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Carbon Dioxide Fixation by Extracts of *Streptococcus faecalis* var. *liquefaciens*

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Extracts of cells of *Streptococcus faecalis* var. *liquefaciens* strain 31 incorporated $^{14}CO_2$ into aspartate. Dialyzed extracts produced radioactive oxalacetate in the absence of exogenously added glutamate and pyridoxal-5'-phosphate and produced radioactive aspartate in the presence of these components. Reduced nicotinamide adenine dinucleotide or reduced nicotinamide adenine dinucleotide phosphate could not be substituted for adenosine triphosphate (ATP); phosphoenolpyruvate even in the presence of nucleoside diphosphates could not replace pyruvate plus ATP; propionate plus coenzyme A (CoA) could not replace pyruvate in supporting CO_2 fixation by cell extracts. Fixation by dialyzed cell extracts required pyruvate, ATP, $MgSO_4$, and was stimulated by biotin, KCl, 2-mercaptoethanol, CoA, and acetyl CoA. Inhibition of fixation occurred when avidin, NaCl, oxalacetate, or aspartate was added to dialyzed extracts. On the basis of the products formed and the effects of substrates and cofactors on the fixation reaction, it was concluded that pyruvate carboxylase is responsible for CO_2 fixation in this microorganism.

The utilization of CO_2 by various species of *Streptococcus* has been reported. Barnes et al. (1) and Wright (20) reported increased dextransucrase synthesis by *S. bovis*. Fixation of CO_2 primarily into aspartic acid has been demonstrated in *S. anginosus* (9) and in *S. faecium* var. *durans* (8). Platt and Foster (11) stated that *S. faecalis* var. *liquefaciens* incorporated CO_2 primarily into acetate, lactate, and cell material.

Carbon dioxide stimulates proteinase biosynthesis by nonproliferating cells of *S. faecalis* var. *liquefaciens* in a synthetic medium (15). Carbon dioxide stimulation of proteinase biosynthesis was eliminated by the addition of aspartate to the medium (18). R. C. Goff (Ph.D. Dissertation, St. Bonaventure University, St. Bonaventure, N.Y., 1969) found that greater than 90% of the radioactivity of whole cells fixing $^{14}CO_2$ occurred as aspartate. The present report describes studies of $^{14}CO_2$ fixation by extracts of *S. faecalis* var. *liquefaciens* designed to discover the enzyme system responsible for fixation in this microorganism.

MATERIALS AND METHODS

Chemicals. $NaH^{14}CO_3$ was prepared from $Ba^{14}CO_3$ (17) obtained from International Chemical and Nuclear Corp., Irvine, Calif. Tris(hydroxymethyl)aminomethane (Tris), primary standard; semicarbazide·HCl; KCl; $NaHCO_3$; and potassium acid phthalate were obtained from Fisher Scientific Co., Fair Lawn, N. J. Oxalacetic acid, KH_2PO_4, NaCl, and $AlK(SO_4)_2 \cdot 12H_2O$ were purchased from J. T. Baker Chemical Co., Phillipsburg, N.J. Biotin and L-aspartic acid were purchased from General Biochemicals, Inc., Chagrin Falls, Ohio. $MgSO_4 \cdot 7H_2O$ was purchased from Merck and Co., Inc., Rahway, N.J. Pyruvic acid (K salt, type III) and 2-mercaptoethanol (type I) were purchased from Sigma Chemical Co., St. Louis, Mo. Adenosine 5'-triphosphate dipotassium (ATP), guanosine 5'-triphosphate Na(GTP), inosine 5'-triphosphate $Na \cdot H_2O$(ITP), 95 to 100% adenosine 5'-diphosphate Na(ADP), guanosine 5'-diphosphate Na(GDP), inosine 5'-diphosphate Na(IDP), 90% reduced nicotinamide adenine dinucleotide (NADH), 90% reduced nicotinamide adenine dinucleotide phosphate (NADPH), 90+% coenzyme A (CoA), acetyl coenzyme A (acetyl CoA), pyridoxal-5'-phosphate·H_2O, phosphoenolpyruvate tricyclohexylammonium salt (PEP), synthetic propionic acid, and egg avidin were purchased from Mann Research Laboratories, New York, N.Y. Solutions were prepared with glass-distilled water.

Organism. The organism used in this study was *S. faecalis* var. *liquefaciens* strain 31. Stock cultures were maintained at −23 C in litmus milk.

