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Autotrophic and Heterotrophic Metabolism of *Hydrogenomonas*: Regulation of Autotrophic Growth by Organic Substrates

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The effects of a number of organic substrates on the autotrophic metabolism of *Hydrogenomonas eutropha* were examined. Dual substrate (mixotrophic) cultivation in the presence of hydrogen plus either fructose or alanine allowed autotrophic growth to begin immediately after the exhaustion of the organic substrate. On the other hand, the presence of acetate, pyruvate, or glutamate caused a lengthy lag to occur before autotrophic growth commenced. With acetate or pyruvate this lag (plateau) in the dicyclic growth curve was due to the repression of ribulose diphosphate carboxylase (RDPC) synthesis during mixotrophic growth. During heterotrophic growth with glutamate, RDPC was partially repressed; however, during mixotrophic growth, RDPC activity was high. Thus the delay of autotrophic growth was not due to a repression of RDPC by glutamate. The data suggest that glutamate interferes with autotrophic metabolism by repressing the incorporation of inorganic nitrogen. The repression of these vital autotrophic functions by acetate, pyruvate, and glutamate occurred both in the presence and absence of hydrogen, i.e., during both heterotrophic and mixotrophic cultivation. The derepression of the affected systems during the plateau phase of the dicyclic growth curves was demonstrated. Carbon dioxide assimilation by whole cells agreed well with the RDPC activity of extracts from cells grown under similar conditions.

A number of organic compounds, utilizable as carbon and energy sources for heterotrophic growth, can affect the autotrophic metabolism of hydrogen bacteria. In cells grown heterotrophically, these effects were observed as decreased levels of the two vital autotrophic functions, the hydrogen-oxidizing and carbon dioxide-fixing systems. Among the members of the genus *Hydrogenomonas*, *H. flava* (9), *H. facilis* (13, 20, 23), *H. ruhlandii* (16), and *Hydrogenomonas* H 1 (2) possess inducible or repressible hydrogenases. Only *H. eutropha* seems to lack this regulatory mechanism (Bovell, Ph.D. Thesis, University of California, Davis, 1957; Stukus, M.S. Thesis, The Catholic University of America, Washington, D.C., 1966).

The carbon dioxide-fixing system plays perhaps an even more critical role in the control of autotrophic metabolism. In *Hydrogenomonas*, as in other autotrophic bacteria, considerable evidence implicates ribulose diphosphate carboxylase (RDPC, EC 4.1.1.39) as the primary catalyst for the assimilation of carbon dioxide during autotrophic growth (1, 3, 7, 8, 14, 17, 19, 21). The activity of this enzyme in a number of facultative autotrophs after heterotrophic cultivation was reported to vary from 0 to 100% of the autotrophic level, depending upon the growth conditions employed. Growth on pyruvate (8), acetate, or malate (5) apparently represses RDPC in *Chromatium*. A similar repression by acetate was observed with *Micrococcus denitrificans* (10) and *H. facilis* (15), suggesting that RDPC plays a critical role in the control of CO_2 assimilation during heterotrophic growth. *H. ruhlandii*, when grown on lactate, possessed only 2% of the autotrophic level of RDPC (22), whereas *H. eutropha* contained much higher levels (18). Growth on fructose produced decreasing levels of RDPC with *Hydrogenomonas* H 16 (6) but not with *H. facilis* (15). More recently, other environmental conditions, including the degree of aeration, were implicated in the maintenance of high RDPC activity during heterotrophic growth on fructose (11).

