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EXPERIMENTAL ARTICLES

On the Mechanism of Autotrophic Fixation of CO_2 by *Chloroflexus aurantiacus*

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Abstract—The activity of two carboxylating enzymes was studied in the green filamentous bacterium *Chloroflexus aurantiacus*. The carboxylation reaction involving pyruvate synthase was optimized using $^{14}CO_2$ and cell extracts. Pyruvate synthase was shown to be absent from cells of *Cfl. aurantiacus* OK-70 and present (in a quantity sufficient to account for autotrophic growth) in cells of *Cfl. aurantiacus* B-3. Differences in the levels of acetyl CoA carboxylase activity were revealed between cells of the strains studied grown under different conditions. The data obtained confirm the operation of different mechanisms of autotrophic CO_2 assimilation in *Cfl. aurantiacus* B-3 and *Cfl. aurantiacus* OK-70: in the former organism, it is the reductive cycle of dicarboxylic acids, and in the latter one, it is the 3-hydroxypropionate cycle.

Key words: *Chloroflexus aurantiacus*, CO_2 assimilation

Most phototrophic bacteria are able to grow using CO_2 as the sole carbon source. Currently, three pathways of CO_2 fixation by autotrophic microorganisms are recognized: the reductive pentose phosphate cycle, the reductive tricarboxylic acid cycle, and the noncyclic acetyl CoA pathway [1].

Chloroflexus aurantiacus is a filamentous thermophilic bacterium able to grow autotrophically utilizing H_2 or H_2S as electron donors [2, 3]. In this organism, the key enzymes of the aforementioned CO_2 fixation pathways have not been revealed [1, 4, 5]. The data available indicate that *Cfl. aurantiacus* employs a novel pathway of autotrophic CO_2 assimilation [4–8]. Two models of this pathway have been suggested (Fig. 1). The first model implies carboxylation reactions catalyzed by pyruvate synthase and phosphoenolpyruvate carboxylase [5]. According to the second model, the major carboxylating enzymes are acetyl CoA carboxylase and propionyl CoA carboxylase [8]. In both cases, acetyl CoA regeneration occurs as a result of malyl CoA cleavage involving malyl CoA lyase. The main products of the cycle, according to both schemes, is glyoxylate. This compound can then be transformed to 3-phosphoglyceroaldehyde in reactions of the serine and glycerate pathways [5, 9]. The 3-hydroxypropionate cycle of CO_2 fixation was proposed based on the study of *Cfl. aurantiacus* OK-70, and the reductive dicarboxylic acid cycle was postulated to operate in *Cfl. aurantiacus* B-3.

The aim of the present work was to further study the pathways of CO_2 assimilation in *Cfl. aurantiacus* strains OK-70 and B-3.

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MATERIALS AND METHODS

The subjects of study were the two thermophilic strains *Cfl. aurantiacus* B3 and OK-70, isolated, respectively, from a hot spring near Lake Baikal and from a hot spring in Oregon, the United States [3, 10]. The bacteria were grown at 55–60°C on Castenholz medium [11] with 0.1% bicarbonate and 0.01% yeast extract (as a vitamin source). In some cases, the medium was supplemented with 0.1% sodium acetate.

The cultures were grown aerobically at an illumination of 1000 lx in sealed flasks completely filled with the medium or in flasks sealed with glass stoppers fitted with taps. The latter flask were filled with medium to 1/3 of their volume; the air in the gas phase was replaced with molecular hydrogen. Cultivation lasted 3 days, which corresponded to the middle of the exponential growth phase.

To determine the activity of carboxylating enzymes, cells were separated from the cultivation medium by centrifugation at 6–8°C, washed in 0.05 M Tris–HCl buffer (pH 7.8), and resuspended in a buffer of the following composition: 50 mM Tris–HCl, 5 mM $MgCl_2$, 5 mM DTT, and 1 mM EDTA (pH 7.8). Then, cells were disrupted in an X-press (LKB) at a pressure of 10000 kg. The homogenate was centrifuged under an atmosphere of argon. The extract obtained was used to determine enzymatic activities.

Enzymatic activities were determined at 55°C. Anaerobic conditions were maintained using H_2. The protein content in the reaction mixture was 0.5–2.0 mg/ml.

