Immunological Studies of Lipogenic Enzymes during Aging

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Summary The effects of aging on enzyme qualities of glucose-6-phosphate dehydrogenase, malic enzyme and acetyl-CoA carboxylase were investigated. The Ouchterlony double-diffusion pattern showed that the enzymes from livers of rats of different ages are immunologically similar to each other. In addition, immunochemical titration demonstrated that the changes in the levels of activity of rats of different age are accompanied by proportionate changes in the quantity of the enzyme protein. The K_m s of the enzymes for substrates were not altered by aging. It is suggested that the age-dependent impairments of the enzyme induction (Iritani, N., Fukuda, H., and Fukuda, E. (1981) *Biochim. Biophys. Acta*, **665**, 639) can be ascribed to decreases in the rise of enzyme protein quantities.

Key Words lipogenic enzymes, aging, immunochemical analysis, K_m

In our previous experiments (1), when 1-, 2- and 9-month-old rats previously adapted to a commercial stock diet were fed on a fat-free diet (induction), the turnovers of lipogenic enzymes in liver were measured by following the time courses of the changes of the enzyme activities. The magnitudes of the induction of the enzymes were very high in 1-month-old rats and then decreased with aging. In this case, the rates of synthesis of the enzymes markedly decreased with increasing age, whereas the rate constants of degradation were rather raised with growth up to 2 months and then reduced in 9-month-old rats, but not so much as those of synthesis. It is suggested that the age-dependent impairments of the enzyme induction may be due to some alterations in the enzyme-forming system. Further, the effects of aging on enzyme qualities were investigated kinetically and immunologically in the present experiment.

MATERIALS AND METHODS

Treatment of animals. Male Wistar rats of three different ages were used for the

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experiments: body weights were 128 ± 6.5 g, 374 ± 10 g and 551 ± 38 g for 1- (5-week), 2- (8–10 weeks) and 9- (8–10 months) month-old rats, respectively. The rats were fed and watered *ad libitum*, and maintained under an automatic lighting schedule from 7 a.m. to 7 p.m. at 24°C. They were allowed to adapt themselves to a fat free-high carbohydrate diet. The fat-free diet contained 67.4% sucrose, 18% casein, 9.5% cellulose, 5% salt mixture(2), 0.1% choline chloride and vitamin mixture(2). Rats were killed by decapitation between 10 and 11 a.m. The liver was quickly removed to measure the activities of lipogenic enzyme in the $105,000 \times g$ supernatant.

Enzyme assays. Glucose-6-phosphate dehydrogenase [EC 1.1.1.49] was assayed according to Glock and McLean (3) and malic enzyme [EC 1.1.1.40] was assayed according to Ochoa (4). Acetyl-CoA carboxylase activity [EC 6.4.1.2] was assayed by the $H^{14}CO_3^-$ -fixation method (5). One mUnit is defined as 1 nmol substrate utilized for glucose-6-phosphate dehydrogenase and malic enzyme, and product formed for acetyl-CoA carboxylase per min at 37°C.

Immunological procedures. As described previously, antiacetyl-CoA carboxylase serum was prepared and the immunoglobulin was isolated by ammonium sulfate precipitation (6). Glucose-6-phosphate dehydrogenase and malic enzyme were purified from rat liver, essentially according to Matsuda and Yugari (7) and Rutter and Lardy (8), respectively. For further purification, each enzyme was chromatographed on an affinity column containing immobilized N^6 -(6-aminohexyl)-adenosine 2',5'-bisphosphate and eluted specifically by NADP, essentially according to Yeung and Carrico (9). The antisera and the immunoglobulins of the enzymes were prepared as described previously (6). Ouchterlony double-diffusion analyses (10) with the use of antibodies against the enzymes gave single precipitation bands. The enzyme preparations of glucose-6-phosphate dehydrogenase, malic enzyme and acetyl-CoA carboxylase were the precipitate fractions of $105,000 \times g$ liver supernatant resulting from 70%, 70% and 30% saturation with ammonium sulfate, respectively.

For immunochemical titrations, increasing amounts of the enzyme preparations were added to the antibodies. After standing overnight at 4°C, the activities of the supernatant were assayed following precipitation.

