GLUTAMIC ACID SYNTHESIS FROM ACETATE UNITS AND BICARBONATE IN EXTRACTS OF PHOTOSYNTHETIC BACTERIA

C. R. Benedict and R. W. Rinne
Department of Chemistry
Wayne State University
Detroit, Michigan 48202

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There have been several proposals for the photometabolism of acetate and bicarbonate in the photosynthetic bacteria:

(1) Anaerobic tricarboxylic acid cycle (Gest, et.al., 1962).

(2) Pathway involving citramalic acid (Arnon, et.al, 1960). (Losada, et.al., 1960) (Benedict, 1962).

(3) Production of a [C_3] intermediate in amino acid synthesis from acetate and bicarbonate (Elsden, 1962).

(4) Initial photometabolism of acetate into the organic acids by reactions other than the TCA cycle (Glover, et.al., 1952).

Gas exchanges have been used with success in studies of anaerobic substrate oxidations in resting cell suspensions and have resulted in the proposal of TCA reactions to account for the stoichiometric amounts of CO_2 and H_2 recovered from succinate, fumarate or acetate photooxidations (Gest, et.al., 1962). Utilizing C^{14} tracer techniques to follow radioactivity into the organic acids during acetate-C^{14} or bicarbonate-C^{14} photometabolism have yielded few tricarboxylic acid intermediates, suggesting the TCA reactions are not involved in initial acetate metabolism (Glover, et.al., 1952). However, a noticeable accumulation of radioactive glutamic acid does occur.

Degradation of this radioactive glutamate yields a labeling pattern which suggests a carboxylation of the C-1 of acetate to yield a $[C_3]$ intermediate in amino acid synthesis (Elsden, 1962). Studies of acetate utilization in cell free extracts of *Chromatium* has shown an active formation of citramalic acid from acetyl-CoA and pyruvate which suggests the production of an intermediate in glutamate synthesis in this organism, (Arnon, et.al., 1960) (Losada, et.al., 1960). The condensation reaction of acetyl-CoA and pyruvate has been extended to extracts of *Rhodospirillum rubrum*, (Benedict, 1962). This paper is an attempt to access some of these reactions in soluble enzymatic preparations of *R. rubrum*.

Methods

Rhodospirillum rubrum (strain 1) was grown anaerobically on malate-$(NH_4)_2SO_4$ or malate-glutamate carbon sources in an illuminated H_2O bath by a procedure described by Kohmiller and Gest, 1951. Sonicated cells were prepared for enzymatic studies by removing the cell debris and chromatophores from the supernatant by centrifugation at 144, 000 x G for 2 1/2 hours. Glutamic acid formation from acetate or bicarbonate in the soluble supernatant was followed by measuring the amount of radioactivity incorporated into the individual organic acids and amino acids from acetate-C^{14} or bicarbonate-C^{14}.

Results

The co-factors and substrates necessary for the formation of radioactive glutamate from acetate-1-C^{14} in dialyzed soluble extracts of *R. rubrum* is seen in Table 1. Omission of ATP and coenzyme A,

Table 1

The Cofactor and Substrate Requirements for Acetate-C^{14} Incorporation into Glutamic Acid

Reaction Mixture	Radioactive Glutamic Acid (c.p.m. x 10^{-3})
Complete	111.1
-ATP, CoA	6.8
-Pyruvate	19.7
-Bicarbonate	28.4

The reaction mixture contained in microcoles: CoA, 0.2; ATP, 10; GSH, 5, $MgCl_2$, 10; potassium phosphate buffer, 100, pH 7.5; potassium acetate, 10; potassium pyruvate, 10; potassium bicarbonate, 10; 20 microcuries of acetate-1-C^{14}; alanine, 5; 50 µg pyridoxal phosphate and 0.5 ml R. rubrum supernatant (dialyzed 9 hours against 0.01 M potassium phosphate buffer pH 7.0). Reaction incubated at 37°C for 60 minutes.

bicarbonate or pyruvate from the reaction mixture leads to a decrease of radioactive glutamate. Presumably acetate-C^{14} is activated to acetyl-C^{14}-CoA by an active endogenous aceto-CoA-kinase, ATP and CoA.

