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EXPERIMENTAL ARTICLES

A Study of the Mechanism of Acetate Assimilation in Purple Nonsulfur Bacteria Lacking the Glyoxylate Shunt: Enzymes of the Citramalate Cycle in *Rhodobacter sphaeroides*

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Abstract—The mechanism of acetate assimilation by the purple nonsulfur bacterium *Rhodobacter sphaeroides*, which lacks the glyoxylate shunt, has been studied. In a previous work, proceeding from data on acetate assimilation by *Rba. sphaeroides* cell suspensions, a suggestion was made regarding the operation, in this bacterium, of the citramalate cycle. This cycle was earlier found in *Rhodospirillum rubrum* in the form of an anaplerotic reaction sequence that operates during growth on acetate instead of the glyoxylate shunt, which is not present in the latter bacterium. The present work considers the enzymes responsible for acetate assimilation in *Rba. sphaeroides.* It is shown that this bacterium possesses the key enzymes of the citramalate cycle: citramalate synthase, which catalyzes condensation of acetyl-CoA and pyruvate and, as a result, forms citramalate, and 3-methylmalyl-CoA lyase, which catalyzes the cleavage of 3-methylmalyl-CoA to glyoxylate and propionyl-CoA. The regeneration of pyruvate, which is the acetyl-CoA acceptor in the citramalate cycle, involves propionyl-CoA and occurs via the following reaction sequence: propionyl-CoA (+CO_2) ⟶ methylmalonyl-CoA ⟶ succinyl-CoA ⟶ succinate ⟶ fumarate ⟶ malate ⟶ oxaloacetate (–CO_2) ⟶ phosphoenolpyruvate ⟶ pyruvate. The independence of the cell growth and the acetate assimilation of CO_2 is due to the accumulation of CO_2/HCO_3^- (released during acetate assimilation) in cells to a level sufficient for the effective operation of propionyl-CoA carboxylase.

Key words: Rhodobacter sphaeroides, citramalate cycle, tricarboxylic acid cycle, acetate.

In the purple nonsulfur bacterium *Rhodospirillum rubrum*, which lacks the glyoxylate shunt, acetate assimilation involves the operation of the citramalate cycle (CM cycle) [1–4]. In this cycle, acetyl-CoA undergoes condensation with pyruvate and, as a result, citramalate is formed, which, via several intermediate products, is converted to glyoxylate and propionyl-CoA. Glyoxylate is involved in the tricarboxylic acid cycle (TCA cycle) via a malate synthase reaction, whereas propionyl-CoA is oxidized to pyruvate and regenerates the acceptor, acetyl-CoA. This conversion involves propionyl-CoA carboxylase and occurs via the following reaction sequence: propionyl-CoA (+CO_2) ⟶ methylmalonyl-CoA ⟶ succinyl-CoA ⟶ succinate ⟶ fumarate ⟶ malate ⟶ oxaloacetate (–CO_2) ⟶ phosphoenolpyruvate ⟶ pyruvate. The net formula of this cycle is acetate ⟶ glyoxylate + 4[H]. Although the latter formula does not include CO_2, *Rsp. rubrum* shows high rates of growth and acetate assimilation only when there is a high bicarbonate concentration in the medium. The dependence of the CM cycle on CO_2 is due to the fact that one of its enzymes, propionyl-CoA carboxylase, has a low affinity to HCO_3^- ($K_m \cong 5 \times 10^{-3}$ M [5]). When there is a low content of bicarbonate in the medium, the reaction catalyzed by propionyl-CoA carboxylase is the limiting step in the cycle. In an accompanying paper [6], we show that acetate assimilation by *Rhodobacter sphaeroides* is independent of the presence of bicarbonate in the medium, although there are grounds to assume the operation of the CM cycle in this bacterium. Based on these data, it is reasonable to suggest the operation of a modified CM cycle lacking the propionyl-CoA carboxylase reaction in relation to *Rba. sphaeroides*. Currently, there are three known CO_2-independent pathways of propionyl-CoA oxidation that can result in pyruvate formation [7, 8]:

(1) The methylcitrate pathway, which operates in fungi and many aerobic bacteria. In this pathway, propionyl-CoA and oxaloacetate undergo condensation, which results in the formation of methylcitrate. Methylcitrate, via methylaconitate, is then converted to methylisocitrate. The latter compound is cleaved into pyruvate and succinate.

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(2) The oxidation of propionyl-CoA to pyruvate via acryloyl-CoA, lactoyl-CoA, and lactate. This pathway has been found in the strictly anaerobic bacteria *Clostridium propionicum* and *Megasphaera elsdenii.*

(3) The direct transfer of CO_2 from oxaloacetate to propionyl-CoA, with the resulting formation of methylmalonyl-CoA and pyruvate. This reaction, known to occur in propionic acid bacteria, is catalyzed by methylmalonyl-CoA carboxytransferase.

It is possible, however, that acetate assimilation in *Rba. sphaeroides* occurs via the same form of the CM cycle as was described for *Rsp. rubrum* but with certain physiological features of *Rba. sphaeroides* providing compensation for the deficiency of CO_2 in the cultivation medium.

The aims of the present work were to check for the presence of the CM cycle enzymes in cell extracts of *Rba. sphaeroides* and to study the distinctive features of the operation of the CM cycle during acetate assimilation in this bacterium.

