CELL-FREE SYNTHESIS OF ACETYL COENZYME A CARBOXYLASE BY LIVER POLYSOMES FROM RATS UNDER DIFFERENT DIETARY CONDITIONS

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1. Introduction

Acetyl coenzyme A carboxylase (acetyl-CoA: carbon-dioxide ligase (ADP-forming), EC 6.4.1.2) plays a critical role in the regulation of long-chain fatty acid biosynthesis [1]. The level of activity of this enzyme in animal tissues varies in accord with the rate of fatty acid synthesis under different metabolic conditions. Fasted rats exhibit a subnormal level of hepatic acetyl-CoA carboxylase activity, whereas rats fasted and subsequently refed a fat-free diet show a level of activity higher than the normal value [2,3]. Immunochemical studies have demonstrated that these variations in the activity level are due to corresponding changes in the quantity of the enzyme protein [4–6]. Furthermore, it has been shown by combined immunochemical and isotopic techniques that the increase in the enzyme content in refed rats can be ascribed solely to an increase in the rate of synthesis of the enzyme, while the decrease in the enzyme content in fasted rats is due to both diminished synthesis and accelerated degradation of the enzyme [5,6].

Recently, we have been able to identify specific polysomes involved in the synthesis of acetyl-CoA carboxylase by the binding of ^{125}I-labelled anti(acetyl-CoA carboxylase) to mammalian liver polysomes [7,8]. Using this binding technique, we have demonstrated that the relative content of acetyl-CoA carboxylase–synthesizing polysomes in the liver correlates well with the rate of hepatic synthesis of the enzyme in animals under different metabolic conditions.

In the present investigation, we utilized a cell-free protein-synthesizing system derived from rabbit reticulocytes to assess, in another way, the hepatic content of specific polysomes synthesizing acetyl-CoA carboxylase. One of the products formed by cell-free translation of rat liver polysomes was identified as acetyl-CoA carboxylase by indirect immunoprecipitation followed by electrophoretic analysis of the dissociated immunoprecipitate. The relative amount of acetyl-CoA carboxylase synthesized by polysomes from livers of rats subjected to different dietary manipulations was shown to vary in accord with the rate of hepatic synthesis in vivo of the enzyme.

2. Materials and methods

Male Wistar rats weighing 150–200 g were subjected to different dietary manipulations as reported previously [7]. Rat liver acetyl-CoA carboxylase was purified to homogeneity, and antisera against this enzyme were prepared as described previously [7]. Anti(acetyl-CoA carboxylase), control immunoglobulin G from non-immunized rabbits and goat anti(rabbit immunoglobulin G) were purified by ammonium sulfate fractionation [7] followed by passing through a DEAE-cellulose column [9]. Reagents were obtained as described previously [10].

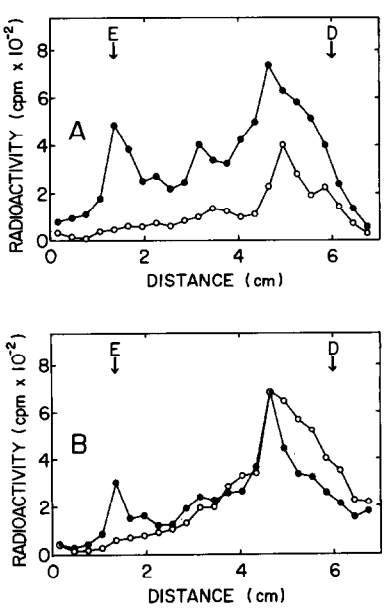
Total polysomes from rat liver were prepared as described previously [7] and were concentrated as follows. All operations were performed at 0–4°C. Polysomes, which were collected from the 1.0–2.5 M interface of the discontinuous sucrose gradient, were diluted with 4 vol. of 50 mM Tris–HCl, pH 7.7, containing 25 mM NaCl, 5 mM $MgCl_2$ and 0.5 mg/ml sodium heparin, overlaid onto 2 ml of 1.0 M sucrose

containing 50 mM Tris–HCl, pH 7.7, 25 mM NaCl and 5 mM $MgCl_2$ and centrifuged at 368 000 × g_{max} for 90 min. The resulting pellet was suspended by gentle homogenization in 50 mM Tris–HCl, pH 7.5, containing 25 mM KCl and 5 mM $MgCl_2$ to yield a concentration of 200–300 A_{254} units/ml. Free polysomes, which were derived from the post-membrane fraction, i.e., the supernatant resulting from centrifugation of the liver homogenate at 27 000 × g_{max} for 10 min, were prepared and concentrated in the same manner.

