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Aseptic radiorespirometry has been used to examine the utilization of external carbon sources by proliferating Thiobacillus thioparus cells. These studies reveal that glucose, galactose, mannose, fructose, ribose, DL-glutamate, and L-aspartate were not utilized by this chemoautotrophic organism. However, it has been shown that trace amounts of acetate, glycine, DL-serine, DL-alanine, succinate and fumarate can be utilized by T. thioparus cells.

To elucidate the nature of the biosynthetic pathways operative in this bacteria, proliferating cell cultures were allowed to metabolize specifically ^{14}C labeled substrates. The resulting ^{14}C labeled cells were subsequently hydrolyzed, their amino acids isolated and subjected to degradation experiments.

Examination of the respective fates of the label in DL-alanine-2-^{14}C, acetate-1-^{14}C, or acetate-2-^{14}C in the

cellular metabolism revealed that the Krebs Cycle pathway is not functioning as a respiratory mechanism in T. thioparus. However, most of the reactions of the Krebs Cycle pathway are involved in the biosynthesis of carbon skeletons for various amino acids. A CO_2 fixation pathway of the C_3+C_1 type is instrumental in providing C_4 dicarboxylic acids and those amino acids derived therefrom. Acetate can be incorporated into α-ketoglutarate and those amino acids derived therefrom, but cannot be incorporated into the C_4 dicarboxylic acids.

It appears that the absence of the enzyme α-ketoglutaric acid oxidase complex accounts for the lack of operation of the Krebs Cycle pathway as the terminal respiratory mechanism. These findings also suggest that the Glyoxylate Cycle pathway is inoperative in this organism.

THE ROLE OF SOME OF THE KREBS CYCLE REACTIONS IN THE BIOSYNTHETIC FUNCTIONS OF *THIOBACILLUS THIOPARUS*

by

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This Thesis is Dedicated To my Wife, Kay,

and our Children, Denise, Kirk, and Carrie Rae

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THE ROLE OF SOME OF THE KREBS CYCLE REACTIONS IN THE BIOSYNTHETIC FUNCTIONS OF THIOBACILLUS THIOPARUS

INTRODUCTION

The physiological behavior of autotrophic organisms is unique in many respects. Photosynthetic organisms derive their energy requirements primarily from light in contrast to chemoautotrophic organisms which utilize the chemical energy liberated by enzymatically catalyzed reactions involving inorganic substrates. In both instances CO_2 serves as the sole carbon source giving rise to primary products, which are in turn converted to other cellular constituents. The key mechanism by which autotrophic organisms reduce carbon dioxide to other carbonaceous compounds is in the nature of the reaction sequence described by Calvin et al. (1, 14, 20, 25, 26, 27). Thus, photoautotrophic life and chemoautotrophic life, using different energy sources, both rely on two key reactions which are peculiar to autotrophic life: the phosphorylation of ribulose-5-phosphate to ribulose-1,5-diphosphate and the subsequent carboxylation of ribulose-1,5-diphosphate with the resulting formation of 3-phosphoglyceric acid.

The photoautotrophs, being facultative in nature, may in turn use 3-phosphoglyceric acid either as an energy source or as a source of carbon from which to build their cellular constituents. Hence, it is possible

to maintain proliferation of photosynthetic organisms in the dark, provided they are furnished with the appropriate organic and inorganic requirements. This is not the case with the strictly chemoautotrophic Thiobacilli species. Consequently, the conventional approach of tracing the fate of metabolic intermediates to study the carbon metabolism has so far failed when applied to these nitrifying and sulfur oxidizing bacteria in their intact form. Although there have been reports in the literature describing the stimulating effect of some complex organic materials on the rate of inorganic substrate utilization as well as on the growth of the Thiobacilli, there has been no evidence that any carbonaceous compound, other than CO_2, serves as the useable carbon source for these chemoautotrophic bacteria (2, 3, 5, 6, 22, 23, 29, 30). This unique chemoautotrophic characteristic has thus prevented efforts to elucidate the fate of 3-phosphoglyceric acid once it is formed by the primary CO_2 fixation processes.