MATERIALS AND METHODS

Bacteria and cultivation conditions. The subjects of this study were *Rba. sphaeroides* 2R and *Rsp. rubrum* 1R, which were taken from the culture collection of the Microbiology Department of Moscow State University. The cultures were grown photoheterotrophically on an Ormerod medium according to the method described in [6].

Studies of acetate assimilation and CO_2 evolution. Experiments on [2-^{14}C]acetate assimilation were performed according to the procedure described in [6]. Determination of the rate of $^{14}CO_2$ formation from 1-[^{14}C]acetate was carried out in flasks with appendages that contained 0.5 ml of an alkaline trapping solution (0.5 M NaOH). The air in the flasks was replaced with molecular nitrogen. The reaction was terminated after 60 min of incubation by the addition of 0.5 ml of 10% trichloroacetic acid to the suspension (3 ml). After further overnight incubation at room temperature, the activity of the CO_2 absorbed by the trapping solution was measured in an LKB RacBeta 1127 liquid scintillation counter.

Preparation of cell extracts. The extracts to be used in the determination of enzymatic activity were obtained according to the procedure described in [6]. The extracts intended for HPLC identification of the products of condensation of acetyl-CoA with pyruvate or propionyl-CoA with glyoxylate were prepared anaerobically in a French press. For this purpose, 2 g of washed biomass was resuspended in 2 ml of a 20 mM MOPS buffer (pH 7.5) containing 2 mM $MgCl_2$, 5 mM dithiothreitol, and trace amounts of DNAse I. The biomass was passed through the French pressure cell at a pressure of 137 MPa and then centrifuged (100000 *g*, 60 min, 4°C). The supernatant was either used immediately or stored anaerobically at –80°C.

The determination of enzyme activity was performed at room temperature. The reaction mixture contained 0.5–1.5 mg of protein/ml. The enzyme activity was expressed in nmol/(min mg protein).

The enzymes of the TCA cycle and glyoxylate shunt were assayed spectrophotometrically using standard methods [1].

Pyruvate kinase was determined spectrophotometrically from the oxidation of NADH in the presence of lactate dehydrogenase [9].

Phosphoenolpyruvate (PEP) carboxylase, PEP carboxykinase, and PEP carboxytransphosphorylase were determined radiochemically according to the procedure described in [10].

Propionyl-CoA carboxylase was determined radiochemically from the propionyl-CoA-dependent fixation of CO_2 [4].

Methylcitrate synthase was determined spectrophotometrically (412 nm, $\varepsilon = 13.6\ mM^{-1}\ cm^{-1}$) from the oxaloacetate-dependent production of CoA from propionyl-CoA in the presence of 5,5'-dithiobis-(2-nitrobenzoate) (DTNB) [7]. The reaction mixture contained 50 mM Tris–HCl (pH 7.5), 1 mM DTNB, 0.2 mM propionyl-CoA, and 5 mM $MgCl_2$. The reaction was initiated by the addition of oxaloacetate (2 mM).

Acrylyl-CoA hydratase was determined spectrophotometrically from the formation of lactate [11]. The amount of lactate formed was determined from the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) in the presence of phenazine methosulfate in a lactate dehydrogenase reaction.

Methylmalonyl-CoA carboxytransferase was determined from the methylmalonyl-CoA-dependent formation of oxaloacetate [8].

Citramalate synthase was determined using two methods: (1) A photometric determination in a discontinuous assay via the pyruvate-dependent formation of CoA from acetyl-CoA in the presence of DTNB. The reaction mixture contained 50 mM MOPS (pH 8.0), 2 mM $MgCl_2$, 2 mM $MnCl_2$, and 1 mM acetyl-CoA. The reaction was initiated by the addition of pyruvate (20 mM). Every 5 min, 50-μl samples were taken and transferred to a cuvette containing 1 ml of a 50 mM Tris–HCl buffer (pH 7.8) and 0.4 mM DTNB. Absorbance was measured immediately after the introduction of the sample into the DTNB-containing buffer. (2) Determination from pyruvate diminution in the presence of acetyl-CoA. The concentration of pyruvate was determined photometrically with phenylhydrazine in a discontinuous assay. The reaction mixture contained 50 mM MOPS (pH 8.0), 2 mM $MgCl_2$, 2 mM $MnCl_2$, and 8 mM pyruvate. The reaction was initiated by the addition of acetyl-CoA (2 mM). After 0, 30, and 60 min, 10-μl samples were taken and transferred into 2 ml of a solution containing 3.5 mM phenylhydrazine and 2.5 mM citric acid (pH 5.0). After 15 min of incubation, the absorbance was measured at 324 nm ($\varepsilon = 11.5\ mM^{-1}\ cm^{-1}$).

