Protein Turnover and 4'-Phosphopantetheine Exchange in Rat Liver Fatty Acid Synthetase*

(Received for publication, November 27, 1970)

JOHN TWETO,† MARCIA LIBERATI, AND ALLAN R. LARRABEE
From the Department of Chemistry, University of Oregon, Eugene, Oregon 97403

SUMMARY

The rate of turnover of rat liver fatty acid synthetase has been measured in animals tested under steady state conditions. The value obtained for the half-life varied from 71 to 108 hours. The exchange rate of the covalently bound prosthetic group, 4'-phosphopantotheine, with unbound pantothetan compounds was found to be more than an order of magnitude greater than the half-life of the enzyme complex. A model is proposed in which the prosthetic group, or some small portion of the enzyme complex containing the prosthetic group, is periodically removed from and replaced on the larger fatty acid synthetase moiety, such action taking place many times before an individual complex is catabolized. Moreover, this exchange is not part of the catalytic function of an enzyme complex, but may be a means of control of over all fatty acid synthetase activity.

The de novo fatty acid-synthesizing mechanism of rat and pigeon liver has been shown to reside within a multichain peptide complex (1). This activity probably exists as a complex of enzymes, each catalyzing a reaction analogous to those described by Vagelos and Wakil (2) in Escherichia coli. None of these activities has been dissociated from the complex. Each mole of the rat and pigeon liver complexes contains a single 4'-phosphopantetheine group, which, in the case of the pigeon liver preparation, has been shown to be involved in the formation of a thioester with the growing fatty acid chain in a manner analogous to bacterial acyl carrier protein (3, 4).

Although work is in progress on the mechanism of synthesis and the control of fatty acid metabolism (5-10), little is known about control at the level of maintenance of integrity of the intact complex. In an effort to elucidate the contribution to control of fatty acid synthetase by mechanisms at this level, we have sought to obtain a value for the average half life of fatty acid synthetase proteins in rat liver under steady state conditions, and to compare this value with the exchange rate of the prosthetic group with unbound pantothetan compounds.

This report shows that the processes of synthetase turnover and of prosthetic group exchange are, on the average, rather widely separated in time, a fact which may indicate the existence of a mechanism for control of synthetase activity by disruption of the intact complex. The timing difference for these processes could be taken to imply the presence of an enzyme which removes the prosthetic group, as in the case of E. coli (11), or the removal of a protein portion of the complex containing the prosthetic group, or both.

MATERIALS AND METHODS

Materials—Malonyl-CoA, acetyl-CoA, TPNH, and phosphotransacetylase were purchased from Nutritional Biochemicals. Acetyl-CoA was also synthesized by the method of Simon and Shemin (12). Sigma Chemical Company provided acetylphosphate. DEAE-cellulose, purchased from Schwarz-Mann, was pretreated before use with base and acid as suggested by H. Reeve Angel and Company, New York, New York, for their Whatman products. Enzyme grade ammonium sulfate was also purchased from Schwarz-Mann. Calcium 4H-pantothenate (specific activity 580 mCi per mmole) was prepared by catalytic exchange by New England Nuclear from Ca2-pantothenate purchased from Calbiochem. Guanidino-14C-l-arginine (specific activity 4.6 mCi per mmole) was also obtained from New England Nuclear. Calcium phosphate gel was prepared by the method of Keilin and Hartree (13) and aged for 5 months, or alternatively, purchased from Calbiochem.

Animals—Animals utilized for this work were male Sprague-Dawley rats, weighing 150 g (± 15 g) at the time of purchase from Don B Lab Animals, Chatsworth, California. Animals were flown to Eugene, Oregon, and immediately fed Purina laboratory chow. The diet utilized by the supplier had the same protein and fat content as Purina laboratory chow (not less than 24% protein and 4% fat). Approximately 24 hours elapsed between the time of shipment and arrival in Eugene. All animals were kept at least 4 days before use. During the period of experimentation, the weight of the animals increased less than 10%.

Purification of Fatty Acid Synthetase and CoA—At appropriate times, rats were killed by decapitation. Liver supernatant solutions and fatty acid synthetase were purified as described by Burton, Haavik, and Porter (14) with the exception that the animals were not fasted and refed a fat-free diet and the gel filtration step was eliminated. The experiments reported here were performed on animals eating and drinking ad libitum. Different preparations of purified enzyme were in various states of dissociation, as evidenced by sedimentation velocity experi-
ments, and consequently the specific activity (nanomoles of TPNH oxidized per min per mg of protein) varied with different preparations. However, the lack of contaminating protein in purified preparations of fatty acid synthetase was verified by ultracentrifuge studies (14).

