Regulation of synthesis of hepatic fatty acid synthetase:
Binding of fatty acid synthetase antibodies to polysomes

(fatty acid synthetase structure/enzyme induction/animal nutrition)

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Contributed by F. Roy Vagelos, August 4, 1975

ABSTRACT  Mammalian fatty acid synthetase was shown to be composed of two peptides, molecular weight 240,000, after dissociation with sodium dodecyl sulfate. Rat liver polysomes that synthesize fatty acid synthetase were identified by sucrose gradient analysis of polysomes that had been reacted with 125I-labeled antibody against fatty acid synthetase. The binding of 125I-labeled antibody to polysomes was found to correlate with the rate of hepatic fatty acid synthesis in various nutritional conditions.

Fatty acid synthetase (FAS) has been identified in all biological systems that catalyze the de novo synthesis of long chain fatty acids from acetyl CoA and malonyl CoA (1–3). Although the mechanisms of the sequential reactions that constitute the FAS are similar in various biological systems, the structures of FAS differ dramatically in prokaryotes and eukaryotes (1). Thus, the Escherichia coli FAS is composed of at least seven peptides which represent the individual enzyme components and the acyl carrier protein (1, 2, 4). The yeast and animal FAS (molecular weight 2.2 × 10^6 and 4.8 × 10^5, respectively), on the other hand, readily dissociate into only two high-molecular-weight peptide components (5–7). Harsh dissociation conditions have been reported to produce lower molecular weight peptides from the yeast (8) and animal FAS (9, 10).

Another distinguishing feature of the animal system is the response of FAS to nutritional and hormonal perturbations. Thus, FAS activity is depressed in starved animals; upon refeeding a fat-free diet the enzyme activity increases to super high levels (1, 11–14). Low levels of FAS activity are also found in diabetic animals, and this condition is reversed by the administration of insulin (15, 16). The changes in enzyme activity noted during starvation and diabetes are due to changes in the amount of FAS and not to activation or inactivation of existing enzyme (11, 12–16). The amount of FAS present in both liver and adipose tissue is largely regulated by the rate of FAS synthesis in these organs (11, 14, 17).

What are the controlling factors involved in the synthesis of FAS? To get some insight into this, we have studied the components of protein synthesis at the molecular level. The approach that was taken initially involved the identification of polysomes synthesizing FAS and was based on the methods developed in Schimke’s laboratory (18–21). In that work, polysomes from hen oviduct synthesizing ovalbumin (18–20) and conalbumin (20) and from rat liver synthesizing albumin (21) were identified by the specific binding of purified 125I-labeled antibodies to the nascent chains of these proteins on the polysomes.

This study contributes information concerning the peptide structure of mammalian FAS. Furthermore, it shows that purified 125I-labeled antibody against rat FAS binds to rat liver polysomes, presumably by recognition of the nascent chains of FAS. The binding of 125I-labeled antibody to polysomes was readily demonstrated in livers that were actively synthesizing fatty acid synthetase.

MATERIALS AND METHODS

FAS from Chang liver cells and from rat liver were purified by a procedure modified from that described by Maitra and Kumar (22). Briefly, livers from rats starved for 48 hr and then refed a fat-free diet were homogenized in 3 volumes of 0.2 M potassium phosphate buffer, pH 7.0, containing 1 mM dithiothreitol and 1 mM EDTA. The 100,000 × g supernatant from the homogenate was subjected to ammonium sulfate precipitation at 35% saturation. The resulting precipitate was dissolved in the homogenizing buffer and diluted with the buffer to a final protein concentration of 10 mg/ml. To this was added 6 volumes of alumina C7 in 1 mM dithiothreitol at a concentration of 5 mg/ml. After stirring 5 min at room temperature, the suspension was centrifuged and the pellet discarded. The supernatant was adjusted with solid ammonium sulfate to 45% saturation and the resulting precipitate was dissolved in a minimal volume of homogenizing buffer. This was then applied to a sepharose-6 B column equilibrated with 0.25 M sodium phosphate, pH 7.0, containing 1 mM dithiothreitol and 1 mM EDTA. The fractions from the column with constant specific activity were pooled, and the FAS was recovered by precipitation with ammonium sulfate at a final concentration of 45% saturation. The resulting precipitate was suspended in a small volume of 45% saturated ammonium sulfate in the column buffer and stored in liquid N2.