Bacterial growth. Cells were grown in A-C broth (12) at 37 C in stationary flasks. The final growth medium (1 liter) was inoculated with a 10% (v/v) inoculum. The cells were harvested at the end of exponential growth (3 hr) by centrifugation at 4 C and

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18,000 × *g* (Sorvall RC2-B) and used immediately. The cell yield was approximately 1.5 g (wet weight).

Cell extracts. In the preparation of cell extracts, cells (1.5 g, wet weight) were suspended in 15 ml of 0.1 M potassium phosphate buffer (*p*H 6.6), and 1.5 g of alumina powder was added. Cells were disrupted with a Branson Sonifier Cell Disrupter, model W-185C, at an output of 100 w for 3 min. Cellular debris was removed by centrifugation at 4 C and 33,000 × *g* for 20 min. The extracts were stored at −23 C prior to use. Dialyzed extracts were prepared by dialyzing fresh extract in cellulose tubing against five 20-volume changes of 0.1 M potassium phosphate buffer, *p*H 6.6, during a 5-hr period at 4 C with constant agitation of the dialysis bag. Protein concentration was determined by the biuret method (5) by using bovine serum albumin as the standard.

Assay for CO_2 fixation. To assay for CO_2 fixation, reaction components as designated in the tables were added to test tubes (13 by 100 mm). A 0.2-ml amount of extract or dialyzed extract was added to each tube. As soon as the reaction mixture warmed to 37 C, 0.15 ml of the $NaH^{14}CO_3$ solution was added and the tubes were immediately stoppered. Incubation was for 30 min at 37 C. Protein was precipitated by the addition of 0.15 ml of 50% trichloroacetic acid and removed by centrifugation at 4 C and 33,000 × *g* for 20 min. Portions (0.4 ml) were removed, flushed with CO_2 for 10 min, and then assayed for radioactivity. Preliminary studies showed that the assay was linear with time at least through 45 min. Controls were run with each experiment in which the trichloroacetic acid was added prior to the $NaH^{14}CO_3$. The radioactivity of these controls was similar to background and was subtracted from the values reported in the tables. Radioactivity reported in the tables is the corrected radioactivity of the 0.4-ml portion.

Assay for radioactivity. Radioactivity was assayed in a Beckman model L-S 100 liquid scintillation counter. The samples were added to 10 ml of a solution containing 50 g of napthalene and 2.5 g of 2,5-diphenyloxazole, and were diluted to 500 ml with 1,4-dioxane. The samples were dark-adapted for 18 hr prior to measurement. The external ratio was approximately 3.0 for all samples, and therefore quenching corrections were not applied.

Chromatographic analysis. Radioactive aspartate was identified by descending chromatography on Whatman no. 1 paper. Reaction mixtures (Tables 7 and 8) were deproteinized by the addition of 0.2 ml of 2 N HCl and centrifugation. A 0.5-ml portion was dried under a stream of CO_2, suspended in 50 μliters of cold ethanol, and left overnight at 4 C. Five microliters of the mixture was spotted on a strip, developed with the solvent systems described in Table 7, exposed to Kodak X-ray film (type BB-54) for 72 hr to locate the radioactivity, and sprayed with 0.5% ninhydrin in 90% *n*-butanol.

Decarboxylation. Decarboxylation was carried out according to the method of Krebs and Eggleston (7), which is specific for the beta carboxyl of oxalacetate. A 0.3-ml sample of each reaction mixture was added to a Warburg vessel containing 1.7 ml of H_2O. In one side arm was added 0.7 ml of saturated solution of $AlK(SO_4)\cdot 12H_2O$, and in the second side arm was was added 0.7 ml of a phthalate buffer (7.6 g KHphthalate + 0.9 g NaOH in 50 ml). Carbon dioxide was absorbed by 0.05 ml of 10% KOH impregnated on filter paper in the center well. Immediately before incubation, 0.2 ml of 2 N HCl was added to the vessel. The components were combined and incubated for 2 hr at 25 C. The filter paper and a 0.4-ml sample of the liquid were assayed for radioactivity.

RESULTS

Table 1 contains data from a preliminary experiment to determine the optimal level *p*H for CO_2 fixation by the cell extract. According to the data, the *p*H optimum for fixation is 8.5. Subsequent experiments were therefore carried out at this *p*H level.

