Note

The Metabolic Pathway of Propionate in *Rhodospseudomonas sphaeroides* S

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Utilization of propionate is one of the criteria in classification of photosynthetic bacteria. *Rhodospirillum rubrum*¹⁻³⁾ and *Chlorobium thiosulfatophilum*⁴⁾ utilize propionate as sole carbon source and metabolize it via the methylmalonyl-CoA pathway. *Rhodospseudomonas sphaeroides* can not utilize propionate as sole carbon source.⁵⁾ Previously, I reported that propionate inhibits the growth of *R. sphaeroides* S on glucose medium under aerobic-dark conditions.⁶⁾ This inhibition could be restored by the addition of acetate or NaHCO₃ to the culture medium. The bacterium assimilated propionate on acetate-propionate medium.⁷⁾ This paper describes some results concerning the metabolic pathway of propionate in *R. sphaeroides* S.

Previously, I showed the decarboxylation pattern of propionate carbons in this bacterium.⁶⁾ The data suggested that propionate was metabolized via methylmalonyl-CoA to succinyl-CoA or via α -hydroxyglutarate to succinyl-CoA. The methylmalonyl-CoA pathway includes CO₂ fixation reaction catalyzed by propionyl-CoA carboxylase. Incorporation of ¹⁴CO₂ was examined.

R. sphaeroides S⁸⁾ was grown at 30°C under aerobic conditions in the dark. The culture medium used consisted of 0.2% glucose and basal medium which contained KH₂PO₄, 0.5 g; K₂HPO₄, 0.5 g; (NH₄)₂HPO₄, 0.8 g; MgSO₄·7H₂O, 0.2 g; NaCl, 0.1 g; FeSO₄·7H₂O, 1 mg; nicotinic acid, 1 mg; thiamine-HCl, 1 mg; biotin, 0.04 mg; trace elements solution,⁹⁾ 1 ml; distilled water, 1 liter. Cells were harvested by centrifugation, and resuspended in basal medium ((NH₄)₂HPO₄ was omitted) to give a cell density of O.D.₆₆₀ 0.225. The cell suspension was used in experiments of ¹⁴CO₂ incorporation. Figure 1 shows that incorporation of ¹⁴CO₂ was activated in medium containing propionate, while succinate had no effect. These results suggest that CO₂ was fixed via the methylmalonyl-CoA pathway in the presence of propionate.

The intermediate metabolites of 1-¹⁴C-propionate were identified on paper chromatogram by autoradiography. Cell suspension was prepared as described

FIG. 1. Effects of Propionate and Succinate on Incorporation of ¹⁴CO₂.

Medium for incorporation of ¹⁴CO₂ consisted of 8 ml cell suspension (equivalent to 2.6 mg dry cells), 8 mg 0.5 μCi NaH¹⁴CO₃, and 8 mg Na-propionate (O—O) or 8 mg Na-succinate (Δ—Δ). The control (×—×) omitted either Na-propionate or Na-succinate. The media were placed in L-shaped tubes and shaken by Monod's shaker at 30°C. At appropriate intervals, 0.5 ml of the medium was removed and filtrated by 0.45 μ membrane filter (Millipore). Radioactivity on the filter was counted by a liquid scintillation counter (Beckman).

above, and optical density (660 nm) was adjusted to 4.2. Two milliliters of the cell suspension was incubated with 5 μCi 1-¹⁴C-propionate (specific activity, 57 mCi/mmol) at 30°C. After incubation for 30 sec, one drop of 12 N HCl was added to the suspension. The mixture was immediately transferred to a boiling-water bath and incubated for 15 min. The precipitate was removed by centrifugation. The supernatant was lyophilized to remove 1-¹⁴C-propionate. The lyophile was resolved in 0.5 ml water, and 100 μl of this solution was chromatographed with authentic succinate, fumarate, malate, methylmalonic acid (Tokyo Chemical Industry Co.), or α -hydroxyglutarate (Sigma Chemical Co.) on Toyo No. 51 filter paper. Authentic materials were detected by spraying bromophenol blue solution on the filter paper. As shown in Fig. 2, radioactive spots were identified to be methylmalonic acid, succinate, fumarate and malate. These findings indicate that propionate uptake was metabolized via methylmalonyl-CoA to succinyl-CoA. Three unidentified-spots of low R_f values were also found. In these experiments, a radioactive spot which gave the same R_f value as authentic α -hydroxyglutarate (R_f0.45) could not be detected. Metabolic overloading technique¹⁰⁾ was employed to detect α -hydroxyglutarate. The cell suspension was incubated with unlabelled α -hydroxyglutarate (final concentration 0.2%) and 5 μCi 1-¹⁴C-propionate (specific activity, 57 mCi/mmol), for 30 seconds, and then the radioactive intermediates formed were

FIG. 2. Paper chromatograms of radioactive intermediates formed from 1-¹⁴C-propionate. Solvent system was 1-pentanol-formate-water (20: 1: 12, v/v).

identified as described above. Radioactive α -hydroxyglutarate, however, could not be detected in these experiments (data not shown), though α -hydroxyglutarate permeated the membrane of this bacterium because the bacterium grew on α -hydroxyglutarate medium as the sole carbon source.

Thus, it might be concluded that propionate was metabolized *via* the methylmalonyl-CoA pathway by this bacterium, while the α -hydroxyglutarate pathway did not work.

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