We previously described the dicyclic growth

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patterns which occurred when *H. eutropha* was grown under dual substrate (mixotrophic) conditions (4). When the organic substrate allowed simultaneous autotrophic and heterotrophic metabolism during the initial growth phase, as evidenced by increased cell yields, a rapid changeover to exclusively autotrophic growth occurred at the point of organic substrate exhaustion. With three of the tested organic substrates, glutamate, acetate, and pyruvate, the initial growth phase seemed to represent heterotrophic rather than simultaneous growth, and with these substrates a lengthy changeover period of 5 to 8 hr preceded the initiation of autotrophic growth. It was suggested that both the lack of simultaneous growth and the lag (plateau) before autotrophic growth commenced were due to a repression of some autotrophic function by these three substrates. Since hydrogenase is constitutive in *H. eutropha*, and since decreased levels of RDPC were reported in some facultative autotrophs after heterotrophic growth on glutamate, acetate, and pyruvate, it seemed likely that these substrates may be repressing RDPC in *H. eutropha*, both in the presence and absence of the autotrophic atmosphere.

The present investigation was undertaken to determine whether the growth patterns observed under dual substrate conditions could be correlated with RDPC activity. In addition, $^{14}CO_2$ assimilation by cells grown under various conditions was compared to their RDPC activities to confirm that this enzyme is the major catalyst for autotrophic carbon dioxide-fixation in *H. eutropha*.

MATERIALS AND METHODS

Special chemicals. 3-Phosphoglyceric acid kinase-3-phosphoglyceraldehyde dehydrogenase, and adenosine triphosphate (ATP) were obtained from Calbiochem, Inc., Los Angeles, Calif.; 2,5-diphenyloxazole (PPO), dimethyl 1,4-bis-2(5-phenyloxazolyl)-benzene (POPOP), and Triton X-100 were from Packard Instrument Co., Downers Grove, Ill.; $NaH^{14}CO_3$ was from Schwarz BioResearch Inc., Orangeburg, N.Y.; dibarium ribulose-1,5-diphosphate and reduced nicotinamide adenine dinucleotide (NADH) were obtained from Sigma Chemical Co., St. Louis, Mo.; Nessler's reagent was from Arthur H. Thomas Co., Philadelphia, Pa.

Media and growth conditions. Autotrophic and dual substrate cultivation of *H. eutropha* were as previously described (4). For heterotrophic growth, organic substrates which had been autoclaved separately in distilled water were added to the specified concentrations, and incubation was under air. Because of the influence of shaker speed on RDPC activity noted by Kuehn & McFadden (11), RDPC was determined in extracts from cultures which had been shaken at both slow (130 gyrations/min) and rapid (325 gyrations/min) speeds. Growth was monitored by following the increase in optical density (OD) at 540 nm by using a Spectronic-20 colorimeter (Bausch and Lomb, Inc., Rochester, N.Y.). An OD of 1.0 corresponds to a cellular dry weight of 0.32 mg/ml, and this relationship is linear throughout exponential growth.

Cell-free extracts. Cells were grown under the specified conditions, harvested by centrifugation at 2 C, and suspended in 0.05 M tris (hydroxymethyl) aminomethane (Tris)-hydrochloride buffer (*p*H 7.9) containing 12 mM of β-mercaptoethanol, 50 mM $NaHCO_3$, and 1.5 mM ethylenediaminetetraacetic acid. The cell suspensions were disrupted by sonic treatment for 2 min in an ice bath by using a Biosonik II sonifier (Bronwill Scientific Co., Rochester, N.Y.) at maximum power. The disrupted suspensions were centrifuged at 20,000 × *g* for 20 min at 2 C. The supernatant fractions were dialyzed overnight at 4 C against 4 liters of the same buffer solution in which the cells had been suspended. These dialyzed extracts were used in the RDPC assay.

