CARBON DIOXIDE FIXATION AND THE SYNTHESIS OF ASPARTATE BY MICROBACTERIUM THERMOSPHACTUM

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Carbon dioxide was fixed by Microbacterium thermosphactum. The primary amino acid synthesized was aspartic acid.

Microbacterium thermosphactum was originally isolated from pork sausages in 1953 (9) and has since been observed as one of the prominent bacteria in packaged pork (4). Davidson et al. (2) has reviewed the pragmatic importance of this bacterium in pork and other meats. Gardner and Carson (3) in 1967 showed that the growth of M. thermosphactum was stimulated by low concentrations of CO_2. Such stimulation suggests the possible fixation of CO_2 as the method of selectively favoring its growth on meats. Despite this observation the fixation of CO_2 by M. thermosphactum has not been reported. The purpose of this investigation was to show CO_2 fixation and the mode of fixation as a preliminary step in a more detailed study of the mechanism of fixation.

METHODS

The strain of M. thermosphactum used was isolated from beef and identified by comparison tests with a known strain. M. thermosphactum was grown in trypticase soy broth until late exponential phase, harvested by centrifugation, reinoculated into fresh trypticase soy broth and exposed to $C^{14}O_2$.

The objectives of the following methods were to measure the uptake of $C^{14}O_2$ into whole cells, cold trichloroacetic acid (TCA) insoluble material and pools. Also desired was the distribution of radioactivity in the separate amino acids in the pool material and in synthesized protein.

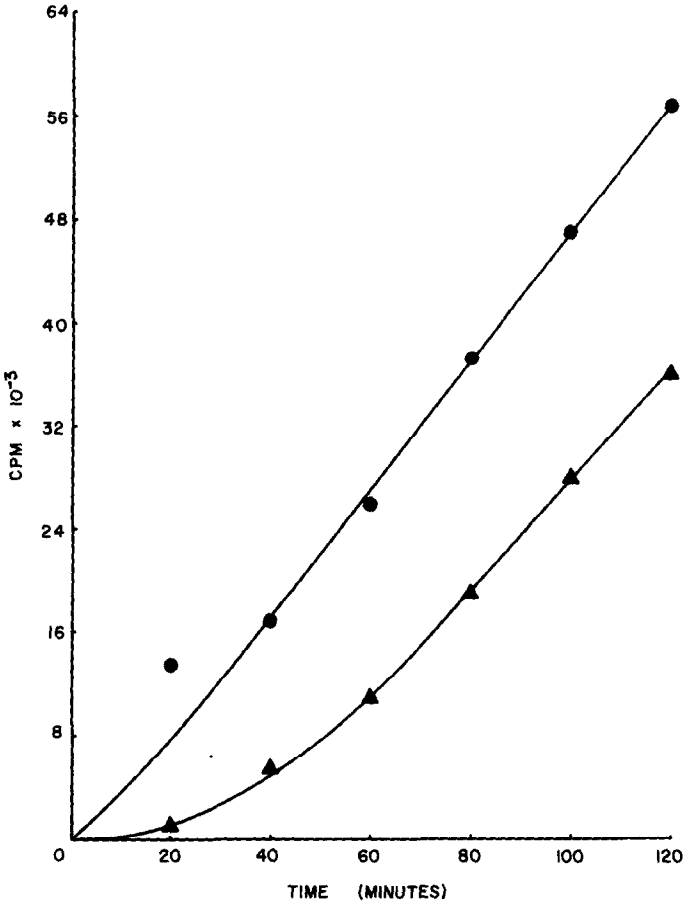

Fig. 1. Uptake of $C^{14}O_2$ by Microbacterium thermosphactum at 20 C where ●——● is the total cellular incorporation and ▲——▲ is is incorporation into the protein and nucleic acid fraction.