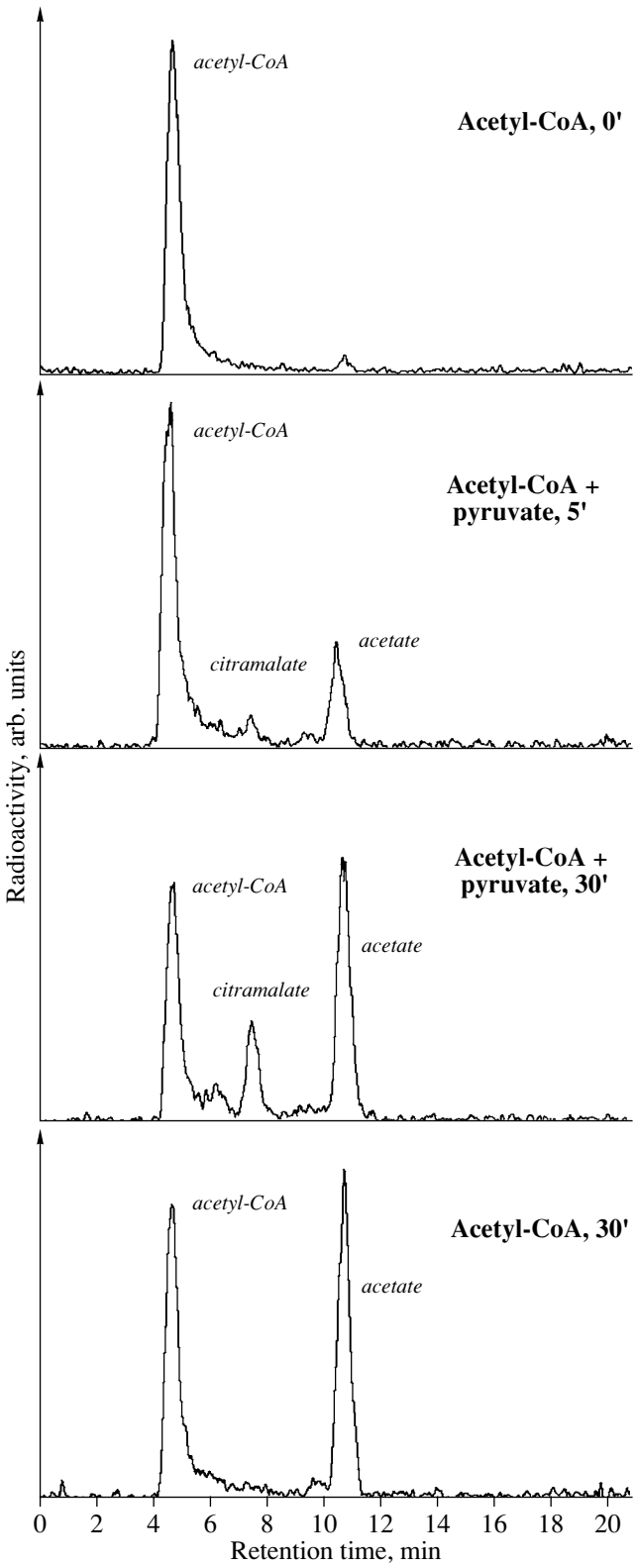

Fig. 1. Products resulting from the condensation of [^{14}C]acetyl-CoA and pyruvate in extracts of the *Rba. sphaeroides* cells grown on acetate. The samples were analyzed on an organic acid–separating Aminex column. The protein content in the reaction mixture was 2.1 mg/ml.

The activity of the enzyme catalyzing the condensation of propionyl-CoA and glyoxylate (3-methylmalate synthase or 3-methylmalonyl-CoA lyase) was determined from glyoxylate diminution in the presence of propionyl-CoA. The glyoxylate concentration was determined photometrically with phenylhydrazine in a discontinuous assay. The reaction mixture contained 50 mM MOPS (pH 8.0), 2 mM $MgCl_2$, 2 mM $MnCl_2$, and 2 mM glyoxylate. The reaction was initiated by the addition of propionyl-CoA (1 mM). After 0, 5, 15, and 30 min, 40-μl samples were taken and transferred into 2 ml of a solution containing 3.5 mM phenylhydrazine and 2.5 mM citric acid (pH 5.0). After 15 min of incubation, the absorbance was measured at 324 nm ($\varepsilon = 17\ mM^{-1}\ cm^{-1}$).

Analysis of the products from the condensation of [^{14}C]acetyl-CoA with pyruvate and [^{14}C]propionyl-CoA with glyoxylate was performed by HPLC. The reaction mixture (100 μl) contained, in the first case, 100 mM NH_4HCO_3 (pH 7.8), 5 mM $MgCl_2$, 0.5 mM $MnCl_2$, 2 mM acetyl-CoA, 100000 cpm of [^{14}C]acetyl-CoA, and 10 mM pyruvate and, in the second case, 100 mM NH_4HCO_3(pH 7.8), 5 mM $MgCl_2$, 0.5 mM $MnCl_2$, 2 mM propionyl-CoA, 100000 cpm of [^{14}C]propionyl-CoA, and 2 mM glyoxylate.

The reaction was initiated by the addition of the cell extract (1.5–2.0 mg of protein/ml) and terminated after various periods of time by the addition of 20 μl of 25% HCl to 100 μl of the reaction mixture. This sample was then divided into two 60-μl portions.

For the analysis of CoA esters, a sample, containing about 30000 cpm and neutralized with 1 M NH_4HCO_3, was loaded onto a column with a C_{18}-reversed phase (LiChristopher 100, endcapped, 5 μm, 125 × 4 mm, Merck, Darmstadt, Germany). Separation was performed in a 2–8% gradient of acetonitrile dissolved in a 50 mM K–phosphate buffer (pH 6.7); elution was performed for 30 min at a rate of 1 ml/min. The eluate was analyzed with a UV detector (210 nm) and a detector of radioactivity (^{14}C). The retention time was 1.5 min for organic acids (acetate, propionate, 3-methylmalate, etc.), 7.5 min for CoA and methylmalonyl-CoA, 8 min for mesaconyl-CoA, and 20 min for propionyl-CoA.