In the case of heterotrophic bacteria, it is recognized that the Krebs Cycle is not only the key pathway in energy production but also plays a key role in the biosynthesis of carbon skeletons for a great variety of cellular constituents. In Thiobacilli, Johnson and Peck (9) have reported that a cell-free

preparation was capable of coupling phosphorylation and CO_2 fixation at the expense of sodium thiosulfate. This fact, examined in the light of the strict autotrophic nature of T. thioparus, strongly suggests that this organism relies exclusively on sulfur metabolism for its energy requirement. In view of this exclusive role played by the sulfur metabolism in energy production, one is led to consider whether the Krebs Cycle pathway is playing any kind of role in T. thioparus. Recently, Cooper (4) has shown that several enzymic reactions of the Krebs Cycle are operative in a cell-free preparation of T. thioparus. Oxalacetic acid, pyruvic acid and acetic acid were found to be converted to α-ketoglutarate and further, malic dehydrogenase and succinic dehydrogenase were found to be active. These findings led Cooper to suggest that the Krebs Cycle pathway is operative in T. thioparus, despite the fact that the presence of α-ketoglutaric acid oxidase complex and fumarase are not demonstrated in the cell-free preparation. However, it is possible that the Krebs Cycle reactions may play an important role in the biosynthetic function of this organism.

In order to clarify this situation, interest in this laboratory has been focused on the nature of the biosynthetic pathway functioning in T. thioparus,

leading from 3-phosphoglyceric acid to other carbon skeletons of cellular constituents, particularly that of the amino acids. The study was conducted, with proliferating cell cultures growing under optimum physiological conditions, by means of radiotracer techniques to observe the intracellular conversions of the carbon skeletons originally synthesized from CO_2.

EXPERIMENTAL

Materials

The organisms used in this study were Thiobacillus thioparus Beijerinck, isolated by R. L. Starkey and obtained from the American Type Culture Collection (ATCC-8158). The cultures were maintained at 26°C on liquid thiosulfate media as described by Jones and Happold (10). This same cultural medium was used in all phases of the study whether the cultures were aerated by shaking or sparging. The cells and inorganic sulfur, which is produced during the growth of these bacteria, were harvested by centrifugation, by means of a Sent-Gyorgi and Blum continuous flow centrifuge.

Radiochemical Substrates

The radiochemical substrates used were obtained from commercial sources. Acetate-1-^{14}C, acetate-2-^{14}C, glycine-1-^{14}C, glycine-2-^{14}C, DL-serine-1-^{14}C, DL-serine-3-^{14}C, L-serine-U-^{14}C, succinate-1,4-^{14}C, succinate-2,3-^{14}C, and glucose-U-^{14}C were purchased from New England Nuclear Corporation.

DL-alanine-1-^{14}C, DL-alanine-2-^{14}C, DL-alanine-3-^{14}C, galactose-1-^{14}C, mannose-1-^{14}C, fructose-1,6-^{14}C, ribose-U-^{14}C,DL-glutamate-1-^{14}C, DL-glutamate-2-^{14}C and DL-glutamate-5-^{14}C were purchased from Nichem, Inc.

Fumarate-1,4-^{14}C was purchased from Volk Radiochemical Corporation, fumarate-2,3-^{14}C was purchased from Nuclear Chicago Corporation while L-aspartate-4-^{14}C was purchased from the California Corporation for Biochemical Research.

The purity and identity of these radiochemical substrates were substantiated by paper chromatography and autoradiography.

Radiorespirometric Experiments

The radiorespirometric techniques used to detect the conversion of ^{14}C labeled substrates to $^{14}CO_2$ by proliferating cells were carried out in the same manner as described by Wang and Krackov (31), with the exception that the respirometer was modified so that the bacterial suspension was maintained in a sterile condition throughout the experiment. The experiments were carried out at 26^{o} C under aerobic conditions. It should be noted that T. thioparus is an obligate aerobic organism although oxygen is presumably required for sulfur metabolism exclusively (9). At the termination of each of the experiments, the respirometer flasks were assayed so that any heterotrophic contamination could be detected. Cell viability of the culture in each of the respirometric flasks was verified by streaking the cell suspension on thiosulfate agar plates and subsequent incubation at 26^{o} C for growth observations.

