

A Proposed Pathway for Catabolism of Propionate in Methanogenic Cocultures

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A metabolic pathway for the catabolism of propionate is proposed. This pathway incorporates a transcarboxylation reaction involving propionyl coenzyme A and oxaloacetate and a carboxylation of pyruvate to regenerate oxaloacetate. Data indicated that the proposed pathway is reversible. The proposed pathway and its apparent reversibility provide a reasonable explanation of observations obtained from metabolism of labeled substrate.

In previous studies of the catabolism of propionate in methanogenic cocultures, it was observed that the product, acetate, was labeled in either the 1 or 2 position regardless of whether propionate labeled at the 2 or 3 position was used as the substrate (3). This observation indicates that propionate is catabolized via a randomizing pathway. Two known pathways have succinate, which could provide this randomization of label in acetate, as an intermediate (2). These are the methylmalonyl coenzyme A (CoA) pathway and the 2-OH-glutarate pathway. Studies performed in our laboratory have suggested that methylmalonyl-CoA is involved in the catabolism of propionate (4). The experiments which indicated that methylmalonyl-CoA was an intermediate showed that [1,2-¹³C]succinate was produced in culture samples that metabolized [2-¹³C]propionate and [1,4-¹³C]succinate. Thus, it was apparent that a carboxyl group from the [1,4-¹³C]succinate was transferred to the 2 position of the [2-¹³C]propionate, and it was assumed that subsequent rearrangement via methylmalonyl-CoA yielded the [1,2-¹³C]succinate. Some features of the pathway that need further study are how the carboxyl group of succinate is transferred to the propionate, how CO₂ enters the pathway, and how the randomization of the methylene and methyl carbons of propionate occurs. Boone (1) has addressed the last issue and has offered the explanation of a possible carboxyl exchange between propionate and succinate, i.e., that succinate produced from propionate and CO₂ is subsequently decarboxylated to form propionate and CO₂. In this communication, I propose a pathway that could explain these observations and that addresses the issues listed above. The basic features, but not all details, of the pathway are as follows: propionate + CoA ↔ propionyl-CoA + oxaloacetate ↔ pyruvate + methylmalonyl-CoA ↔ succinyl-CoA ↔ succinate ↔ fumarate ↔ malate → CO₂ + pyruvate + CoA → acetyl-CoA + CO₂ → acetate + CoA. This pathway incorporates the transcarboxylation reaction that converts propionyl-CoA and oxaloacetate to methylmalonyl-CoA and pyruvate. To provide a mechanism for the entry of CO₂, we thought it reasonable to propose that pyruvate carboxylase might also function in this pathway by converting pyruvate to oxaloacetate.

The description, procedures, and analyses for the digestors and for digester operation and performance have been previously described (6, 7). The methods used to establish the enriched coculture and the methods used for nuclear magnetic resonance analyses of samples from this

digester have also been previously described (4). The nuclear magnetic resonance chemical shifts of CoA esters have been identified elsewhere (8), and the method for enhancing the detection of metabolic intermediates has been previously described (5).

The major result of this study was the demonstration that the pathway was, at least in part, freely reversible. Metabolism of 20 mM [2-¹³C]succinate yielded [2-¹³C]succinyl-CoA, [2-¹³C]propionate, [3-¹³C]-propionate, [2-¹³C]acetate, and [1-¹³C]acetate (Fig. 1). The [1-¹³C]acetate resonance at 182 ppm is not shown. The results clearly indicate the reversibility of this propionate catabolic pathway (Fig. 1). The fact that labeled succinate yielded both [2-¹³C]propionate and [3-¹³C]propionate indicates that the previously observed randomization of label derived from either [2-¹³C]propionate or [3-¹³C]propionate metabolism by propionate-degrading bacteria could be explained by reversibility

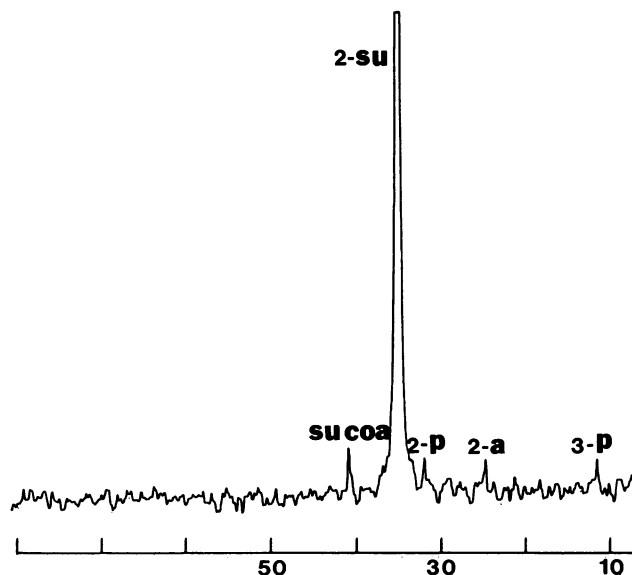


FIG. 1. Nuclear magnetic resonance spectrum obtained from 1,800 pulses over 1 h of 20 mM [2-¹³C]succinate metabolism. The resonance frequencies indicated at the bottom of the figure are in parts per million relative to tetramethylsilane at 0 ppm. Abbreviations: 2-a, [2-¹³C]acetate; 2-P and 3-P, [2-¹³C]propionate and [3-¹³C]propionate, respectively; 2-su, [2-¹³C]succinate; sucoa, [2-¹³C]succinyl-CoA.

of this pathway. This reversibility in conjunction with the proposed pathway could also explain the observations of Boone (1) that indicated that [1-¹⁴C]propionate was removed from the propionate pool at a faster rate than [2-¹⁴C]propionate. Some of the carboxyl group of [1-¹⁴C]propionate would be transferred to other metabolite pools, and some would be lost by conversion to acetate. Propionate labeled in position 2 would be lost from the propionate pool only by conversion to acetate. How CO₂ enters the pathway remains to be demonstrated, as does the mechanism of transcarboxylation. Thus, the pathway proposed here has two hypothetical reactions, namely, the pyruvate carboxylase and the propionyl-CoA-oxaloacetate transcarboxylase reactions. The proposed pathway does, however, explain the observation that [1,4-¹³C]succinate and [2-¹³C]propionate produced [1,2-¹³C]succinate. The [1,4-¹³C]succinate would produce oxaloacetate labeled in position 4, i.e., the carboxyl group used to carboxylate propionyl-CoA. Some of this labeled oxaloacetate would function to produce labeled methylmalonyl-CoA and, subsequently, succinate. Thus, adjacent labeled carbon atoms in succinate would be produced from [2-¹³C]propionate and [1,4-¹³C]succinate. On the basis of energetics, the well-known transcarboxylation reaction involving oxaloacetate and propionyl-CoA seems to be a better explanation of this pathway than a direct transfer of a carboxyl group from succinate to propionyl-CoA.

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