The organic acids were analyzed using an Aminex column. The samples (50 μl, ~30000 cpm) were eluted with 5 mM H_2SO_4 at a flow rate of 0.8 ml/min. In some cases, the column was loaded with samples that had been subjected to an alkaline hydrolysis of CoA esters, which was carried out in the presence of 1 M KOH at 85°C for 60 min. The eluate was analyzed with a UV detector (210 nm) and a detector of radioactivity (^{14}C). The retention time was 4.5 min for CoA and its esters, 7.5 min for citramalate and 3-methylmalate, 11 min for acetate, 12.5 min for propionate, and 15 min for mesaconate.

Protein determination was performed by the Lowry method. Bovine serum albumin was used as the standard.

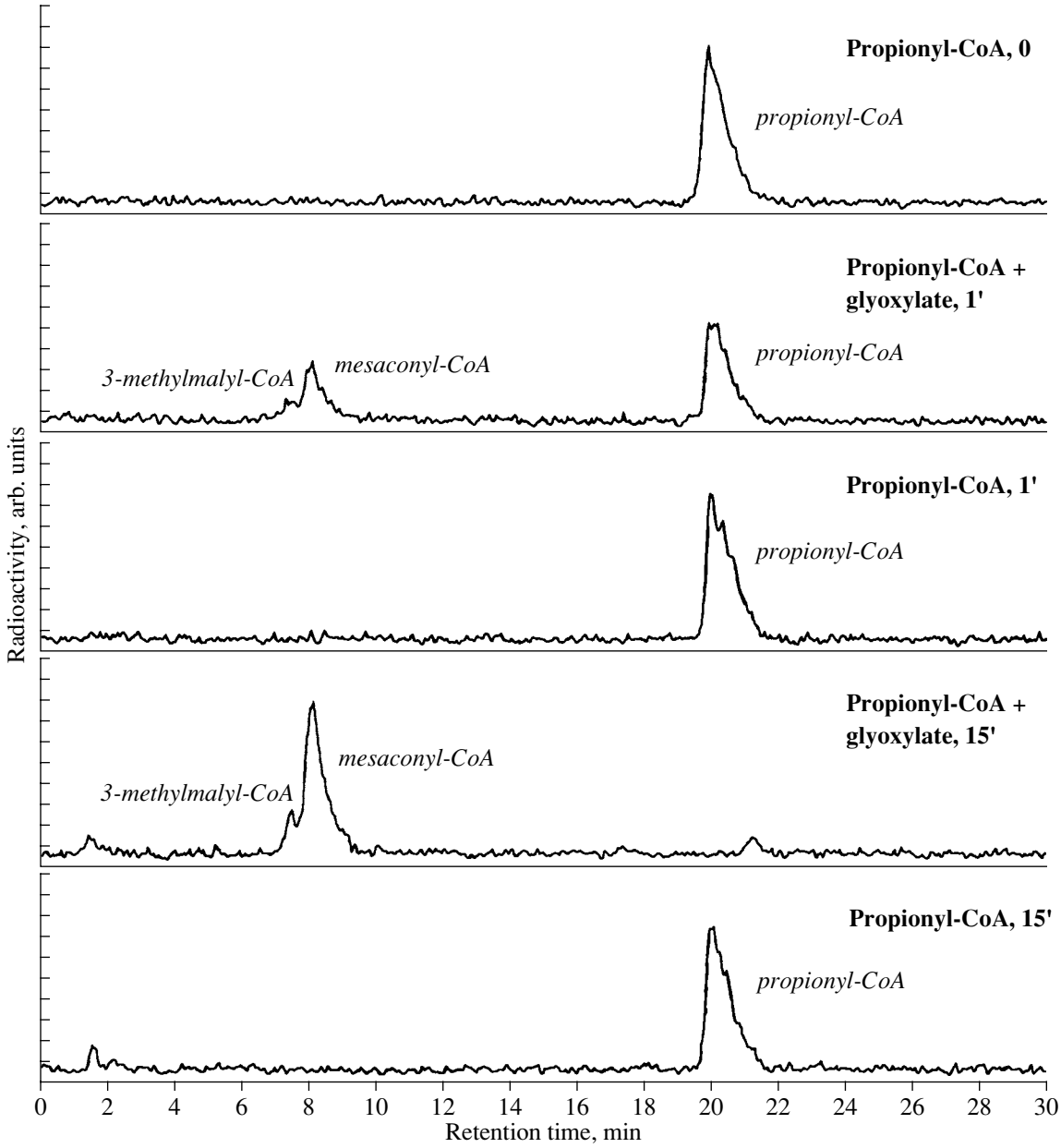

Fig. 2. Products resulting from the condensation of [^{14}C]propionyl-CoA and glyoxylate in extracts of the *Rba. sphaeroides* cells grown on acetate. The samples were analyzed on a CoA ester–separating RP_{18} column. The protein content in the reaction mixture was 0.5 mg/ml.

Synthesis of U-[^{14}C]acetyl-CoA and 2-[^{14}C]propionyl-CoA was performed enzymatically [12].

RESULTS AND DISCUSSION

Enzymes involved in the conversion of acetyl-CoA into glyoxylate. The key reactions of the CM cycle are the citramalate synthase and 3-methylmalyl-CoA lyase reactions. The stimulation of pyruvate consumption by the addition of acetyl-CoA testified to the presence of an enzyme catalyzing the condensation of pyruvate with acetyl-CoA in the *Rba. sphaeroides* cells grown on a medium with acetate. The activity of this enzyme was 36.0 nmol/(min mg protein). After growth on a medium with malate, the activity of this enzyme was somewhat lower (25.8 nmol/(min mg protein)). Moreover, the pyruvate-induced stimulation of CoA release from acetyl-CoA was shown (27.8 nmol/(min mg protein)).