Incorporation of Acetate-1-^{14}C, Acetate-2-^{14}C, and DL-Alanine-2-^{14}C

When the utilization of certain carbonaceous substrates was ascertained by means of the radiorespirometric experiments, the fate of the ^{14}C labeled carbon atom of selected metabolic intermediates in proliferating T. thioparus cells were studied by a series of incorporation experiments. Thus, DL-alanine-2-^{14}C, acetate-1-^{14}C, or acetate-2-^{14}C was administered to individual two-liter cultures and inoculated with cells in the first one-third of the logarithmic phase of cell proliferation (4.6 μg of total cellular nitrogen per two liters of media). The cultures were sparged with sterile air and the effluent gas scrubbed to remove CO_2 so that the production of metabolic $^{14}CO_2$ could be examined. Cell proliferation was demonstrated by following the change in the turbidity of the cell suspension and the pH of the media. The proliferating cultures were allowed to metabolize the administered substrates until the yield of respiratory $^{14}CO_2$ had declined to a steady low-level and the cell mass had increased to 20 mg in dry weight equalling approximately 0.8 mg of cellular nitrogen per two liters of culture media.

Upon termination of each of the experiments, the cells and sulfur were harvested by centrifugation with a

Sent-Gyorgi and Blum continuous flow centrifuge. The cell and sulfur mass was washed and dried in vacuo over P_2O_5.

The cell samples obtained from the incorporation experiments were hydrolyzed individually with 20% HCl (in sealed 10 cc tubes) at 110° C for 24 hours. The free sulfur in the sample remained unchanged during the hydrolysis and was removed by filtration. The filtered hydrolysates were repeatedly evaporated to dryness in vacuo to remove the HCl, taken up in a defined amount of water and further processed for the separation of amino acids.

Separation of Amino Acids

Quantitative amino acid analyses were performed on buffered ion exchange resin columns (15, 21) with a Beckman model 120-B amino acid analyzer. The column effluent was monitored for ^{14}C radioactivity by means of a Tri-Carb flow monitor. This reveals the specific activity of each of the amino acids (expressed as μc per μmole of amino acid). The results were further verified by individual determination of the specific activity of several of the isolated key amino acids by means of liquid scintillation counting of the radioactivity and a colorometric assay of the respective amino acids according to the method of Yemm and Cocking (34).

The bulk of the cellular amino acids needed for degradation studies were isolated from the cell-hydrolysate by means of resin column chromatography according to the procedures described by Hirs, Moore and Stein (7). The identity and purity of each isolated cellular amino acid were verified by means of paper chromatography (19). For degradation studies each of the amino acids was diluted with carrier to a suitable quantity.

Degradation Procedures for Glutamic Acid

The distribution of ^{14}C label in the samples of cellular glutamate was determined by means of the following chemical reactions: (A) Radioactivity of the whole glutamic acid molecule was determined by means of a persulfate combustion (11) of the compound to $^{14}CO_2$; (B) the ninhydrin decarboxylation reaction (28) was used to convert C-1 of glutamate to CO_2 for radiochemical assay; (C) 1,4-diaminobutyric acid, obtained by means of a Schmidt decarboxylation of glutamate (18), was combusted to CO_2 by the persulfate oxidation reaction (11) yielding the ^{14}C content from C-1, C-2, C-3, and C-4. The radiochemical content of C-2, C-3 and C-4 of glutamate was calculated by difference since the ^{14}C of C-1 is known from the ninhydrin reaction (28); and (D) C-5 of glutamate was converted to CO_2 via the Schmidt reaction using a modification of the procedure

described by Katz, et al. (12).

Degradation Procedures for Aspartic Acid

The distribution of radiocarbon in cellular aspartic acid was determined by the following chemical operations: (A) Radioactivity of the whole molecule was assayed by a persulfate combustion of the compound to CO_2 (11); (B) C-1 and C-4 of aspartate were converted to CO_2 by the ninhydrin decarboxylation reaction (28); and, (C) the radioactivity of C-2 and C-3 was calculated by difference.