The rabbit reticulocyte lysate, which was prepared according to the procedure described by Palmiter [11], was further centrifuged at 145 000 × g_{max} for 70 min, and the resulting post-ribosomal supernatant was used for cell-free translation experiments. Unless otherwise stated, the reaction mixture for cell-free protein synthesis (total vol, 0.1 ml) contained 25 mM Tris–HCl, pH 7.5, 80 mM KCl, 3 mM $MgCl_2$, 2 mM dithiothreitol, 20 μM hemin, 1 mM ATP, 0.2 mM GTP, 8 mM creatine phosphate, 40 μg/ml creatine phosphokinase (EC 2.7.3.2), 0.1 mM each of 19 unlabelled amino acids, 0.1 mCi/ml L-[4,5-^{3}H]leucine (specific radioactivity, 57 Ci/mmol), 0.6 ml/ml reticulocyte post-ribosomal supernatant and 20–30 A_{254} units/ml liver polysomes. After incubation at 25°C for 90 min, the reaction was terminated by the addition of 0.12 ml of a solution composed of 10 mM sodium phosphate, pH 7.2, 0.15 M NaCl, 1.8% sodium deoxycholate, 1.8% Triton X-100 and 20 mM unlabelled leucine. The diluted mixture was then centrifuged at 145 000 × g_{max} for 60 min, and the resulting post-ribosomal supernatant was collected. For indirect immunoprecipitation, 0.2 ml of this supernatant was mixed with 28 μg of anti(acetyl-CoA carboxylase) or control immunoglobulin G. The mixture was incubated at 25°C for 60 min and allowed to stand at 4°C for 5–10 h. Then, a sufficient amount of goat anti(rabbit immunoglobulin G) was added to precipitate the anti(acetyl-CoA carboxylase) or control immunoglobulin G quantitatively. After storage of the mixture at 4°C overnight, the resulting immunoprecipitate was washed twice by a procedure essentially identical to that of Maurer et al. [12]. The washed immunoprecipitate, as well as the post-ribosomal supernatant from the reaction mixture, was analyzed by electrophoresis on 4% polyacrylamide gel containing 0.1% sodium dodecyl sulfate according to the method of Weber and Osborn [13] as described previously [10]. For the determination of radioactivity, the gel was divided into 3 mm slices, which were incubated with 0.6 ml of NCS solubilizer-water (9:1, by vol.) at 45°C overnight and then counted in 5 ml of toluene scintillator fluid with a liquid scintillation spectrometer.

3. Results and discussion

Rat liver polysomes were translated in a cell-free system containing the post-ribosomal supernatant derived from rabbit reticulocytes. The translation products were analyzed by indirect immunoprecipitation followed by dodecylsulfate–polyacrylamide gel electrophoresis of the dissociated immunoprecipitate. The results of the electrophoretic analyses are presented in fig.1A. The immunoprecipitate formed with anti (acetyl-CoA carboxylase) contained a labelled product which migrated with a mobility identical to that of the subunit polypeptide of authentic rat liver acetyl-CoA carboxylase (mol. wt, 230 000 [14]). This slowly moving product was absent in the immunoprecipitate resulting from incubation of the same translation products with control immunoglobulin G. Further evidence for the identity of the slowly moving product was provided by a competition experiment as shown in fig.1B. Prior addition of an excess amount of



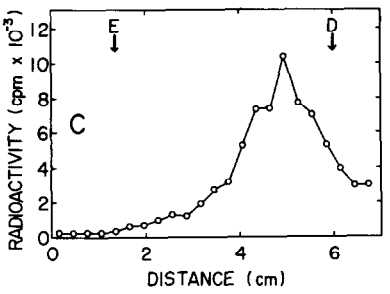


Fig.1. Dodecylsulfate–polyacrylamide gel electrophoresis of cell-free translation products. (A) Free polysomes (2.4 A_{254} units) from livers of refed rats were translated, and the post-ribosomal supernatant from the reaction mixture was subjected to indirect immunoprecipitation with anti(acetyl-CoA carboxylase) (•——•) or control immunoglobulin G (○——○). The immunoprecipitates were analyzed electrophoretically. (B) The post-ribosomal supernatant from a similar reaction mixture was subjected to indirect immunoprecipitation with anti(acetyl-CoA carboxylase) in the presence (○——○) or absence (•——•) of 40 μg of unlabelled purified rat liver acetyl-CoA carboxylase. The immunoprecipitates were analyzed electrophoretically. (C) An aliquot (10 μl) of the post-ribosomal supernatant from a similar reaction mixture was subjected to electrophoresis without prior immunoprecipitation. Experimental details were as described in Materials and methods. E and D indicate the positions to which the subunit of rat liver acetyl-CoA carboxylase and the tracking dye (bromophenol blue) migrated, respectively.