The products resulting from the condensation of acetyl-CoA with pyruvate in the cell extracts of *Rba. sphaeroides* were analyzed by HPLC on the organic acid–separating Aminex column. Citramalate was shown to be formed from [^{14}C]acetyl-CoA and pyruvate (Fig. 1). According to the HPLC data, the rate

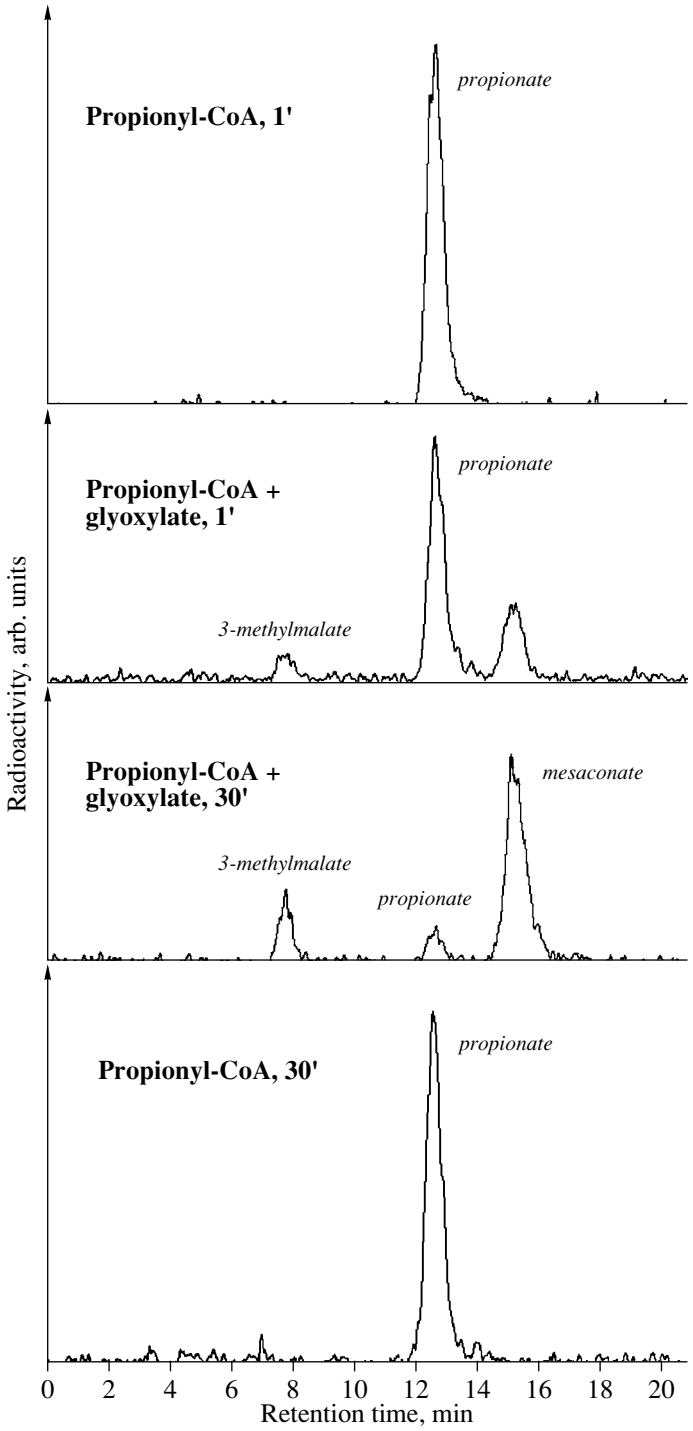

Fig. 3. Products resulting from the condensation of [^{14}C]propionyl-CoA and glyoxylate in extracts of the *Rba. sphaeroides* cells grown on acetate. The samples were analyzed on an organic acid–separating Aminex column after alkaline hydrolysis. The protein content in the reaction mixture was 0.5 mg/ml.

of condensation was 20 nmol/(min mg protein). An HPLC analysis that used the CoA ester–separating RP_{18} column failed to reveal citramalyl-CoA, which is the product of the citramalyl-CoA lyase reaction (data not shown). Thus, our results suggest that the condensation of acetyl-CoA with pyruvate in the cell extracts of *Rba. sphaeroides* involves citramalate synthase, which catalyzes the following reaction:

$$\text{acetyl-CoA + pyruvate} \longrightarrow \text{citramalate + CoA.}$$

The rate of propionyl-CoA and glyoxylate condensation in the cell extracts of *Rba. sphaeroides*, deter-

mined from glyoxylate consumption in the presence of propionyl-CoA, was 166.0 nmol/(min mg protein) for the cells grown on acetate and 46.0 nmol/(min mg protein) for the cells grown on malate.