Measurement of Radioactivity

The respiratory CO_2 from cells utilizing ^{14}C labeled substrate was absorbed in 10 cc of 1:2, 2-amino-ethanol:ethanol (100%) housed in a CO_2 trap (31, 32). The trap solution was replaced hourly and the radio-activity contained therein assayed by means of liquid scintillation counting. Usually 5 cc aliquots of the trapped solution were mixed with 10 cc of toluene con-taining terphenyl (3g/l) and 1,4-bis-2 (5-phenyloxa-zolyl)-benzene (30 mg/l), in a 20 cc glass counting vial. Countings were carried out with a Tri-Carb series 314-EX2 liquid scintillation counter in the manner described by Wang (31). The radioactivity of the cell samples was also assayed by means of liquid scintillation counting

using gel sample preparations according to the method of White and Helf (33). The efficiency of radioactivity assay for each type of counting sample was determined by the use of internal standard techniques.

The $^{14}CO_2$ samples obtained in the degradation experiments were converted into $Ba^{14}CO_3$ and mounted on aluminum planchets by means of the centrifugation technique (8). Determination of radioactivity was carried out with a gas-flow Geiger-Muller counter. Corrections of counting data for background and self-absorption were applied in the conventional manner.

All of the counting operations described above were carried out over a specified duration so that the relative standard deviation of the counting data is no greater than two percent.

Radioactivity in the effluent resulting from ion exchange column chromatography was detected by the use of a Tri-Carb flow monitor, using an anthracene-packed cartridge.

RESULTS AND DISCUSSION

Previous studies have characterized the physiology of T. thioparus as an obligate chemoautotroph relying on carbon dioxide as the sole source of carbon and the oxidation of sulfur compounds as the sole energy source (16, 24, 29). The predominant mechanism of CO_2 fixation, as demonstrated in a cell-free preparation from T. thioparus, is the fixation pathway of the C_5+C_1 type, which relies on the enzyme carboxydismutase (20) to yield 3-phosphoglyceric acid as the key intermediate. However, it is not known by what pathway 3-phosphoglyceric acid is converted to the other carbon skeletons of various cellular constituents in T. thioparus.

Much interest in the sulfur metabolism of these unique bacteria is reflected by careful studies in several laboratories designed to elucidate the relationship of the sulfur metabolism and the energy metabolism (13, 17). The report by Johnson and Peck (9), describing the ability of a cell-free preparation of T. thioparus to couple phosphorylation and carbon dioxide fixation at the expense of thiosulfate, is indicative of the exclusive role played by sulfur metabolism in providing the energy required for the biosynthesis of carbonaceous cellular constituents. Cooper (4) demonstrated the presence of a weak succinic dehydrogenase activity in

whole cells of T. thioparus and the conversion of oxalacetic acid, pyruvic acid and acetic acid to α-ketoglutarate, in a cell-free preparation of T. thioparus. These findings led the author to speculate that a complete Krebs Cycle pathway may be operative in T. thioparus.

However, if the energy requirement in T. thioparus is satisfied by the action of sulfur metabolism, one is led to consider the exact role of the Krebs Cycle pathway and its relation to energy production in this bacteria. With heterotrophic bacteria, it is recognized that the Krebs Cycle pathway plays a key role in both energy production and biosynthesis of carbon skeletons for a great variety of cellular constituents.

In order to understand better the function of the Krebs Cycle pathway, if operative, in T. thioparus the present work was undertaken to elucidate biosynthetic pathways leading from 3-phosphoglyceric acid to carbon skeletons of cellular constituents, particularly several key amino acids. Emphasis has been particularly placed on the participation of the reactions of the Krebs Cycle pathway in cellular biosynthesis.

