activation — as is seen using dominant-negative *ras* in mammalian cells[4,8,9]. Similarly, overactivation of one of the two input pathways with an activated oncogene can lead to (at least) partial activation of the downstream elements, as exemplified by the effect of v-*ras* on MAPK[3]. Variation in (for instance) the efficacy of v-*ras* in activating MAPK in different cell types might reflect changes in the relative importance of input signals leading to choices between branches of a complex network rather than fundamental changes in the basic wiring of the network itself. □

Thomas M. Roberts is at the Dana-Farber Cancer Institute, 44 Binney Street, Harvard Medical School, Boston, Massachusetts 02115, USA.

POLYMER SCIENCE —

Tender morsels for microbes

Paul Calvert

POLY[(R)-3-HYDROXYBUTYRATE] is a microbial storage polyester which forms in intracellular granules about 0.5 micrometres in diameter. The polymer has been commercialized as a biodegradable fibre-forming material with properties similar to those of polypropylene. Curiously, *in vivo* the polymer is soft and readily degradable, but after extraction it becomes a tough, stiff fibre. Why this change occurs has led to a disagreement. Sanders and co-workers, at Cambridge, suggest[1,2] that some clever biological plasticization occurs *in vivo*, but de Koning and Lemstra argue[3] that the small particles found in microbes are simply too small to crystallize and remain rubbery even when 150 °C below the melting point.

To put the difference in perspective it is worth considering the plant polymers cellulose and starch, which are both based on glucose. Cellulose, whose function is structural, has a regular arrangement with β-linked glucose units, which allow the polymer chains to be densely packed and highly crystalline. The impenetrability of the structure makes chemical and enzymatic digestion very slow. The α-linkage in starch, the energy-storage version of the polymer, drives the chain into an open helix, and frequent chain branching in the amylopectin fraction prevents extensive crystallization. The resulting material is soluble in water and readily degradable. Against this background, one would hardly expect poly[(R]-3-hydroxybutyrate] (PHB) to be an unbranched and readily crystallizable polymer, if it is to serve its biological function. But its behaviour when extracted shows otherwise. *In vivo*, some mechanism must operate to prevent crystallization.

Sanders and colleagues have shown[1] that PHB is an amorphous rubber in its native state within cells of *Alcaligenes eutrophus*. Carbon-13 NMR spectra show narrow lines, and measurements of spin–lattice relaxation times, (T_1), correspond to those of a rubbery polymer with a glass transition temperature of about −40 °C. X-ray diffraction shows a completely amorphous polymer which becomes crystalline if the cells are washed with acetone and dried at 100 °C. A further paper[2] shows that mobility is partly lost on freeze-drying, although the polymer remains amorphous, and mobility is regained on rehydration. Careful rupturing of cells has no effect, but centrifugation of the granules causes them to crystallize. On this basis the group argues for some limited plasticization by water, which explains the changes on freeze-drying. In addition, the existence of an unknown hydrophobic nucleation inhibitor is postulated, either at the surface or distributed in the granule.

Lauzier *et al.* find[4] that isolation of the granules results in a crystalline 'skin' on each granule but leaves a soft amorphous core. They attribute the persistence of the core to the presence of triacetin and other lipids, which could prevent further growth of the skin. But these lipids make up only about 0.5 weight per cent of the granule, which is nowhere near enough to solubilize a significant amount of the polymer. On the other hand, Lauzier *et al.* also find that the core stays soft down to liquid-nitrogen temperatures, which suggests that it is very plasticized.

de Koning and Lemstra[3] have carried out scanning calorimetry on granules and freeze-dried cells. The glass transition of wet granules occurs at about −5 °C and moves up by about 5 °C on drying. This suggests that plasticization due to water is only modest. The difference in glass transition temperature reported by Sander's group and de Koning and Lemstra is not serious, as the NMR estimate involves a long extrapolation. It does, however, contradict the plasticized core deduced by Lauzier *et al.* Other evidence against the granule being heavily plasticized *in vivo* comes from Ceccorulli *et al.*[5], who find that PHB can be plasticized with dibutylphthalate with a large reduction in the glass transition, but that there is a consequent increase in the crystallization rate at room temperature.

It is the rate of nucleation that de Koning and Lemstra hold responsible for the pliability of the biological form of PHB. They estimate from nucleation-rate data that the typical time for a granule 0.5 μm across (like a natural granule) to nucleate for crystallization is 10^{12} seconds — over 30,000 years — at 20 °C. Compaction by centrifugation, as done in Sander's laboratory, welds the granules together into a single volume 10^{12} times larger, with a commensurate decrease in the time to nucleation. Once nucleated, the whole particle can be expected to crystallize. Indeed, this has been a problem in commercial forms of PHB, as the crystallized polymer tends to comprise a small number of large crystalline spherulites, owing to the low nucleation rate, and is therefore opaque and brittle. An extensive trial-and-error search has identified saccharin as a good nucleating agent (possibly because of a close match of intermolecular spacings) which improves the quality of the polymer[6,7].

Lastly, the granules *in vivo* are surrounded by some kind of coating, or possibly a lipid membrane. Treatments that damage the granule surface also cause crystallization. de Koning and Lemstra attribute this effect to adventitious particles which then contact the polymer surface. The earlier search for nucleating agents suggests that suitable particles are quite rare. The alternative explanation is that nucleation can occur at a water–polymer interface, whereas the membrane surface is non-nucleating, if not inhibitory. We know very little about the nucleating effects of different liquid–liquid surfaces, so anything is possible.

There has been little work on the crystallization behaviour of very small particles of synthetic polymers. Surface effects must be very important and there may be strange plasticization effects arising from the small size. The bacteria do seem to have hit upon a simple method for keeping polymers rubbery (and edible) at far below the melting point. If we could do this too, it would be a terrific way of making tough coatings. □

Paul Calvert is in the Arizona Materials Laboratories, 4715 East Fort Lowell Road, Tucson, Arizona 85712, USA.

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