NET SYNTHESIS OF METHYLMALONYL-CoA BY ADP-, ATP-, AND Mg-INDEPENDENT TRANSCARBOXYLATION CATALYZED BY PROPIONYL CARBOXYLASE *

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Evidence indicating that propionyl carboxylase catalyzes an exchange between C^{14}-propionyl-CoA and methylmalonyl-CoA, which is independent of Mg^{++}, ADP, ATP, or P_i, has been provided by Halenz and Lane, 1961, and by Friedman and Stern, 1961. The following reaction is in accord with these findings:

(1) Methylmalonyl-CoA + enzyme-biotin $\rightleftharpoons$ enzyme-biotin-CO_2 + propionyl-CoA

The present report provides evidence that highly-purified mitochondrial propionyl carboxylase can catalyze the formation of methylmalonyl-CoA from ethylmalonyl-CoA and propionyl-CoA in the absence of added Mg^{++}, ADP, ATP, or P_i. It seems likely that the transcarboxylation reaction proceeds via an "enzyme-biotin-CO_2" intermediate as shown in Reactions 2 and 3:

(2) Ethylmalonyl-CoA + enzyme-biotin $\rightleftharpoons$ enzyme-biotin-CO_2 + butyryl-CoA

(3) Enzyme-biotin-CO_2 + propionyl-CoA $\rightleftharpoons$ methylmalonyl-CoA + enzyme-biotin

Bovine liver mitochondrial propionyl carboxylase (specific activity, 554 μmoles HCO_3^- fixed per hour per mg. of protein) was prepared as described by Lane et al., 1960. Chemically-synthesized ethylmalonyl-CoA (prepared according to the method of Wieland and Rueff, 1953) and propionyl-CoA-1-C^{14} (prepared according to the method of Simon and Shemin, 1953) were employed in these studies

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to avoid adenine nucleotide contaminants. As shown in Table I, the formation of C^{14}-methylmalonyl-CoA from propionyl-CoA-1-C^{14} was dependent upon the presence of both carboxylase and ethylmalonyl-CoA, but not upon Mg^{++} or ADP and P_i.

TABLE I

Net Synthesis of C^{14}-Methylmalonyl-CoA From Propionyl-CoA-1-C^{14} and Ethylmalonyl CoA

Additions to or deletions from the basic reaction mixture*	Propionyl-CoA-1-C^{14} incorporation into methylmalonyl-CoA**
	c.p.m. x 10^{-3}***
None	24.0
- Enzyme	2.0
- Ethylmalonyl-CoA	2.0
+ Avidin	2.4
+ $MgCl_2$	26.1
+ $MgCl_2$ + ADP + P_i	12.6

* Basic reaction mixture included (in μmoles): Tris, pH 8.5, 100; glutathione, 5; propionyl-CoA-1-C^{14} (6.2 x 10^5 c.p.m. per μmole), 0.3; ethylmalonyl-CoA, 0.31; and propionyl carboxylase (554 units per mg.), 59 units. Other additions: avidin (2.5 units per mg.), 10 mg.; $MgCl_2$, 4 μmoles; ADP, 4 μmoles; and K_2HPO_4, 4 μmoles. Total volume, 1.1 ml.

** Reaction mixtures were incubated for 30 minutes at 37°. After alkaline hydroly (1N NaOH) of thioesters and acidification, free acids were quantitatively extract paper chromatographed with unlabeled methylmalonic acid as reference (n-amylformate-water-formic acid solvent system), and the methylmalonic acid spot quantitatively eluted and counted as described by Halenz and Lane, 1960. Radioactivity on chromatograms located with a strip counter corresponded exactly to the methylmalonic acid reference spot.

*** Average of duplicates.

Pretreatment of propionyl carboxylase with avidin for 10 minutes at 0° completely inhibited the transcarboxylation reaction. The addition of Mg^{++}, ADP, and P_i partially inhibited transcarboxylation. This is in agreement with our previous report (Halenz and Lane, 1961) that ADP and P_i inhibits C^{14}-propionyl-CoA-methylmalonyl-CoA exchange in the presence, but not in the absence, of Mg^{++}. In the cases in which transcarboxylation proceeded maximally (see Table I), approximately 0.04 μmoles of methylmalonyl-CoA were synthesized. This corresponds to 26 per cent "carboxyl transfer" from added ethylmalonyl-CoA. Presumably, only 0.15 μmole of the enzymatically active isomer of ethylmalonyl-

CoA was added, since, according to Beck and Ochoa, 1958, only 50 per cent of chemically-synthesized methylmalonyl-CoA is enzymatically active.

It has also been demonstrated in our laboratory that the propionyl carboxylase-catalyzed exchange between C^{14}-propionyl-CoA and methylmalonyl-CoA (Halenz and Lane, 1961) is inhibited both by avidin and p-hydroxymercuribenzoate.

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