Analysis of the products resulting from the condensation of propionyl-CoA and glyoxylate showed that this reaction is catalyzed by 3-methylmalyl-CoA lyase, since 3-methylmalyl-CoA is the product of the reaction. 3-Methylmalyl-CoA is further converted into mesaconyl-CoA, whose amount increases over time (Fig. 2). In order to identify the products, the samples were also subjected to alkaline hydrolysis and loaded onto the organic acid–separating Aminex column. In the presence of glyoxylate, [^{14}C]mesaconate and [^{14}C]methylmalate were detected in the samples in addition to [^{14}C]propionate (Fig. 3). These data suggest the occurrence of the following reaction in the extracts:

propionyl-CoA + glyoxylate
$\longrightarrow$ 3-methylmalyl-CoA $\longrightarrow$ 3 mesaconyl-CoA.

A decrease in the activity of citramalate synthase and 3-methylmalyl lyase in the *Rba. sphaeroides* cells grown on malate indicate that these enzymes are involved in acetate assimilation. The involvement of citramalate synthase in acetate metabolism in *Rba. sphaeroides* is confirmed by the early appearance of citramalate among the labeled products of [^{14}C]acetate assimilation [13].

Summing up, it can be suggested that, in acetate-grown *Rba. sphaeroides* cells, as in *Rsp. rubrum*, the conversion of acetyl-CoA into glyoxylate may occur via the following reaction sequence:

acetyl-CoA + pyruvate $\longrightarrow$ citramalate
$\longrightarrow$ mesaconate $\longrightarrow$ mesaconyl-CoA
$\longrightarrow$ 3-methylmalyl-CoA $\longrightarrow$ propionyl-CoA
$\longrightarrow$ 3 glyoxylate.

Oxidation of propionyl-CoA to pyruvate in *Rba. sphaeroides*. For acetate to be oxidized in the CM cycle, pyruvate, the acetyl-CoA acceptor, must be regenerated from the propionyl-CoA formed in the methylmalyl-CoA lyase reaction. Our attempts to find CO_2-independent mechanisms of propionate metabolism, namely, the methylcitrate cycle, acrylate pathway, or transcarboxylase pathway, in *Rba. sphaeroides* led us to a conclusion that the key enzymes of these pathways, i.e., methylcitrate synthase, acrylyl-CoA hydratase, and methylmalonyl-CoA carboxytransferase, are absent from this bacterium.

In *Rsp. rubrum*, propionyl-CoA oxidation to pyruvate in the CM cycle involves propionyl-CoA carboxylase. The succinyl-CoA formed in the propionyl-CoA carboxylase pathway is oxidized to oxaloacetate by the TCA cycle enzymes. The decarboxylation of oxaloacetate to PEP and the conversion of PEP to pyruvate complete the cycle. In the extracts of the *Rba. sphaeroides* cells grown on acetate, we found the activity of all the enzymes necessary for this reaction sequence (Table 1).

Table 1. Enzymes involved in acetate assimilation by *Rba. sphaeroides* cells grown on a medium with acetate

Enzyme	Activity, nmol/(min mg protein)
Acetyl-CoA synthase	206.2
Citrate synthase	39.5
Aconitase	217.1
Isocitrate dehydrogenase	100.6
2-Oxoglutarate dehydrogenase	21.8
Succinate dehydrogenase	18.9
Fumarase	165.0
Malate dehydrogenase	517.1
Malate synthase	23.3
Propionyl-CoA carboxylase	9.6
PEP carboxylase	14.6
PEP carboxykinase	6.9
PEP carboxytransphosphorylase	<0.1
Pyruvate kinase	12.4

Table 2. Assimilation of [1-^{14}C]acetate and concomitant release of $^{14}CO_2$ by *Rba. sphaeroides* and *Rsp. rubrum* (nmol/(min mg protein))

Rate of	*Rba. sphaeroides*	*Rsp. rubrum*
Acetate assimilation	204.1	34.2
CO_2 release	82.8	13.6

Table 3. The effect of bicarbonate on the rate of acetate assimilation (nmol/(min mg protein)) by *Rba. sphaeroides* cells as dependent on acetate concentration and culture age

Substrate in the suspension	Acetate concentration, mM	Culture age, h		
		8	18	24
Acetate	5.0	231.1	393.9	271.4
Acetate + $NaHCO_3$		212.3	344.9	241.5
Acetate	0.5	111.0	134.8	92.9
Acetate + $NaHCO_3$		152.9	113.0	93.7
Acetate	0.2	86.0	107.6	35.5
Acetate + $NaHCO_3$		103.6	107.1	35.9
Acetate	0.1	52.4	75.4	16.7
Acetate + $NaHCO_3$		68.3	80.3	18.1
Acetate	0.05	32.8	32.3	8.5
Acetate + $NaHCO_3$		50.6	42.9	8.6

Note: The cell concentration was 0.03 mg of protein/ml in the experiments with 8- and 18-h cultures and 0.14 mg/ml for the 24-h culture.

