PROPIONYL APOCARBOXYLASE ACTIVATION CATALYZED BY CELL-FREE ENZYME EXTRACTS*

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It was recently reported (Kosow and Lane, 1961) that rapid restoration of propionyl carboxylase activity, depressed by biotin deficiency, could be accomplished by incubating liver slices from biotin-deficient rats with d-biotin. This present report describes the ATP-dependent formation of propionyl carboxylase from propionyl apocarboxylase and d-biotin which is catalyzed by cell-free enzyme extracts.

Whole liver acetone powders, prepared from biotin-deficient rats (Kosow and Lane, 1961), were extracted with dilute Tris buffer, pH 7.3, and the clear supernatant retained after centrifugation (13,000 x g for 10 minutes). Incubation of extracts prepared from normal or biotin-deficient liver for 10 hours at 30° and pH 7.3 resulted in no loss of propionyl carboxylase activity. Extracts were fractionated with ammonium sulfate between 0 and 45% saturation (0-45% fraction) and between 45 and 85% saturation (45-85% fraction) and were then dialyzed against dilute Tris buffer, pH 7.3. In the experiments to be described the criterion for apocarboxylase activation is a biotin-dependent increase in carboxylase activity above the endogenous level of the enzyme preparation tested. Propionyl carboxylase assays were conducted as previously described (Halenz and Lane, 1960). As shown in Table I, a net increase

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TABLE I

Activation of Propionyl Apocarboxylase by Cell-free Enzyme Preparations

Enzyme preparation tested[1]	Enzyme protein added	d-biotin added	Propionyl carboxylase activity after incubation[2] (propionyl-CoA-dependent $HC^{14}O_3^-$ fixation)	
			Total	Due to d-biotin addition
	mg	µg	µmoles/hour/mg protein	
A. P. extract[3]	20.4	0	.07	---
A. P. extract	20.4	100	.18	.11
0-45% fraction	11.2	0	.13	---
0-45% fraction	11.2	100	.27	.14
45-85% fraction	7.0	0	.02	---
45-85% fraction	7.0	100	.03	.01
0-85% fraction	18.2	0	.09	---
0-85% fraction	18.2	100	.15	.06

[1] Enzyme preparations were incubated for 6 hours at 30° with the following components (in µmoles): sodium phosphate, pH 7.0, 80; ATP-$MgCl_2$ (1 µmole of each added at 0, 2, and 4 hours), 3; and GSH, 2.5. Final volume, 2.0 ml. Following incubation a 0.2 ml aliquot of the diluted (1:5) reaction mixture was assayed for propionyl carboxylase activity according to the method described by Halenz and Lane, 1960.

[2] It has been established in other experiments with the same enzyme preparations that carboxylase activity does not change during incubation in the absence of added biotin.

[3] Acetone powder extract.

in propionyl carboxylase activity occurs when the acetone powder extract or 0-45% ammonium sulfate fraction is incubated with d-biotin in the presence of phosphate buffer, ATP, $MgCl_2$, and glutathione. It is apparent that the 0-45% fraction contains all of the components necessary for apocarboxylase activation. Table II summarizes a kinetic study of propionyl apocarboxylase activation catalyzed by the 0-45% fraction. The data indicate that the enzymatic reaction is essentially complete after a 4-hour incubation period.

TABLE II

Kinetics of Propionyl Apocarboxylase Activation

Length of incubation	Propionyl carboxylase activity (propionyl-CoA-dependent $HC^{14}O_3^-$ fixation)	
	Basic system[1]	Basic system[1] + 100 μg d-biotin
hours	μmoles/hour/mg protein	μmoles/hour/mg protein
0	.12	.10
2	.12	.17
4	.12	.20
6	.10	.21

[1] Basic system and procedure were the same as in Table I. Each tube contained 11.2 mg of the 0-45% fraction.

The requirements for apocarboxylase activation catalyzed by the 0-45% fraction were investigated by noting the effect of deletions from or additions to the complete system consisting of phosphate, ATP, $MgCl_2$, GSH, d-biotin, and enzyme. The results, shown in Table III (Experiment 1), reveal that d-biotin and ATP are absolute requirements for this system. It is of interest to note that addition of coenzyme A had no effect and the deletion of $MgCl_2$ stimulated apocarboxylase activation. In order to determine whether d-biotin becomes protein-bound during apocarboxylase activation, an experiment similar to that just described, in which d-biotin-1-C^{14} replaced unlabeled d-biotin, was conducted simultaneously. The same enzyme preparation was used in both experiments (Table III, Experiments 1 and 2). The enzymatic reaction in tubes containing C^{14}-biotin was terminated with trichloroacetic acid (TCA). The protein precipitate, collected by centrifugation, was washed 3 times with 5% TCA, 2 times with ethanol, once with ethanol-ether (1:1), and once with ether. The protein was dissolved in methanolic Hyamine (1 M) and counted using a liquid scintillation counter. The data, summarized in Table III (Experiment 2), show that the enzymatic binding of C^{14}-biotin to protein requires ATP, is increased by deletion of $MgCl_2$, and is unaffected by coenzyme A addition.

TABLE III

Requirements of the Propionyl Apocarboxylase Activating and C^{14}-biotin-binding Systems

Deletions from or additions to the complete reaction mixture[1]	Experiment 1	Experiment 2
	Propionyl carboxylase activity after incubation (propionyl-CoA-dependent $HC^{14}O_3^-$ fixation)	Biotin-1-C^{14} bound to protein
	μmoles/hour/mg protein[2]	mμg/mg protein[2]
None	.26	4.45
-biotin	.11	.05[3]
-ATP	.11	.11
-$MgCl_2$	.28	6.10
+ Coenzyme A	.23	4.00

[1] The complete reaction mixture was the same as in Table I except that 7.3 mg of the 0-45% fraction were used and in Experiment 2, 5.2 μg of d-biotin-1-C^{14} (specific activity, 0.094 μc per μg) replaced unlabeled biotin. Coenzyme A addition was 0.4 μmole. Final volumes were 1.75 ml and 1.30 ml in Experiments 1 and 2, respectively. Incubated for 6 hours at 30°. Following incubation aliquots were taken for carboxylase assays (Experiment 1) as described in Table I and the amount of C^{14}-biotin bound to protein (Experiment 2) determined as described in text.

[2] Average of duplicates.

[3] d-biotin-1-C^{14} added after termination of reaction with 5% TCA following incubation.

It is significant that C^{14}-biotin binding follows the same pattern as propionyl apocarboxylase activation in this system. Approximate calculations based on the biotin content and specific activity of highly-purified liver mitochondrial propionyl carboxylase (Lane and Halenz, in press) indicate that more C^{14}-biotin was bound than could be accounted for by binding to propionyl carboxylase alone. It seems likely that C^{14}-biotin binding to other apocarboxylases occurred.

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