T_{g_2} are very far apart.[14,15]

From the results of Figures 1 and 2 it turns out that DBP, a well-known plasticizer for synthetic commodity polymers,[9] displays a plasticizing effect toward the biopolymer (P(3HB) very similar to that previously found for poly(vinyl chloride),[15] with an analogous T_g/composition dependence. The present results indicate that the amount of DBP needed to lower the glass transition of P(3HB) from the "pure" polymer value (6 °C) to −40 °C—the estimated T_g of the polymer "in vivo"[5]—is about 30%. Of course, depending on the species that actually play the role of plasticizer in vivo, this amount will differ: the lower the plasticizer T_g, the stronger the plasticizing effect and the lesser the amount needed to bring the polymer T_g to −40 °C.

As already mentioned, plasticization of P(3HB) in the native granules has been proposed as the reason for the observed mobility[3] and absence of crystallinity[4] of the polymer in vivo. However, plasticization does not imply an inability of the macromolecules to crystallize. On the contrary, as shown in the DSC curves of Figure 3, P(3HB) is able to crystallize above T_g during the thermal scan, not only in the pure (undiluted) state but also—and closer to room temperature—in the presence of the DBP diluent: a crystallization exotherm is observed at temperatures that decrease with an increase in the DBP content. The finding that the crystallization temperature decreases as the plasticizer content increases is not surprising and can be attributed to the concomitant decrease of the glass

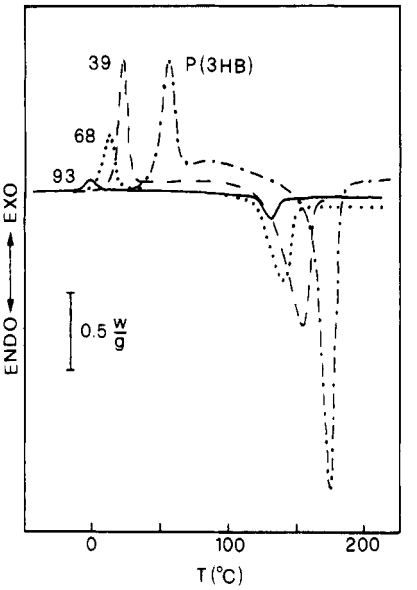

Figure 3. Influence of different amounts of DBP (numbers on the curves are the percent weight fractions) on P(3HB) crystallization and melting.

transition temperature, above which the macromolecules acquire enough mobility to rearrange and crystallize. An estimate of the enthalpy associated with the crystallization exotherms of Figure 3 shows that the change of area correctly correlates with the decrease of the P(3HB) content in the different polymer–diluent mixtures. This indicates that the crystallizing ability of P(3HB) does not decrease in the presence of plasticizer.

Results analogous to the present ones are found in the literature[18] for blends of bacterial P(3HB) and poly(ethylene oxide) (PEO), where the PEO component (T_g = −59 °C) behaves like a high molecular weight plasticizer: with an increase in the PEO content, parallel to a lowering of the T_g of P(3HB), a decrease of the crystallization temperature of the bacterial component is reported. Though it might be objected that neither DBP nor PEO has direct relevance as a low or high molecular weight plasticizer to the in vivo situation of P(3HB) granules, it is worth pointing out that the observed phenomena reflect a quite general behavior in polymer plasticization and are expected to occur whatever the nature of the plasticizer.

The obvious consequence of the predicted decrease of the crystallization temperature of P(3HB) in the presence of the in vivo plasticizing agent is that the polymer should very easily crystallize at the normal microorganism fermentation temperatures. This leads to the conclusion that, although plasticization by water or by other cellular material certainly contributes to P(3HB) mobility in vivo, different mechanisms need to come into play to explain the prevention of P(3HB) crystallization.

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