The uptake of $C^{14}O_2$ into whole cells and into the cold TCA insoluble fraction was measured by the filter method of Britten and McClure (1). The millipore filter discs were placed in toluene, POP, dimethyl POPOP scintillation fluid and the radioactivity measured on a Packard Tricarb liquid scintillation spectrometer (model 3320).

To determine the distribution of the labeled carbon into protein the incorporation was allowed to proceed for 2 hours, the cells were harvested by centrifugation and the cell pellet was treated with 5% TCA. The resulting TCA precipitate was centrifuged washed with phosphate buffer, pH 7.0, and hydrolyzed with 6 N HCl for 15 hrs. at 110 C in sealed tubes. The hydrolyzed protein was prepared for chromatography by the

method of Kay and Grondlund (6) which include HCl removal, drying, washing on a Dowex 50 (H+ form)column, elution, and redrying. The residue was dissolved in distilled water, spotted on cellulose EK thin layer plates, and developed with n-butyl alcohol-acetic acid water (4:1:1, v/v). Developed plates were cut into 2cm strips and scanned for radiolabel on a radiochromatograph scanner (Packard model 7201). The amino acids were identified by comparison to the mobility of both scanned standard radioactive and ninhydrin detected non-radioactive amino acids.

For the detection of $C^{14}O_2$ incorporation into pool material and its distribution in the amino acids of pool material M. thermosphactum was grown in the presence of $C^{14}O_2$ as previously described and sampled at 10 minute intervals for 100 minutes. The cold TCA soluble pool material

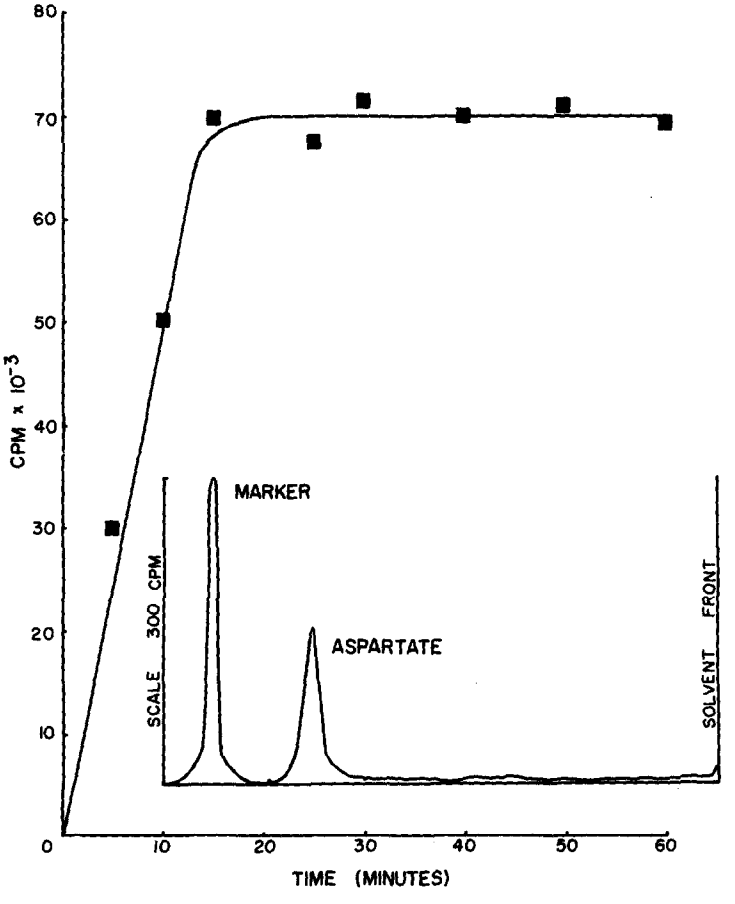

Fig. 2. Uptake of $C^{14}O_2$ into the bacterial pool by Microbacterium thermosphactum. The insert figure of the radiochromatograph scan shows the distribution of labeled amino acids found in the pool after 20 min exposure.

after filtration was taken up in dioxane scintillation fluid and the radioactivity determined. The radioactivity of separate pool amino acids was determined by evaporative removal of TCA and preparing the residue for chromatography by the same method used for the hydrolyzed protein of the TCA insoluble fraction. Also, the thin layer plates were developed, scanned and the radiolabeled amino acids were identified as previously described.