Chang liver cell FAS was obtained from cells adapted to grow in suspension. Two liters of culture (10^8 cells per ml) were used. Cells were cultured and FAS was induced as previously described (23) except that [3H]pantothenic acid was substituted for unlabeled pantothenic acid in the medium. Cell-free extracts were prepared as previously described (23). FAS was purified from these extracts in the same manner as was used with rat liver. The FAS obtained from both sources, assayed as previously described (23), had specific activities of 750–800 units per mg of protein and appeared homogeneous, as determined by sodium dodecyl sulfate (NaDodSO4)-polyacrylamide gel electrophoresis (24) (see Fig. 1).

Purification of Rabbit Antibody Against Rat FAS. Antisera to rat liver FAS was obtained from Dr. Joseph Volpe (Washington University) and purified by ammonium sulfate precipitation and DEAE-Sephadex chromatography.

Abbreviations: FAS, fatty acid synthetase; NaDodSO4, sodium dodecyl sulfate.

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Peptide structure of mammalian FAS

Prior to our studies of the mechanism of control of the synthesis of FAS in mammals, it was important to understand the peptide structure of the mammalian FAS, which has a molecular weight of 480,000 (7). To do this, Chang liver cells were grown in culture in the presence of [3H]pantothenic acid to label the FAS prosthetic group, 4'-phosphopantetheine, and the enzyme was purified to apparent homogeneity. Electrophoresis of FAS, after dissociation of the peptides with NaDodSO4, was carried out on NaDodSO4-polyacrylamide gels. As shown in Fig. 1, only one protein band was seen when the gel was stained with Coomassie blue. When this gel was sliced and radioactivity was determined, 97% of the applied radioactivity derived from [3H]pantothenic acid was found in the region of the gel exactly corresponding to the protein band. Myosin, with a molecular weight of 207,000, migrated somewhat ahead of FAS. Similar results were obtained with homogeneous rat liver FAS. From similar experiments using peptides of known molecular weights, the molecular weight of the peptides of rat and human FAS was calculated to be 240,000. Although we noted the appearance of lower molecular weight peptides upon prolonged exposure to NaDodSO4, as previously reported (17, 26), these peptides did not appear when phenylmethyl sulfon fluoride was included in the NaDodSO4 solution during the procedure utilized to dissociate FAS. It is thus concluded that mammalian FAS is composed of two peptides of similar molecular weight.

Binding of 125I-labeled antibody against FAS to rat liver polysomes

Antibody prepared against purified rat liver FAS specifically precipitated peptides from crude extracts of rat liver that migrated on NaDodSO4-polyacrylamide gels as a single band with the same mobility as purified FAS (data not shown). When this antibody was purified by affinity chromatography and then iodinated with 125I, at least 50% of the radioactivity was immunoprecipitated by FAS at its equivalence point. The iodinated antibody was used to identify the rat liver polysomes that synthesize FAS. Fig. 2 demonstrates the binding of 125I-labeled antibody against FAS to poly-

