CARBON DIOXIDE FIXATION AND THE SYNTHESIS OF ASPARTIC ACID BY S. FAECIUM VAR. DURANS[1]

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Carbon dioxide is stimulatory or required by various streptococci, including the "minute" streptococci (Streptococcus anginosus[6], S. pyogenes[8], and certain of the enterococci[2] Streptococcus bovis requires CO_2 for growth in a synthetic medium containing ammonium salts as the sole nitrogen source.[10] Even in a rich medium, CO_2 is needed by S. bovis for optimum growth.[11] Studies by C. W. Langston (cited by Deibel[2]) indicate that growth of S. faecalis is stimulated by CO_2 in a defined medium.

The major portion of the glucose supplied to enterococci is used as an energy source and is metabolized through the Embden-Meyerhof pathway to produce lactic acid. A small quantity of the glucose carbon, however, is assimilated.[1] The pathway that the assimilated glucose carbon takes has not been elucidated, but CO_2 fixation seems to be involved in the process. We feel that CO_2 fixation is needed for the incorporation of carbon from glucose into aspartic acid via the forma-

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tion of oxalacetate from pyruvate. This hypothesis is based on the findings that enterococci do not require an exogenous supply of aspartate and that radioactivity from glucose-U-^{14}C is incorporated into cellular aspartate. The essential data is reported here; further experiments and details will be presented elsewhere.

We observed that cell suspensions from exponentially-grown cells of S. faecium var. durans ATCC 349 in Brain Heart Infusion broth incorporated radioactivity from ^{14}C-bicarbonate, mostly in the trichloroacetic acid (TCA)-soluble and protein fractions. Fig. 1 shows that at 5-min incubation, 70% of the radioactivity of the cells was conta. in the cold TCA-soluble fraction. The specific activity of this fraction diminished with time and a corresponding increase of activity in the protein residue ensued, surpassing the cold TCA-soluble fraction in activity after 20 min of incubation. Aspartic acid, identified by its chromatographic behavior, was the only radioactive compound detected in these fractions. When samples of ^{14}C aspartic acid, isolated from the protein and cold TCA-soluble fractions by preparative chromatography, were degraded with the use of aspartate-glutamate decarboxylase[7], more than 90% of radioactivity from each sample was released, showing that ^{14}C was primarily in the β-carboxyl carbon of aspartic acid.

It is generally accepted that glutamate is synthesized via a $C_3 + CO_2$ condensation and the Krebs cycle reactions followed by the amination or transamination of α-ketoglutarate. That no other amino acid, except aspartic acid, contained ^{14}C may mean the absence of a citric acid cycle in S. faecium. Another possibility is that the rich medium in which this organism was grown may have repressed formation of the Krebs cycle enzymes and, consequently, their synthetic function. The Krebs cycle has been demonstrated[3] in Staphylococcus aureus; yet $^{14}CO_2$ is incorporated into aspartic acid but not into glutamate or any intermediates of the Krebs cycle[4]. Very recently, the repression

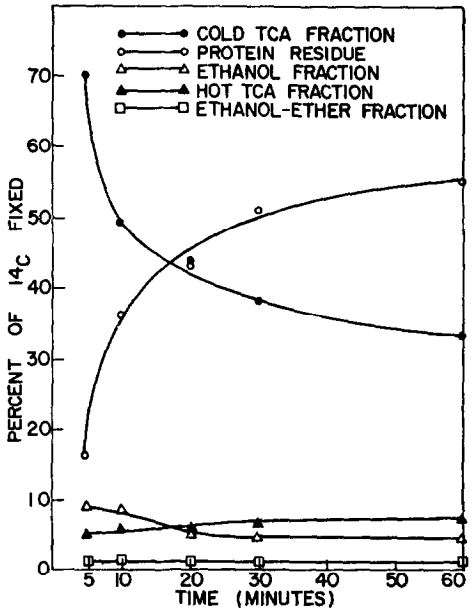

Fig. 1. Kinetics of ^{14}C distribution after $^{14}CO_2$ fixation by S. faecium Five tubes were prepared, each containing 120 mg wet weight of washed cells suspended in 0.1 M phosphate buffer (pH 7.5) which contained 5 mM glucose and 185 μM $NaH^{14}CO_3$ (1.2×10^6 counts/min) in a final volume of 5 ml. The tubes were capped immediately and incubated at 37°C; one tube was removed at each time shown in the figure. The cells were killed by the addition of 2 N HCl and were washed with the phosphate buffer. Chemical fractionation of the cells was carried out according to the methods of Roberts et al.[9] Aliquots of the fractions were assayed for radioactivity with a gas flow-Geiger counter at a constant geometry.

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of aconitase in Bacillus subtilis and B. licheniformis and the repression of isocitric dehydrogenase and fumarase in E. coli were demonstrated when glutamate was added to a minimal glucose medium[5].

The presence of exogenous aspartate in the cell suspension of

S. faecium did not alter significantly the uptake of $^{14}CO_2$, as shown in Table 1. The cells were also observed to have difficulty in assimilating ^{14}C-aspartic acid. The presence of insufficient 'permease' in S. faecium may have rendered the cells less inclined to utilize exogenous aspartate at a rate sufficient to permit maximum protein synthesis. Instead, it may be more efficient for the cells to synthesize aspartate via the carboxylation of a three carbon unit, which is then transaminated to form aspartate.

Table 1. Effect of aspartate on the incorporation of $^{14}CO_2$ by cell suspensions of S. faecium*

L-Aspartate	Activity fixed (counts/min/0.1 ml cell suspension)
0	1451
1.5 mM	1372
3.0 mM	1378
50.0 mM	1014

* Experimental conditions were the same as in Fig. 1 except for the added L-aspartate.

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