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The rapid appearance of glycolate during photosynthesis in *Rhodospirillum rubrum*

Photosynthetic CO_2 fixation occurs in green plants *via* the reductive pentose phosphate cycle. Enzymes of the cycle also occur in the chemosynthetic and photosynthetic bacteria. Ribulose-diphosphate carboxylase (3-phospho-D-glycerate carboxy-lyase (dimerizing), EC 4.1.1.39) is present and functions in microbial forms[1-3]. However, in the bacterial systems, several additional carboxylations are known to occur[2,4]. In addition the enzymes concerned with carbon assimilation are under metabolic control[2] and the method of growth of any one organism would be expected to affect both the activity of individual enzymes and the kinetic pattern of CO_2 fixation.

Since the rate of photosynthetic CO_2 fixation is much lower in the bacteria than in green plants or algae, analagous experiments using short-term kinetic analysis to show the operation of the reductive pentose phosphate cycle under either photoautotrophic or photoheterotropic conditions have not been performed. In a series of experiments designed to study the control of photometabolism in one of the non-sulfur photosynthetic bacteria, *Rhodospirillum rubrum*, we have found that under certain photoheterotrophic conditions the first apparent stable product during CO_2 fixation is not phosphoglyceric acid, but glycolic acid. It would appear that an alternate metabolic pathway of CO_2 assimilation may be operative in this organism during photosynthesis under certain photoheterotrophic conditions and that the first stable intermediate of this pathway is glycolic acid.

R. rubrum, strain S-1, was grown photoheterotrophically on 0.005 M L-glutamate, 0.05 M L-malate, mineral salts medium[5]. Growth was measured by turbidity at 650 mμ using a Zeiss spectrophotometer. The products of $^{14}CO_2$ incorporation were analyzed chromatographically using the techniques of BENSON, BASSHAM AND CALVIN[6].

The results of a $^{14}CO_2$ fixation experiment with cells cultured on L-malate, glutamate media are shown in Figs. 1a, b and c. After 3 sec 69% of the activity incorporated was found in glycolic acid. A strongly negative slope is obtained when the percent incorporation into this compound is plotted against time, indicating that it is an early product of CO_2 fixation. A plot of percent incorporation of $^{14}CO_2$ into phosphate esters has a positive slope; incorporation into glycolate therefore apparently precedes and is independent of the formation of phosphate esters. In 3 sec activity was incorporated into 2 other compounds which appeared to be phosphoglyceric acid and phosphoenolpyruvate. (The slopes for percent incorporation into these compounds were positive.) Activity was found in malate in 7 sec; the percent incorporation was negative with time suggesting that malate might arise from glycolate *via* known pathways. Plots of time *versus* percent incorporation of activity into alanine, succinic, fumaric, and citric acids gave negative slopes, indicating that these compounds may be derived from glycolate or malate or by independent carboxylation reactions. Activity was not found in these compounds until 15 sec after the introduction of $^{14}CO_2$.

In Fig. 1d radioactivity found in glycolate and in the phosphate ester area on

the chromatogram, corrected to give counts/min in the total extract, is plotted against time. No counts were found on the chromatogram in a zero time control. There is net fixation into both phosphate esters and into the spot corresponding to glycolic acid. The low total incorporation into the latter spot indicates that the pool of glycolate is small.

The extreme volatility on chromatograms of the compound corresponding to glycolic acid and the low total activity found in the compound make difficult a rigorous