To undertake such a task, it is necessary to first devise a method to study the carbon metabolism of a bacteria that is reportedly relying on CO_2 as the sole

carbon source. One possible approach is to use the enormous magnifying power of the radiotracer techniques so that if a minute amount of a certain labeled carbonaceous compound could be given entry into the organism, it would be mixed with the unlabeled intracellular compounds, thereby making it possible to trace the metabolic fate of such a compound. The radiorespirometric method has been used in this regard to detect substrate utilization, as reflected by the production of respiratory $^{14}CO_2$. In anticipation of the slow rate of substrate utilization, the design of the radiorespirometer previously described (32) was modified to permit the execution of experiments for an extended duration under aseptic conditions.

Preliminary findings in a series of radiorespirometric experiments revealed that glucose, galactose, mannose, fructose, ribose, DL-glutamate and L-aspartate were not utilized by T. thioparus cells. These experiments were carried out for 100 hours during which time there was active cell proliferation and the cultures were shown to be viable at the end of each of these experiments.

Given in Table I are the data relative to the utilization of a number of substrates by proliferating T. thioparus cells. The data were those observed at

Table I

^{14}C Labeled Substrates Utilized by Proliferating Thiobacillus thioparus Cells

Substrate	Level μMole	Experimental Duration Hrs	Distribution of Substrate ^{14}C (percent) Resp. CO_2	Cells
Acetic acid-1-^{14}C	10	36	0.6	88
Acetic acid-2-^{14}C	10	36	Trace	89
Glycine-1-^{14}C	1	27	13	12
Glycine-2-^{14}C	1	27	Trace	22
DL-Alanine-1-^{14}C	1	46	2	Trace
DL-Alanine-2-^{14}C	1	46	Trace	Trace
DL-Alanine-3-^{14}C	1	46	Trace	1
DL-Serine-1-^{14}C	1	37	4	4
DL-Serine-3-^{14}C	1	32	Trace	5
L-Serine-U-^{14}C	1	34	3	11
Succinic acid-1, 4-^{14}C	3	42	5	Trace
Succinic acid-2, 3-^{14}C	3	42	1	2
Fumaric acid-1, 4-^{14}C	3	42	7	Trace
Fumaric acid-2, 3-^{14}C	3	42	3	1

Each flask contained proliferating cells (approximately 0.14 mg cellular nitrogen per flask) which had been harvested during the first one-third of the exponential phase of growth. These cells were incubated at 26° C in 20 cc of thiosulfate media and aerated at 60 cc per minute with sterile air.

the end of the respective time courses of utilization as indicated by the fact that the production of respiratory $^{14}CO_2$ had declined to an insignificant level. It was noted that even with a dense cell suspension, the rate of substrate utilization was very low, ranging from 1 μmole to 3 μmole of labeled substrate over a period of 30 hours. An induction period as long as 28 hours prior to the active utilization of the C_4 dicarboxylic acids was also observed.

Encouraged by the findings in the preliminary experiments, the experimental conditions were modified to increase the net amount of substrate utilization. Labeled substrates were introduced into an incubation medium, which had been inoculated with a very small amount of cells. The cells were then permitted to undergo active proliferation over an extended duration. Under these conditions, the amount of substrate utilization was found to be significantly increased when compared with a similar experiment with a dense cell suspension. In fact, the total amount of labeled substrate incorporated into the cells was respectable in magnitude when the cells were harvested at the terminal side of the exponential growth curve.

From the list of carbonaceous substrates found to be utilized by T. thioparus cells, DL-alanine-2-^{14}C,

acetate-1-^{14}C, and acetate-2-^{14}C were selected as tracing substrates because of their close relation with 3-phosphoglycerate metabolism. Pyruvate-2-^{14}C, arising from DL-alanine-2-^{14}C may either be decarboxylated to yield CO_2 and acetate-1-^{14}C or enter into the C_3+C_1 type of carbon dioxide fixation pathway to provide C_4 dicarboxylic acids required for the biosynthesis of a number of key amino acids. Using specifically ^{14}C labeled acetate samples to trace the role of C_2 metabolism should also give insight into the exact role played by the Krebs Cycle reactions in this organism.