Since many CO_2-fixing enzymes are stimulated by potassium ions and by reduced oxidation-reduction (OR) potentials, the effect of KCl and 2-mercaptoethanol on CO_2 fixation by the cell extract was studied. The data in Table 2 demonstrate that both KCl and mercaptoethanol are stimulatory to CO_2 fixation.

To determine the extent to which each of a number of possible CO_2-fixing enzymes might be

TABLE 1. *Effect of pH on the fixation of CO_2 by cell extracts*[a]

*p*H of Tris buffer	Radioactivity[b]
7.0	4,343
7.5	16,228
8.0	27,200
8.5	30,651
9.0	23,654

[a] Reaction mixtures contained (micromoles): Tris, 200; pyruvate, 10; adenosine 5'-triphosphate, 2.5; $MgSO_3$, 3; biotin, 8×10^{-6}; $NaHCO_3$, 3; $NaH^{14}CO_3$, 9 μc; protein, 2 mg; total volume, 1.5 ml.

[b] Expressed as counts per minute.

TABLE 2. *Effect of KCl and 2-mercaptoethanol on CO_2 fixation by cell extracts*[a]

Additions to reaction mixture[b]	Radioactivity[c]
KCl (100)	33,555
Mercaptoethanol (5)	44,375
KCl (100) + mercaptoethanol (5)	60,850
None	20,278

[a] Basic reaction mixture contained (micromoles): Tris (*p*H 8.5), 200; pyruvate, 10; adenosine 5'-triphosphate, 2.5; $MgSO_4$, 3; biotin, 8×10^{-6}; $NaHCO_3$, 3; $NaH^{14}CO_3$, 9 μc; protein, 1.9 mg; total volume, 1.5 ml.

[b] Values in parentheses indicate micromoles added.

[c] Expressed as counts per minute.

operating in *S. faecalis* var. *liquefaciens*, a series of combinations of different cofactors and substrates was added to the basic reaction mixture. Table 3 shows the amount of CO_2 fixation that was achieved by the cell extract in these different reaction mixtures. Apparently the fixation enzyme requires ATP as the energy source. Both GTP and ITP supported only minimal fixation. This low level of fixation could have resulted from the formation of ATP from these other cofactors. Neither NADH nor NADPH was able to substitute for ATP. PEP was not able to substitute for pyruvate and ATP, either alone, or in the presence of the nucleoside diphosphates, ADP, GDP, and IDP. Finally propionic acid and CoA were not able to substitute for pyruvate.

Table 4 contains the data from a series of studies with different buffers that were used to dialyze the extract. It can be seen that only dialysis against 0.1 M phosphate buffer (pH 6.6) provided for an active enzyme preparation. Tris-hydrochloride buffer (0.1 M, *p*H 7.2) and phosphate buffer (0.001 M, *p*H 6.6) inactivated the enzyme. It is interesting to note further that when 0.001 M phosphate buffer was enriched with 0.1 M KCl, the enzyme was again inactivated. Finally, when the extract, dialyzed against 0.001 M phosphate buffer (*p*H 6.6), was added to a reaction mixture which compensated for the dilution of the phosphate as a result of the dialysis against the weak phosphate buffer, no CO_2 fixation was observed. There appears to be a unique property of the 0.1 M potassium phosphate buffer (*p*H 6.6) that is required to preserve the activity of the CO_2-fixing enzyme in the extract during dialysis.

TABLE 3. *Effect of additions and deletions in the reaction mixture on CO_2 fixation by cell extracts*[a]

No.	Deletions	Additions[b]	Radioactivity[c]
1	ATP	None	881
2	ATP	GTP (2.5)	3,308
3	ATP	ITP (2.5)	3,368
4	ATP, pyruvate	PEP (10)	455
5	ATP, pyruvate	PEP (10), ADP (2.5)	1,675
6	ATP, pyruvate	PEP (10), IDP (2.5)	1,500
7	ATP, pyruvate	PEP (10), GDP (2.5)	705
8	ATP	NADH (2.5)	1,752
9	ATP	NADPH (2.5)	1,553
10	Pyruvate	Propionate (10), CoA (0.6)	1,824
11	None	None	30,851

[a] Basic reaction mixture contained (micromoles): Tris (*p*H 8.5), 200; pyruvate, 10; adenosine 5′-triphosphate (ATP), 2.5; $MgSO_4$, 3; biotin, 8×10^{-6}; $NaHCO_3$, 3; $NaH^{14}CO_3$, 9 μc; KCl, 100; mercaptoethanol, 5; protein, 2.5 mg; total volume, 1.5 ml. Abbreviations: GTP, guanosine 5′-triphosphate; ITP, inosine 5′-triphosphate; PEP, phosphoenolpyruvate; ADP, adenosine 5′-diphosphate; IDP, inosine 5′-diphosphate; GDP, guanosine 5′-diphosphate.