RDPC assay. The method employed was a modification of that described by Hurlbert and Lascelles (8). The assay consisted of two parts. The first part involved the formation of 3-phosphoglyceric acid (3-PGA); the second part was the spectrophotometric determination of the amount of 3-PGA formed in part one measured by the oxidation of NADH. The reaction mixture for part one contained 12 μmoles of $MgSO_4$, 12 μmoles of reduced glutathione, 80 μmoles of $NaHCO_3$, 1 μmole of ribulose diphosphate, 100 μmoles of Tris-hydrochloride buffer (*p*H 7.9), and the amount of cell extract containing 1.0 mg of protein, in a final volume of 2.0 ml. The mixture was incubated at 37 C for 5 min and then placed in a boiling water bath for 3 min to stop the reaction. After cooling, the mixture was clarified by filtration through a Swinnex 13 membrane filter unit (Millipore Corp., Bedford, Mass.) with a pore size of 0.45 μm. The filtered material was used in the spectrophotometric assay which was performed by using a Hitachi Perkin-Elmer 139 spectrophotometer equipped with a Coleman-Hitachi 165 recorder. For the assay, the cuvettes contained 17 μmoles of $MgSO_4$, 20 μmoles of L-cysteine, 2 μmoles of ATP, 0.4 μmoles of NADH, 150 μmoles of Tris-hydrochloride buffer (*p*H 7.9), 210 μg of 3-phosphoglyceraldehyde dehydrogenase, and 1.0 ml of filtered material, in a final volume of 3.0 ml. The reaction was started by the addition of 2.2 μg of 3-PGA kinase. The oxidation of NADH was measured as the decline in OD at 340 nm until there was no further decrease, and the amount of 3-PGA in the sample was calculated from the amount of NADH oxidized. A unit of enzyme activity is defined as the amount catalyzing the formation of 1 nmole of 3-PGA per min which corresponds to a decrease in OD of 0.0045. Specific activity is defined as units per milligram of protein. Protein was determined by the biuret method.

Fixation of labeled bicarbonate. Cells were grown under the specified conditions, harvested at the desired growth phase by centrifugation at 2 C, and resuspended to 4.0 OD in 0.04 M potassium phosphate

buffer (*p*H 7.2). Incorporation of $^{14}CO_2$ was performed by using double side-arm Warburg flasks. The main compartment of the flask contained 1.0 ml of the cell suspension and 1.5 ml of phosphate buffer. Into one side arm was placed a 0.4-ml amount containing 4 μc of $NaH^{14}CO_3$ (5.3 mc/mmole); the other sidearm contained 0.3 ml of 20% trichloroacetic acid. The flasks were incubated with shaking in a Warburg apparatus at 30 C. One series of flasks was incubated under an atmosphere of 80% H_2 and 20% O_2, with a duplicate series being incubated under 80% N_2 and 20% O_2. After equilibrating for 15 min, the $NaH^{14}CO_3$ was tipped into the main compartment, and the reaction was allowed to proceed for 1 hr. The flasks were then removed from the water bath, and filter paper saturated with 40% KOH was placed into each center well. The flasks were stoppered, and the trichloroacetic acid was tipped into the main compartment. After allowing the remaining dissolved CO_2 to evolve, 1.0-ml samples were removed from the main compartment and assayed for radioactivity by using a Packard Tri-Carb liquid scintillation spectrometer. The scintillation fluid in each vial consisted of 3.0 ml of Triton X-100 and 6.0 ml of a toluene solution containing 0.4% PPO and 0.01% POPOP. The results were corrected for controls incubated under N_2 and O_2.

Nitrogen determination. Inorganic nitrogen uptake was followed using Nessler's reagent. Cells were harvested by centrifugation at 2 C, washed, and suspended to 2.0 OD in media containing 0.01% $MgSO_4 \cdot 7\ H_2O$, 0.001% $Fe(NH_4)_2SO_4 \cdot 6\ H_2O$, and 106 μmoles $(NH_4)_2SO_4$ in 0.04 M potassium phosphate buffer (*p*H 6.0). The cell suspension (50 ml) was added to flasks and incubated under an atmosphere of 70% H_2, 20% O_2, 10% CO_2. Samples were withdrawn at the indicated time intervals and chilled in an ice bath to stop nitrogen uptake. The suspensions were then centrifuged at 2 C to remove the cells, and the clear supernatant fluid was assayed for nitrogen. Treatment of the suspensions with trichloroacetic acid rather than chilling yielded no significant difference.