The absence of any bound radioactive CoA in the fatty acid synthetase preparations was verified by employing the procedure described by Larrabee et al. (15) for the identification of 4'-phosphopantetheine as prosthetic group. More than 90% of the label bound to a sample of enzyme was accounted for as 4'-phosphopantetheine and none was identified as CoA.

In certain experiments, when it was desired to isolate a sample of CoA, a 1- to 2-g aliquot of liver was taken when the animals were killed, and an acetone powder was made immediately as described by Brown (16). After storage over P2O5, this powder was extracted (16), and the CoA isolated on a DEAE-cellulose column (17). CoA was determined by assay with phosphotransacetylase (CoA 96%, from Nutritional Biochemicals, as standard (18)).

Assays—Protein determinations of purified rat liver fatty acid synthetases were made spectrophotometrically (19) or by use of the biuret reaction (20). Determination of protein by either procedure afforded similar values. The presence of 2H and 14C in all experiments was measured with a Nuclear-Chicago 720 series liquid scintillation system. Absolute efficiencies were determined with the use of internal standards. Protein samples were dissolved by the use of NCS (Nuclear-Chicago Corporation).

Supernatant Fraction—When it was desired to measure the amount of isotope in a protein-free supernatant fraction, a sample of tissue was homogenized in 10% perchloric acid and the radioactivity in this supernatant fraction was determined after centrifugation.

Ultracentrifuge Studies—Analytical ultracentrifuge velocity sedimentation determinations were carried out in a Spinco model E ultracentrifuge. The rotor temperature was 25° and the rotor speed was 59,750 rpm.

RESULTS AND DISCUSSION

Protein Turnover Studies—Recent work has emphasized the importance of both protein synthesis and degradation in the regulation of enzyme activities in mammals (22). Schimke (23) has described a technique, utilizing arginine labeled in the guanidino group, for the determination of the rate of turnover of various enzymes in rat liver. He has shown that proteins with half-lives significantly longer than 2 hours can be effectively pulse-labeled by administration of this amino acid in a single intraperitoneal injection. The problem of recycled label derived from protein degradation is minimized by the fact that arginine is rapidly removed via the urea cycle. By observing the rate of loss of label from the liver protein of rats injected with arginine, and with the assumption of first order kinetics, he was able to determine a rate of degradation of those proteins (23).

In order to determine the average rate of turnover of fatty acid synthetase, Schimke's method was employed. Each rat was maintained in an approximate steady state and was exposed to 15 μCi of 14C-guanidino arginine and 1.1 mCi of calcium-3H pantothenate via a single intraperitoneal injection. After specified times, two rats were killed, their livers pooled, and the fatty acid synthetase prepared. The natural logarithm of the specific activity (disintegrations per min per 14C per mg) of enzyme was plotted as a function of time up to 48 hours after injection, and a value for the half-life of fatty acid synthetase of 108 hours was calculated. Since the above experiment did not extend beyond even one half-life of the complex, the experiment was repeated (14C-guanidino arginine as the only injected compound) over a time period of 6 days.

Calculations based on the data shown in Fig. 1 yield a value of 7 1 hours for the mean half-life. The discrepancy between determination does not alter the interpretation placed on the data (see below), although 71 hours is probably the better figure. This value represents the mean for all the enzymes of the complex, leaving the question of different half-lives for different peptide chains unanswered.

![Fig. 1](left). Loss of label from fatty acid synthetase (FAS) as a function of time. At the times indicated after injection of 14C-guanidino-arginine (15 μCi), four animals were killed and the livers from two animals were pooled. Enzyme was purified from each pool of two livers as described under "Materials and Methods."

![Fig. 2](right). The specific radioactivity of fatty acid synthetase (FAS) and CoA as a function of time after a single injection of calcium-3H-pantothenate (see text). The actual specific radioactivity values for fatty acid synthetase and CoA at 48 hours were 2500 and 7600 dpm of 3H per umole, respectively.
Allmann, Hubbard, and Gibson (24) and Butterworth et al. (25) have conducted extensive studies on the disappearance of fatty acid synthetase in mammalian and avian liver during periods of fasting. Upon refeeding a fat-free diet, the levels of the multi-enzyme complex rise to suprannominal levels which were 40 to 60 times the level after fasting for 2 days. It was shown that during the refeed period, 14C-leucine was taken up into protein containing synthetase activity (26). They concluded that in fasted animals, only very small amounts of synthetase remain, and that during the refeed period, the complex was synthesized at a rapid rate, reaching maximum activity after 40 hours. If their conclusion is valid and the loss of activity represents total degradation of synthetase complex, the rapid rate of synthesis of complex they observed must be much greater than the steady state rate. This is clear, since in the case of the steady state, only half of the normal complement of fatty acid synthetase is replaced in 71 hours.

