PHOTOSYNTHETIC CONVERSION OF FORMATE AND CO_2 TO GLUTAMATE BY RHODOPSEUDOMONAS PALUSTRIS*

D. C. Yoch and E. S. Lindstrom

Department of Microbiology, The Pennsylvania State University

University Park, Pennsylvania

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Rhodopseudomonas palustris was shown under photosynthetic conditions to assimilate carbon dioxide and formate in a yeast extract medium with thiosulfate as an electron donor (Van Niel 1944). In 1966 Rolls and Lindstrom demonstrated that the addition of thiosulfate to a formate medium increased the cell yield. With $Na_2S_2O_3$ slow photoautotrophic growth occurs unless p-aminobenzoate is excluded from the medium. The relative generation time is 40 to 50 hours compared to 10 hours when grown on pyruvate.

This communication presents evidence that R. palustris contains a formic dehydrogenase which converts formate to CO_2. A high percentage of the total activity from labeled CO_2 appeared initially in glutamic acid with most of the activity in the C-1 position. In the presence of CO_2 succinate, pyruvate, or acetate assimilation is greatly stimulated. These data are compatible with Arnon's reductive carboxylic acid cycle.

Methods and Materials

R. palustris, strain ATCC 11168, was grown anaerobically at 30° C with a light intensity of 4,000 ft candles. The medium of Cohen-Bazire et al. (1957) was modified by the addition of 0.2mg/l PABA and substitution of NH_4Cl and either sodium formate or bicarbonate for the diammonium malate.

Cells in the logarithmic growth phase harvested by centrifugation were washed, resuspended in 0.05 M sodium phosphate buffer pH 7.0 containing

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0.001 M $MgSO_4$, and the suspension sonified with a Bronson 10 kc sonic oscillator for three 30 sec periods with 1 min intervals for cooling. The extract was centrifuged at 144,000 x g for 30 min to give a straw yellow supernatant soluble enzyme fraction and a red pellet which was resuspended in an equal volume of buffer to give a particulate enzyme fraction.

Formic dehydrogenase activity was measured either by conversion of $H^{14}COONa$ to $^{14}CO_2$ (Malavolta et al. 1962) or by reduction of 2,6-dichlorophenol-indophenol (DCIP). The millimolar extinction coefficient of DCIP at 600 mμ was taken as 19.1 (Basford and Huenuekens 1955). One unit of enzyme activity was the amount of enzyme required to catalyse the reduction of 25 nmoles of DCIP/min. Protein was determined by the biuret reaction (Gornall et al. 1949).

Cells exposed in the light to labeled substrates were extracted with ethanol as described by Hoare (1963), the labeled products separated by paper chromatography (Benson et al. 1950) and identified by co-chromatography with the authentic compounds.

Results and Discussion

Formic dehydrogenase activity of R. palustris extracts was found in the soluble fraction when measured by either indophenol reduction or $^{14}CO_2$ production from labeled formate (Table I).

The product of formate metabolism was CO_2, therefore, the products of formate and CO_2 assimilation were compared. When formate-grown cells were exposed to $H^{14}COOH$ for 30 sec and the ethanol soluble material extracted and chromatographed, over 80% of the radioactivity appeared in glutamic acid (Fig. 1). Photoautotrophic $^{14}CO_2$ fixation for 30 sec yielded less activity in glutamate but more in several other amino acids. In both cases only about 5% of the activity appeared in phosphate esters. Chloramine-T decarboxylation showed the C-1 position of glutamate to have over 70% of the activity at 30 sec.

TABLE I. FORMIC DEHYDROGENASE ACTIVITY OF R. PALUSTRIS

Assay	Substrate treatment	Enzyme	Specific activity	cpm CO_2/10 min
DCIP reduced[1]	Formate	Soluble	210[2]	
	Formate	Particulate	0	
$^{14}CO_2$ formed[3]	Formate-C^{14} (92,000 cpm)	Soluble		69,911[2]

[1]The reaction mixture for DCIP reduction consisted of phosphate buffer (pH 6.8), 200 μmoles; $MgCl_2$, 10 μmoles; DCIP, 0.2 μmoles; formate, 20 μmoles; cell protein, 1.09 mg and water to 3.0 ml.

[2]Endogenous control substracted (DCIP red. = 5; $^{14}CO_2$ formed = 4087 cpm).

[3]Assay described by Malavolta et al. 1962 using 8.25 mg protein.