RESULTS

The growth curves obtained when *H. eutropha* was cultured heterotrophically and mixotrophically in a glutamate-salts medium are shown in Fig. 1. Similar curves were obtained when acetate (4) or pyruvate (Fig. 2) was substituted for glutamate. With these substrates, heterotrophic growth rates and cell yields are about equal to the growth rates and yields during the initial phase of mixotrophic incubation. The fact that glutamate was exhausted at the initiation of the plateau period seen in Fig. 1 was confirmed by bioassay. Thus these three substrates probably do not allow simultaneous growth to occur, as was observed with some other organic substrates plus hydrogen (4, 18). The plateaus observed in the dicyclic curves shown in Fig. 1 and 2 indicate that some enzyme(s) required for autotrophic growth is repressed by these substrates.

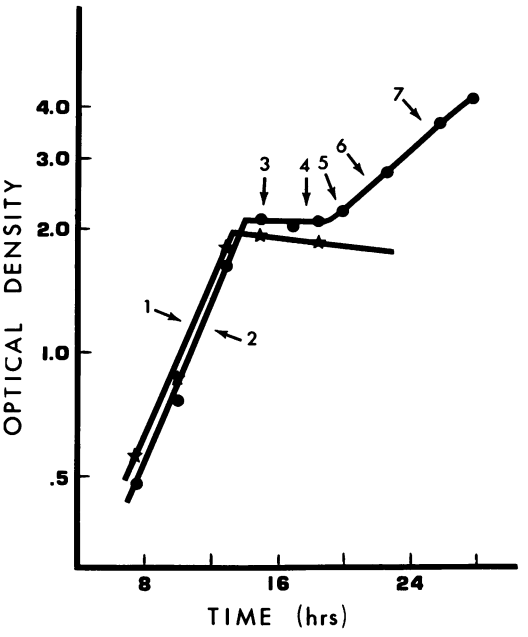

FIG. 1. *Growth of Hydrogenomonas eutropha in mineral-salts media containing 0.2% L-glutamate. Incubation was under 70% H_2, 20% O_2, 10% CO_2 (●) or under air (★). The arrows indicate the points at which culture samples were withdrawn and assayed for their ability to incorporate ammonia (see Fig. 3). Ordinate is a logarithmic scale.*

Cells grown heterotrophically in the presence of acetate or pyruvate did not possess significant RDPC activity (Table 1). Under dual substrate conditions, there also was no significant activity of RDPC until the acetate or pyruvate was consumed; however, as expected, enzyme activity was high during the second (autotrophic) phase of growth. During mixotrophic cultivation with pyruvate, the derepression of RDPC during the plateau period can be observed by comparing the points at which culture samples were withdrawn (Fig. 2) with their corresponding enzyme levels (Table 1).

The addition of acetate or pyruvate to an autotrophic extract had little effect on the RDPC activity (Table 2), providing evidence that acetate and pyruvate do not directly inhibit the enzyme.

Of the organic substrates tested, only acetate and pyruvate repressed RDPC during dual substrate incubation. Thus, although RDPC levels were low during heterotrophic growth on glutamate, the high enzyme levels present during the first phase of dual substrate incubation precluded the repression of this enzyme being responsible for the plateau observed with glutamate.