4'-Phosphopantetheine Exchange Studies—The prosthetic group of fatty acid synthetase in higher organisms is functionally analogous to that of the ACP of E. coli (2), which also contains 1 mole of 4'-phosphopantetheine. Edelsohn and Vagelos (27) have demonstrated that CoA is the immediate precursor of the 4'-phosphopantetheine prosthetic group of E. coli ACP, and that in exponentially growing cells of E. coli, the rate of turnover of the 4'-phosphopantetheine group is 4 times the rate of growth of the ACP pool (28). In order to pursue the analogy further, the work described here examines the possibility that once the prosthetic group is added to the multi-enzyme complex, it remains in metabolic equilibrium with unbound pantothenate compounds.

In Fig. 2, a characteristic time course of the incorporation of tritium-labeled pantothenate into both CoA and into purified fatty acid synthetase complex is presented. Although the qualitative shape of the curve representing specific activity rises with respect to time is quite reproducible, especially in the case of the protein, little significance can be attached to the quantitative values of specific activity obtained, since these vary between experiments, due presumably to the differing sizes of animals and alteration of precise nutritional states. In experiments of longer duration, the CoA specific activity can be seen to decline after 40 to 50 hours, while the protein curve remains essentially flat between 24 and 140 hours.

Two facts are evident from the curves in Fig. 2. First, the results of this time course experiment are consistent with the implication of CoA as a precursor to the prosthetic group of fatty acid synthetase. This suggestion derives from the timing of the rise of specific activity of the CoA curve, which parallels that of the enzyme labeling profile. However, since the existence of separate, slowly exchanging CoA pools cannot be ascertained, the precursor-product explanation may not be unique. Moreover, a model describing an assembly of pantothenate compounds (perhaps including CoA) acting as a precursor of the prosthetic group is not excluded. It should be reiterated here that once the prosthetic group is added to the multi-enzyme complex, it remains in metabolic equilibrium with unbound pantothenate compounds.

A third feature of the curve in Fig. 2 is the pronounced lag prior to the abrupt increase in specific activity. We are unable to interpret this phenomenon in terms of simple requirements for precursor labeling or the possibility of delayed penetration of the liver with label. The former explanation seems unlikely, since one expects that the gradual build-up of label in precursor would yield a low but definite increase in labeling of the complex from the time of injection. The latter explanation is unlikely, since other experiments demonstrate that the blood supernatant level of labeled compounds rises rapidly and subsequently reaches its lowest value within 2 hours after injection, whereas label in the liver supernatant rises hyperbolically from the time of injection. In addition, Fig. 1 demonstrates that the arginine label is quickly incorporated in the liver.

Further work is in progress in this laboratory to determine whether all the individual enzymes of the multi-enzyme complex have the same turnover rate. If mammalian ACP exists as a small molecule relative to the 540,000 weight of fatty acid synthetase (bacteria, plant (29), and yeast ACP molecules (30) all have molecular weights of approximately 9,500 to 16,000), it is possible for the protein of ACP to turn over at a rate equal to that of the prosthetic group while the bulk of the protein of the complex is turned over at a much slower rate.

Since the complex cannot be yet be fractionated into its component enzymes, we are attempting to measure the rates of turnover of isolated peptides from an enzymatic digest of purified labeled complex. This approach should be especially fruitful, since labeling of fatty acid synthetase with both tritiated pantothenate and 14C-labeled amino acids allows an easy identification of certain peptides derived from ACP.

1 The abbreviation used is: ACP, acyl carrier protein.
Acknowledgments—We wish to thank Dr. Frank Reithel and Judy Holt for their assistance with the ultracentrifuge studies.

REFERENCES

Protein Turnover and 4'-Phosphopantetheine Exchange in Rat Liver Fatty Acid Synthetase

John Tweto, Marcia Liberati and Allan R. Larrabee


Access the most updated version of this article at http://www.jbc.org/content/246/8/2468

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/246/8/2468.full.html#ref-list-1