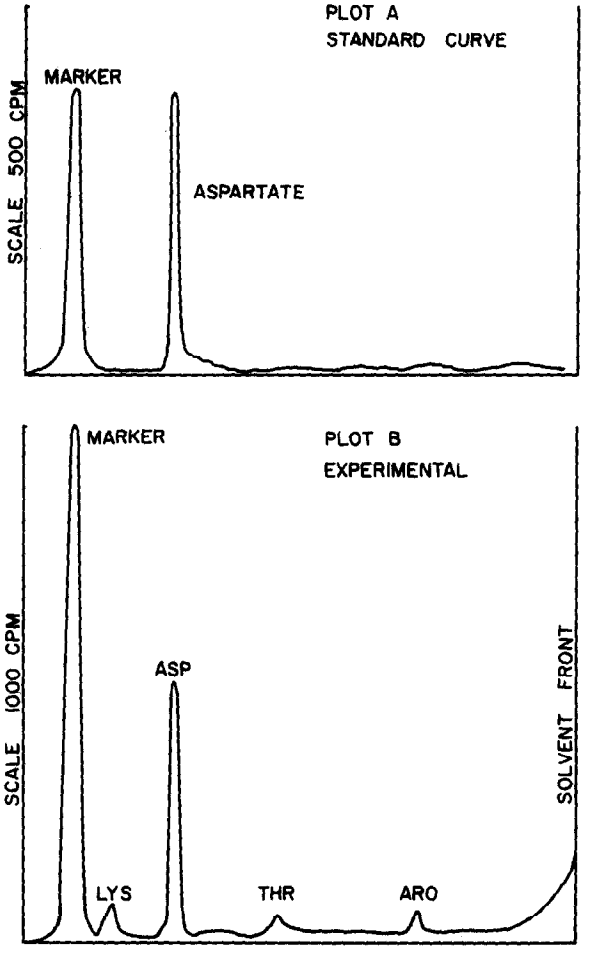

Fig. 3. Distribution of fixed $C^{14}O_2$ in the protein amino acids of Microbacterium thermosphactum determined by radiochromatographs of labeled amino acids from hydrolyzed protein which were separated on cellulose EK thin layer plates. The development solvent was n-butanol-acetic acid-water. The chart speed was 0.5 cm/min and the gas flow was 300 cc/min. Plot A is a radiochromatograph of known aspartate. Plot B is a radiochromatograph of amino acids labeled through $C^{14}O_2$ fixation and included aspartate (ASP), lysine (LYS), threonine (THR), and aromatic amino acids (ARO).

RESULTS AND DISCUSSION

The uptake of $C^{14}O_2$ into whole cells of M. thermosphactum and into the cellular constituents of the cold TCA insoluble fraction is shown in Fig. 1. This uptake into both whole cells and cellular constituents established the ability of M. thermosphactum to fix CO_2. The $C^{14}O_2$ was rapidly incorporated into the pool and was used primarily to synthesize aspartate which was the only prominently radiolabeled amino acid of the pool amino acids (Fig. 2). The distribution of the fixed $C^{14}O_2$ in the protein amino acids is shown in Fig. 3. Again the radioactivity was found almost entirely in aspartate.

Lachica (7) in his review concluded that the CO_2 fixed by heterotrophic microorganisms was primarily found in aspartate, glutamate, and arginine. Several bacteria, including Staphylococcus aureus (5) and Lactobacillus arabinosa (8) have been shown to incorporate CO_2 almost exclusively into aspartate as was true of the M. thermosphactum in this investigation. Such exclusive incorporation suggested that the Krebs cycle was either not present or inoperative.

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