CARBON DIOXIDE FIXATION AND CARBOXYDISMUTASE IN THIOBACILLUS NOVELLUS*

M. I. H. Aleem and Elizabeth Huang

Research Institute for Advanced Studies

1450 South Rolling Road, Baltimore, Maryland 21227

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Thiobacillus novellus is a facultative autotroph capable of growing either heterotrophically using organic substrates such as glutamate or autotrophically utilizing the energy of the oxidation of an inorganic sulfur compound in the reduced form such as thiosulfate. The extent of CO_2 fixed under these conditions involving the reductive pentose phosphate pathway would naturally be regulated by the level of carboxydismutase. The experiments reported here reveal that the autotrophic cells catalyze CO_2 fixation involving reactions of the carbon reduction cycle. A drastic reduction in the level of carboxydismutase occurs in the heterotrophic cell. However, the phospho-enol-pyruvate carboxylase activity is virtually unchanged under autotrophic or heterotrophic growth conditions. In the autotrophic extracts ATP and DPNH provide the carbon reduction power.

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Abbreviations: ATP and AMP, adenosine tri- and mono-phosphate; DPNH, reduced form of diphosphopyridine nucleotide; PGA, 3-phosphoglyceric acid; PEP, phospho-enol-pyruvic adid; R_5P, ribose-5-phosphate; RuDP, ribulose 1,5-diphosphate.

METHODS

Thiobacillus novellus was grown under autotrophic or heterotrophic conditions in culture media described by Vishniac and Santer (1957). The cell-free extracts were prepared as described by Aleem (1965). The fixation of CO_2 and products of fixation were obtained involving techniques used previously in case of Nitrobacter agilis (Aleem, 1965). The experimental details are given in appropriate tables.

RESULTS AND DISCUSSION

Washed intact cells of T. novellus grown autotrophically catalyzed active CO_2 fixation (Table I). A marked stimulation in the carbon assimilation occurred in the presence of added thiosulfate indicating that thiosulfate oxidation was coupled to generate energy and reducing power necessary for the endergonic reduction of CO_2.

TABLE I

Rate of ^{14}C Incorporation from $NaH^{14}CO_3$ into T. novellus cells

Time Seconds	Total $^{14}CO_2$ Assimilated	
	$+ S_2O_3^=$	$- S_2O_3^=$
	cpm x 100	
10	320	70
30	1,740	100
60	3,100	120
180	8,800	180
600	12,000	370

Reaction mixture in a total volume of 1.0 ml contained 33 µl wet-packed intact cells, 30 mM phosphate buffer pH 7.2, 10 mM S_2O_3, and 50 µc $NaH^{14}CO_3$. Reaction was run at 30°C.

The Products of CO_2 Fixation. Under the conditions of Table I the pattern of carbon dioxide fixation as revealed by chromatography and radioautography, resembled the autotrophic carbon reduction pathway outlined in case of Nitrobacter agilis (Aleem, 1965). Thus after a 30 second exposure of

the cells to $^{14}CO_2$, 50% of the total radioactivity appeared in PGA followed by a rapid and progressive decrease in radioactivity in this compound with increased time intervals. The radioactivity in sugar phosphates and glutamic acid increased with time. After a 30 second exposure of T. novellus cells to $^{14}CO_2$, about ten radioactive compounds were detected containing 50% of the total radioactivity in PGA, 17% in sugar phosphates, 13% in aspartic acid, 5% in glutamic acid, and 3 to 5% in each succinic acid, α-ketoglutaric acid, glutamine, serine, glycine, and alanine.

Table II shows the levels of carboxydismutase and PEP carboxylase in cell extracts obtained from T. novellus grown under autotrophic or heterotrophic conditions. The heterotrophic cells were obtained by growing the autotrophic strain in a culture medium containing glutamate as the energy source. Almost all of the RuDP carboxylase activity was lost in the heterotrophic cell extracts and little fixation of CO_2 was observed when these

TABLE II

Carboxydismutase and PEP Carboxylase Activity in Autotrophic and Heterotrophic Extracts