Pyruvate synthase (EC 1.2.7.1) was determined by the reaction of pyruvate synthesis and by the reaction of pyruvate–$^{14}CO_2$ exchange. In the former case, the reac-

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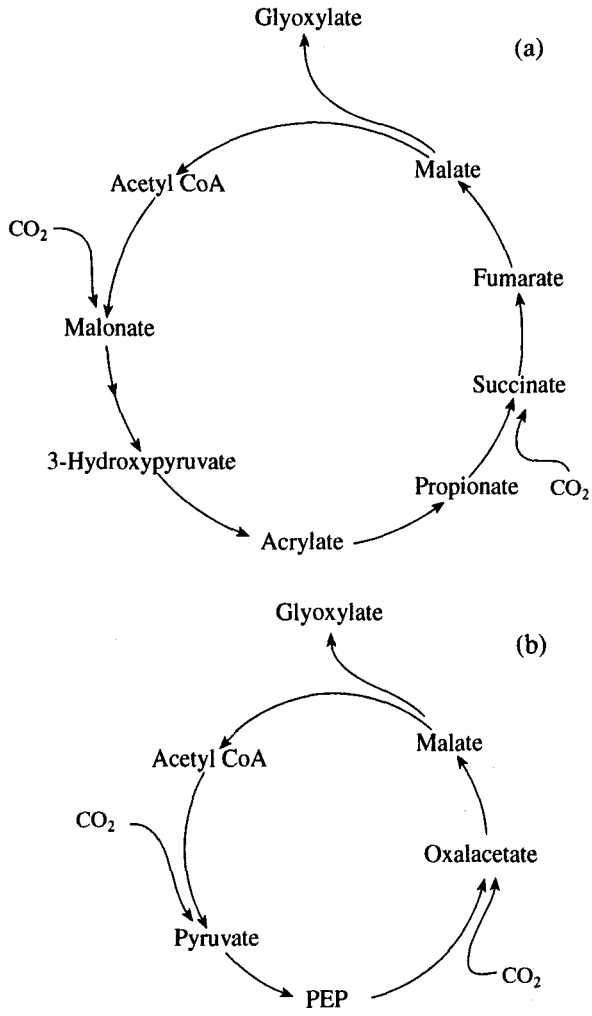

Fig. 1. Pathways of autotrophic CO_2 assimilation in *Cfl. aurantiacus*: (a) the 3-hydroxypropionate cycle; (b) the reductive dicarboxylic acid cycle.

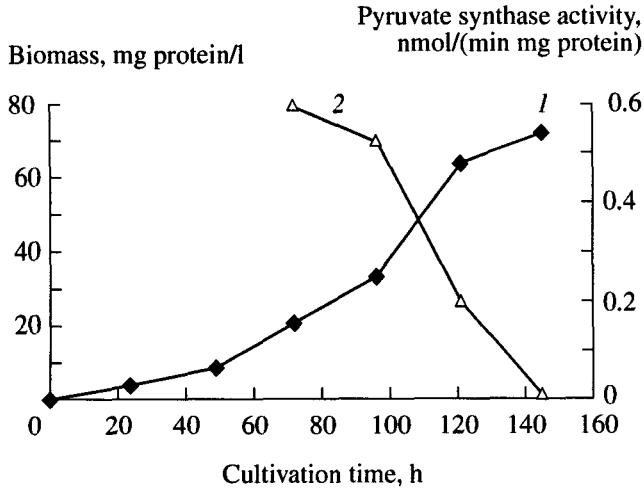

Fig. 2. Effect of culture age on the activity of pyruvate synthase of *Cfl. aurantiacus* B-3. (*1*) Culture growth; (*2*) pyruvate synthase activity.

tion mixture contained 50 mM Tris–HCl, 5 mM $MgCl_2$, 5 mM DTT, 2 mM methyl viologen (MV) reduced with metallic zinc, 5 mM Na acetate, 0.1 mM CoA, 5 mM AMP, 2 mM serine, 10 mM $NaHCO_3$, 0.04 MBq of $NaH^{14}CO_3$, and 0.5–1.0 mg/ml protein (pH 8.0). In the case of the exchange reaction, the reaction mixture contained 50 mM Tris–HCl, 5 mM $MgCl_2$, 5 mM DTT, 10 mM Na pyruvate, 0.1 mM acetyl CoA, 10 mM $NaHCO_3$, 0.04 MBq of $NaH^{14}CO_3$, and 0.5–1.0 mg/ml protein (pH 8.0).