![Fig. 2. Binding of 125I-labeled antibody against FAS to rat liver polysomes. Two levels of rat liver polysomes (6 and 12 A260 units in 0.5 ml) were incubated with 6.5 μg of 125I-labeled antibody for 1 hr at 0°C and then dialyzed for an additional 3 hr against Buffer A at 0°C. After dialysis the polysomes were centrifuged on sucrose gradients, the gradients were fractionated, A260 was monitored, and radioactivity was determined (see Materials and Methods). Upper and lower solid lines are the A260 profiles of the higher and lower levels of polysomes. Radioactivity of 125I-labeled antibody against FAS centrifuged in absence of polysomes (A); with 6 A260 units of polysomes (●); with 12 A260 units of polysomes (○).](https://example.com/figure2.png)
Fig. 3. Effect of rat liver FAS on the binding of $^{125}$I-labeled antibody against FAS to polysomes. $^{125}$I-Labeled antibody (6.5 μg) was incubated with polysomes (6.0 $A_{260}$ units) from livers of rats that were starved and then refed the fat-free diet for 16 hr in the presence and absence of rat liver FAS (35 μg) and with rat liver FAS in the absence of polysomes and analyzed as in Fig. 2. The positions of the 80S monosome peak and the peak fraction of polysomes are indicated. $^{125}$I-labeled antibody against FAS incubated with polysomes (●); incubated with polysomes and FAS (○); incubated with FAS (▲).

Fig. 4. Effect of concentration of $^{125}$I-labeled antibody against FAS on binding of $^{125}$I-labeled antibody to polysomes. Polysomes (6 $A_{260}$ units) from livers of rats that were starved and then refed the fat-free diet for 16 hr were incubated with three levels of $^{125}$I-labeled antibody against FAS and analyzed on sucrose gradients as in Fig. 2. The positions of the 80S monosome peak and the peak fraction of polysomes are indicated. Polysomes incubated with 3.25 μg (●), 6.5 μg (○), and 15 μg of $^{125}$I-labeled antibody (▲).

Fig. 5. Effect of nutritional state on the binding of $^{125}$I-labeled antibody against FAS to polysomes. $^{125}$I-Labeled antibody (6.5 μg) was incubated with polysomes (6 $A_{260}$ units) prepared from livers of normally fed rats, starved rats, and from starved rats that were refed the fat-free diet for 16 hr and then analyzed as in Fig. 2. The $A_{260}$ was determined on the fractions from the three gradients and then normalized to the 80S monosome peak (fraction 20). Absorbance at 260 nm for the three gradients (▲). $^{125}$I Labeled antibody cpm with polysomes from normally fed rats (●); from starved rat (○); from starved and then refed rats (▲).

to sucrose gradient centrifugation. Antibody was bound to the large polysomes, and this binding was almost completely suppressed when excess free FAS was included in the incubation mixture. When labeled antibody was incubated with synthetase in the absence of polysomes, no radioactivity was found in the polysome region.

Fig. 4 demonstrates that binding of $^{125}$I-labeled antibody against FAS was dependent on the concentration of the antibody. With increasing levels of antibody, increased binding to polysomes was noted.

Effect of nutritional state of animal on binding of $^{125}$I-labeled antibody against FAS to liver polysomes

It has been shown that starved animals synthesize FAS at approximately 18% the rate of rats fed normal rat chow, while rats fed a fat-free diet synthesize FAS approximately 14 times faster than rats fed a normal diet (14). The differences in rates of FAS synthesis were reflected in the amount of labeled antibody bound to the polysomes, suggesting that more FAS nascent peptides were associated with polysomes under these conditions (Fig. 5). A constant amount of $^{125}$I-labeled antibody against rat FAS was reacted with equal concentrations of polysomes obtained from a starved animal, a normally fed animal, and an animal that had been starved and then refed the fat-free diet. In all three cases the polysome profiles were similar. Polysomes from refed animals bound the expected quantities of labeled antibody. Although very few counts were associated with the polysomes from the starved animals, a radioactive peak was found with the polysomes from normally fed animals. Thus, the binding of antibody against rat FAS to polysomes reflects the nutritional state of the animal.

Further studies were carried out to determine whether the binding of antibody to polysomes correlated with the time of appearance of increased levels of FAS in the liver. Animals were starved and then refed the fat-free diet for varying periods of time, after which they were sacrificed. The specific activity of FAS was determined at each time point (Fig. 6). After a lag of approximately 4 hr, there was a rapid rise in FAS activity which continued during the entire course of the experiment to a specific activity of 54 at 44 hr. At 27 hr one rat was switched to a normal diet. Liver FAS...
activity decreased from 36 at 27 hr to 29 at 44 hr on this diet.