Figure 1 represents the chromatogram scans of the C^{14}-organic acid and C^{14}-amino acid fractions resulting from bicarbonate-C^{14} utilization in soluble dialyzed extracts. In reaction mixtures containing bicarbonate-C^{14}, ATP and pyruvate there is an incorporation of the label into malic acid but little radioactivity is found in glutamate or

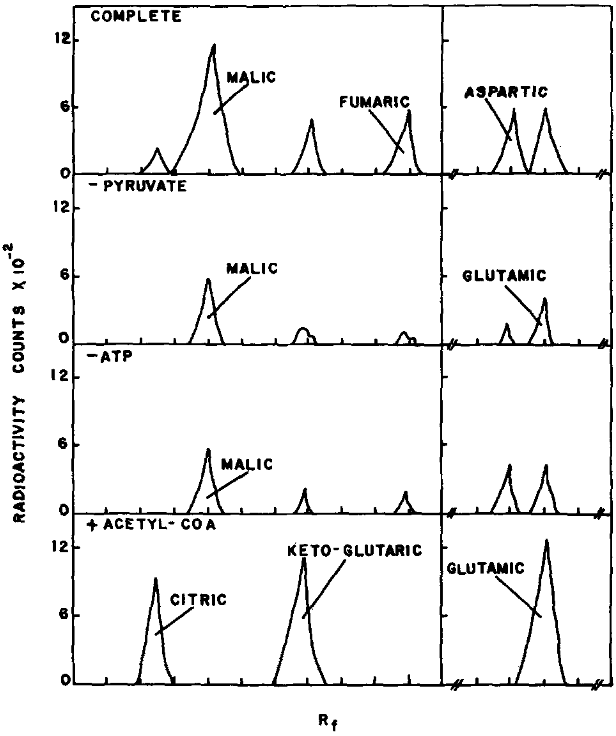

Figure 1:

Chromatogram scan of radioactive organic acids and amino acids. The reaction mixture contained in micromoles: ATP, 10; $MgCl_2$, 10; potassium pyruvate, 5; potassium bicarbonate, 10 and 20 microcuries of $NaHC^{14}O_3$; potassium phosphate buffer, 100, pH 7.5; and 0.4 ml of soluble dialyzed extract of R. rubrum. 1.0 mg of acetyl-CoA was added to complete reaction mixture. Reaction incubated at 37°C for 30 minutes. Solvents for organic acids and amino acid were 1 N pentanol; 5 N formic acid 50:50 V/V and methanol-NH_4OH-H_2O 90:5:5, V/V/V respectively.

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aspartate. Malic-C^{14} is decreased in mixtures devoid of ATP or pyruvate. Addition of acetyl-CoA to the complete reaction mixture results in a marked increase in radioactive glutamate. In this latter mixture with acetyl-CoA the C^{14} in the organic acids is diverted from malic-C^{14} to citric-C^{14} and alpha ketoglutaric-C^{14}. Dialysis of the extracts for periods of over 4 hours at $0^{\circ}C$ leads to a decrease in bicarbonate-C^{14} utilization.

The incorporation of acetate-C^{14} into the organic acids and amino acids in soluble extracts containing ATP, CoA, oxalacetate and acetate-C^{14} (Figure 2A) shows that the organic acids are synthesized prior to the appearance of radioactivity into the amino acids. Glutamic acid accounts for over 90% of the radioactivity in the amino acid fraction. Figure 2B records the changes in the individual organic acids. In 15 minutes the order of activity in the acids is citric, α-ketoglutaric and succinic. Other observations noted in extracts of R. rubrum are: the presence of an active TPN-linked isocitric dehydrogenase; the formation of radioactive glutamic acid from bicarbonate-C^{14}, ATP, pyruvate and acetyl-CoA labels predominantly the C-1 carboxyl group of glutamic acid.