[b] Values in parentheses indicate micromoles added.

[c] Expressed as counts per minute.

TABLE 4. *Effectiveness of various buffers for dialysis of cell extracts*[a]

Dialysis buffer	Reaction mixture[b]	Radioactivity[c]
Extract, no dialysis	Basic	17,671
0.1 M Phosphate (K), *p*H 6.6	Basic	17,900
0.001 M Tris, *p*H 7.2	Basic	250
0.1 M Tris, *p*H 7.2	Basic	201
0.001 M Phosphate (K), *p*H 6.6	Basic	280
0.001 M Phosphate (K), *p*H 6.6, 0.1 M KCl	Basic	580
0.001 M Phosphate (K), *p*H 6.6	Basic + 100 μmoles of KCl	222
0.001 M Phosphate (K), *p*H 6.6	Basic + 100 μmoles of KCl + 20 μmoles of phosphate (K)	105

[a] Dialysis of the extract was carried out against five 20-volume changes at hourly periods at 4 C.

[b] Basic reaction mixture contained (micromoles): Tris (*p*H 8.5), 200; pyruvate, 10; adenosine 5′-triphosphate, 2.5; $MgSO_4$, 3; biotin, 8×10^{-6}; $NaHCO_3$, 3; acetyl CoA, 0.6; $NaH^{14}CO_3$, 9 μc; protein, 2 mg; total volume, 1.5 ml.

[c] Expressed as counts per minute.

The effect of additions and deletions in the reaction mixture on CO_2 fixation by the dialyzed extract is shown in Table 5. Pyruvate, ATP, and $MgSO_4$ are essential for significant CO_2 fixation. Mercaptoethanol, KCl, CoA, and acetyl CoA are stimulatory. NaCl is inhibitory.

Yeast pyruvate carboxylase is inhibited by aspartate and by oxalacetate (10). Biotin-containing enzymes are inhibited by avidin. The data in Table 6 demonstrate that oxalacetate and, to a lesser extent, aspartate are inhibitory to the fixation process. Likewise, avidin is inhibitory

both in the presence and absence of exogenous biotin. Biotin stimulates CO_2 fixation.

In an effort to identify the product formed by the extract during the fixation of CO_2, chromatography of the reaction mixtures as shown in Table 7 was performed. It can be seen that, when the cell extract fixes CO_2, it forms a compound with the R_F of aspartic acid in both solvent systems. Co-chromatography of the reaction mixture with aspartic acid revealed that the zone of radioactivity had the same shape as the zone of the ninhydrin reacting material at the location of aspartic acid.

Table 8 contains the data from an experiment to determine the susceptibility of the radioactive product formed by a cell extract and by dialyzed extracts to decarboxylation by a method which is specific for the beta-carboxyl group of oxalacetate (8). The dialyzed extract fixed CO_2 in the basic reaction mixture and also in a reaction mixture to which pyridoxal phosphate and glutamate, compounds normally associated with transamination, were incorporated. It was postulated that the aspartate formed by the extract resulted from the transamination of oxalacetate which is the primary CO_2 fixation product. On the basis of this hypothesis, one would expect the product formed by the extract to be resistant to decarboxylation, the product formed by the dialyzed extract in the basic reaction mixture to be susceptible to decarboxylation, and the product formed by the dialyzed extract in the reaction mixture to which pyridoxal

TABLE 5. *Effect of additions and deletions in the reaction mixture on CO_2 fixation by a dialyzed extract*[a]

No.	Deletions	Additions[b]	Radioactivity[c]
1	None	None	50,705
2	CoA	Acetyl CoA (0.6)	54,550
3	None	Mercaptoethanol (5)	61,765
4	CoA	None	33,725
5	Pyruvate	None	1,100
6	ATP	None	355
7	$MgSO_4$	None	897
8	KCl	None	43,632
9	KCl	NaCl (100)	30,855

[a] Basic reaction mixture contained (micromoles): Tris (*p*H 8.5), 200; pyruvate, 10; adenosine 5'-triphosphate, 2.5; $MgSO_4$, 3; biotin, 8×10^{-6}; KCl, 100; CoA, 0.6; $NaHCO_3$, 3; $NaH^{14}CO_3$, 9 μc; protein, 2 mg; total volume, 1.5 ml.