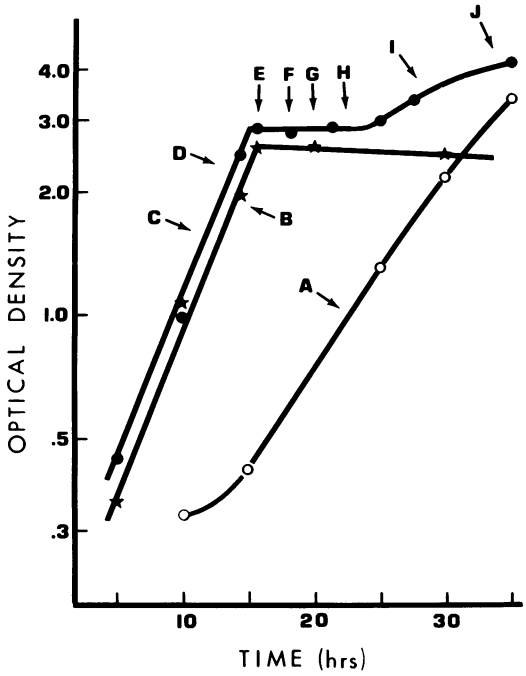

FIG. 2. *Growth of Hydrogenomonas eutropha under autotrophic* (○), *heterotrophic* (★), *and mixtotrophic* (●) *conditions. For heterotrophic and mixotrophic growth 0.3% pyruvate was added to the mineral-salts medium. Incubation was under air for heterotrophic growth and under 70% H_2, 20% O_2, 10% CO_2 for autotrophic and mixotrophic growth. The arrows indicate the points at which culture samples were withdrawn and assayed for ribulose diphosphate carboxylase (see Table 1).*

The shaker speed at which cultures were incubated had no noticeable effect on RDPC levels when acetate or pyruvate was present. With cells grown either heterotrophically or mixotrophically on fructose, glutamate or alanine levels of RDPC were generally somewhat lower when rapid shaking was used. The activities in Table 1 were therefore determined with cultures incubated with slow shaking. The specific activity of RDPC from autotrophic cultures grown with slow shaking was 48.3 ± 5.1 units per mg protein. Autotrophic cultures grown with rapid shaking showed slightly lower specific activities (43.4 ± 5.6).

Dual substrate cultivation with fructose as the organic substrate gave a dicyclic growth curve lacking a plateau and similar to that seen with alanine (4). The high levels of RDPC present during mixotrophic cultivation with fructose or alanine are therefore in agreement with the growth patterns.

Cells cultured under conditions similar to those used for RDPC assay were also tested for their ability to assimilate labeled bicarbonate (Table 1). The results confirmed that heterotrophic and mixotrophic growth with pyruvate and acetate prevented autotrophic CO_2 fixation, whereas cells cultured mixotrophically in the presence of the other organic substrates allowed fixation to occur at high levels.

Since cells cultivated mixotrophically with glutamate contained a high level of RDPC, the plateau period after glutamate exhaustion must be explained in terms of some other function. One major difference between heterotrophic growth with glutamate and autotrophic growth is the assimilation of nitrogen. During autotrophic growth, *H. eutropha* must produce its organic nitrogen by the assimilation of ammonia, apparently to form glutamate. When grown on glutamate, inorganic nitrogen is not required and, in fact, may be somewhat inhibitory to growth (Strenkoski, *unpublished data*). It was reasoned, therefore, that the presence of glutamate may inhibit the assimilation of inorganic nitrogen during both heterotrophic and dual substrate incubation, and that the plateau after glutamate exhaustion may be the time required for the formation of a nitrogen-assimilating system. To investigate this possibility, cells harvested from various stages of the (glutamate) mixotrophic and heterotrophic growth curves (Fig. 1) were tested for their ability to take up nitrogen (Fig. 3). Cells harvested during heterotrophic growth or the first phase of mixotrophic culture showed little or no nitrogen uptake during the 80-min test period. Cells harvested from sequential points along the plateau showed increasing abilities to incorporate nitrogen, and a rate approaching the autotrophic level was reached during the second phase of the dicyclic growth curve. Cells grown heterotrophically or mixotrophically with pyruvate yielded nitrogen uptake rates similar to those of autotrophic cultures.

To demonstrate that the suppression of nitrogen uptake in cells grown on glutamate was not due to some accumulating inhibitor or other function of the culture medium, cells were harvested from various points along the dicyclic growth curve, washed, and inoculated into fresh autotrophic media. This procedure had no effect on the duration of the total lag before autotrophic growth commenced. Cells harvested previous to or at the beginning of the plateau required about 7 hr before growth began. Cells harvested at the end of the plateau showed little or no additional lag, whereas cultures from sequential points along the plateau showed decreasing lag times in fresh media. The total lag between the exhaustion or removal of glutamate, and the initiation of autotrophic growth was about 7 hr in all cases.

