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Hydrogen Metabolism by *Rhodomicrobium vannielii*

DEREK S. HOARE AND S. LOUISE HOARE
Microbiology Department, The University of Texas, Austin, Texas 78712

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Under appropriate cultural conditions, cell suspensions of *Rhodomicrobium vannielii* effect two distinct photoreactions involving molecular hydrogen: (i) the photoreduction of carbon dioxide, and (ii) the photoproduction of hydrogen.

Rhodomicrobium vannielii (2) is a unique photosynthetic bacterium which reproduces by budding. Its morphology is similar to that of the chemoheterotroph *Hyphomicrobium vulgare* (5). Not much is known about its physiological properties apart from its characterization as a strictly anaerobic photoheterotroph. As part of a comparative study of the photoassimilation of organic compounds by the *Athiorhodaceae*, we found that *R. vannielii* could photoreduce carbon dioxide with hydrogen. This evidently implied that, contrary to the findings of Ackrell, Asato, and Mower (1), *R. vannielii* contained a hydrogenase. This note reports the ability of cell suspensions of *R. vannielii* to effect two distinct photoreactions involving molecular hydrogen: (i) the photoreduction of carbon dioxide, and (ii) the photoproduction of hydrogen.

R. vannielii was obtained from H. C. Douglas, Microbiology Department, University of Washington, Seattle. Cultures were grown anaerobically in the light (120 ft-c light intensity) at 28 C in a medium (2) with sodium acetate (0.2%, w/v) as organic carbon source. Cultures (1 liter) were gassed and sealed under nitrogen and carbon dioxide or under hydrogen and carbon dioxide (95:5 parts by vol).

Cell suspensions of acetate-grown *R. vannielii* were tested manometrically (3) for their ability to photoassimilate organic compounds. Cell suspensions (10 to 20 mg, dry weight) in 25 mM sodium bicarbonate buffer were incubated under nitrogen and carbon dioxide (95 to 5 parts by volume) at 30 C in the light (460 ft-c light intensity) with 20 μmoles of organic substrate. Cell suspensions assimilated the following substrates in the light: butyrate, acetate, propionate, valerate, succinate, malate, lactate, caproate, and β-hydroxybutyrate. Butyrate was assimilated most rapidly with a rate of 22 μliters of gas consumed per hr per mg (dry weight). Formate, glyoxylate, oxalate, and pyruvate were not assimilated.

When cell suspensions were incubated in the light without organic compounds under an atmosphere of hydrogen and carbon dioxide (95:5 parts by vol), a rapid but variable gas uptake ensued. The photoreduction of carbon dioxide with hydrogen proceeded most rapidly in cell suspensions which had been preincubated anaerobically in the light. The photoassimilation of hydrogen by freshly harvested cell suspensions frequently developed only after a lag period. The effect of preincubation conditions on the ability of cell suspensions to photoassimilate hydrogen is summarized in Table 1. When cell suspensions were assayed after anaerobic incubation in the dark, the reaction was initially rapid but it was followed by a sharp break leading to a slower rate of gas uptake (Fig. 1). This faster initial rate is attributed to the photoassimilation of metabolic products which were released from the cells during the dark incubation; if the cell suspension was centrifuged after incubation in the dark and was then resuspended in buffer, the gas uptake corresponded to the lower rate. Cell suspensions incubated in the light also photoassimilated hydrogen at the same lower rate. If acetate was added to the reaction mixture, there was an increased rate of gas consumption (Fig. 1).

Cell suspensions of *R. vannielii*, like *Rhodospirillum rubrum* (4), can also produce hydrogen in the light. The cultural conditions for the photoproduction of hydrogen are distinct from those for the photoassimilation of hydrogen. The photoproduction of hydrogen by *Rhodomicrobium vannielii*, as in *Rhodospirillum rubrum*, is negligible in cells harvested from media containing ammonium salts but it is high in cells harvested at later stages of growth when this nitrogen source is depleted from the medium. Cultures grown with glutamate as nitrogen source rapidly evolved hydrogen in the light. Cell suspensions which photoproduced hydrogen were unable to photoassimilate it. Similarly, cell suspensions which photoassimilated hydrogen did not photoproduce it. The photoproduction of hydrogen was inhibited by nitrogen and by ammonium salts, and was comparatively in-