[b] Values in parentheses indicate micromoles added.

[c] Expressed as counts per minute.

TABLE 6. *Effect of inhibitors on CO_2 fixation by dialyzed cell extracts*

Reaction mixture[a]	Inhibitor	Radioactivity[b]
Basic	None	83,683
Basic	Aspartate[c]	58,128
Basic	Oxalacetate[c]	15,588
Basic minus biotin	None	67,413
Basic minus biotin	Avidin[d]	49,381
Basic	Avidin[d]	53,237

[a] Basic reaction mixture contained (micromoles): Tris (*p*H 8.5), 200; pyruvate, 10; adenosine 5'-triphosphate, 2.5; $MgSO_4$, 3; biotin, 8×10^{-6}; CoA, 0.6; KCl, 100; mercaptoethanol, 5; protein, 2 mg; $NaHCO_3$, 3; $NaH^{14}CO_3$, 9 μc; total volume, 1.5 ml.

[b] Expressed as counts per minute.

[c] Ten micromoles added.

[d] Four-hundred micrograms added.

TABLE 7. *Chromatography of the radioactive amino acid formed by the cell extract*

Solvent system	Chromatographed sample			
	Reaction mixture[a]		Reaction mixture plus aspartate	
	Ninhydrin spot (R_F)	Radioactive spot (R_F)	Ninhydrin spot (R_F)	Radioactive spot (R_F)
A. Phenol-H_2O (4:1), vapor of 10% NH_4OH	0.17 (tr)[b] 0.24 (tr)	0.17	0.18 0.24 (tr)	0.18[c]
B. Methyl isobutyl ketone-88% formic acid-H_2O (100:30:15)	0.04 0.13 (tr) 0.23 (tr)	0.13	0.04 0.13 0.24 (tr)	0.13[c]

[a] Basic reaction mixture consisted of the following components (micromoles): Tris (*p*H 8.5), 200; pyruvate, 10; adenosine 5'-triphosphate, 2.5; $MgSO_4$, 3; biotin, 8×10^{-6}; CoA, 0.6; KCl, 100; $NaHCO_3$, 3; $NaH^{14}CO_3$, 9 μc; protein, 2 mg; total volume, 1.5 ml.

[b] Trace.

[c] The radioactive spot had the same shape as the ninhydrin spot.

TABLE 8. *Decarboxylation of the radioactive product formed by the cell extract and dialyzed extract*[a]

Enzyme preparation	Reaction mixture	Radioactivity		
		Added to vessel	Liquid	Paper
		counts/min	%	%
Extract	Basic	42,950	87	13
Dialyzed extract	Basic	33,445	0	100
Dialyzed extract	Basic + PLP + glutamate	32,999	86	14

[a] Basic reaction mixture contained (micromoles): Tris (*p*H 8.5), 200; pyruvate, 10; adenosine 5'-triphosphate, 2.5; $MgSO_4$, 3; biotin, 8×10^{-6}; KCl, 100; CoA, 0.6; $NaHCO_3$, 3; $NaH^{14}CO_3$, 9 μc; protein, 2 mg; total volume, 1.5 ml. Where noted above, pyridoxal phosphate (PLP) was incorporated at a concentration of 0.007 μmoles and glutamate at 10 μmoles. Incubation of the reaction mixtures to promote CO_2 fixation was as usual. Portions of deproteinized reaction mixtures were added to Warburg vessels for decarboxylation. Radioactivity remaining in the liquid was resistant to decarboxylation. Radioactivity of the paper was due to absorption of radioactive CO_2. Total recovery of the radioactivity after decarboxylation was 95 to 100% of the total radioactivity added to the Warburg vessel.

phosphate and glutamate were added to be resistant to decarboxylation. The data completely support the hypothesis. The total radioactivity fixed by the dialyzed extract in the basic reaction mixture and in the modified reaction mixture was the same. However, in the absence of the components promoting transamination, all of the fixed radioactivity was decarboxylated, whereas, in the presence of these components, essentially all of the radioactivity was resistant to decarboxylation. Similarly the radioactivity fixed by the extract was essentially resistant to decarboxylation.