TABLE 1. *Relative levels of ribulose diphosphate carboxylase (RDPC) and CO_2 fixation during heterotrophic and mixotrophic growth of Hydrogenomonas eutropha*

Organic substrate	Atmosphere	Growth phase	$H^{14}CO_3$ Incorporation	RDPC activity
	Autotrophic[a]	Exponential[b] (A)[c]	1.00	1.00
Acetate	Air	Exponential	0.08	0
Acetate	Autotrophic	I[d]	0	0
Acetate	Autotrophic	II[e]	0.89	0.64
Pyruvate	Air	Exponential (B)	0	0.01
Pyruvate	Autotrophic	I (C)	0	0.02
Pyruvate	Autotrophic	I (D)	NA[f]	0.04
Pyruvate	Autotrophic	Plateau (E)	0	0.02
Pyruvate	Autotrophic	Plateau (F)	0.14	NA
Pyruvate	Autotrophic	Plateau (G)	NA	0.43
Pyruvate	Autotrophic	Plateau (H)	NA	0.64
Pyruvate	Autotrophic	II (I)	NA	0.96
Pyruvate	Autotrophic	II (J)	0.72	0.88
Fructose	Air	Exponential	0.55	0.64
Fructose	Autotrophic	I	0.89	0.77
Fructose	Autotrophic	II	0.85	1.00
Glutamate	Air	Exponential	0.29	0.36
Glutamate	Autotrophic	I	NA	0.74
Alanine	Air	Exponential	0.61	0.27
Alanine	Autotrophic	I	0.92	0.84

[a] 70% H_2, 20% O_2, 10% CO_2.
[b] Since only one substrate was present, activities were determined with samples from the exponential portions of the standard growth curves.
[c] Letters A through J correspond to the points in Fig. 2 at which the samples were harvested.
[d] Harvested during the initial (mixotrophic) phase of dicyclic growth.
[e] Harvested during the second (autotrophic) phase of dicyclic growth.
[f] Not assayed.

DISCUSSION

A number of previous studies showed that autotrophic bacteria grown in the presence of organic compounds often contain decreased levels of autotrophic enzymes. In most cases these decreased enzyme levels were observed in the absence of the inorganic substrate; it was usually not determined if the enzymes would be similarly repressed in the presence of both organic and inorganic substrates. In one study, however, the facultative autotroph *Chromatium* contained low levels of RDPC during growth on pyruvate, but maintained high enzyme levels when both pyruvate and thiosulfate were present. The thiosulfate apparently prevented the repression of RDPC by pyruvate (8). Our results with *H. eutropha* demonstrate that the repression of RDPC by pyruvate or acetate occurs in the presence as well as the absence of the autotrophic atmosphere, and this repression is not due to a direct effect at the enzyme level.

During simultaneous cultivation with hydrogen and all tested organic substrates except glutamate, there was a correlation between the capacity of the cells to grow autotrophically and their RDPC activity. Substrates such as alanine and fructose which supported high levels of RDPC during the first phase of mixotrophic growth allowed autotrophic growth to commence rapidly when the organic substrate was consumed. With acetate and pyruvate the repression of RDPC during the first growth phase necessitated the synthesis of this enzyme before autotrophic growth could begin.

TABLE 2. *Effect of acetate and pyruvate on the activity of ribulose diphosphate carboxylase (RDPC) in an autotrophic extract of Hydrogenomonas eutropha*

Additions	RDPC (specific activity)
None	40.6
0.05% Pyruvate	33.0
0.1% Pyruvate	40.6
0.2% Pyruvate	42.4
0.1% Acetate	32.0
0.2% Acetate	30.2

McFadden and Tu (15) reported that when *H. facilis* was grown heterotrophically on glutamate it contained approximately 3% of the autotrophic level of RDPC. We found, however, that *H. eutropha* grown on glutamate possessed

