ENZYME CATALYZED EXCHANGE OF 1-C^{14}-PROPIONYL CoA AND METHYLMALONYL CoA

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The participation of an "enzyme-biotin-CO_2" intermediate in the ATP-dependent carboxylation of acyl-CoA compounds was demonstrated by Lynen, Knappe, Lorch, Jutting and Ringelmann (1959) for the bacterial β-methylglutaconyl-CoA carboxylase. On the basis of experiments showing that this enzyme catalyzed (a) the Mg^{++}-dependent exchange of P_i and ATP which was inhibited by bicarbonate, (b) the exchange of C^{14}-labelled β-methylglutaconyl-CoA with non-labelled β-methylcrotonyl-CoA in the absence of Mg^{++} and (c) the carboxylation of free biotin, the following three-step mechanism was proposed:

(1) ATP + biotin-enzyme $\overset{Mg^{++}}{\rightleftharpoons}$ ADP-biotin-enzyme + P_i

(2) ADP-biotin-enzyme + CO_2 $\overset{Mg^{++}}{\rightleftharpoons}$ CO_2-biotin-enzyme + ADP

(3) CO_2-biotin-enzyme + β-methylcrotonyl-CoA $\rightleftharpoons$ biotin-enzyme + β-methylglutaconyl-CoA

Subsequent studies with propionyl-CoA carboxylase have shown that the formation of "enzyme-biotin-CO_2" intermediate does not occur by the separate successive Reactions 1 and 2 but by a concerted mechanism (Kaziro, Leone and Ochoa 1960; Lane, Halenz, Kosow and Hegre 1960). This report provides evidence that a partly purified propionyl-CoA carboxylase from ox liver catalyzes the following partial reaction, which is analogous to Reaction 3:

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

The occurrence of Reaction 4 was tested by use of 1-C^{14}-propionyl-CoA

prepared by reaction of 1-C^{14}-propionic anhydride and CoA. 1-C^{14}-Propionic acid was removed by ether extraction at acid pH. The carboxylase was a 30-fold purified preparation from ox liver (Stern and Friedman 1960) which by radioassay catalyzed the carboxylation of 0.46 μmole of propionyl-CoA per mg. of protein in 30 minutes at 30°. The reaction mixture contained (in μmoles): Tris buffer pH 7.0 (100), glutathione (5), methylmalonyl-CoA (0.6), total 1-C^{14}-propionyl thioester (0.8 μmole, of which 0.6 μmole was 1-C^{14}-propionyl-CoA) 152,000 counts per minute per μmole thioester, and 2.6 mg. of enzyme protein, final volume 1.0 ml. Avidin (0.5 unit) when used was preincubated with the enzyme in buffer solution for 10 minutes at 0°, then the other reaction components were added, and finally (where indicated) d-biotin (1.5 μmole) was added at 15 minutes, just prior to the incubation. After 30 minutes incubation at 30°, the reaction was terminated by addition of 1.5 ml. of 2 N KOH and the mixture heated for 10 minutes in a boiling water bath to hydrolyze thioesters. Carrier propionic acid (60 μmoles) and methylmalonic acid (30 μmoles) were added, the acids extracted with ethanol and chromatographed on a celite column using chloroform and chloroform-n-butanol mixture as eluant (Swick and Wood 1960). Propionic and methylmalonic acids were eluted as separate peaks with coincidence of C^{14} content and acid titration values. Some losses of propionic acid but not of methylmalonic acid occurred during concentration of the acids prior to chromatography.

As shown in Table I, the carboxylase catalyzed an exchange of 1-C^{14}-propionyl-CoA with methylmalonyl-CoA. This exchange occurred in the absence of Mg^{++} ions, essential for the over-all carboxylation reaction and for the P_i and ADP exchange reactions with ATP. Avidin, in an amount (0.5 unit) which gave almost complete inhibition of the carboxylation reaction as determined by radioassay, caused 84 per cent inhibition of the propionyl-CoA exchange. Moreover the subsequent addition of d-biotin to the avidin-treated enzyme resulted in essentially complete reversal of the avidin inhibition of the propionyl-CoA exchange reaction. This result is most surprising

Table I

Exchange of 1-C^{14}-Propionyl-CoA with Methylmalonyl-CoA

System	Propionic Acid	Methylmalonic Acid	Inhibition of exchange
	c.p.m.	c.p.m.	per cent
Complete	69,900	14,900	
No enzyme	118,000	240	
Complete + avidin	100,000	2,360	84
Complete + avidin + d-biotin	69,400	14,500	0

since under similar conditions we find that avidin inhibition of the over-all carboxylation reaction, as well as of the P_i-ATP exchange, is reversed little, if at all, by later addition of d-biotin.

Avidin is a very basic protein and occurs naturally bound to deoxyribonucleic acid (Fraenkel-Conrat et al. 1952). It is possible that when nucleotide is bound to the enzyme, as in the intermediate ATP (or ADP)-biotin-enzyme, inhibition by avidin involves its binding not only by the biotin but also by the adenine nucleotide. This could account for the failure of d-biotin to reverse avidin inhibition of the carboxylation reaction and the P_i-ATP exchange reaction. When nucleotide is not bound to the enzyme, as in the propionyl-CoA exchange reaction, avidin inhibition is reversed by addition of d-biotin in excess.

The avidin-sensitive exchange of propionyl-CoA and methylmalonyl-CoA has been observed in extracts of Propionibacterium shermanii (Stadtman et al. 1960). However it was not determined whether this exchange was catalyzed by propionyl-CoA carboxylase or by methylmalonyl-CoA transcarboxylase, which would be predicted also to catalyze this exchange. Both enzymes occur in P. shermanii extracts (Swick and Wood 1960). Direct radioassay for methylmalonyl-CoA transcarboxylase in the ox liver fraction was negative (cf. Swick and Wood), so the propionyl-CoA exchange reaction can be attributed to propionyl-CoA carboxylase. Furthermore, we find that crystalline heart

propionyl-CoA carboxylase, kindly supplied by Dr. S. Ochoa, also catalyzes this avidin-sensitive propionyl-CoA exchange reaction under the above conditions. This enzyme has been shown to catalyze an exchange of C^{14}-methylmalonyl-CoA with non-labelled propionyl-CoA (Y. Kaziro and S. Ochoa, personal communication).

The velocity of the exchange Reaction 4 relative to that of the forward (carboxylation) and back (decarboxylation) over-all Reaction 5 is a critical consideration in deciding whether Reaction 4 can be a partial reaction in the over-all propionyl-CoA carboxylase reaction.

(5) Propionyl-CoA + ATP + $CO_2 \rightleftharpoons$ methylmalonyl-CoA + ADP + P_i

The rate of the exchange reaction can be calculated from the data in Table I to be 8.4 per cent of the carboxylation rate. The rate of the back Reaction 5 has been determined by indirect means to be about 2.7 per cent of the forward reaction. Thus the rate of Reaction 4 is rapid enough to be a step in the over-all Reaction 5. Indeed it is the only partial reaction to proceed at a rate greater than that of the back reaction. Thus, it can be calculated from published data (Lynen et al.; Kaziro et al.; Lane et al.) that the P_i-ATP exchange and the ADP-ATP exchange occur at rates that are only 0.1 to 0.4 per cent of the carboxylation reaction and hence are considerably less than the rate of the back reaction. Until this discrepancy can be resolved, the propionyl-CoA exchange reaction remains the only exchange rapid enough to reflect a partial reaction in the mechanism of the carboxylation reaction.

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