Acetyl CoA carboxylase (EC 6.4.1.2) was determined in a reaction mixture containing 100 mM Tris–HCl, 4 mM $MgCl_2$, 5 mM DTT, 1 mM NADPH, 2 mM ATP, 0.4 mM acetyl CoA, 10 mM creatine phosphate, 0.1 mM CoA, 10 mM $KHCO_3$, 0.04 MBq of $NaH^{14}CO_3$, 7 units of creatine kinase, and 1.0–2.0 mg/ml of protein (pH 8.0).

The activity of serine–pyruvate aminotransferase (EC 2.6.1.51) was determined from the formation of hydroxypyruvate [12].

Radioactivity was measured on an LKB RacBeta 1127 scintillation counter. The rate of assimilation of labeled compounds by cells was expressed in nanomoles of radiocarbon in 1 min per 1 mg of cell protein (nmol/(min mg protein)).

Protein was determined by the Bradford method [13], using bovine serum albumin as the standard.

RESULTS AND DISCUSSION

Earlier, it was shown that *Cfl. aurantiacus* OK-70 and B-3 cells grown autotrophically contain all the enzymes necessary for the operation of the postulated pathways of autotrophic CO_2 assimilation [4, 5, 8]. The activity of the specific enzymes in cell-free extracts was sufficient to account for the cell growth rate observed under autotrophic conditions [4, 5]. The only exception was pyruvate synthase. The specific activity of this enzyme in *Cfl. aurantiacus* B-3 cells grown autotrophically was 0.3 nmol/(min mg protein) as measured in the reaction of pyruvate synthesis [5]. This enzyme is highly unstable, and, when measured in vitro, its activity is often underestimated [4, 5]. Therefore, we optimized the conditions for the reaction of pyruvate synthesis (carboxylation involving pyruvate synthase) in a cell-free extract. The level of pyruvate synthase activity depended on the growth phase of *Cfl. aurantiacus*. As can be seen from Fig. 2, high activity was observed in 3- and 4-day cultures (mid-exponential growth phase). Younger cultures were difficult to use in the optimization studies because the biomass accumulated was too low. The optimal pH for the reaction studied was found to be 7.5–8.0 (Fig. 3). It turned out that, along with the reaction studied, decarboxylation of the pyruvate synthesized also occurred, which can be explained by the presence of decarboxylating enzymes in the extract (Fig. 4). To suppress pyruvate decarboxylation, we added transaminase substrates to the reaction mixture;

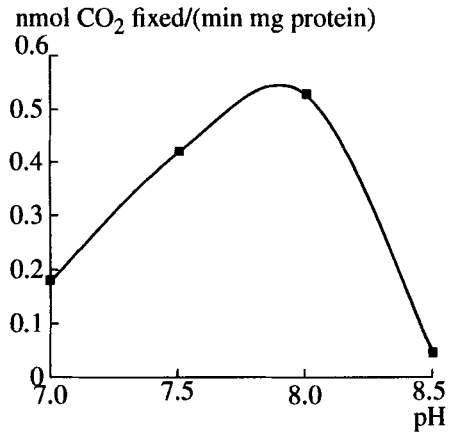

Fig. 3. Dependence of the activity of *Cfl. aurantiacus* B-3 pyruvate synthase on the pH of the reaction mixture.

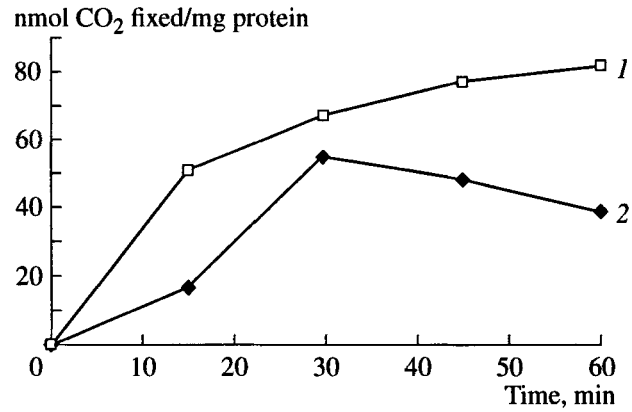

Fig. 4. Determination of the activity of *Cfl. aurantiacus* B-3 pyruvate synthase: (*1*) reaction mixture containing 2 mM serine; (2) control (without serine).

serine proved to be the additive that most efficiently prevented pyruvate decarboxylation (Fig. 4 and Table 1). Indeed, in *Cfl. aurantiacus* B-3 cells, serine–pyruvate aminotransferase is rather active (5 nmol/(min mg protein)). Serine does not stimulate carboxylation but allows decarboxylation processes, masking the carboxylation reaction, to be avoided (Table 2).

