Poly-β-hydroxybutyrate in the Chemolithotrophic Bacterium Ferrobacillus ferrooxidans

W. S. WANG and D. G. LUNDGREN
Department of Bacteriology and Botany, Syracuse University, Syracuse, New York 13210

Received for publication 6 November 1968

Poly-β-hydroxybutyric acid (PHB) has been demonstrated in a wide range of bacteria, including both chemolithotrophic and photolithotrophic organisms. In the chemolithotrophic group, Schlegel, Gottschalk, and von Bartha (8) reported the formation and utilization of PHB in Hydrogenomonas, Lundgren et al. (4) extracted PHB from Ferrobacillus ferrooxidans, and Tobback and Laudelout (10) chemically identified PHB in Nitroacter. In thin sections of the photosynthetic bacterium Rhodospirillum rubrum, Vatter and Wolfe (11) reported fat vacuoles in the cell’s cytoplasm which Cohen-Bazire and Kunisawa (2) later identified as PHB granules. The existence of a limiting membrane surrounding the PHB granule was reported by Boatman (1); it was a single membrane about 4 nm wide. Pfister and Lundgren (6) also reported a membrane around PHB granules in Bacillus cereus. The membrane was seen on isolated PHB granules examined as carbon replicas (5). In this report, morphological evidence is given for the presence of PHB granules in F. ferrooxidans grown heterotrophically with glucose as the primary energy source.

F. ferrooxidans was grown in the 9K salts medium of Silverman and Lundgren (9) with 0.5% glucose as the energy source instead of iron. The cultural conditions were those reported in the earlier report of Remens and Lundgren (Bacteriol. Proc., p. 33, 1963). The cells were harvested, washed with a solution of ethylenediaminetetraacetaetate (2 × 10⁻⁴ M), fixed with 0.5% glutaraldehyde in collidine buffer (0.5 M, pH 7.6) for 30 min, and postfixed with 1% osmium tetroxide in distilled water overnight. Sections were poststained with 1% uranyl acetate for 60 min at 60 °C, followed by lead citrate (7) for 5 min at room temperature. Electron micrographs were taken with an RCA EMU-2D electron microscope operating at 50 kV.

An increase in cell number was observed when F. ferrooxidans was grown under heterotrophic conditions, and some differences in gross morphology and fine structure were apparent when these cells were compared with iron-grown cells. Figure 1 shows the typical wavy or rugose surface of iron-grown cells, whereas glucose-grown cells, although still rugose, possess many structures which cause the cell surface to bulge (Fig. 1b, arrows); these structures are the PHB storage granules.

In thin sections (Fig. 2a), iron-grown cells possess the typical multilayered cell envelope of gram-negative organisms with a cytoplasm containing a dispersed nucleus, ribosomes, and electron-dense bodies; the latter were previously reported by Dugan and Lundgren (3). In thin sections, glucose-grown cells (Fig. 2b) show many electron-transparent areas, typical of PHB storage bodies. The PHB granule is surrounded by a single membrane which appears as a dense line of 2.5 to 3.5 nm. In addition, another structural difference in heterotrophically grown cells is the absence of electron-dense cytoplasmic bodies. The reasons for the accumulation of PHB and the loss of cytoplasmic bodies in ferrobacilli that have been grown heterotrophically are being investigated.

ACKNOWLEDGMENTS

This work was supported by the Federal Water Pollution Control Administration, Department of the Interior, grant no. 14010 DAY.

LITERATURE CITED

FIG. 1. Cells negatively stained with ammonium molybdate (1%), dried, and shadowed at an angle of 1:3 with platinum-carbon. (a) Iron-grown cells showing the wavy surface. The rod-shaped cells cast a slightly higher shadow at the poles. (b) Glucose-grown cells shadowed as above. Cells are rounder and possess bulging spheres identified as PHB granules (arrows). Markers indicate 1 μm.
Fig. 2. Thin sections of F. ferrooxidans. (a) Iron-grown cells showing the wavy cell envelope, dispersed nucleus (N), and electron-dense bodies (arrows). (b) Glucose-grown cells showing a similar fine structure, as in (a), but with PHB granules and no dense cytoplasmic bodies. Markers indicate 0.5 μm.