TRANSCARBOXYLATION AND CO_2 "EXCHANGE" CATALYZED BY PURIFIED PROPIONYL CARBOXYLASE

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Recent investigations (Lynen, Knappe, Lorch, Jütting, and Ringelmann, 1959) have demonstrated the formation of an "enzyme-biotin-CO_2" intermediate which appears to be the "active CO_2 donor" in many ATP-dependent carboxylation reactions. This report provides evidence that propionyl carboxylase can catalyze the ATP-independent transcarboxylation reaction described below presumably via an "enzyme-biotin-CO_2" intermediate:

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

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

Ammonium sulfate-purified mitochondrial propionyl carboxylase was prepared as described by Halenz and Lane (1960). As shown in Table I, incubation of enzymatically-prepared, carboxyl-labeled methylmalonyl CoA (Halenz and Lane, 1960) and butyryl CoA or carboxyl-labeled ethylmalonyl CoA (Hegre, Halenz, and Lane, 1959) and propionyl CoA with carboxylase, resulted in the formation of labeled ethylmalonyl CoA and methylmalonyl CoA, respectively. The possibility that carboxylation rather than transcarboxylation occurred was excluded by quantitative removal of unreacted ATP with glucose and hexokinase. In addition, any $C^{14}O_2$ from decarboxylation would have been greatly diluted with unlabeled HCO_3^- added to the reaction mixture. It is significant that after incubation the percentage distribution of radioactivity in methylmalonic and ethylmalonic acids was the same regardless of the direction from which equilibrium was approached (reactions 1 and 2). Recently a transcarboxylation reaction involving methylmalonyl CoA and pyruvate in the formation of oxalacetate and propionyl CoA

was reported by Swick and Wood (1960).

TABLE I

Transcarboxylase Activity of Propionyl Carboxylase

Donor substrate* ($-C^{14}O_2H$)	Acceptor Substrate	Radioactivity following incubation**		
		Total	Methylmalonic acid	Ethylmalonic acid
		c.p.m.		
Methylmalonyl CoA	none	86,600	100	---
Methylmalonyl CoA	butyryl CoA	83,000	37	63
Ethylmalonyl CoA	none	41,500	---	100
Ethylmalonyl CoA	propionyl CoA	45,900	41	59

* Carboxyl-labeled methylmalonyl CoA and ethylmalonyl CoA free of ATP and $HC^{14}O_3^-$ were prepared by incubating 0.35 μmole of propionyl CoA or butyryl CoA for 20 minutes at 37° with tris, pH 8.5, 28 μmoles; ATP and $MgCl_2$, 1.9 μmoles; glutathione, 2.4 μmoles; $KHC^{14}O_3$, 0.5 μmoles (2.4 μcuries); carboxylase, 3 mg. After acidification to pH 2 at 0°, CO_2, followed by N_2, was bubbled through the reaction mixture to remove excess $HC^{14}O_3^-$. The reaction mixture was neutralized to pH 8.5 with 120 μmoles of tris, preincubated for 10 minutes at 30° with 40 μmoles of glucose and 200 units of yeast hexokinase which quantitatively removed ATP, and then incubated 30 minutes at 37° with 10 μmoles of $KHCO_3$ and 2.5 mg. of carboxylase in the presence and absence of butyryl CoA or propionyl CoA (0.5 μmoles), as shown.

** After alkaline hydrolysis of thioesters and acidification, free acids were quantitatively extracted, paper chromatographed, and the radioactivity eluted and determined as previously described by Halenz and Lane (1960).

An investigation (Table II) of "$HC^{14}O_3^-$ exchange" catalyzed by propionyl carboxylase revealed that maximal incorporation of radioactivity into methylmalonyl CoA occurred only in the presence of both ADP and P_i. The relatively small but significant amount of incorporation when ADP alone was added was apparently a result of traces of P_i contaminating the basic system. In light of these observations it appears unlikely that either an "enzyme-ADP" or "enzyme-phosphate" intermediate is involved in the carboxylation mechanism. It seems likely that $HC^{14}O_3^-$ incorporation into methylmalonyl CoA resulted from a reversal of the over-all reaction whereby propionyl CoA was made available for direct carboxylation. The inability of ATP to catalyze $HC^{14}O_3^-$ incorporation confirms this. That a reversal of the carboxylation reaction can occur under these conditions is shown by the data in Table III. In the presence of relatively high concentrations of ADP and

P_i, 83 per cent of the methylmalonyl CoA added was decarboxylated.

It is interesting to note that an equimolar quantity of arsenate can significantly replace phosphate both for $HC^{14}O_3^-$ incorporation into methylmalonyl CoA (Table II) and decarboxylation of methylmalonyl CoA (Table III). In other experiments (Lane, 1959), arsenate added at similar levels did not affect the rate of the forward reaction, i.e., propionyl CoA carboxylation.

TABLE II

Incorporation of $HC^{14}O_3^-$ into Methylmalonyl CoA

	$HC^{14}O_3^-$ incorporation into methylmalonic acid**	
	c.p.m.	
Experiment 1		
Basic system*	28	49
Basic system + P_i	184	104
Basic system + ADP	418	522
Basic system + P_i + ADP	3,315	3,444
Basic system + ATP	301	412
Basic system + arsenate + ADP	2,521	2,171
Basic system + arsenate + P_i + ADP	4,317	4,582
Experiment 2		
Basic system	38	68
Basic system - methylmalonyl CoA + P_i + ADP	114	258
Basic system + P_i + ADP	5,054	4,914
Basic system + P_i + ADP + avidin	30	38

* Basic system contained: tris, pH 8.5, 80 μmoles; $MgCl_2$, 4 μmoles; $KHC^{14}O_3$, 1 μmole (1,350,000 c.p.m. per μmole); methylmalonyl CoA, 0.55 μmole; carboxylase, 1.5 mg. Other additions: ADP, K_2HPO_4, Na_2HAsO_4, and ATP, 4 μmoles; avidin, 9.2 mg. (2,500 units per g.). Incubated 20 minutes at 37°.

** Determined as non-volatile c.p.m. after acidification (Halenz and Lane, 1960). That all non-volatile c.p.m. fixed were present as methylmalonic acid was verified paper chromatographically by methods previously described (Halenz and Lane, 1960).

TABLE III

Enzymatic Decarboxylation of Methylmalonyl CoA

Additions to basic system*	Methylmalonyl CoA ($-C^{14}O_2H$)		
	Radioactivity remaining after 30-minute incubation		% Decarboxylated
	c.p.m.		
0	57,300	58,100	0
ADP	53,600	56,600	5
ADP + P_i	9,100	10,000	83
Arsenate	43,200	43,300	25
ADP + arsenate	25,100	27,900	54

* Basic system contained: tris, pH 8.5, 100 μmoles; glutathione, 5 μmoles; $MgCl_2$, 4 μmoles; enzymatically-synthesized methylmalonyl CoA ($-C^{14}O_2H$), 0.033 μmoles (59,000 c.p.m.); carboxylase, 1.5 mg. Other additions: ADP, K_2HPO_4, and Na_2HAsO_4, 4 μmoles. Incubated at 37°.

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