Table II

Distribution of Substrate Radioactivity in Incorporation Experiments with Thiobacillus thioparus Cells

Substrate	Level mg	Experimental Duration Hrs	Distribution of Substrate Radioactivity (Percent)		
			Resp. CO_2	Cells	Medium
$CH_3{}^{14}COOH$	1.20	22	0.6	88	11
$^{14}CH_3COOH$	1.20	22	Trace	89	11
$CH_3{}^{14}CHNH_2COOH$	2.41	50	Trace	72	28

Each flask contained two liters of thiosulfate media into which was inoculated proliferating cells (4.6 μg of total cellular nitrogen) in the first one-third of the exponential phase of growth. These cells were incubated at 26° C in the presence of the ^{14}C substrates and aerated with sterile air at a rate of 260 cc per minute.

In Table II the inventory of radioactivity from the respective ^{14}C labeled substrates is given in experiments with T. thioparus under active proliferating conditions. It is noted that a significant amount of ^{14}C compound has gained entry into the cells. The fact that yields of respiratory $^{14}CO_2$ are extremely low in magnitude provide a clue that these substrates are not involved, to any great extent, in energy producing processes.

In order to elucidate the nature of the biosynthetic pathways operative in this bacteria, efforts were then directed to investigation of whether the respective substrates, upon entry into the cells, were engaged in further metabolic processes. The ^{14}C labeled cells were hydrolyzed and processed for the separation of cellular amino acids. The amino acids were separated by means of resin column chromatography and their respective radioactivities assayed by means of a liquid scintillation flow counter.

It is evident, from the data present in Table III, that the labeled substrate has entered the pool of intracellular constituents and engaged in active metabolism giving rise to carbon skeletons of several amino acids.

It is interesting to note that the variety of cellular amino acids derived from DL-alanine-2-^{14}C is in sharp contrast to that derived from ^{14}C labeled

Table III

Incorporation of the Labeled Atom of DL-Alanine-2-^{14}C, Acetic Acid-1-^{14}C, and Acetic Acid-2-^{14}C into the Cellular Amino Acids of Thiobacillus thioparus

Cellular Amino Acid	μc/ μMole Cellular Amino Acid		
	DL-Alanine-2-^{14}C	Acetic Acid-1-^{14}C	Acetic Acid-2-^{14}C
Glutamic acid	.005	3.27	2.84
Proline	.005	3.98	3.41
Arginine	.005	3.55	3.12
Leucine	.009	3.41	3.12
Valine	.007	0	0
Aspartic acid	.002	0	0
Threonine	.002	0	0
Lysine	.005	0	0
Isoleucine	.005	0	0
Methionine	.003	0	0
Alanine	.108	0	0
Serine	.001	0	0

Substrate level:

DL-Alanine-2-^{14}C, 27 μMoles at 2.09 μc/ μMole; Acetic acid-1-^{14}C and Acetic acid-2-^{14}C, 20 μMoles at 25 μc/ μMole.

acetates. In the alanine-2-^{14}C experiment, the bulk of the radioactivity is found in cellular alanine. The magnitude is such that it may reflect that D-alanine-2-^{14}C has undergone epimerization to L-alanine-2-^{14}C prior to its incorporation into cellular proteins. The remaining radioactivity of the alanine-2-^{14}C is found to be distributed among a great number of amino acids. On the other hand, one finds that in the experiments with ^{14}C labeled acetates, labeling is confined to only four amino acids, all related directly to α-ketoglutarate. However, a close examination of the amino acids that were preferentially labeled from alanine-2-^{14}C revealed that they are related either directly to pyruvic acid (e.g., serine and valine), or to oxalacetic acid which is presumably derived from pyruvic acid by a CO_2 fixation process of the C_3+C_1 type.

One is therefore led to conclude, from these findings, that in this organism there exists no pathway leading from acetate to a C_4 dicarboxylic acid. This contention implies that neither a complete Krebs Cycle pathway nor the Glyoxylate Cycle pathway is operative in this organism. Both are the important known mechanisms in converting acetate to C_4 carbon skeletons.

To verify these speculations, cellular glutamic acid and aspartic acid obtained in these incorporation

experiments were subjected to degradation studies in order to gain information on the intramolecular distribution pattern of the ^{14}C label derived from the respective ^{14}C labeled substrates.