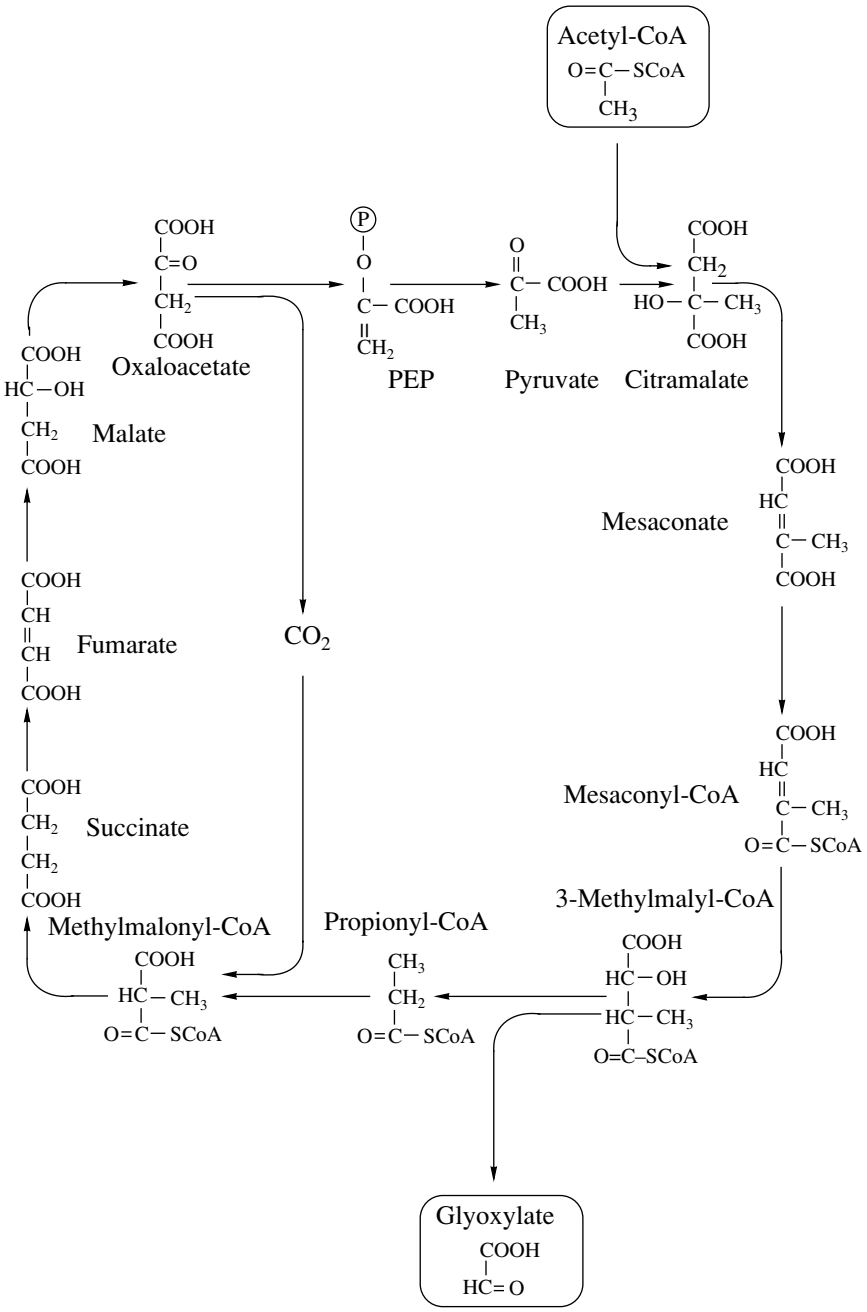

Fig. 4. The citramalate cycle operating in *Rba. sphaeroides* during acetate assimilation.

A scheme of the CM cycle based on the data that we obtained is shown in Fig. 4.

The features of *Rba. sphaeroides* metabolism that account for the independence of acetate assimilation from the presence of CO_2 in the medium. The results obtained in this work indicate possible involvement of the CM cycle in acetate assimilation by *Rba. sphaeroides*. Taking into account the lack of a CO_2-independent alternative mechanism of propionyl-CoA oxidation in this organism (the methylcitrate cycle, acrylate pathway, or transcarboxylase pathway), our data are indicative of the existence of some physiological features in *Rba. sphaeroides* that can compensate for bicarbonate deficiency in the cultivation medium and provide for the efficient operation of propionyl-CoA carboxylase and, thus, of the CM cycle as a whole. During the photoheterotrophic growth of *Rba. sphaeroides* and *Rsp. rubrum*, acetate is not only the carbon source but also the single electron donor. Its oxidation via the reactions occurring in the TCA cycle is accompanied by a release of a significant amount of CO_2. In *Rba. sphaeroides*, the rate of acetate assimilation and