Determination of the activities of pyruvate synthase and acetyl CoA carboxylase revealed significant differences between the two strains of *Cfl. aurantiacus* studied. In extracts of *Cfl. aurantiacus* OK-70 cells grown under various conditions, we failed to detect pyruvate synthase activity in the reaction of pyruvate synthesis. Only in the exchange reaction was a weak activity of pyruvate synthase recorded (Table 3). In *Cfl. aurantiacus* B-3 cells, pyruvate synthase plays an important role in the process of autotrophic CO_2 fixation. This is indicated by the activity of this enzyme, sufficiently high (2.5 nmol/(min mg protein)) to account for autotrophic growth (Table 3). In this strain, pyruvate synthase is synthesized not only under photoautotrophic but also under photoheterotrophic conditions. The highest activity of this enzyme was recorded in extracts of cells grown on acetate and CO_2. Under these conditions, pyruvate synthase most probably participates in the assimilation of exogenous acetate via reductive carboxylation, as this occurs in other phototrophic bacteria and fermentative bacteria [15].

In both *Cfl. aurantiacus* strains studied, we found a sufficiently high activity of acetyl CoA carboxylase (Table 3). However, the changes in the activity of this enzyme in response to changes in cultivation conditions were different in these strains. In *Cfl. aurantiacus* B-3, the activity showed little changes upon the transition from photoautotrophic to photoheterotrophic growth, suggesting that the function of the enzyme in this strain is restricted to fatty acid synthesis [14]. In *Cfl. aurantiacus* OK-70, the activity of acetyl CoA synthase was relatively low after growth on medium with acetate as a sole carbon source and considerably higher after photoautotrophic growth or photoheterotrophic growth on medium with acetate and CO_2, confirming the involvement of acetyl CoA carboxylase in autotrophic CO_2 assimilation and suggesting the participation of this enzyme in acetate assimilation during growth on medium with acetate and CO_2. Thus, the absence of pyruvate synthase from *Cfl. aurantiacus* OK-70 and its high activity in *Cfl. aurantiacus* B-3, as well as data suggesting the involvement of acetyl CoA carboxylase in autotrophic acetate assimilation by *Cfl. aurantiacus*

Table 1. Effect of transamination substrates on the apparent activity of pyruvate synthase of *Cfl. aurantiacus* B-3 as measured in the reaction of pyruvate synthesis (the culture was grown autotrophically)

Transamination substrate	Activity, nmol/(min mg protein)
Control (no transamination substrates)	0.38
Serine (2 mM)	1.1
Glycine (2 mM)	0.43
Aspartic acid (2 mM)	0.05

Table 2. Dependence of the rate of the reaction catalyzed by pyruvate synthase of *Cfl. aurantiacus* B-3 on the composition of the reaction mixture

Composition of the reaction mixture	Activity, nmol/(min mg protein)
+ CoA + acetate + serine	1.4
+ CoA + acetate – serine	0.73
+ CoA – acetate + serine	1.0
– CoA + acetate + serine	0.6
– CoA – acetate + serine	<0.1

5 mM ATP.

Table 3. Activity of pyruvate synthase and acetyl CoA carboxylase of *Cfl. aurantiacus* B-3 and *Cfl. aurantiacus* OK-70, nmol/(min mg protein)

Substrates in cultivation medium	Pyruvate synthase				Acetyl CoA carboxylase	
	the reaction of synthesis		the exchange reaction			
	B-3	OK-70	B-3	OK-70	B-3	OK-70
Acetate	3.4	<0.1	33.2	1.2	9.2	7.4
$H_2 + CO_2$	2.5	<0.1	10.1	2.7	9.0	31.8
Acetate + CO_2	5.7	<0.1	16.9	0.5	4.3	52

OK-70, are in agreement with the earlier suppositions about different mechanisms involved in autotrophic CO_2 assimilation by different strains of *Cfl. aurantiacus* (the 3-hydroxypropionate cycle in *Cfl. aurantiacus* OK-70 [8] and the reductive dicarboxylic acid cycle in *Cfl. aurantiacus* B-3 [5]).