Fig. 7 shows the binding of iodinated antibody to polysomes prepared from the livers of these animals. There was little difference in binding in the zero and 2-hr animals and only a slight increase after 4 hr (Fig. 7A). However by 5 hr, the time point when FAS specific activity began to increase in the liver, there was greatly enhanced binding of antibody to polysomes. These results suggest that during the first 4 hr after refeeding there is little synthesis of immunologically reactive FAS peptides.

Synthesis of FAS was still continuing in the 44-hr fat-free reared rat, as indicated by the binding of antibody against FAS to polysomes (Fig. 7B). However, relatively little binding was obtained with the polysomes from the animal that had been switched to a normal diet at 27 hr, indicating that enzyme synthesis was greatly diminished.

**DISCUSSION**

*E. coli* FAS consists of at least seven peptides that catalyze the sequence of reactions leading to the formation of palmitate (1, 2, 4). The same set of reactions in yeast is catalyzed by a high-molecular-weight FAS complex, which had been shown by Sweitzer *et al.* (6) to consist of only two large dissimilar peptides with molecular weights of 185,000 and 180,000 on NaDodSO₄-polyacrylamide gel electrophoresis. 4'-Phosphopantetheine, the covalently bound prosthetic group of acyl carrier protein, was present on the larger peptide. The presence of additional smaller peptides in yeast FAS, reported in earlier work (8), was shown to be due to proteolysis.

Animal FAS obtained from various sources is a protein complex with a molecular weight of approximately 480,000 which, under certain conditions, dissociates into 240,000 molecular weight subunits (1, 7, 27, 28). Workers in several laboratories have reported the isolation from animal FAS of peptides containing 4'-phosphopantetheine ranging in molecular weight from 6,000 to 100,000 (9, 10, 17, 29). Using highly purified FAS prepared from either rat or human liver or from Chang liver cells grown in culture, we have been able to identify only a single peptide with a molecular weight of 240,000 on NaDodSO₄-polyacrylamide gels. It therefore appears that mammalian FAS is composed of only two peptides of essentially identical molecular weight. The formation of lower molecular weight peptides probably occurs by proteolysis during the procedure utilized to dissociate the FAS. Similar conclusions have been reported recently from Waki's laboratory (30).

The binding of ¹²⁵I-labeled antibodies against several different proteins to polysomes has been used to identify the polysomes synthesizing those proteins (18–21). Applying the technique with ¹²⁵I-labeled antibody against rat FAS to polysomes obtained from rats under different nutritional conditions permitted the identification of the liver polysomes that synthesize FAS. There was good correlation between the time of appearance in liver of increased levels of FAS at 5 hr after refeeding the fat-free diet and the binding of the antibody to the polysomes. In addition, when an animal was switched to normal diet after a high level of FAS was attained in the liver, the rise of liver FAS stopped, and a decrease in the amount of antibody bound to polysomes was noted.

Correlative evidence that ¹²⁵I-labeled antibody against FAS is bound to the nascent chains of FAS on the polysomes has been obtained by using an in vitro translation system with the polysomes (31). In these studies the nascent chains were completed in vitro, and the products were precipitated with antibody against FAS and identified by NaDodSO₄-polyacrylamide gel electrophoresis. There was excellent agreement between the time when there was antibody-precipitable protein after completion of nascent chains and the
binding of $^{125}$I-labeled antibody to polysomes, as described in this communication. The major antibody-precipitable peptide that was synthesized in these studies had similar molecular weight, 240,000, as the subunits of native rat-liver FAS.

We thank Mr. Duane Martin for the iodination of the antibody. These studies were supported by Grant HL 10406 from the National Institutes of Health and by Grant GB-38676X from the National Science Foundation. A.W.S. is a postdoctoral fellow of the National Institutes of Health (AM 00280-01).