CARBON DIOXIDE FIXATION IN THE CHEMOAUTOTROPH, FERROBACILLUS FERROOXIDANS[1]

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Over the past decade, the mechanism of CO_2 fixation has been elucidated for green plants, algae, photosynthetic bacteria, and some of the obligate and facultative chemoautotrophic bacteria. In each case, CO_2 fixation was found to proceed, principally, via the Calvin photosynthetic cycle. Little attention has been given to CO_2 fixation in the iron bacterium, Ferrobacillus ferrooxidans, because of the inability to obtain large masses of clean, metabolically active cells. The present communication describes the method used for obtaining sufficient cells for the identification of metabolic intermediates formed from the fixation of CO_2. The data suggests that the Calvin scheme is a route for CO_2 fixation by the iron-oxidizing cells.

F. ferrooxidans, strain TM, was originally supplied by W. W. Leathen (Leathen and Braley, 1954) and cultured at 28°C in 9K liquid medium (Silverman and Lundgren, 1959) with a starting pH of 1.6 in a forced aeration system. This system permitted maximal cell growth, free from the voluminous ferric precipitate that normally accompanied growth. The cells were harvested at 60,000 x g by Sharples centrifugation and the resulting cell paste washed twice in 100 ml of cold H_2SO_4 at a pH of 2.6. Clean cell masses (43.01 - 363.30 mg N) were recovered from 10 -

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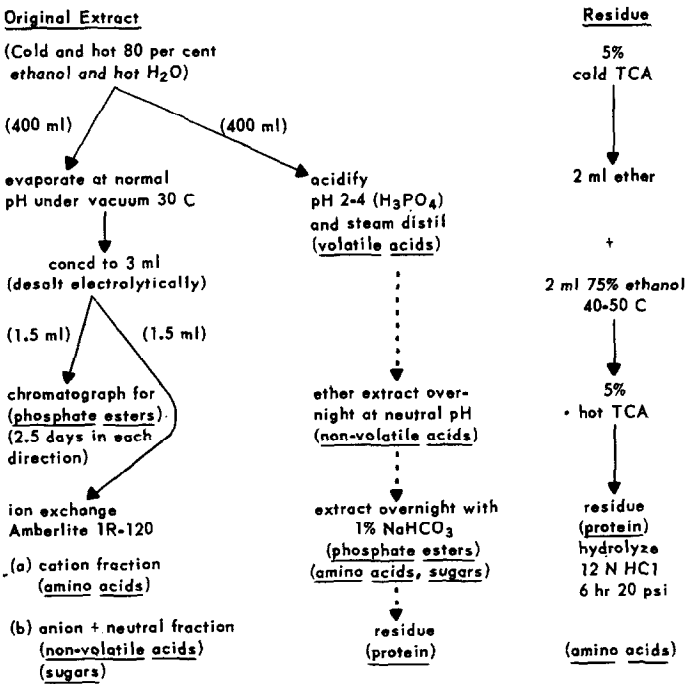
105.6 liters of 9K medium.

To 14.3 x 10^{12} washed logarithmic-phase cells (0.274 mg N) suspended in 30 ml H_2SO_4 at a pH of 3.0 were added 100 µg Mg^{++} (as $MgSO_4 \cdot 7H_2O$) plus 800 µM Fe^{++} (as $FeSO_4 \cdot 7H_2O$). The suspension was then aerated 10 min at 30°C to allow the cells to reach a steady state of CO_2 fixation. After CO_2-free air was bubbled through the cell suspension for 5 min to reduce the CO_2 level, 1 mM $NaHC^{14}O_3$ (sp. act. 1 mc/mM) and 180 uc P^{32} were added to the suspension from a side arm of the reaction vessel. After 5.0 min, 200 ml absolute ethanol were added to the reaction mixture through a 15-mm bore stopcock to stop the reaction. The contents of the reaction vessel were then subjected to the fractionation procedure shown in Fig. 1.

Figure 1
OUTLINE OF FRACTIONATION PROCEDURE



- - - - - - Broken lines represent alternate route.