TABLE 1. *Effect of storage conditions on the photoreduction of carbon dioxide with hydrogen by cell suspensions of Rhodomicrobium vannielii*[a]

Expt.	Storage conditions	Activity[b]	
		Before	After
1	Hydrogen, light, 28 C	2.0	9.6
	Hydrogen, dark, 28 C	2.0	30.8, then 9.6
2	Nitrogen, light, 28 C	2.0	10.6
	Nitrogen, dark, 28 C	2.0	29.0, then 9.6
3	Hydrogen, dark, 2 C	4.0	3.2
	Hydrogen, dark, 28 C	4.0	27.6, then 11.4
	Hydrogen, dark, 28 C, washed and resuspended before assay	4.0	12.3
	Hydrogen, light, 28 C	4.0	12.3

[a] Cell suspensions [6 to 8 mg (dry weight) per ml] in 5 mM KH_2PO_4 buffer (*p*H 7.0) were incubated for 18 to 20 hr in Thunberg tubes as indicated. Suspensions were then assayed for the photoreduction of carbon dioxide with hydrogen.

[b] Expressed as microliters of total gas consumption (i.e., hydrogen + carbon dioxide) per hour per milligram (dry weight) of cells.

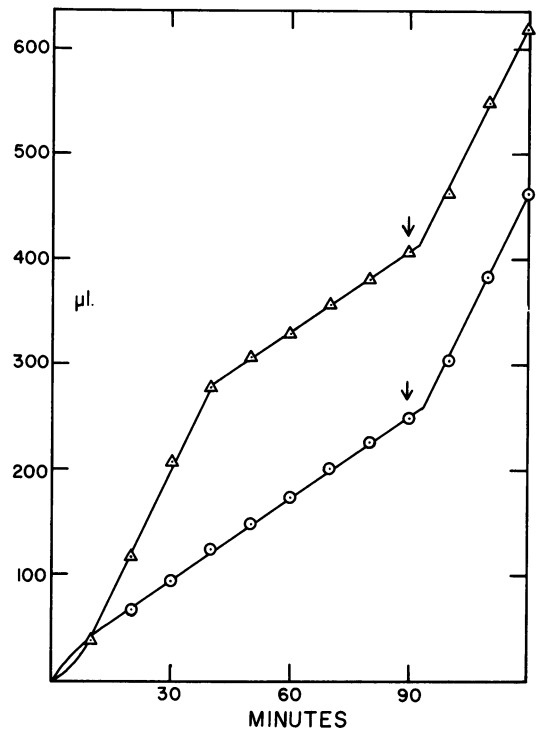

FIG. 1. *Photoreduction of carbon dioxide with hydrogen by suspensions of R. vannielii after storage for 18 hr at 28 C under hydrogen in the dark (△) and in the light (○). Suspensions [15 mg (dry weight) per 3 ml of 0.025 M $NaHCO_3$] were assayed for photoreduction under an atmosphere of H_2 + CO_2 (95:5, v/v) in the light at 30 C; 10 µmoles of sodium acetate (in 0.025 M $NaHCO_3$) was added after incubation for 90 min.*

sensitive to inhibition by *ortho*-phenanthroline (OP). In contrast, the photoassimilation of hydrogen and carbon dioxide was inhibited by OP (58% inhibition by 2×10^{-4} M), but it was not inhibited by nitrogen or ammonium salts. Photoproduction of hydrogen (with malate as substrate) was completely inhibited by 10^{-4} M OP in *R. rubrum* (4).

These results show that under appropriate conditions cell suspensions of *Rhodomicrobium vannielii* can photoreduce carbon dioxide with hydrogen and can photoproduce hydrogen. *R. vannielii*, therefore, contains a hydrogenase. Since the two reactions involving molecular hydrogen are manifested under different conditions and show different sensitivities to inhibitors, it is conceivable that *R. vannielii* produces two hydrogenases, one involved in the photoassimilation of hydrogen, and another involved in the photoproduction of hydrogen. The physiological significance of these reactions is not yet clear. Attempts to grow *R. vannielii* photoautotrophically with hydrogen have been unsuccessful.

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