Additions	$^{14}CO_2$ Fixed/mg Protein	
	Autotrophic	Heterotrophic
	cpm	
Extract	382	240
Extract + RuDP	90,000	670
Extract + R_5P	53,000	330
Extract + R_5P + ATP	77,000	980
Extract + ATP	2,000	700
Extract + PEP	108,000	100,000

Reaction mixture in a total volume of 1.0 ml contained 0.2 ml cell-free fraction supernatent 144,000 x g containing 1.0 mg enzyme protein, 40 mM Tris pH 8.0, 10 μc $NaH^{14}CO_3$, 5 mM $MgCl_2$, and where shown 1 mM RuDP, 10 mM R_5P, 1 mM ATP and 10 mM PEP. Incubation period 30 minutes at 25°C.

extracts were primed with exogenous RuDP or R_5P and ATP. The autotrophic extracts under these conditions on the other hand, catalyzed very active CO_2 fixation. In short term incubation experiments lasting 4 to 5 minutes the examination of the products of the RuDP primed carboxylations was conducted by two-dimensional paper chromatography and radioautography. About 92 to 96% of the ^{14}C-fixed accounted for phosphoglyceric acid. Similar results have been reported by McFadden and Tu (1965) in a facultative autotroph, Hydrogenomonas facilis. Of particular interest is the observation that the autotrophic or heterotrophic extracts contained active PEP carboxylase, the level of which appears to be independent of the mode of growth of T. novellus.

Table III illustrates the effect of AMP on the reductive CO_2 assimilation, carboxydismutase, and PEP carboxylase systems of Thiobacillus novellus and an obligatory autotroph, Thiobacillus X. The observations of Johnson and Peck (1965 a,b) indicate that AMP played a potent role in controlling the autotrophic CO_2 fixation and McFadden and Tu (1965) have recently obtained confirming evidence in Hydrogenomonas facilis. In Table III (Expt. 1) the data indicate that similar to the Nitrobacter system (Aleem and Nason, 1963) the ATP and DPNH provided the assimilatory power in both T. novellus and Thiobacillus X.

A 1 mM concentration of AMP exhibited 82 and 96% inhibition of the reductive CO_2 assimilation by T. novellus and T. X. respectively. Similar results were obtained when the cell extracts were primed with R_5P and ATP. The RuDP carboxylase system was observed to be less sensitive at 1 mM AMP concentration and was not completely inhibited even in the presence of 5 mM AMP (Expt. II). In view of these observations it may be stated that AMP might be causing a competitive inhibition in systems where ATP has been used. The PEP carboxylase was not inhibited by AMP and as a matter of fact AMP caused stimulation in the CO_2 fixed.

In chemosynthetic bacteria there are at least two active sites for the entry and fixation of CO_2 controlled and catalyzed by the level of carboxy-

TABLE III

Effect of AMP on the Carboxylation Systems of Thiobacillus novellus and Thiobacillus X.

Treatment	CO_2 Acceptor	Total $^{14}CO_2$ Assimilated (cpm)	
		T. novellus	T. X.
Expt. I			
Cell extract	None	3,700	2,700
" + ATP	None	4,400	13,800
" + ATP + DPNH	None	28,000	128,000
" + ATP + DPNH + AMP	None	4,400	4,200
" + R_5P	R_5P	58,000	240,000
" + R_5P + ATP	R_5P	150,000	427,000
" + R_5P + ATP + AMP	R_5P	11,000	104,000
" + RuDP	RuDP	107,000	211,000
" + RuDP + AMP	RuDP	89,000	201,000
Expt. II			
Cell extract	None	4,000	3,000
" + RuDP	RuDP	85,000	36,000
" + RuDP + AMP	RuDP	28,000	13,000
Expt. III			
Cell extract	None	8,000	---
" + PEP	PEP	206,000	---
" + PEP + AMP	PEP	365,000	---

Experimental conditions were the same as in Table II except where indicated 2 μmoles of DPNH was used. Expt. I contained 1 mM AMP and Expt. II 5 mM AMP. Experiment III contained 5 mM AMP.

dismutase and PEP carboxylase. In the facultative chemoautotrophs while the PEP carboxylase level remains unchanged under autotrophic or heterotrophic growth conditions the level of RuDP carboxylase plays an important role in controlling the CO_2 fixation.

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