Lipophilic Soluble Protein Represents Labile Protein in Rat Liver

Atsushi Sato,1 Yuzo Hiroi,2 and Yasuo Natori1

1Department of Nutritional Chemistry, School of Medicine, The University of Tokushima, Tokushima 770, Japan
2Department of Food and Nutrition, Nakamura Gakuen College, Jonan-ku, Fukuoka 814, Japan

(Received January 31, 1992)

Summary Lipophilic proteins, having higher turnover rates than the average of the total soluble protein pool, were separated from soluble rat-liver proteins on alkyl agarose column. The quantitative immunoprecipitation, using the antibody against the lipophilic proteins, showed that the soluble proteins from the liver of fed rats contained about twice as much lipophilic proteins as that of 3-day-starved rats. This result indicates that the lipophilic proteins, at least in part, represent labile protein in rat liver.

Key Words labile protein, lipophilic protein, rat liver

It has long been recognized that there exists a labile reserve of body protein which accumulates under favorable nutritional conditions and is readily dissipated under adverse nutritional circumstances. This labile material has been referred to as "labile protein" or "reserve protein" (1). Differences in rates of loss of proteins from various tissues during starvation have been noted by many investigators; in starving rats, the liver loses its initial protein content more rapidly and extensively than most other organs (1). This observation has led several investigators to look for a discrete fraction of liver protein having the characteristic of "labile protein". Luck (2) separated the proteins of rat liver chemically into four fractions and compared the effect of protein depletion on the total protein content of each fraction. His experiment, however, provided no evidence of a chemically discrete store lost on depletion. This negative finding has been confirmed by other methods of protein separation (3–5). The possible existence of specific reserve or preferentially labile protein in the liver is, therefore, a still unsettled question.

Segal et al. (6) and Bohley et al. (7) separated soluble rat-liver proteins according to their lipophilic affinities and found that the more lipophilic proteins had higher degradation rates in vivo. We separated the lipophilic protein from soluble rat-liver proteins on an alkyl agarose column and raised the anti-serum against the lipophilic protein. The relative contents of the lipophilic protein in the
total pool of soluble proteins were estimated by quantitative immunoprecipitation. It was found that the soluble proteins from the liver of starved rats contain relatively less amount of the lipophilic protein than those from the liver of fed rats. This result indicates that the lipophilic proteins, at least in part, represent labile proteins in rat liver.

MATERIALS AND METHODS

Animals. Male Wistar rats weighing about 225 g were housed in individual cages in a controlled environment (temperature 23±1°C, and light from 0700 to 1900 h). One group of the animals was fed a commercial diet (CE-2, Japan Clea Co., Tokyo) and the other group was starved for 3 days. Drinking water was given ad libitum.

Preparation of soluble rat-liver proteins. Rats were sacrificed and the livers perfused thoroughly with cold 0.15 M NaCl, then homogenized in 3 vol. of the homogenizing buffer (0.15 M NaCl in 0.05 M sodium phosphate buffer, pH 7.4) with a Potter-Elvehjem homogenizer. The homogenate was centrifuged for 15 min at 3,000 × g and the supernatant fluid therefrom recentrifuged at 104,000 × g for 60 min to obtain the soluble protein fraction.

Lipophilic chromatography of soluble proteins. CNBr-activated Sepharose 4B (Pharmacia Fine Chemicals, Uppsala) was coupled with hexamethylenediamine to prepare aminohexyl agarose as described by Shaltiel and Er-el (8). Ten ml of the soluble liver-protein fraction, containing 200 mg of protein, were added to the aminohexyl agarose column (5.0 × 1.5 cm), equilibrated with the homogenizing buffer, and then developed with the same buffer. Approximately 1.5 ml fractions were collected. After the non-adsorbed protein had flowed through the column, as monitored by a return of the 280 nm absorbance to the baseline, the adsorbed lipophilic protein was eluted by a linear NaCl (0.05–1.0 M) gradient.

Double isotope labeling of soluble proteins. In order to distinguish turnover rates in the protein pool, the double isotope technique of Arias et al. (9) was employed. Rats were fasted 18 h before an intraperitoneal injection of 5 µCi of L-[U-14C]leucine (330 Ci/mol, Daiichi Pure Chemicals Co., Tokyo). Food supply was restored 4 h later. After 3 days the same schedule was repeated, except that the animals were each injected with 10 µCi of L-[4,5-3H]leucine (32 Ci/mmol, Daiichi Pure Chemicals Co., Tokyo). The animals were sacrificed 4 h after the second injection and the soluble liver proteins were prepared as described above. Protein samples were precipitated with the addition of 2 vol. of 10% (w/v) trichloroacetic acid and washed with 5% (w/v) trichloroacetic acid. The samples were dissolved in Soluene 100 (Packard Instrument Co., Illinois), mixed with toluene-based scintillator fluid and counted in an Aloka LSC-700 liquid scintillation counter.

Quantitative immunoprecipitation of lipophilic protein. The antiserum was raised against the lipophilic proteins prepared by lipophilic chromatography as described above. Male New Zealand white rabbits were immunized with the
Lipophilic proteins dissolved in 10 mM Tris-HCl buffer (pH 7.4) and emulsified in complete Freund's adjuvant (1:1 v/v). Each animal received subcutaneous injections on days 1, 7 and 15; the dose was 2.0 mg of the lipophilic proteins in a total volume of emulsion of 0.5 ml. Booster injections of 2.0 mg lipophilic proteins in the emulsion of 50% (v/v) Freund's incomplete adjuvant were given 1 week later. The animals were bled weekly after the booster injection. Gamma globulin fraction was isolated from the antiserum by ammonium sulfate precipitation as described by Garvey et al. (10). The quantitative immunoprecipitation of the lipophilic proteins was performed as described also by Garvey et al. (11) except that the protein was determined by the method of Lowry et al. (12), with bovine serum albumin as a standard.

