

# Amorphous polymer granules

**SIR** — Poly-*R*-3-hydroxybutyrate (PHB), a hydrophobic storage polymer found in bacteria, is used commercially as a biodegradable thermoplastic<sup>1</sup>. It is stored *in vivo* in granules whose structure has long been debated. Isolated PHB is highly crystalline, and it was originally believed that the native granules were also crystalline. It is now clear, however, that the polymer is stored in cells as a mobile elastomer<sup>2</sup>. Many

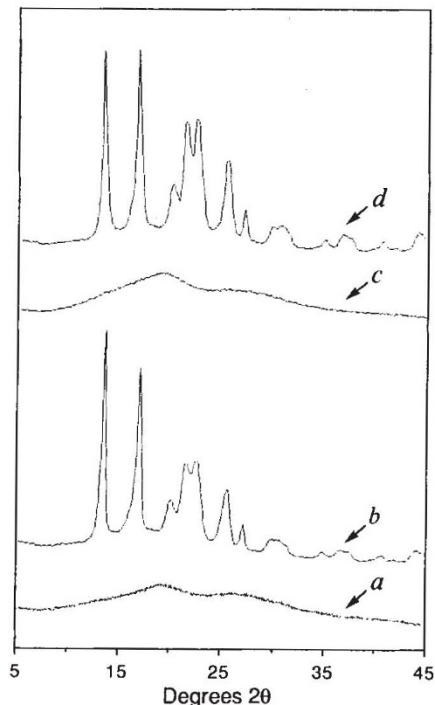


FIG. 1 Wide-angle X-ray diffraction patterns for *a*, whole *A. eutrophus* cells; *b*, crystalline PHB powder; *c*, artificial amorphous PHB granules; *d*, artificial granules dried overnight at 125 °C.

hypotheses have been advanced to explain the metastable amorphous state of the polymer *in vivo*<sup>3</sup>, most recently that it is simply the result of the slow nucleation kinetics that are operative for small particles<sup>4,5</sup>. We now provide experimental support for that explanation by preparing artificial granules which effectively contain only PHB and are virtually indistinguishable from native storage granules.

The essence of the *in vivo* kinetic model is that polymer that is newly biosynthesized in an amorphous mobile form within the granule will only crystallize as a result of homogeneous (spontaneous) nucleation. In the absence of heterogeneous nucleation or granule coalescence, an ensemble of 0.25- $\mu$ m granules should have a crystallization half-life of more than 1,000 yr at 30 °C, whereas bulk samples of the polymer

quenched to 30 °C from the melt typically crystallize within a few minutes. It should therefore be possible to reconstitute stable amorphous PHB granules *in vitro*<sup>6,7</sup>.

Accordingly, a solution of PHB (99.9% pure) in CHCl<sub>3</sub> (5% wt/v) was sonicated at 20 kHz with 20 vol of an aqueous solution of cetyl trimethylammonium bromide (CTAB, 5 mM). The resulting emulsion was dialysed exhaustively over 48 h against additional 5 mM CTAB to remove the organic solvent. Laser light scattering measurements confirm that the remaining PHB is particulate, with median diameter 0.3–0.4  $\mu$ m. Nycodenz gradients show that the PHB particles have density 1.18 g cm<sup>-3</sup>, the same as native granules and pure amorphous PHB; crystalline PHB powder has a density of 1.241 g cm<sup>-3</sup> (ref. 8). The surfactant-coated, amorphous PHB granules were collected by centrifugation for further studies.

The wide-angle X-ray diffraction patterns obtained from pastes of either artificial granules or PHB-rich whole cells of *Alcaligenes eutrophus*<sup>9</sup> show only an amorphous halo, whereas crystalline PHB powder gives a series of sharp peaks (Fig. 1). In addition, the NMR spectra of the reconstituted granules show the same signals and temperature dependence as the polymer contained in whole cells (Fig. 2)<sup>2</sup>, implying essentially identical mobility properties. No signals are detectable from CTAB associated with the artificial granules. Isolated PHB powder, which is about 70% crystalline, has no <sup>13</sup>C-NMR spectrum at these temperatures. Artificial amorphous PHB granules prepared as described are stable in aqueous suspension at 30 °C for at least 6 months.

We have thus established that PHB mobility *in vivo* can be explained solely on the basis of the intrinsic material properties of the polymer. Figure 1*d* demonstrates that by a simple drying treatment the artificial amorphous granules can be caused to coalesce and returned to their original highly crystalline

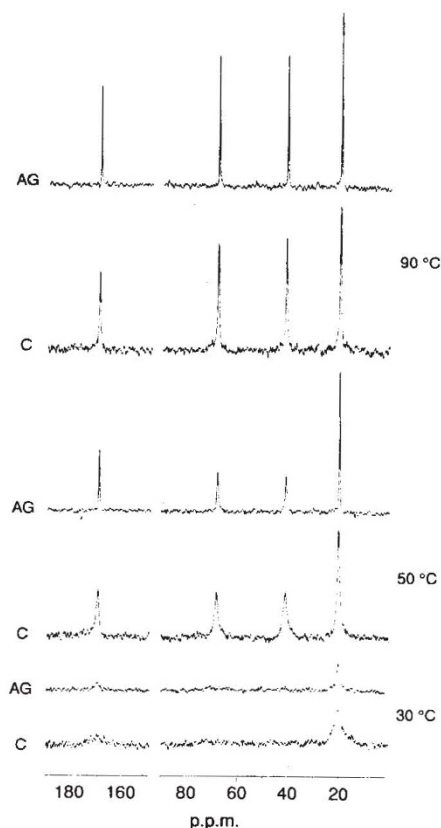


FIG. 2 Natural abundance 100-MHz <sup>13</sup>C-NMR spectra at three temperatures of artificial PHB granules (AG) and whole cells of *A. eutrophus* (C). Artificial granules were collected by centrifugation (33,000g), re-suspended in D<sub>2</sub>O and examined in a NMR tube containing a d<sub>6</sub>-benzene capillary.

state. Presumably, the removal of surface water destabilizes the surfactant-polymer interactions that keep the granules physically separate. The findings we report here have interesting implications for the preparation of polymer coatings.

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