The labeled compounds were separated on Schleicher and Schuell (Keene, New Hampshire) 2043a acid-washed chromatography paper and their presence revealed by autoradiography. The P^{32} labeled phosphate esters were distinguished by the dark spots they produced on both sheets of Kodak No-Screen x-ray film overlaying the chromatography paper while compounds labeled solely with C^{14} produced dark spots only on the film in direct contact with the paper.

The labeled compounds were eluted from the paper chromatograms and transferred to new papers for further R_f value determinations using two to three different solvent systems (Hirsch, 1963). Authentic compounds were often developed simultaneously in parallel channels with the unknowns on the same chromatograms and the spots were revealed with suitable detection reagents. The phosphate esters were treated with 0.1% polidase at 37°C for 2 days and the parent compounds were further identified by paper chromatography. The volatile acids were converted to their hydroxamic acid derivatives (Thompson, 1951a,b) which were stable in acidic solvents, before being compared chromatographically.

Following positive identification of the isolated compounds, aliquots of the eluates were placed in stainless steel planchets, dried, and counted in a windowless, gas-flow, proportional counter. The per cent of fixed radiocarbon present in each of the isolated compounds is shown in Table I. The maximum $C^{14}O_2$ fixed in 5.0 min in our system was 0.668%.

The observed pattern of radiocarbon incorporation into products of the Calvin cycle suggests that this fixation mechanism may be operative when iron acts as the reducing agent for CO_2 reduction. However, definitive proof of the CO_2 fixation mechanisms involved may be realized only after additional experimentation to meet the criteria proposed by Elsden (1962).

TABLE I

PRODUCTS OF 5.0 MINUTE $C^{14}O_2$ FIXATION

BY INTACT IRON-OXIDIZING F. FERROOXIDANS

Compound	% of Total $C^{14}O_2$ Fixed[1]	Compound	% of Total $C^{14}O_2$ Fixed[1]
Fructose-6-phosphate +		Glucose	6.7
Ribose phosphate	10.3	Fructose	2.7
Phosphoglyceric acid	8.0		
Glucose-1-phosphate	6.8		
Glucose-6-phosphate	6.3		
Ribulose phosphate	3.4	Glutamic acid	6.4
(Ribulose-1,5-diphosphate)[2]		Aspartic acid	6.1
Ribose phosphate	1.9	Alanine	4.3
(Ribose-5-phosphate)[2]		Serine	3.1
Fructose-1,6-diphosphate	1.3	Glycine	2.4
Phosphoenolpyruvic acid	1.2	Valine	2.2
Uridine diphosphoglucose	1.0	Arginine	1.4
Dihydroxyacetone phosphate	0.7	Leucine	1.4
Sedoheptulose phosphate	0.2	Isoleucine	1.3
Unidentified phosphate	0.1	Threonine	1.1
Orthophosphate	-	Phenylalanine	1.0
		Proline	0.5
		Histidine	0.4
Glyceric acid	5.0	Lysine	0.4
Malic acid	2.0	Peptides	1.6
Citric acid	0.9		
Acetic acid	0.7	Unanalyzed[3]	5.2
Formic acid	0.3		

[1] Total $C^{14}O_2$ fixed = 14,010,900 cpm.

[2] Compound in parentheses was identified from R_f values reported in the literature.

[3] Hot and cold trichloroacetic acid fraction, ether-alcohol fraction.

No explanation of the radiocarbon levels in the amino and organic acids can be made at this time; however, it is likely that they could be

accounted for either through drain-off points in the Calvin scheme or through a Wood-Werkman reaction as Suzuki and Werkman (1958) postulated in Thiobacillus thiooxidans to account for their radiocarbon levels in aspartic and glutamic acid.

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References

Elsden, S. R., In "The Bacteria - A Treatise on Structure and Functions," III, Academic Press, New York, 1962.
Hirsch, P., Archiv fur Mikrobiol. 46 53 (1963).
Leathen, W. W., and Braley, S. A., Bacteriol. Proc. 44 (1954).
Silverman, M. P., and Lundgren, D. G., J. Bacteriol. 77 642 (1959a).
Suzuki, I., and Werkman, C. H., Iowa State Coll. J. Sci. 32 475 (1958).
Thompson, A. R., Australian J. Sci. Res. B4 180 (1951a).
Thompson, A. R., Australian J. Sci. Res. B4 282 (1951b).