The label distribution patterns of the cellular amino acids derived from DL-alanine-2-^{14}C, acetate-1-^{14}C, and acetate-2-^{14}C are given in Table IV along with the labeling patterns anticipated, assuming the operation of several possible sets of reaction sequences. It can be readily concluded that cellular glutamate is derived from acetate by way of: citrate $\longrightarrow$ cis-aconitate $\longrightarrow$ isocitrate $\longrightarrow$ α-ketoglutarate $\longrightarrow$ glutamate. Cellular aspartate is derived from oxalacetate which in turn is derived from pyruvate by way of a CO_2 fixation process of the C_3+C_1 type. The fact that acetate was found to be converted to glutamate but not to aspartate suggests strongly that the enzyme α-ketoglutaric acid oxidase complex is not present in this organism and thereby forbids the operation of the Krebs Cycle pathway in a cyclic manner.

These results indicate that enzymes responsible for several reactions of the Krebs Cycle pathway are operative in T. thioparus, but they function only as biosynthetic reactions. Given in Figure 1 is a graphic summary of the understandings derived from the present

Table IV

Labeling Patterns of Cellular Glutamic Acid and Aspartic Acid Derived from ^{14}C Labeled Substrates

Pathway Followed by the Labeled Carbon Atoms	Expected and Observed Isotopic Distribution Pattern (Percent)				
Glutamic Acid					
	COOH	CH_2	CH_2	$CHNH_2$	COOH
Acetate-1-^{14}C					
Via Krebs Cycle at steady state	67	0	0	0	33
Via Krebs Cycle reactions to α-ketoglutarate	100	0	0	0	0
Observed	100		0		0
Acetate-2-^{14}C					
Via Krebs Cycle after two turns	0	50	25	25	0
Via Krebs Cycle at steady state	0	29	29	29	14
Via Krebs Cycle reactions to α-ketoglutarate	0	100	0	0	0
Observed	0		100		0
DL-Alanine-2-^{14}C					
Via pyr → acetate → α-ketoglutarate	100	0	0	0	0
Via pyr → OAA; OAA+acetate (derived from pyr) → α-ketoglutarate	50	0	50	0	0
Observed	65		35		0
Aspartic Acid					
	COOH	CH_2	$CHNH_2$		COOH
DL-Alanine-2-^{14}C					
Via pyr → acetate → Krebs Cycle → OAA	50	0	0		50
Via $C_3+C_1 \rightarrow C_4$ acid (unsymmetrical) → OAA	0	0	100		0
Observed	0		100		0

