RIBULOSE DIPHOSPHATE CARBOXYLASE AND CO_2 INCORPORATION IN EXTRACTS OF *HYDROGENOMONAS FACILIS*[*]

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Little work describing the direct detection of ribulose-1,5-diphosphate (RuDP) carboxylase in chemosynthetic bacteria has been published, although its presence can be inferred from results obtained in many laboratories. In facultative autotrophs such as hydrogen bacteria the level of this enzyme, which is one of the catalysts that uniquely functions in the reductive pentose phosphate path for CO_2 fixation, may regulate the relative amounts of CO_2 fixed by this and other paths during heterotrophic and autotrophic growth.

We wish to report data on the variation of levels of RuDP carboxylase in *Hydrogenomonas facilis* as a function of mode of culture. In addition, variation in the DPNH-, ATP-dependent incorporation of $^{14}CO_2$ by extracts is described. The correlation of these two sets of data strongly implicates the RuDP carboxylase-catalyzed step as a major control point in adjusting the flow of CO_2 through the reductive pentose phosphate cycle during heterotrophic growth.

METHODS

Culture and Preparation of Cells and Extracts. Autotrophic and heterotrophic culture of *H. facilis* were exactly as described by McFadden and

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Homann (1965). Provision of H_2, O_2 and CO_2 during autotrophic growth was as defined by McFadden and Atkinson (1957). After harvest, the cell mass was washed in buffer and suspended in 0.1 M Tris, pH 7.2, containing 2 mM GSH. In some cases the buffer also contained 20 mM $MgCl_2$. The cell suspension was treated for 20 sec with a 20 kc Biosonik oscillator at less than 10° C. The suspension was then centrifuged (2° C) at 20,000 g for 20 min and the supernatant used in the experiments described. Protein concentration was estimated by the method of Warburg and Christian (1941) with a correction for GSH absorbancy as necessary.

Assays. After apparently optimal conditions for catalysis by RuDP carboxylase had been established, assay reaction mixtures (0.25 ml) at pH 7.5 contained, in addition to cell extract, the following components (in μmoles): $MgCl_2$, 3; RuDP, 0.15; $NaH^{14}CO_3$, 5; GSH, 0.3; Tris, 15. All components except RuDP were preincubated for 30 min at 0° and the temperature was then brought to 30° during a 5 min interval. Then RuDP (or water in the case of controls) was added to initiate the reaction. After 5 min, the reaction was stopped by the addition of 0.1 ml 60% (w/v) trichloroacetic acid (TCA). Early studies at pH 8.0 revealed that the amount of aerobic uptake of $H^{14}CO_3^-$ was about 80% that of anaerobic fixation. Aerobic assays were subsequently conducted for convenience. Two-dimensional paper chromatography and subsequent radioautography revealed that the major acid-stable product accounting for ca. 95% of the ^{14}C fixed was phosphoglycerate for both aerobic and anaerobic experiments.

Apparently optimal conditions for CO_2 fixation in the presence of reducing power and ATP were also established. Then extracts from H. facilis grown in various ways were assayed for CO_2 incorporation using a reaction mixture (pH 7.5) of 0.5 ml containing in addition to extract the following components (in μmoles): $MgCl_2$, 6; $NaH^{14}CO_3$, 12.5; DPNH, 2; ATP, 1.5; GSH, 0.6; Tris, 45. Incubations were conducted at 30° under N_2 in Warburg flasks. Reactions were initiated by adding $H^{14}CO_3^-$, ATP and DPNH simul-

taneously and stopped after 30 min by the addition of 0.2 ml 60% (w/v) TCA.

Just before all experiments the specific radioactivity of the $H^{14}CO_3^-$ used was determined by the method of Bray (1960). Subsequent measurements of incorporation were made using the same method. In all cases, specific incorporation (or RuDP carboxylase) activities were calculated in a region of first-order kinetic dependence upon protein concentration.

RESULTS AND DISCUSSION

Table 1 shows the dependence of RuDP carboxylase activity upon several factors.