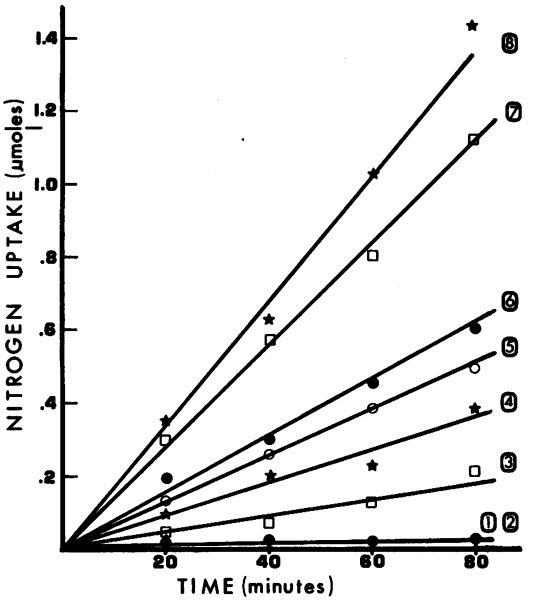

FIG. 3. *Nitrogen uptake by Hydrogenomonas eutropha cells grown heterotrophically or mixotrophically on glutamate. The assays were performed under autotrophic conditions with cells which had been washed and resuspended in the mineral medium. Nitrogen uptake represents ammonia consumed per milliliter of OD 2.0 culture [0.64 mg (dry weight)/ml]. Curves 1 through 7 represent uptake by cells harvested at the corresponding points in the growth curves of Fig. 1. Curve 8 represents uptake by an autotrophically grown culture.*

36% of the autotrophic level of RDPC, and, when grown mixotrophically on glutamate plus hydrogen, the RDPC level increased to 74%. Thus a repression of RDPC by glutamate could not account for the plateau observed in the growth curves during dual substrate incubation. However, heterotrophic and mixotrophic growth on glutamate does directly or indirectly interfere with nitrogen uptake or assimilation, and this deficiency may be responsible for the temporary inability to grow autotrophically.

Of the major nitrogen assimilating catalysts, only glutamate dehydrogenase has been found in *H. eutropha* (Strenkoski, *unpublished data*). Its properties are similar to those reported for *Thiobacillus novellus* (12), and it catalyzes both the synthesis and degradation of glutamate in vitro. Since cells grown on glutamate possess high levels of glutamate dehydrogenase which could presumably function biosynthetically after glutamate exhaustion, the control point for nitrogen assimilation in *H. eutropha* is probably not the assimilating enzyme itself but may be either the nitrogen uptake mechanism or the synthesis of 2-oxoglutarate. Heterotrophic or mixotrophic growth on glutamate might therefore suppress either of these processes.

The correlation between the relative activities of RDPC and resting cell CO_2 fixation, using cells grown both heterotrophically and mixotrophically, strongly implicates RDPC as the major catalyst for knallgas-supported CO_2 fixation in *H. eutropha*. This correlation can be compared with the parallel levels of RDPC and cell-free reductive CO_2 fixation observed with *H. facilis* (15) and suggests a similarity between the pathways of CO_2 assimilation and their regulation in these two species.