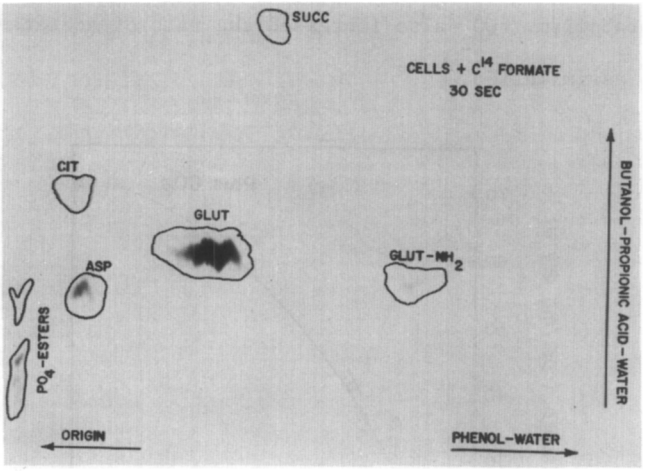

Figure 1. Radioautograph of ethanol extract from cells of R. palustris after 30 sec exposure to C^{14}-formate. Abbreviations: CIT, citrate; ASP, aspartate; GLU-NH_2, glutamine; GLUT, glutamate; SUCC, succinate; PO_4-ESTERS, phosphate esters.

Samples taken 15 sec to 8 min after $H^{14}COOH$ was added showed glutamic acid to have a negative slope when representing the percentage of total activity fixed. No more than 6% of the activity in any sample appeared as phosphate esters. These data also suggest glutamate as the earliest stable product of CO_2 fixation and formate assimilation in R. palustris.

Photoheterotrophic $^{14}CO_2$ fixation with malate-grown cells however shows a labeling pattern indicative of the reductive pentose cycle with large percent-

ages of labeled phosphate esters in short term experiments. These results differ from those of Anderson and Fuller (1967a, 1967b) who compared autotrophic and heterotrophic CO_2 fixation in Rhodospirillum rubrum and found as early products phosphate esters and glycolate respectively.

Fig. 2 demonstrates a presumed $C_4 + C_1$ condensation though glutamate but not 2-oxoglutarate has been radioautographically identified. Cells grown on malate plus $S_2O_3^=$ were depleted of endogenous carbon reserves by 3 hrs of starvation. The "depleted cells" were then incubated in the light with succinate-C^{14} for fixed periods of time. The addition of CO_2 greatly stimulated the incorporation of succinate-C^{14} into the 10% trichloroacetic acid soluble pool (Fig. 2) suggesting a carboxylation as the primary step in succinate metabolism. CO_2 also increased the rate of acetate-C^{14} and pyruvate-C^{14} assimilation.

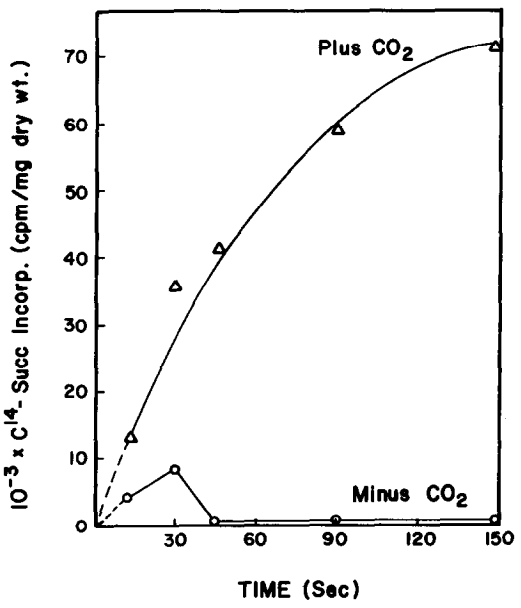

Figure 2. Time-course of incorporation of succinate-2,3-C^{14} into the TCA-soluble fraction of R. palustris in the presence and absence of $NaHCO_3$. Both cultures had a helium atmosphere.

Our labeling patterns confirm that R. palustris has ribulose diphosphate carboxylase activity when grown photoheterotrophically (Lascelles 1960).

These patterns are distinctly different from those obtained from photoautotrophic CO_2 fixation. The rapid labeling and position of the label in glutamate suggests that the major path of $^{14}CO_2$ fixation during photoautotrophic growth or photoheterotrophic growth on formate is not the reductive pentose cycle but suggests a process similar to the reductive carboxylic acid cycle (Evans et al. 1966).

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