TABLE 1

RuDP Carboxylase Activity in Autotrophic Extracts

Component varied and its final concn.[a]		d.p.m. $H^{14}CO_3^-$ fixed
Mg^{+2}		
	none added	1,666
	0.01 M	9,600
	0.1 M	10,265
RuDP		
	none added	74
	0.4 mM	711
	0.6 mM	831
$H^{14}CO_3^-$		
	5 mM	2,080
	10 mM	2,800
	15 mM	2,860

[a]Data for different components are not comparable since different experiments were conducted. Experimental protocol was essentially as described in the text except for the component varied.

Table 2 presents data which establish that in the absence of exogenous RuDP, $H^{14}CO_3^-$ fixation is markedly stimulated by DPNH plus ATP and is inhibited by 10^{-3} M AMP.

Replacement of DPNH by TPNH in the otherwise complete system resulted in 3% as much incorporation emphasizing the marked specificity for reductant. Incubations conducted aerobically resulted in ca. 30% as much fixation

and were therefore routinely run under anaerobic conditions. H. facilis is known to have a potent DPNH oxidase (unpublished observation, H. R. Homann).

TABLE 2

Reductive CO_2 Fixation by Autotrophic Extracts

Incubation conditions*	c.p.m. $H^{14}CO_3^-$ fixed
Mg^{+2} varied	
10^{-3} M	1,000
10^{-2} M	14,024
10^{-1} M	15,845
ATP varied	
none added	183
2 mM	870
3 mM	1,170
DPNH varied	
none added	163
3 mM	3,531
4 mM	3,982
$H^{14}CO_3^-$	
20 mM	96,564
25 mM	110,454
Complete	17,486
1 mM AMP	5,552

*See footnote in Table 1.

Table 3 illustrates the changes which occur in specific activity of RuDP carboxylase and reductive CO_2 fixation as a result of different modes of culture.

Of interest is the high specific activity of RuDP carboxylase in fructose-grown cells, which is significantly different from the findings of Gottschalk et al. (1964) for Hydrogenomonas H16. Of major interest, however, is the qualitative parallel between reductive CO_2 fixation and RuDP carboxylase activity. The observed CO_2 fixation has the properties

TABLE 3

Variations in RuDP Carboxylase and Reductive CO_2 Fixation

Cultured	Reductive, ATP-dependent[a] CO_2 fixation μmoles CO_2 fixed/min/ mg protein x 10^3	RuDP carboxylase[b] specific activity μmoles CO_2 fixed/min/ mg protein x 10^3	
	A	B	
Autotrophically	4.75	8.8	(15.2)[c]
On D-fructose	4.83	5.5	(15.4)
" D-glucose	1.65	2.67	(5.7)
" D-ribose	2.07	3.06	(6.7)
" D,L-lactate	1.73[d]	0.21	(1.5)
" L-glutamate	0.85	0.07	(1.3)
" succinate	0.24	0.04	(0.9)
" acetate	0.41	0.27	(0.5)

[a] All data are corrected for results with controls which lacked DPNH and ATP.

[b] All data are corrected for results with controls which lacked RuDP.

[c] Carboxylase activity in these experiments (bracketed numbers) was measured at pH 8.0 under essentially the same conditions as described in the text. All other values (columns A and B) were obtained for a single extract under conditions described in the text.

[d] This fixation was inhibited about 70% by 10^{-3} M AMP.

expected of incorporation via the pentose phosphate cycle used by plants (for a review see Calvin, 1962), except that DPNH is the reductant instead of TPNH. Apparently DPNH also serves specifically as a reductant during $^{14}CO_2$ fixation by extracts of Nitrobacter (Aleem and Nason, 1963) so this may be a fundamental difference between chemosynthetic and oxygen-evolving photosynthetic species. Further supporting the supposition that the bulk of the CO_2 fixation observed involved the pentose phosphate cycle and therefore catalysis by RuDP carboxylase is the inhibition by 10^{-3} M AMP (cf. Tables 2 and 3). On the basis of evidence obtained with Thiobacillus thioparus, Chromatium and spinach leaves, Johnson and coworkers (1965a,b) have recently proposed that AMP regulation of CO_2 fixation may represent a basic control mechanism in autotrophic metabolism.

In conclusion, our data establish that one important control point

for CO_2 fixation by facultative chemosynthetic organisms such as the hydrogen bacteria is the level of RuDP carboxylase.

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