Rat-liver fatty-acid-synthesizing complex

P. M. GILLEVET and K. DAKSHINAMURTI

Department of Biochemistry, Faculty of Medicine,
University of Manitoba, Winnipeg, Canada R3E 0W3

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Under conditions favoring lipogenesis, a high-
molecular-weight species of acetyl-CoA carboxylase was
isolated that did not co-sediment with the in vitro
polymerized enzyme. Assays for ATP-citrate lyase,
acetyl-CoA carboxylase, and fatty acid synthetase
indicated that all three enzymes were associated
together as a high-molecular-weight complex and that
under low-lipogenic conditions the level of these
enzymes decreased. Phosphorylation of the isolated
complex shifted it toward a lower molecular weight.

Acetyl-CoA carboxylase (EC 6.4.1.2) has been recognized to
catalyze the rate-limiting step of lipogenesis. The enzyme is under
diverse control mechanisms (1) such as feedback inhibition by
long-chain fatty acyl-CoA (2) and the in vitro conversion of the
enzymatically inactive protomer to the active polymer in the presence
of citrate (3). Recent reports suggest that the enzyme activity is
regulated by a phosphorylation-dephosphorylation mechanism (4,5).
Acetyl-CoA carboxylase (ACC) is generally considered to be a
cytosolic enzyme. However, Easter and Dils (6) have demonstrated
that a species of ACC is sedimented with the microsomes in lactating
rabbit mammary gland. We reported earlier on the in vivo distribution
of ACC from rat liver between a cytosolic species and one that
sedimented with the microsomal pellet (7). Similar observations were
subsequently reported by Witters et al. (8).

We now present evidence which suggests that the high-molecular-
weight polymer of ACC observed in vitro does not exist in vivo under
conditions favoring lipogenesis. In addition the form of the enzyme
generally referred to as the microsomal form is actually associated in
a fatty-acid-synthesizing complex containing fatty acid synthetase and
ATP-citrate lyase (EC 4.1.3.8). This complex seems to be regulated
by a cAMP-dependent phosphorylation mechanism.

Materials and Methods

Materials

D-[1-14C]Pantothenic acid (57 mCi/mmole) was obtained from New
England Nuclear Corporation (Boston, MA) and D-[carbonyl-14C]biotin
(51 mCi/mmole) from Amersham Corporation (Oakville, Ont.).
Electrophoretic reagents were obtained from Sigma Chemical Company
(St. Louis, MO).
Preparation of animals

Male Long-Evans rats (150 g) were starved for 48 h and then refed a high-carbohydrate diet for another 48 h. During refeeding, the rats were injected with either $[{}^{14}\text{C}]$biotin or $[{}^{14}\text{C}]$pantothenate (10 $\mu$Ci/day). The rats were then divided into two groups, one of which was fasted overnight, the other being kept on the high-carbohydrate diet. They were then sacrificed by decapitation. The livers were homogenized in 0.25 M sucrose, 50 mM phosphate buffer pH 7.5, 1 mM EDTA, and 5 mM $\beta$-mercaptoethanol at room temperature. The homogenate was centrifuged at 10 000 g for 20 min at 21°C. The supernatant was filtered through cheesecloth and applied on the sucrose gradients.

Sucrose gradient

Aliquots of the low-speed supernatant were centrifuged through a 20- to 40% sucrose gradient, containing 50 mM phosphate buffer pH 7.5, 1 mM EDTA, and 5 mM $\beta$-mercaptoethanol for 2.5 h at 60 000 r.p.m. at 21°C in a Beckman 70Ti fixed-angle rotor. Fractionation was accomplished through the use of a peristaltic pump to remove the gradient from the bottom of the tube.

SDS/gel electrophoresis

A 50- $\mu$L aliquot of each of the above fractions was denatured in 1% SDS and 5% $\beta$-mercaptoethanol by heating at 100°C for 5 min and was then subjected to SDS/gel electrophoresis on slab gel with a 3% acrylamide stacking gel and a 5% acrylamide running gel according to the method of Laemmli (9).

Enzyme assay

ACC activity was determined by the $[{}^{14}\text{C}]$bicarbonate fixation assay of Dakshinamurti and Desjardins (10), after the enzyme had been preincubated at 37°C for 30 min in the presence of 20 mM potassium citrate. Fatty acid synthetase (FAS) activity was assayed by following the incorporation of $[{}^{14}\text{C}]$malonyl-CoA into long-chain fatty acids (11). The ATP-citrate lyase (CL) activity was monitored by following the incorporation of label from $[{}^{1,4}\text{C}]$citrate into fatty acid through a coupled assay with FAS (10,11).

Results and Discussion

Distribution of enzyme activities on sucrose gradients

The distribution of ACC, CL, and FAS activities in the low-speed supernatant prepared from rats fed a high-carbohydrate diet, on 20-40% sucrose density gradients is given in Fig. 1. ACC activity sedimented as a single peak between the major peaks of cytosolic and microsomal proteins. The in vivo form of ACC formed under high lipogenic conditions did not sediment with the purified, polymerized avian enzyme which sedimented with the microsomes. The activity of