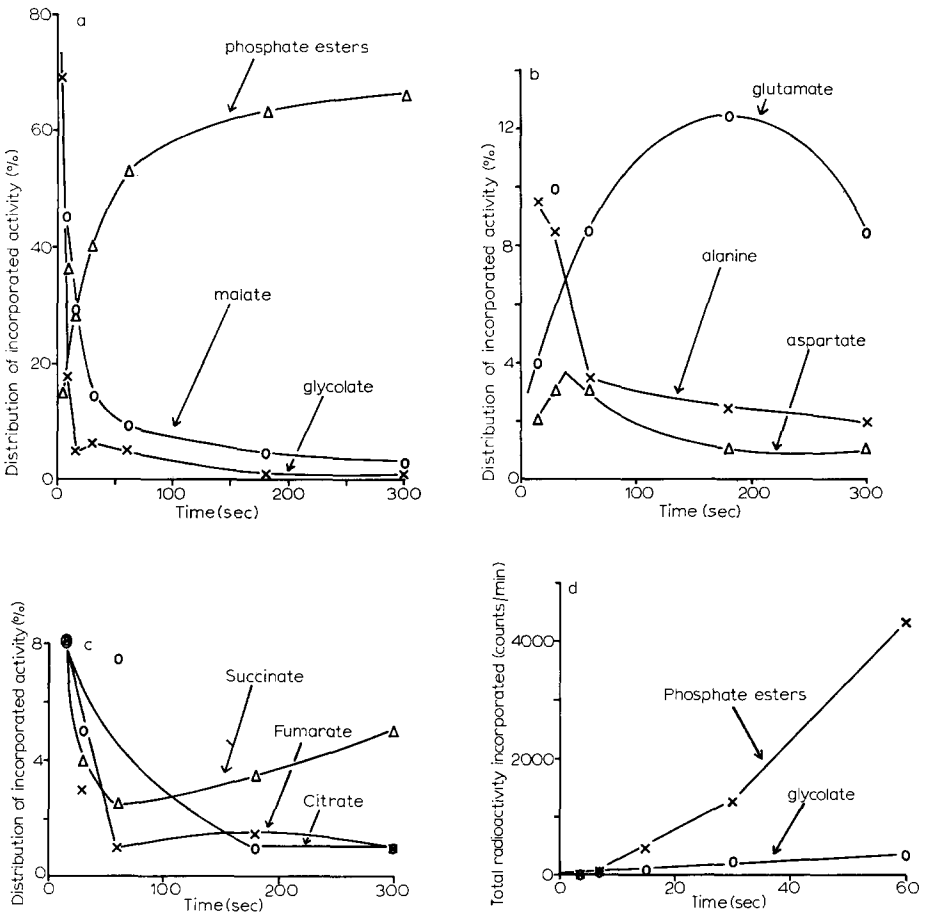

Fig. 1. (a) Distribution of activity incorporated from $^{14}CO_2$ into phosphate esters, glycolic acid, and malic acid with time in cells cultured on L-malate, L-glutamate media. Each manometer flask contained, in 2 ml: 10 μmoles L-malic acid, 10 μmoles $NaHCO_3$, and cells equivalent to 16.5 mg dry weight suspended in dilute buffer solution. After 30 min preincubation under He in the light 2 μmoles $NaH^{14}CO_3$ (100 μC) were added to the side arm and tipped. The $A_{650m\mu}$ of the cells when harvested was 1.12. After 1 min 156000 disint./min had been incorporated, 8% of this being in the ethanol- and water-insoluble precipitate. (b) Distribution of activity incorporated into the amino acids, alanine, glutamate and aspartate. Activity was not noted in any of these compounds until 15 sec after the administration of isotope. (c) Distribution of activity incorporated into succinic, fumaric, and citric acids with time. Activity was not found in these compounds until 15 sec after isotope was administered. (d) Counts/min incorporated into glycolic acid and phosphate esters *versus* time. Results are expressed as detectable counts/min on the chromatogram corrected to total counts/min incorporated.

proof that the compound detected on the chromatograms is glycolic acid. The following criteria lead us to identify this compound as glycolic acid: (1) The compound behaves chromatographically in the standard solvent systems as does glycolic acid. In ethyl acetate–formic acid–water (70:20:10) and ethanol–ammonium hydroxide–water (80:5:15) the radioactive compound corresponds to glycolic acid. (2) The compound is steam-volatile on chromatograms. (3) When an aliquot of a sample which had been shown to contain the unknown compound was chromatographed with carrier glycolic acid on a celite, 0.25 M H_2SO_4, ether column[7], recovery was 67 % of the expected counts (based on counts on original chromatogram of the sample). The eluate was concentrated and rechromatographed using the standard solvent system. The only detectable counts were in one spot which chromatographed as glycolic acid. (4) When an aliquot of a sample known to contain the unknown compound was chromatographed on a Dowex acetate column and the eluate concentrated and chromatographed as described by ZELITCH[8] a radioactive compound co-chromatographed with glycolic acid. It would appear therefore that the unknown compound is probably glycolic acid.

Results similar to those presented in Fig. 1 were obtained in experiments with cells grown on L-malate, $(NH_4)_2SO_4$ media. Glycolic acid was not detected in the photosynthate of cells which had been cultured on $(NH_4)_2SO_4$, H_2 and CO_2. The results with autotrophically grown cells are consistent with the operation of the reductive pentose phosphate cycle. A complete report of these experiments will be published elsewhere.

When glycolic acid was first found in the photosynthate of higher plants and algae it appeared to be a secondary product of photosynthesis[9,10]. WARBURG[11] first suggested that glycolic acid might be a first product of photosynthesis on the basis of quantum measurements and later found that under certain conditions (low partial pressures of CO_2 approximating those found in nature) as much as 90 % of the CO_2 fixed eventually appeared in glycolic acid[12]. Large amounts of glycolate were found in Scenedesmus when ethanol was used to inhibit the pentose phosphate reduction pathway[13]. Recently ZELITCH has obtained the first clear-cut experimental evidence for the formation of glycolic acid independent of phosphoglyceric acid. Using leaf disks of *Nicotiana tabacum* in the presence of an inhibitor of glycolic acid oxidation, he found that the specific activity of either carbon of the glycolic acid formed during photosynthetic $^{14}CO_2$ fixation was 10-fold higher than the specific activity of the carboxyl carbon of 3-phosphoglyceric acid[8]. It was concluded that "glycolic acid is synthesized in the light by a carboxylation reaction different from that catalyzed by ribulose-diphosphate carboxylase". Our data are consistent with and substantiate these observations in a bacterial system.

