THE ROLE OF BIOTIN AND VITAMIN B_{12} COENZYME IN PROPIONATE METABOLISM

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Propionic acid formation represents a major metabolic process catalyzed by bacteria belonging to the genus Propionibacteria. In view of the fact that these bacteria contain uniquely high concentrations of vitamin B_{12} and biotin**, experiments have been carried out to determine if these vitamins are involved in propionic acid metabolism. The metabolism of propionate in cell-free extracts of Propionibacterium shermanii was measured by the incorporation of 1-C^{14}-propionate into succinate as was previously described (Phares, Delwiche and Carson, 1956). After treatment with protamine, then with charcoal, followed by dialysis, cell-free extracts lose their ability to catalyze the incorporation of propionate into succinate. This ability is restored by the addition of catalytic amounts of acetyl CoA and a boiled extract of the organism (Table I). The boiled extract is completely replaced with low concentrations (10^{-6} M; Km = 5×10^{-8} M) of pure dimethylbenzimidazole-B_{12}-coenzyme (DMBC) which was kindly supplied by Dr. H. A. Barker.

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** J. Knappe, unpublished data.

Table I

The Influence of DMBC and Avidin on the Incorporation of C^{14}-Propionate into Succinate

	C^{14}-Propionate incorporated into succinate **
Experiment 1	c.p.m.
complete system*	7170
" " -acetyl CoA	231
" " -succinate	615
" " -succinate + methyl malonate	603
" " -DMBC	1274
" " + Boiled cell extract	6912
" " " " " -DMBC	6472
Experiment 2	
complete system	4400
" " -DMBC	990
" " + Biotin, 0.1 µmole	4500
" " + Avidin, 0.32 units	370
" " + Avidin, 0.32 units + biotin 0.1 µmoles, (preincubated 5 min.)	4900

* The complete system contained: (Expt. 1) Potassium succinate (pH 6.5), 25 µmoles; potassium propionate-1-C^{14} (10,000 cpm per µmole), 10 µmoles; DMBC, 1.6×10^{-6} M; acetyl CoA, 0.1 µmole; potassium maleate buffer (pH 6.5), 10 µmoles; potassium-magnesium versenate, 5 µmoles; cysteine, 5 µmoles; Enzyme, 0.52 mg; total volume, 0.5 ml; 37°C, 90 min. (Expt. 2) same as for experiment 1 except acetyl CoA, 0.027 µmoles, 80 min.

** The incorporation of propionate into succinate was determined by measuring the amount of C^{14} fixed into the non-volatile acid fraction. Proof that the non-volatile acid formed is succinate, was obtained by direct isolation of succinic acid from pooled reaction mixtures, followed by recrystallization to constant specific activity. Melting point 188°C.

An additional role of biotin in the propionate exchange system is indicated by the fact that the reactivated enzyme is completely inhibited by avidin but not by avidin which has been pretreated with biotin (Table I, Experiment 2).

Previous studies have shown that propionyl CoA, succinyl CoA and methylmalonyl CoA are intermediates in propionic acid metabolism by enzymes derived from animal tissues (Flavin and Ochoa, 1957) and from bacteria (Whitely, 1953; Swick, 1959). A possible role of biotin in the decarboxylation of methylmalonyl CoA to form propionyl CoA is suggested by the recent observation that avidin inhibits a transcarboxylation from methylmalonyl CoA to pyruvate (reaction 1) which is catalyzed by extracts of P. shermanii (Swick, 1959).

(1) methylmalonyl CoA+pyruvate $\rightleftharpoons$ oxalacetate+propionyl CoA

Moreover the recent report (Smith and Monty, 1959) that the succinyl CoA isomerase activity of rat liver is lowered in vitamin B_{12} deficiency points to the possibility that DMBC is required for the conversion of succinyl CoA to methylmalonyl CoA (reaction 3). In view of these observations, the over-all incorporation of C^{14}-propionate into succinate can be readily explained by the following reaction mechanism:

(2) acetyl CoA + succinate $\rightleftharpoons$ succinyl CoA + acetate

(3) succinyl CoA $\overset{\text{DMBC}}{\rightleftharpoons}$ methylmalonyl CoA

(4) methylmalonyl CoA + biotin.enzyme $\rightleftharpoons$ CO_2-biotin.enzyme + propionyl CoA

(5) propionyl CoA + acetate $\rightleftharpoons$ acetyl CoA + propionate

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sum:

(6) succinate+biotin.enzyme $\rightleftharpoons$ CO_2-biotin.enzyme+propionate

Evidence for reactions 2 and 5 has been presented previously

(Whitely, 1953; Phares, Delwiche and Carson, 1956). Direct experimental support for reaction 3 was obtained by showing that the cell-free extracts catalyze the conversion of methylmalonyl CoA to succinyl CoA. The reaction was followed by taking advantage of the marked differences in heat stability of the two isomers; methylmalonyl CoA is completely stable to heating at 100°C for 2 minutes, pH 6.0 - 7.0, whereas succinyl CoA is completely destroyed by this treatment. Thus, the conversion of methylmalonyl CoA to succinyl CoA is associated with a decrease in heat stable thiolester, which can be measured by the hydroxamic acid method (Stadtman, 1957). Table II summarizes the results of a

Table II

The Effect of DMBC and Avidin on the Conversion of Methylmalonyl CoA to Succinyl CoA

	methylmalonyl CoA disappearance**, μmoles
complete system*	0.29
" " -DMBC	0.06
" " + Avidin, 0.13 units	0.30
" " -Enzyme	0.0

* The complete system contained: potassium maleate buffer (pH 6.5), 25 μmoles; methylmalonyl CoA, 0.66 μmoles; DMBC, 7.8×10^{-8} M; enzyme, 0.105 mg; volume, 0.5 ml. After 30 minutes at 37°C, the samples were heated at 100°C for 2 minutes and the residual methylmalonyl CoA was determined by the hydroxamic acid method.

** The synthetic methylmalonyl CoA preparation is a mixture of two diastereoisomers; therefore, 0.33 μmoles probably represent a quantitative disappearance of the enzymatically active isomer.

typical experiment showing that the disappearance of methylmalonyl CoA is dependent upon the presence of DMBC and is not influenced by avidin. Succinate (in 30% yield) was isolated from the reaction mixtures by ether extraction and was estimated by succinic dehydrogenase, using cytochrome c as the electron acceptor (Beck, Flavin, Ochoa, 1957). No succinate could be detected in incubation mixtures in which DMBC was absent.

According to reaction 4, propionyl CoA should be in equilibrium with methylmalonyl CoA. In agreement with this prediction, it was found that incubation of C^{14}-propionyl CoA with methylmalonyl CoA leads to the formation of a labeled product which, after alkaline hydrolysis (100°C, 0.1 M NaOH, 10 minutes) and then acidification, is not volatile at elevated temperatures. The labeled non-volatile product was isolated from acid solution by ether extraction and was identified as methylmalonic acid by crystallization

Table III

Effect of Avidin on the incorporation of C^{14}-propionyl CoA into Methylmalonyl CoA

	C^{14} incorporated into methylmalonyl CoA
	c.p.m.
complete system	6900
" " -methylmalonyl CoA	0
" " + Avidin, 0.31 units	0
" " + Avidin, 0.31 units + biotin, 0.1 µmole, (preincubated 5 min. at 37°C)	7240

The complete system contained: maleate buffer (pH 6.5), 10 µmoles; 1-^{14}C-propionyl-CoA (10^6 cpm per µmole), 0.07 µmole; methylmalonyl CoA, 0.05 µmoles; enzyme, 46 µg; total volume, 0.5 ml; 37°C, 30 min.

to constant specific activity (m.p. = 133°C) following the addition of unlabeled methylmalonic acid. As can be seen from the data of table III the fixation of C^{14}-propionate is completely inhibited by avidin.

From these results it appears probable that the incorporation of C^{14}-propionate into succinate occurs by the mechanism outlined above.

In view of the fact that methylmalonyl CoA is a reactive intermediate in propionate metabolism, it is noteworthy that free methylmalonate will not replace succinate in the propionate exchange reaction (Table I). Apparently the enzyme preparations are unable to catalyze thioesterification of the free acid.

Since DMBC is definitely required for the isomerization of methylmalonyl CoA to succinyl CoA, it is perhaps significant that this isomerization is analogous to the isomerization of glutamate to form methylaspartate which was previously shown by Barker, Weissbach and Smyth (1958) to require the vitamin B_{12}-coenzyme.

Similarly, the function of biotin as a CO_2-acceptor in the conversion of methylmalonyl CoA to propionyl CoA (reaction 4) is completely analogous to its established role in the transformation of ß-methylglutaconyl CoA to ß-methylcrotonyl CoA (Lynen, Knappe, Lorch, Jütting, Ringelmann, 1959).

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