pyr = pyruvate, OAA = oxalacetate

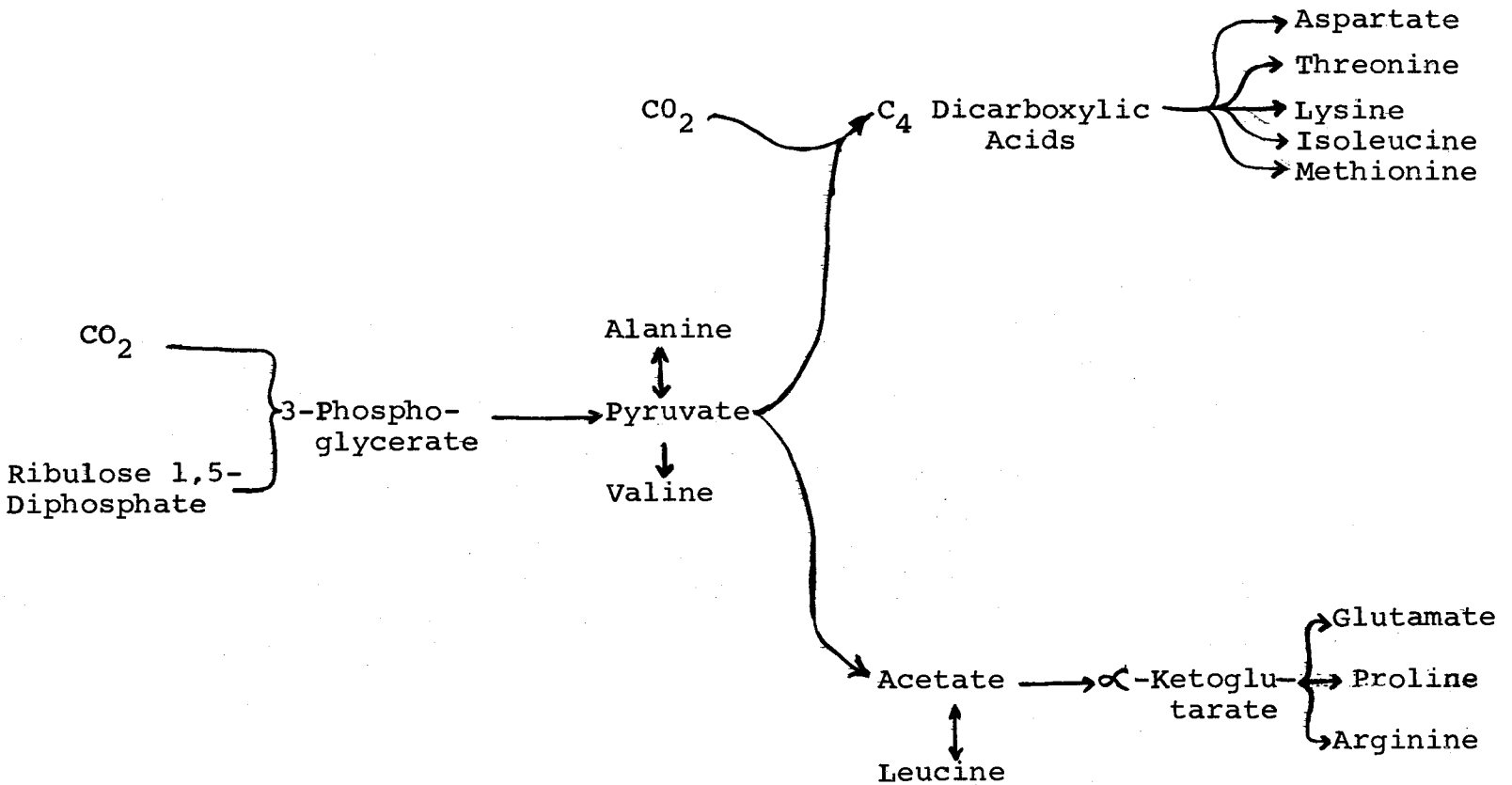

Figure 1. The Relation of 3-Phosphoglycerate Metabolism and the Biosynthesis of Cellular Amino Acids in T. thioparus.

study, relative to the fate of 3-phosphoglyceric acid in the biosynthesis of cellular amino acids found in T. thioparus. The energy required to implement these reactions is derived exclusively from sulfur metabolism.

SUMMARY

Aseptic radiorespirometry has been used to examine the utilization of external carbon sources by proliferating Thiobacillus thioparus cells. These studies reveal that trace amounts of succinate, fumarate, acetate, DL-serine, DL-alanine, and glycine can be utilized by this chemoautotrophic organism.

Examination of the respective fates of the label in DL-alanine-2-^{14}C, acetate-1-^{14}C, or acetate-2-^{14}C in the cellular metabolism revealed that the Krebs Cycle pathway is not functioning as a respiratory mechanism in T. thioparus. However, most of the reactions of the Krebs Cycle pathway are involved in the biosynthesis of carbon skeletons for various amino acids. A CO_2 fixation pathway of the C_3+C_1 type is instrumental in providing C_4 dicarboxylic acid and those amino acids derived therefrom. Acetate can be incorporated into α-ketoglutarate and those amino acids derived therefrom, but cannot be incorporated into the C_4 dicarboxylic acids.

It appears that the absence of the enzyme α-ketoglutaric acid oxidase complex accounts for the lack of operation of the Krebs Cycle pathway as the terminal respiratory mechanism. These findings also suggest that the Glyoxylate Cycle pathway is inoperative in this organism.

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