ACTIVE ACETYL CoA CARBOXYLASE FROM LIVERS OF RATS FED CARBOHYDRATE

W. P. Welbourne, R. F. Swanson*, and H. S. Anker

Department of Biochemistry
The University of Chicago
Chicago, Illinois 60637

Received October 9, 1969

Summary: Enzymatically active and soluble acetyl CoA carboxylase was prepared from livers of rats fed carbohydrate by homogenizing at ca. 38°. The molecular weight of the enzyme prepared from fed rats is several times larger than that of the inactive enzyme present in liver homogenates of rats fasted 16 hours.

Acetyl CoA carboxylase occurs as a soluble but enzymatically inactive protein in the 100,000 g supernatants prepared from the livers of rats which had been fasted for 12 to 18 hours (1, 2). Enzymatically inactive preparations can be converted into several enzymatically active types: by incubation for 4 hours at 32° (2), by incubating for 1 hour after addition of citrate (1, 3, 4), or by adding trypsin and incubating for 5 min (1). The product obtained by incubation with citrate had a much higher molecular weight (ca. 43-45S) than the inactive protein present in the 100,000 g supernatant (ca. 14-18S) or the active materials obtained by the other two procedures (2). The presence of acetyl CoA carboxylase in livers of fed rats has not been demonstrable (5).

We have now found that soluble and active acetyl CoA carboxylase was present in the 100,000 g supernatant prepared from the livers of rats fed carbohydrate by stomach tube, provided that homogenization was carried out in warm (38°) phosphate buffer with a homogenizer prewarmed to 40° (Table I). The enzymic activity was not decreased if the homogenate was cooled in ice immediately after homogenization. Active acetyl CoA carboxylase was absent from a homogenate prepared at the same temperature from the livers of fasted rats (Table I).

*Present address: Carnegie Institution of Washington, Baltimore, Maryland, 21210.

TABLE I

INCORPORATION OF ^{14}C-ACETYL CoA INTO FATTY ACIDS

BY RAT LIVER HOMOGENATES

Animals	Rate of Incorporation nMoles/min/ml*
Fed; homogenized at 37°	
700 g supernatant	43
100,000 g supernatant prepared from above	62
Fasted; homogenized at 37°	
100,000 g supernatant	0.44
Fed; homogenized in the cold:	
700 g supernatant	3.4
100,000 g prepared from above	5.6
700 g supernatant incubated at 32° for 60 min	47
100,000 g supernatant prepared from above	63

* Average of 2 experiments; the protein concentration of the 100,000 g supernatants were 25-30 mg/ml.

Holtzman rats of 150-200 g were maintained on a fat free, carbohydrate rich diet. They were fed 5 ml of a solution containing 15% glucose and 15% fructose by stomach tube and killed 2 hours later or they were fasted for 16 hours. Assays were carried out as previously described: each sample was incubated with ^{14}C-acetyl CoA and the labeled fatty acids isolated (1, 2). Fatty acid synthetase, if not present, was added in excess to each sample.

A soluble and active enzyme was also obtained if the 700 g supernatant from a homogenate prepared at 4° was incubated for 1 hour at 32° (Table I). On centrifugation of the 700 g supernatant, particles sedimenting between 20,000 g and 35,000 g in 15 min yielded a soluble and active preparation if incubated together for 1 hour with the 100,000 g supernatant fraction from the same preparation, but not if incubated in buffer only. Sucrose gradient analysis of acetyl CoA carboxylase preparations from

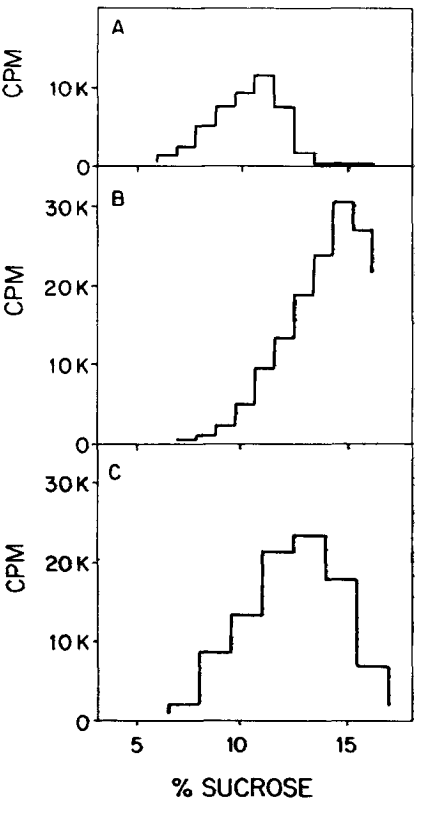

Fig. 1. Rate of incorporation of ^{14}C-acetyl CoA into fatty acids as a function of position in a 5-20% sucrose gradient after centrifugation. To each fraction excess fatty acid synthetase was added and the rate of incorporation of ^{14}C-acetyl CoA into the fatty acid fraction was determined.