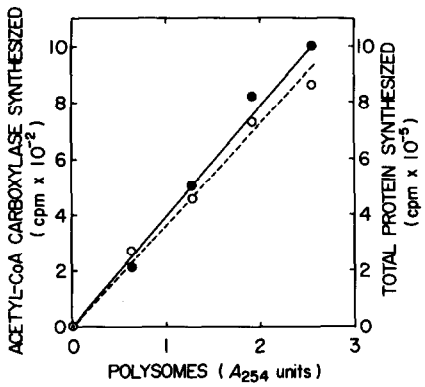


Fig.2. Dependence of the amounts of acetyl-CoA carboxylase (•——•) and total protein (○– – –○) synthesized on the amount of polysomes added. The indicated amounts of free polysomes from livers of refed rats were translated, and the immunoprecipitates formed with anti(acetyl-CoA carboxylase) or control immunoglobulin G were analyzed electrophoretically as described for fig.1A. The radioactivities present in the gel-slices containing the acetyl-CoA carboxylase subunit were totaled. From this amount, the radioactivities found in the corresponding slices of the gel on which the control immunoprecipitate was electrophoresed were subtracted. The radioactivity incorporated into total protein was determined with an aliquot (5 μl) of the post-ribosomal supernatant from the reaction mixture as described by Palmiter [11]. Values represent those per 0.1 ml of the reaction mixture.

unlabelled rat liver acetyl-CoA carboxylase to the translation products resulted in the disappearance of the slowly moving radioactivity band observed upon electrophoresis, whereas the faster moving radioactivity bands were not abolished. When the total translation products were electrophoresed without prior immunoprecipitation, the majority of the labelled proteins migrated with mobilities similar to those of the proteins precipitated with the control immunoglobulin G–anti(immunoglobulin G) complex (fig.1C). These results indicate that the slowly moving radioactivity band actually represents acetyl-CoA carboxylase, while the faster moving radioactivity bands arise from coprecipitation of nonspecific proteins.

In the experiment shown in fig.2, varying amounts of liver polysomes were translated in the cell-free system, and the amounts of acetyl-CoA carboxylase synthesized were measured by indirect immunoprecipitation and gel electrophoresis. The amount of the enzyme produced, as well as that of total protein synthesized, was proportional to the amount of polysomes added up to at least 2.6 A_{254} units of polysomes/assay. The ability to incorporate [^{3}H]leucine into acetyl-CoA carboxylase and into total protein varied from one reticulocyte lysate to another as reported by Palmiter [15] for the synthesis of avian egg-white proteins. However, one and the same batch of reticulocyte lysate incorporated reproducible amounts of [^{3}H]leucine into the enzyme as well as into total protein.

Using the technique mentioned above, we then compared the relative amounts of acetyl-CoA carboxylase synthesized by liver polysomes which were derived from rats fed a balanced diet, rats fasted for 48 h, and rats fasted for 48 h and subsequently refed a fat-free diet for 72 h. The results of such an experiment, presented in table 1, showed that polysomes from fasted rats synthesized about one-third as much acetyl-CoA carboxylase as did polysomes from normal

Table 1
Effects of dietary conditions on the amount of acetyl-CoA carboxylase synthesized

Dietary condition	[^{3}H]Leucine incorporation	
	Total protein (cpm × 10^{-4})	Acetyl-CoA carboxylase (cpm)
Normal	172	248
Fasted	162	72
Refed	188	730

Total polysomes (2.5 A_{254} units) from livers of rats under the different dietary conditions were translated as described in Materials and methods, except that the reaction mixture was scaled up to 0.125 ml. The radioactivities incorporated into acetyl-CoA carboxylase and total protein were measured as described for fig.2. Values represent those per 0.125 ml of the reaction mixture.

rats, whereas polysomes from refed rats produced an about 3-fold larger amount of the enzyme than did polysomes from normal rats. Polysomes from the three types of rats incorporated [^{3}H]leucine into total protein to essentially the same extent. In this particular experiment, total polysomes were used. Similar effects of the dietary manipulations on the cell-free synthesis of hepatic acetyl-CoA carboxylase were demonstrated with free polysomes.

Previous studies have shown that the rate of acetyl-CoA carboxylase synthesis in vivo per liver is diminished about 2-fold in fasted rats as compared with normal rats, whereas it is 3- to 4-fold higher in refed rats than in normal rats [5,6]. Thus, the amount of the enzyme synthesized in vitro by liver polysomes varies in accord with the rate of synthesis in vivo of the enzyme under the different dietary conditions.

It is to be noted that, when aurintricarboxylic acid, an inhibitor of peptide chain initiation [16], was added to the protein-synthesizing system used in the present investigation, [^{3}H]leucine incorporation neither into total protein nor into acetyl-CoA carboxylase was depressed. This indicates that no initiation of protein synthesis occurred in our experiments and that the amino acid incorporation measured was due entirely to the completion of nascent peptide chains on polysomes. Thus, the results reported in the present paper strengthen our previous finding that the relative content of hepatic acetyl-CoA carboxylase-synthesizing polysomes correlates well with the rate of synthesis in vivo of the enzyme in rats subjected to the different dietary conditions [7].

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