DNA–DNA hybridization revealed a DNA homology level of 99% between *Cfl. aurantiacus* strains OK-70 and B-3, unambiguously indicating their affiliation to the same species. Probably, the only difference between these strains is the absence of the gene of pyruvate synthase in one of them (OK-70); this difference has virtually no effect on DNA homology but compels this strain to use an unusual mechanism of autotrophic CO_2 fixation, based on the enzymatic system of fatty acid synthesis, common for all organisms.

REFERENCES

1. Fuchs, G., Alternative Pathways of Autotrophic CO_2 Fixation, *Autotrophic Bacteria*, Schlegel, H.G. and Bowien, B., Eds., Berlin: Sci. Tech., 1989, pp. 365—382.
2. Madigan, M.T. and Brock, T.D., Photosynthetic Sulfide Oxidation by *Chloroflexus aurantiacus*, a Filamentous, Photosynthetic Gliding Bacterium, *J. Bacteriol.*, 1975, vol. 122, pp. 782–784.
3. Keppen, O.I. and Krasil'nikova, E.N., Growth of *Chloroflexus aurantiacus* under Photoautotrophic Conditions, *Mikrobiologiya*, 1986, vol. 55, no. 5, pp. 879–881.
4. Holo, H. and Sirevag, R., Autotrophic Growth and CO_2 Fixation of *Chloroflexus aurantiacus*, *Arch. Microbiol.*, 1986, vol. 145, no. 2, pp. 173–180.
5. Ivanovsky, R.N., Krasilnikova, E.N., and Fal, Yu.I., A Pathway of the Autotrophic CO_2 Fixation in *Chloroflexus aurantiacus*, *Arch. Microbiol.*, 1993, vol. 159, no. 3, pp. 257–264.
6. Holo, H. and Grace, D., Polyglucose Synthesis in *Chloroflexus aurantiacus* Studied by ^{13}C-NMR (Evidence for Acetate Metabolism by a New Metabolic Pathway in Autotrophically Grown Cells), *Arch. Microbiol.*, 1987, vol. 148, no. 4, pp. 292–297.
7. Kondratieva, E.N., Ivanovsky, R.N., and Krasilnikova, E.N., Carbon Metabolism in *Chloroflexus aurantiacus*, *FEMS Microbiol. Lett.*, 1992, vol. 100, pp. 269–272.
8. Strauss, G. and Fuchs, G., Enzymes of a Novel Autotrophic CO_2 Fixation Pathway in the Phototrophic Bacterium *Chloroflexus aurantiacus*: The 3-Hydroxypropionate Cycle, *Eur. J. Biochem.*, 1993, vol. 941, pp. 1–11.
9. Ivanovskii, R.N. and Krasil'nikova, E.N., Glyoxylate Metabolism in *Chloroflexus aurantiacus*, *Mikrobiologiya*, 1995, vol. 64, no. 3, pp. 310–314.
10. Pierson, B.K. and Castenholz, R.W., A Phototrophic Gliding Filamentous Bacterium of Hot Springs, *Chloroflexus aurantiacus* gen. and sp. nov., *Arch. Microbiol.*, 1974, vol. 100, pp. 5–24.
11. Castenholz, R.W. and Pierson, B.K., Isolation of Members of the Family *Chloroflexaceae*, *The Prokaryotes*, Berlin: Springer, 1981, vol. 1, pp. 290–298.
12. Rowsell, E.V., Snell, K., Carnie, J.A., and Al-Tai, A.H., Liver *L*-Alanine–Glyoxylate and *L*-Serine–Pyruvate Aminotransferase Activities: An Apparent Association with Gluconeogenesis, *Biochem. J.*, 1969, vol. 115, pp. 1071–1073.
13. Bradford, M.M., A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein–Dye Binding, *Anal. Biochem*, 1976, vol. 72, pp. 248–254.
14. Metzler, D.E., *Biochemistry*, New York: Academic, 1977.
15. Bassham, J.A. and Buchanan, B.B., Pathway of CO_2 Fixation in Plants and Bacteria, *Photosynthesis*, Govindjee, R., Ed., New York: Academic, 1982, vol. 2, pp. 218–272.