A; 100,000 g supernatant from the liver of a rat fasted for 16 hours and homogenized at 4°. B; 100,000 g supernatant prepared from the liver of a rat fed glucose + fructose by stomach tube and homogenized at 37°. C; 100,000 g supernatant prepared from the liver of a rat fed glucose + fructose by stomach tube, homogenized at 4°, 700 g supernatant incubated at 32° for 1 hour, and centrifuged.

livers of fed rats showed that the enzyme had a high molecular weight both in preparations homogenized at body temperature and in the cold (Fig. 1).

Homogenates from fed and fasted rats contained about equal amounts of fatty acid synthetase. The conditions required for the incorporation of acetyl CoA into long chain fatty acids by the various preparations are summarized in Table II.

It cannot be stated unequivocally in what form acetyl CoA carboxylase is present in the livers of fed rats *in vivo*. It appears unlikely

TABLE II

^{14}C-ACETYL CoA INCORPORATION INTO LONG CHAIN FATTY ACIDS BY DIFFERENT RAT LIVER HOMOGENATES

	Animals	
	Fasted	Fed
Liver homogenized at 4°:		
100,000 g supernatant	-	±
100,000 g supernatant incubated for 1 h at 32°	±	±
100,000 g supernatant incubated for 4 h at 32°	+++	±
100,000 g supernatant incubated for 1 h at 32° with citrate	+++	±
100,000 g supernatant incubated for 5 min with trypsin	+++	±
100,000 g supernatant prepared from a 700 g supernatant which had been incubated for 1 h at 32°	±	+++
Liver homogenized at 37°:		
100,000 g supernatant	±	+++

that an enzyme, if it were present in insoluble form *in vivo*, could be solubilized during a 2 min period of homogenization at body temperature even though it required a whole hour of incubation at 32° for complete solution after homogenization in the cold. It seems much more plausible that the enzyme is present in soluble form *in vivo* and that it became bound, perhaps to a lipid component of the endoplasmic reticulum, during disruption of the cell at low temperature; acetyl CoA carboxylase has been shown to bind to some lipids readily (6).

The present and previous (1, 2) experiments demonstrate that acetyl CoA carboxylase undergoes a reversible transformation: an enzymatically in-

active protein of low molecular weight is present in fasted animals and is converted, after carbohydrate feeding, into an active enzyme of high molecular weight and vice versa. It is at present not clear if the active enzyme isolated from fed animals is identical with the polymeric enzyme obtained by incubation of the inactive protein with citrate. The mechanism of the transformation *in vivo* apparently is not due to citrate since the citrate concentrations in livers from fasted and fed rats were reported to be the same (7).

The active forms of acetyl CoA carboxylase of low molecular weight obtained by incubation with trypsin or by prolonged incubation of the 100,000 g supernatant may be identical since activation during prolonged incubation of the homogenate could be due to the presence of a small quantity of a proteolytic enzyme. These preparations do not seem to have physiologically occurring equivalents.

This work was supported by the Louis Block and Worcester Funds of the University of Chicago and by a grant from the Heart Institute, National Institutes of Health, U.S.P.H.

REFERENCES

1. Swanson, R. F., Currie, W. M., and Anker, H. S., Proc. Natl. Acad. Sci. U.S.A., 58, 1243 (1967).
2. Swanson, R. F., Currie, W. M., and Anker, H. S., Biochem. Biophys. Acta, 159, 390 (1968).
3. Vagelos, P. R., Alberts, A. W., and Martin, D. B., J. Biol. Chem., 238, 533 (1963).
4. Matsuhashi, M., Matsuhashi, S., and Lynen, F., Biochem. Z., 340, 263 (1964).
5. Lowenstein, J. M., Biochem. Soc. Symp. No. 24, 153 (1963).
6. Numa, S., Ringelmann, E., and Lynen, F., Biochem. Z., 343, 243 (1965).
7. Spencer, A. F., and Lowenstein, J. M., Biochem. J., 103, 342 (1967).