Polyhydroxybutyrate: an Intriguing Biopolymer

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INTRODUCTION

Poly-3-hydroxybutyrate (PHB) is a linear polyester of D(-)-3-hydroxybutyric acid which was first discovered in bacteria by Lemoigne in 1925. It is accumulated in intracellular granules by a wide variety of Gram-positive and Gram-negative organisms under conditions of a nutrient limitation other than the carbon source (Dawes and Senior, 1973). The polymer, which serves as a reserve of carbon and energy, is now known to be but one example, albeit the most abundant, of a general class of compounds referred to as polyhydroxyalkanoates and possessing the general formula,

\[
[\begin{array}{c}
R \\
O-CH-CH_2-C \\
\end{array}]_n
\]

where \( R = CH_3 \) in PHB. The principal other polyesters currently identified have \( R = CH_3CH_2 \) (polyhydroxyvalerate, PHV) and \( R = CH_3(CH_2)_4 \) (polyhydroxyoctanoate, PHO).

The molecular weight of PHB differs according to organism, conditions of growth and method of extraction, and can vary from about 50,000 to well over a million. The polymer possesses the important properties of thermoplasticity and
biodegradability and, in consequence, has attracted considerable commercial interest, to which I shall refer in greater detail later.

PHB is an ideal carbon reserve material since it exists in the cell in a highly reduced state as a virtually insoluble polymer exerting negligible osmotic pressure. Marchessault and his colleagues reported that the PHB molecule from *Rhizobium* is a compact right-handed helix with a two-fold screw axis and a fibre repeat of 59.6 nm (Okamura and Marchessault, 1967; Cornibert and Marchessault, 1972). The granules are generally spherical and vary in size according to the organism, e.g. in *Bacillus megaterium* from 0.2 to 0.7 \( \mu \text{m} \) diameter (Ellar et al., 1968). Electron microscopy of native granules isolated from cell-free extracts by density-gradient centrifugation shows that they are bounded by a membrane which is not a typical fluid mosaic membrane and which has associated with it the final enzyme of polymerization, PHB synthase or polymerase. In some, but not all, organisms studied, the initial enzyme system for PHB degradation, the depolymerase, is also entirely associated with the granule membrane.

**BIOSYNTHESIS OF PHB AND ITS REGULATION**

When *Azotobacter beijerinckii* is grown in batch culture fixing atmospheric nitrogen with glucose as the carbon source, it displays no microscopically perceptible granules in the early exponential phase; by mid-exponential phase granules of PHB start to accumulate and by the time the stationary phase is attained the cells are packed with granules, the polymer representing about 75 per cent of the biomass. Chemostat experiments revealed that an oxygen limitation yields a much greater polymer content than a nitrogen limitation, and the amount of polymer accumulated is inversely related to the growth rate (Senior et al., 1972).

When an oxygen limitation is imposed upon a steady-state chemostat culture which is nitrogen limited, a period of unbalanced growth ensues during which the initially very low PHB content of the cells increases until it attains a value characteristic of the new steady state (some 50 per cent of the biomass in the experiments conducted). Conversely, when an oxygen-limited culture has that limitation relaxed to give a dissolved oxygen tension of 2 per cent of air saturation, the PHB content of the cells declines and eventually reaches a much lower value determined by the new steady state. If a redox electrode is inserted into cultures undergoing such transitions of dissolved oxygen tension, interesting observations may be made. Thus when an oxygen limitation is imposed upon a nitrogen-limited culture operating at a dissolved oxygen tension of 10 per cent of air saturation, the redox potential plummets from +15 mV to −50 mV. But as PHB synthesis commences in response to the oxygen limitation, the redox potential increases rapidly and soon exhibits an \( E_h \) of over +30 mV; we thus have the paradoxical situation of an oxygen-limited culture displaying a redox potential higher than that of a culture growing in the presence of excess oxygen. To explain these observations we suggested that PHB synthesis serves as an electron sink for the reducing power which accumulates as a consequence of an oxygen limitation; electrons are no longer able to traverse the electron transfer chain to oxygen at