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LITERATURE CITED

1. Aleem, M. I. H. 1965. Path of carbon and assimilatory power in chemosynthetic bacteria I. *Nitrobacter agilis*. Biochim. Biophys. Acta 107:14–28.
2. Bartha, R. 1962. Physiologische Untersuchungen über den Chemolithotrophen Stoffwechsel neu isolierter *Hydrogenomonas*-Stämme. Arch. Mikrobiol. 41:313–350.
3. Bergmann, F. H., J. C. Towne, and R. H. Burris. 1958. Assimilation of carbon dioxide by hydrogen bacteria. J. Biol. Chem. 230:13–24.
4. DeCicco, B. T., and P. E. Stukus. 1968. Autotrophic and heterotrophic metabolism of *Hydrogenomonas*. I. Growth yields and patterns under dual substrate conditions. J. Bacteriol. 95:1469–1475.
5. Fuller, R. C., R. M. Smillie, E. C. Sisler, and H. L. Kornberg. 1961. Carbon metabolism in *Chromatium*. J. Biol. Chem. 236:2140–2149.
6. Gottschalk, G., U. Eberhardt, and H. G. Schlegel. 1964. Verwertung von Fructose durch *Hydrogenomonas* H16. I. Arch. Mikrobiol. 48:95–108.
7. Hirsch, P., G. Georgiev, and H. G. Schlegel. 1963. CO_2-Fixierung durch Knallgas-bakterien. III. Autotrophe und organotrophe CO_2-Fixierung. Arch. Mikrobiol. 46:79–95.
8. Hurlbert, R. E., and J. Lascelles. 1963. Ribulose diphosphate carboxylase in *Thiorhodaceae*. J. Gen. Microbiol. 33:445–458.
9. Kluyver, A. J., and A. Manten. 1942. Some observations on the metabolism of bacteria oxidizing molecular hydrogen. Antonie van Leeuwenhoek J. Microbiol. Serol. 8:71–85.
10. Kornberg, H. L., J. F. Collins, and D. Bigley. 1960. The influence of growth substrates on metabolic pathways in *Micrococcus denitrificans*. Biochim. Biophys. Acta 39:9–24.
11. Kuehn, G. D., and B. A. McFadden. 1968. Factors affecting the synthesis and degradation of ribulose-1,5-diphosphate carboxylase in *Hydrogenomonas facilis* and *Hydrogenomonas eutropha*. J. Bacteriol. 95:937–946.
12. LeJohn, H. B., and B. E. McCrea. 1968. Evidence for two species of glutamate dehydrogenases in *Thiobacillus novellus*. J. Bacteriol. 95:87–94.
13. Linday, E. M., and P. J. Syrett. 1958. The induced synthesis of hydrogenase by *Hydrogenomonas facilis*. J. Gen. Microbiol. 19:223–227.
14. McFadden, B. A. 1959. Some products of $^{14}CO_2$ fixation by *Hydrogenomonas facilis*. J. Bacteriol. 77:339–343.
15. McFadden, B. A., and C. L. Tu. 1967. Regulation of autotrophic and heterotrophic carbon dioxide fixation in *Hydrogenomonas facilis*. J. Bacteriol. 93:886–893.
16. Packer, L., and W. Vishniac. 1955. Chemosynthetic fixation

of carbon dioxide and characteristics of hydrogenase in resting cell suspensions of *Hydrogenomonas ruhlandii* nov. spec. J. Bacteriol. **70**:216–223.

17. Quayle, J. R. 1961. Metabolism of C_1 compounds in autotrophic and heterotrophic microorganisms. Annu. Rev. Microbiol. **15**:119–152.
18. Rittenberg, S. C., and N. S. Goodman. 1969. Mixotrophic growth of *Hydrogenomonas eutropha*. J. Bacteriol. **98**:617–622.
19. Santer, M., and W. Vishniac. 1955. CO_2 incorporation by extracts of *Thiobacillus thioparus*. Biochim. Biophys. Acta **18**:157–158.
20. Schatz, A., and C. Bovell, 1952. Growth and hydrogenase activity of a new bacterium, *Hydrogenomonas facilis*. J. Bacteriol. **63**:87–98.
21. Trudinger, P. A. 1956. Fixation of carbon dioxide by extracts of the strict autotroph *Thiobacillus denitrificans*. Biochem. J. **64**:274–286.
22. Vishniac, W., and P. A. Trudinger. 1962. Symposium on autotrophy. V. Carbon dioxide fixation and substrate oxidation in the chemosynthetic sulfur and hydrogen bacteria. Bacteriol. Rev. **26**:168–175.
23. Wilson, E., H. A. Stout, D. Powelson, and H. Koffler. 1953. Comparative biochemistry of the hydrogen bacteria. I. Simultaneous oxidation of hydrogen and lactate. J. Bacteriol. **65**:283–287.