The reaction mixture containing the extract and the reaction mixture containing the dialyzed extract with pyridoxal phosphate and glutamate (Table 8) were analyzed by paper chromatography by using solvent system B described in Table 7. The aspartate that was co-chromatographed with the reaction mixtures separated into two ninhydrin reacting zones. One zone had an R_F of 0.08 and the other zone had an R_F of 0.16. It is believed that the use of trichloroacetic acid to precipitate the protein caused the separation of the amino acid, since no such separation occurred when similar reaction mixtures, to which HCl was added, were chromatographed. The zones of radioactivity completely coincided with the two zones of ninhydrin reacting material from the aspartic acid.

It was concluded that the radioactive product formed by the dialyzed extract in the presence of components promoting transamination is aspartate.

DISCUSSION

According to paper chromatographic evidence, the radioactive compound formed by the cell extract as a result of CO_2 fixation is aspartate. Likewise, aspartate is formed from fixation by the dialyzed extract in a reaction mixture containing glutamic acid and pyridoxal phosphate. However, the fixation product formed by the dialyzed extract in the absence of exogenous transaminating components is most probably oxalacetate, since the aluminum decarboxylation procedure shows that a beta ketoacid is formed under these conditions. It is concluded that the primary fixation product in this microorganism is oxalacetate and that the oxalacetate is efficiently converted by transamination to aspartate.

Among the various enzymes that synthesize oxalacetate by CO_2 fixation (19), most can be eliminated from consideration by the results in Table 3. Since NADH or NADPH was not able to substitute for ATP, it appears as if a "malic enzyme" is not responsible for fixation. In view of the finding that PEP, even in the presence of various nucleoside diphosphates, was not able to substitute for pyruvate plus ATP, none of the enzymes that use PEP for fixation, PEP carboxylase, PEP carboxykinase, and PEP carboxytransphosphorylase, appears to be operative in this microbe. Finally, propionyl CoA carboxylase does not appear to function in the indirect synthesis of oxalacetate from fixation, because propionate plus CoA was not able to substitute for pyruvate.

The enzyme which appears to be responsible for CO_2 fixation in *S. faecalis* var. *liquefaciens* is pyruvate carboxylase. It is a biotin-containing enzyme that synthesizes oxalacetate from pyruvate, $CO_2(HCO_3^-)$, and ATP in the presence of Mg^{2+} ions (19). Data in Table 5 show that pyruvate, ATP, and $MgSO_4$ are essential for fixation by a dialyzed extract; Table 6 suggests that biotin is involved in the reaction because of the stimulatory effect of biotin and the inhibitory effect of avidin. The lack of effect of adding biotin on the inhibition promoted by avidin may have been due to the small quantity of biotin compared to the relatively large concentration of avidin added to the reaction mixture.

Pyruvate carboxylase has been implicated in

CO_2 fixation in liver (6, 16), *Chromatium* (3), *Pseudomonas citronellolis* (14), *Aspergillus niger* (2), and in yeast (4, 10, 13). The enzyme in *S. faecalis* var. *liquefaciens* resembles yeast pyruvate carboxylase in that biotin, alkaline *p*H, CoA, acetyl CoA, K^+, and reduced OR potential stimulate the reaction, whereas Na^+, aspartate, and oxalacetate inhibit fixation.

The efficient conversion of the primary fixation product, oxalacetate, to aspartate via transamination explains the finding of Welch and Hartman (18) that the addition of aspartate to a synthetic medium eliminated the requirement for CO_2 in the synthesis of proteinase by *S. faecalis* var. *liquefaciens* and that of Goff (Ph.D. Dissertation, St. Bonaventure University, 1969), who found that whole cells fixing $NaH^{14}CO_3$ incorporated over 90% of the radioactivity into the aspartate component of protein. The inhibition of the fixation reaction by oxalacetate and aspartate may explain the observation by the latter author that aspartate added to a synthetic medium inhibited incorporation of ^{14}C from $NaH^{14}CO_3$ into cell material. Pyruvate carboxylase represents the first enzyme in a series leading to aspartate synthesis from fixation so that the inhibition may represent end-product inhibition. Further work is required to determine whether allosteric interactions are involved in the inhibition of fixation in whole cells.

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