RESULTS

Effect of starvation on body and liver weights of rats

Body and liver weights of rats fed or starved for 3 days are shown in Table 1. At the time of sacrifice, the body and liver weights of the starved rats were 76% and 64% of the fed rats, respectively. The magnitude of the loss in the liver weight upon starvation was greater than that in the total body weight. This observation is consistent with the idea that the liver is an important organ for the storage of labile protein.

Separation of lipophilic protein from soluble rat-liver proteins

Rats were double-labeled with 14C- and 3H-leucine and soluble liver proteins were prepared. The double-labeled soluble proteins were then subjected to lipophilic chromatography on an aminohexyl agarose column. The elution pattern is shown in Fig. 1. After the non-adsorbed protein had flowed through the column, the adsorbed protein was eluted by a linear NaCl gradient. It can also be seen in Fig. 1 that the 3H/14C ratio of the adsorbed fraction was significantly higher than that of the non-adsorbed fraction, indicating that the adsorbed lipophilic fraction indeed represents proteins of higher turnover rates than the average of the total soluble protein pool. The adsorbed protein amounted to 7% of the total protein added to the column.

Table 1. Body and liver weights of rats fed or starved for 3 days.

| Group   | Body weight | Liver weight (g) |
|---------|-------------|------------------|
|         | Initial (g) | Final (g)        |                   |
| Fed     | 225±7       | 235±2            | 14.9±0.2          |
| Starved | 225±4       | 180±8            | 9.5±0.1           |

Values are means±SD for 4 animals in each group.

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Fig. 1. Lipophilic chromatography of soluble rat-liver proteins on aminohexyl agarose column. Double-labeled soluble proteins (200 mg), containing 110,000 dpm of $^{14}$C and 250,000 dpm of $^3$H, were applied on aminohexyl agarose column. Non-adsorbed protein was washed off until the absorbance at 280 nm (○) dropped below 0.05, then a linear NaCl gradient was applied. Fractions (1.5 ml) were collected and their $^3$H/$^{14}$C ratios (●) were determined. Non-retarded (I), slightly-retarded (II) and adsorbed (III) fractions were separately pooled.

Estimation of lipophilic protein content in soluble proteins by quantitative immunoprecipitation

The lipophilic protein fraction (Fraction III in Fig. 1) was pooled and the antiserum was raised in rabbits. Quantitative immunoprecipitation, using anti-lipophilic-protein antibody, was performed with the non-retarded (Fraction I), slightly-retarded (Fraction II) and adsorbed (Fraction III) proteins separated in Fig. 1. The result in Fig. 2 shows that the antibody reacted strongly with the adsorbed protein and weakly with the slightly-retarded protein. The non-retarded protein did not produce any precipitation with the antibody. Thus the antibody appears to specifically recognize the lipophilic protein among the total soluble protein pool. It should be noted, however, that the precipitation reaction with the adsorbed protein (Fraction III) caused precipitation of only a small portion of the added fraction.

Employing the anti-lipophilic-protein antibody, the relative content of lipophilic protein in the rat-liver soluble proteins was estimated by quantitative im-
Fig. 2. Quantitative precipitation reaction between various protein fractions from lipophilic chromatography of soluble rat-liver proteins and antibody against lipophilic protein. Varying amounts of protein fractions were added to the fixed amount (1.5 mg) of anti-lipophilic-protein IgG. I, II and III represent pooled protein fractions I, II and III of Fig. 1, respectively. Error bar denotes ±1 SD of the mean of 4 determinations.

munoprecipitation. As shown in Fig. 3, the point of maximum precipitation with the soluble proteins from the starved rats was about twice that from the fed rats. This result indicates that the relative content of the immunoprecipitable lipophilic protein in the soluble liver protein pool is almost halved by starvation for 3 days.

DISCUSSION

Adverse nutritional conditions, such as starvation, cause a rapid loss of liver proteins. The findings of the present study show that some lipophilic proteins are preferentially lost in the liver during starvation and therefore can be regarded as "labile protein".

Several investigators have looked for a specific fraction of liver proteins having the characteristic of labile protein. These workers fractionated the soluble liver proteins on the basis of salt concentration, pH or electrophoretic mobility, but failed to find any fractions which were more labile than the others (2-5). Our demonstration of the lipophilic protein as labile protein was based on quantitative immunoprecipitation of the double-labeled lipophilic protein with the antibody. Probably the previously employed fractionation methods were not sufficiently specific to detect the labile protein fraction.

A marked correlation has been found between lipophilicity of proteins and...
their degradation rates in vivo (6, 7). The present demonstration of lipophilic protein as labile protein may simply mean that proteins with rapid turnover rates are lost preferentially during nutritional deprivation. A correlation is also known between half-lives of proteins in vivo and their isoelectric points (13). We recently found that, in the liver of neonatal chicks, acidic proteins are more responsive to changes in nutritional states than basic proteins, probably because the turnover rates of acidic proteins are higher than those of basic proteins (14). This observation is also in line with the idea of labile protein as rapidly-turning-over protein.

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