Since glycolic acid appears to be the first stable product of CO_2 fixation under photoheterotrophic conditions in *R. rubrum* cultured on L-malate the operation of a CO_2 assimilation mechanism distinct from the reductive pentose phosphate cycle in this bacterium is strongly implicated. Apparently, 2 pathways for the uptake of CO_2 occur in *R. rubrum*; one, involving phosphoglyceric acid, which accounts for the major part of CO_2 fixation in autotrophically grown cells, and the other, involving glycolic acid, in photoheterotrophically grown cells. Clearly the nature of the carbon source during growth controls and influences both the kinetic pattern of fixation and the photometabolism of CO_2 in this organism.

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Department of Microbiology,
Dartmouth Medical School,
Hanover, N.H. (U.S.A.)

LOUISE ANDERSON
R. C. FULLER

1 R. C. FULLER AND M. GIBBS, *Plant Physiol.*, 34 (1959) 324.
2 R. C. FULLER, R. M. SMILLIE, E. C. SISLER AND H. L. KORNBERG, *J. Biol. Chem.*, 236 (1961) 2140.
3 P. A. TRUDINGER, *Biochem. J.*, 64 (1956) 274.
4 M. C. W. EVANS, B. BUCHANAN AND D. I. ARNON, *Proc. Natl. Acad. Sci., U.S.*, 55 (1966) 928.
5 J. G. ORMEROD, K. S. ORMEROD AND H. GEST, *Arch. Biochem. Biophys.*, 94 (1961) 449.
6 J. A. BASSHAM AND M. CALVIN, *The Path of Carbon in Photosynthesis*, Prentice-Hall, Inc., Englewood Cliffs, N.J., 1957, p. 16.
7 E. F. PHARES, E. H. MOSBACH, F. W. DENISON, JR. AND S. F. CARSON, *Anal. Chem.*, 24 (1952) 660.
8 I. ZELITCH, *J. Biol. Chem.*, 240 (1965) 1869.
9 A. A. BENSON AND M. CALVIN, *J. Exptl. Botany*, 1 (1950) 63.
10 M. CALVIN, J. A. BASSHAM, A. A. BENSON, V. H. LYNCH, C. OUELLET, L. SHOU, W. STEPKA AND N. E. TOLBERT, *Symp. Soc. Exptl. Biol.*, 5 (1951) 284.
11 O. WARBURG, G. KRIPPAHL AND W. SCHRODER, *Z. Naturforsch.*, 10b (1955) 631.
12 O. WARBURG AND G. KRIPPAHL, *Z. Naturforsch.*, 15b (1960) 197.
13 M. LEFRANCOIS AND C. OUELLET, *Can. J. Botany*, 37 (1959) 499.

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2-Amino-1,1,3-tricyanopropene: a new inhibitor of oxygen evolution in photosynthesis

One of the most fundamental problems of photosynthesis is the mechanism of O_2 evolution; despite many studies of this problem, remarkably little is known. Investigations by WESSELS AND VAN DER VEEN[1] showed that some substituted phenylureas (*e.g.* DCMU) act as inhibitors of O_2 evolution; further work (GINGRAS AND LEMASSON[2], IZAWA AND GOOD[3], has been carried out with these inhibitors on the mechanism of inhibition which may throw light upon the O_2 evolution process itself. Some substituted benzimidazoles and aminotriazines also inhibit the oxygen-evolving system of photosynthesis (BÜCHEL *et al.*[4], BISHOP[5]). The purpose of the present work was to search for other compounds capable of specifically inhibiting O_2 evolution. 2-Amino-1,1,3-tricyanopropene (TCAP), previously shown to uncouple phosphorylation in mitochondria (EBERTS[6], PARKER[7]), was found in this study to inhibit selectively O_2 evolution of the photosynthetic reactions studied (ARNON *et al.*[8]).

Fresh spinach leaves obtained commercially were used in the preparation of chloroplasts by a modification of the method of WHATLEY AND ARNON[9]. 50 g of sliced,

Abbreviations: TCAP, 2-amino-1,1,3-tricyanopropene; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DCIP, 2,6-dichlorophenolindophenol; PMS, phenazine methosulphate.