that the latter becomes more effective in catalyzing the formation of Ado-3',5'-*P*.

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Biotin- and adenosine triphosphate-dependent activation of propionyl apocarboxylase

As reported previously, [2-^{14}C] biotin is oxidized to labeled CO_2 and acetoacetate by cell-free preparations of a soil bacterium grown on biotin as sole carbon source[1,2]. A biotin-activating enzyme obtained from the bacterial extracts appears to catalyze the following reactions, as shown by the ATP-dependent conversion of biotin to biotin hydroxamate, stimulated by CoA, and by a [^{32}P]pyrophosphate–ATP exchange which is inhibited by CoA:

$$\text{Biotin} + \text{ATP} \overset{Mg^{2+}}{\rightleftharpoons} \text{biotinyladenylate} + \text{pyrophosphate} \quad (1)$$

$$\text{Biotinyladenylate} + \text{CoA} \rightleftharpoons \text{biotinyl-CoA} + \text{AMP} \quad (2)$$

The present report is concerned with the possibility that carboxyl activation occurs as a step in the incorporation of biotin into proteins as well as in biotin oxidation. In agreement with the findings of KOSOW AND LANE[3], we have shown that activation of the apoenzyme of propionyl-CoA carboxylase (EC 6.4.1.3) and the binding of [^{14}C]biotin to proteins in cell-free extracts of biotin-deficient liver are dependent upon the presence of ATP (Table I)[4]. The carboxylase activity (as measured by $^{14}CO_2$ fixation) when biotin is omitted is a measure of the residual holocarboxylase present in the biotin-deficient extracts. Since the omission of ATP or glutathione gives a similar value, it may be concluded that the effect of biotin in activating the apocarboxylase is almost entirely ATP-dependent and also requires the presence of glutathione, presumably to stabilize the apoenzyme. The overall apoenzyme-activating reaction, which is stimulated by the presence of Mg^{2+} but not consistently by CoA, may be formulated as follows:

$$\text{Biotin} + \text{apocarboxyiase} \xrightarrow[Mg^{2+}]{ATP} \text{carboxylase}$$

The requirements for binding [^{14}C]biotin to protein in the soluble system from biotin-deficient rat liver are also shown in Table I. The reaction is clearly ATP- and magnesium-dependent, whereas glutathione is not required.

TABLE I

REQUIREMENTS FOR ACTIVATION OF APOENZYME OF PROPIONYL-CoA CARBOXYLASE AND BINDING OF [^{14}C]BIOTIN TO PROTEIN

The complete reaction mixture for $^{14}CO_2$ fixation contained 50 μmoles Tris buffer (pH 7.7), 1 μmole biotin, 5 μmoles ATP, 5 μmoles glutathione, 2 μmoles $MgCl_2$, and 0–45% ammonium sulfate fraction of biotin-deficient rat liver (3.0 mg protein) in a final volume of 1.0 ml. After incubation for 3 h at 37° an aliquot (0.2 ml) was assayed for propionyl-CoA carboxylase with $^{14}CO_2$ according to the procedure of TIETZ AND OCHOA[5]. The complete value represents 0.38 μmole CO_2 fixed per hour. The complete reaction mixture for [^{14}C]biotin binding contained 50 μmoles Tris buffer (pH 7.7), 2 mμmoles [1-^{14}C]biotin* (15 300 counts/min), 2 μmoles ATP, 5 μmoles glutathione, 2 μmoles $MgCl_2$, and 0–45% ammonium sulfate fraction of biotin-deficient rat liver (3.7 mg protein). Incubation, 1 h at 37°. Proteins were then precipitated with trichloroacetic acid, resuspended and washed thoroughly 6 times with dilute trichloroacetic acid and assayed in hyamine solution in a scintillation counter. The complete value represents 0.06 mμmole biotin bound.

System	Propionyl-CoA dependent $^{14}CO_2$ fixation (per cent of complete system)	[^{14}C]Biotin bound (per cent of complete system)
Complete	100	100
No biotin	53	
No ATP	65	4
No glutathione	57	135
No $MgCl_2$	81**	6
No enzyme	2	
Complete + CoA (1 μmole)	120	89
Complete (incubated at 0°)		6

* Kindly furnished by Professor O. Wiss of Hoffmann-La Roche Inc. (Basel).
** EDTA (5 μmoles) was included in this experiment.

The complete enzyme system has been resolved into apoenzyme and apoenzyme-activating fractions, as indicated in recent preliminary reports from this laboratory[4] and by KOSOW AND LANE[6].

We have recently found that liver of various species contains a biotin-activating enzyme similar to the bacterial enzyme already described. The enzyme, assayed by the ATP-dependent formation of biotin hydroxamate in the presence of CoA and Mg^{2+}, has been purified about 20-fold from pig-liver extracts. Such preparations, unlike the bacterial preparations, significantly stimulate propionyl-CoA carboxylase formation from the apoenzyme (Table II). Whether interfering enzymes are absent in the bacterial system is not yet clear. A rat-liver apocarboxylase preparation containing only traces of the biotin-activating enzyme was incubated in Expts. 1 and 2 with partially purified biotin-activating enzyme from pig liver and in Expts. 3 and 4 with an activating system from biotin-deficient rat liver.

TABLE II

REQUIREMENT OF TWO ENZYME FRACTIONS IN ACTIVATION OF APOENZYME OF PROPIONYL-CoA CARBOXYLASE

The reaction mixture for determining propionyl apocarboxylase activation was like that in Table I but with enzyme preparations as follows. Rat-liver apoenzyme preparation obtained by DEAE-cellulose chromatography (1.0 mg protein in Expt. 1) or from an alumina C_γ gel supernatant fraction (1.2, 1.9, and 1.2 mg protein in Expts. 2–4). Apocarboxylase-activating system from pig liver (0.8 and 0.2 mg protein in Expts. 1 and 2) or from biotin-deficient rat liver (0.9 and 1.2 mg protein in Expts. 3 and 4). The values in parentheses represent experiments in which biotin was omitted.

Enzyme fraction added	Total counts $^{14}CO_2$ fixed			
	Expt. 1	Expt. 2	Expt. 3	Expt. 4
Apocarboxylase	1750	820 (430)	610	850 (850)
Activating system	0	40 (40)	359	640 (470)
Apocarboxylase + activating system	2960	1410 (500)	1462	2300
Increase due to combining fractions	71%	64% (6%)	51%	54%

These experiments establish a requirement for at least two soluble enzyme fractions in the biotin- and ATP-dependent formation of propionyl-CoA carboxylase from its apoenzyme: (a) the apoenzyme, in extracts of biotin-deficient liver, and (b) an apoenzyme-activating system present in both normal and biotin-deficient tissues. Although the results obtained indicate that a biotin-activating enzyme is present in the latter fraction, a conclusion as to the possible role of biotinyladenylate in apocarboxylase activation must await further enzyme purification.

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