In reaction mixtures containing acetate-C^{14}, CoA, ATP (or acetyl-C^{14}-CoA) and pyruvate, no radioactive glutamate is formed; however, there occurs a large incorporation of radioactivity into the organic acids. 85-90% of this radioactivity can be accounted for by citramalic-C^{14} acid. This acid is made in sizeable amounts but is not metabolized by these extracts. In reaction mixtures containing acetate-C^{14}, CoA, ATP, pyruvate and bicarbonate there is still a noticeable production of citramalic-C^{14} acid but a considerable amount of activity is recovered in citric, α-ketoglutaric and glutamic acids. In mixtures containing ATP, acetyl-CoA, pyruvate and bicarbonate-C^{14} no citramalic-C^{14} is formed but citric, α-ketoglutaric and glutamic acids are

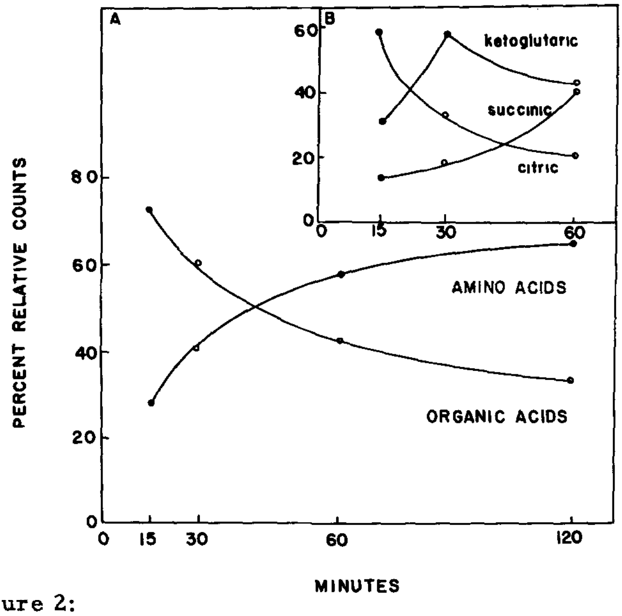

Figure 2:

Acetate-C^{14} incorporation into organic acids and amino acids of R. rubrum extracts. The reaction mixture contained in micromoles: CoA, 0.2; ATP, 10; GSH, 5; $MgCl_2$, 10; potassium phosphate buffer, 100, pH 7.5; potassium acetate, 10; potassium oxalacetate, 5; 20 microcuries Ac-1-C^{14}, alanine, 5; 50 μg pyridoxal phosphate and 0.5 ml R. rubrum supernatant. Reaction incubated at 37°C.

A. Radioactivity in organic acids and amino acids.

B. Radioactivity in individual organic acids.

radioactive. The reaction of pyruvate with acetyl-CoA to form citramalic acid may not be of physiological importance in this organism (C. F. Elsden, 1962).

The protein catalyzing the condensation of pyruvate and acetyl-CoA has been partially purified over 100 fold (Benedict and Rinne, 1963). In the presence of enzyme, acetyl-CoA and pyruvate, oxalacetate or glyoxylate there is a formation of citramalic, citric and malic acid, respectively. The enzymatic condensation of acetyl-CoA with these 3 substrates may be due to the presence of 3 separate condensing enzymes pyruvate transacetase, oxalacetate transacetase and malate synthetase in this partially purified preparation or may be due to a broad substrate specificity of pyruvate transacetase.

The results presented in this communication support the conclusion that glutamic acid formation from acetate units and bicarbonate in soluble extracts of Rhodospirillum rubrum can be accounted for by a carboxylation of pyruvate to oxalacetate followed by TCA reactions.

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