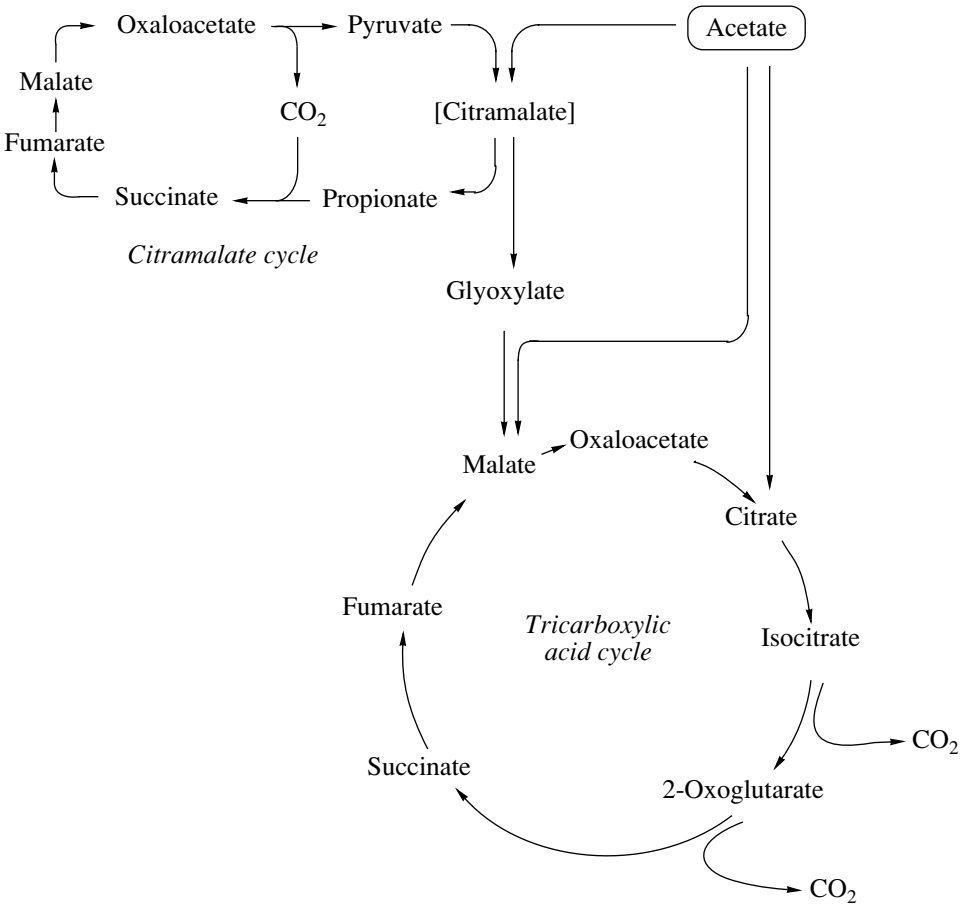

Fig. 5. Acetate metabolism in *Rba. sphaeroides* under photoheterotrophic conditions.

concomitant production of CO_2 was found to be about sixfold higher than that in *Rsp. rubrum* (Table 2). Moreover, it can be assumed that *Rba. sphaeroides* cells, due their mucous capsules [14], are capable of a more efficient retention of the CO_2 (or bicarbonate) formed in the course of acetate assimilation (*Rsp. rubrum* cells do not possess capsules [15]). Due to the above-specified reasons, the concentration of CO_2/HCO_3^- in the acetate-assimilating cells of *Rba. sphaeroides* may reach a level sufficient for the efficient operation of propionyl-CoA carboxylase. Under such conditions, the addition of exogenous bicarbonate would have no effect on cell growth and acetate assimilation.

In order to investigate the effect of the CO_2 released during acetate assimilation, we altered the experimental conditions as follows. The concentration of acetate was gradually decreased from 5 to 0.05 mM in order to decrease the rate of acetate assimilation and, consequently, of CO_2 evolution. The cell concentration was decreased from 0.1–0.2 to 0.03 mg of protein/ml. In addition, in these experiments, we used cultures of different ages (8, 18, and 24 h, which corresponded to the early, mid, and late exponential growth phase). The cells of 8-h cultures were motile, which indicated that they lacked capsules (whose formation leads to the loss of motility).

In an 8-h culture of *Rba. sphaeroides*, the stimulation of acetate assimilation by exogenous bicarbonate was observed for acetate concentrations of 0.5 mM and lower (Table 3). In an 18-h culture, the stimulatory effect of bicarbonate was observed only at acetate concentrations of no higher than 0.1 mM. In dense (0.14 mg of protein/ml) suspensions of cells in a 24-h culture, no bicarbonate-induced stimulatory effect was observed, regardless of the acetate concentration. These data suggest that the stimulatory effect of exogenous bicarbonate can only be revealed when the production rate of endogenous CO_2 decreases to a level insufficient for the efficient operation of propionyl-CoA carboxylase. Moreover, the stimulatory effect of exogenous bicarbonate could be shown only in young cultures, as their cells are devoid of capsules, which hinder efflux of endogenous CO_2. These results are in agreement with data on the cultivation of cells in a photobioreactor [6]. The omission of CO_2 from the composition of the photobioreactor gas phase resulted in a decrease in the growth rate of *Rsp. rubrum.* However, growth did not cease completely, suggesting that the culture can grow at the expense of the CO_2 released in the process of ace-

tate assimilation. An increase in the rate of the nitrogen gas flow through the reactor promoted a more efficient removal of the endogenous CO_2 dissolved in the medium and resulted in a virtually complete cessation of the growth of *Rsp. rubrum.* However, this effect was not observed with *Rba. sphaeroides* [6].

Coupling of the CM cycle reactions with the reactions of the TCA cycle. The glyoxylate formed as result of CM cycle operation can be involved in the TCA cycle via condensation with acetyl-CoA that is catalyzed by malate synthase (Table 1). The operation of the CM cycle also allows additional acetyl-CoA molecules to be involved in the TCA cycle via condensation with oxaloacetate that is catalyzed by citrate synthase. Thus, the CM cycle is an anaplerotic mechanism that replenishes TCA cycle substrates consumed for biosynthetic purposes. It plays the role of the glyoxylate shunt lacking in *Rba. sphaeroides*. An integral scheme showing the place of the CM cycle in the carbon metabolism is shown in Fig. 5.

The data obtained in this work suggest that, as in *Rsp. rubrum*, acetate assimilation in *Rba. sphaeroides* involves the operation of the CM cycle. As in *Rsp. rubrum*, acetate assimilation in *Rba. sphaeroides* is a CO_2-dependent process. However, in *Rba. sphaeroides*, the effect of CO_2 can be recorded only if, during the experiment, the amount of CO_2 in the medium (or in the cells) does not reach a level sufficient for the effective operation of propionyl-CoA carboxylase.

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