RESULTS AND DISCUSSION

As shown by Lineweaver-Burke plots represented in Fig. 1, the K_m of the lipogenic enzymes for their substrates and also the K_m of acetyl-CoA carboxylase for citrate (an effector for activation) were not changed by aging.

We conducted respective immunochemical analyses of glucose-6-phosphate dehydrogenase, malic enzyme and acetyl-CoA carboxylase preparations obtained from rats of the three age groups. The complete connections of the precipitate bands were clearly observed in all age groups as shown in Fig. 2. It was shown by the Ouchterlony double-diffusion pattern that the enzymes from livers of rats of

Fig. 1. Effect of aging on Lineweaver-Burke plots of reaction velocity, and substrate or effector concentration. The enzyme preparations used were the same ones as used for the immunological procedures. See MATERIALS AND METHODS for the enzyme assays and enzyme units. $\circ$, 1-month-old; $\bullet$, 2-month-old; $\triangle$, 9-month-old. A, glucose-6-phosphate dehydrogenase; B, malic enzyme; C and D, acetyl-CoA carboxylase.

different ages are immunologically similar to each other.

In addition, immunochemical titration clarified this information. Figure 3 (A, B, C) show the respective immunochemical titrations of glucose-6-phosphate dehydrogenase, malic enzyme and acetyl-CoA carboxylase of liver supernatants from rats of different age groups adapted to the fat-free diet. Despite the fact that the specific activities of the enzymes were greatly altered by age (shown in the legend of Fig. 3), the equivalence points (the points at which enzyme activities first appeared in the supernatant) were the same for all groups. The results showed that the changes of these enzymes were accompanied by proportionate changes in the quantities of immunochemically reactive proteins. Thus, it is suggested that there are no effects on K_m and immunochemical quantities of lipogenic enzymes caused by aging. Yagil (11) also reported that evidence for the existence of an appreciable part

Fig. 2. Ouchterlony double-diffusion analysis of lipogenic enzymes. (A) and (B) (glucose-6-phosphate dehydrogenase and malic enzyme)—Central well: anti γ -globulin (0.87 mg); upper wells, 1, 2 and 9-mo contained 0.46 mg of enzyme protein of 1-, 2- and 9-month-old rats, respectively, whereas lower wells, 1, 2 and 9-mo contained 0.12 mg of the same enzymes as in the upper wells. (C) (acetyl-CoA carboxylase)—Central well: anti γ -globulin (0.64 mg); wells 1, 2 and 9-mo contained 1.2 mg of enzyme protein of 1-, 2- and 9-month-old rats, respectively whereas the lower wells contained 0.3 mg.

of glucose-6-phosphate dehydrogenase in altered form could not be found from immunoelectrophoresis using aged mice.

The specific activities of glucose-6-phosphate dehydrogenase, malic enzyme and acetyl-CoA carboxylase (used for immunochemical titration) in 2-month-old rats were 53.8, 42.7 and 64.3% of those in 1-month-old rats, respectively, and in 9-month-olds, 26.0, 21.1 and 49.6% of those in 1-month-olds, respectively. In our previous study (1), on feeding a fat-free diet, the rates of synthesis of glucose-6-

Fig. 3. Immunochemical titration of lipogenic enzymes. Increasing amounts of $105,000 \times g$ liver supernatant solutions containing enzyme activities indicated were added to 109 mg (A), 0.21 mg (B) or 0.03 mg (C) of anti γ -globulin. Following completion of precipitation, the supernatant fluids were assayed for enzyme activities. A, glucose-6-phosphate dehydrogenase; 537, 289 and 140 munits/mg protein in 1-($\circ$), 2-($\bullet$) and 9-($\triangle$)month-old rats, respectively. B, malic enzyme; 522, 223 and 110 munits/mg protein, and C, acetyl-CoA carboxylase; 28.0, 18.0 and 13.9 munits/mg protein in 1-, 2- and 9-month-old rats, respectively.

phosphate dehydrogenase, malic enzyme and acetyl-CoA carboxylase in 2-month-old rats were 61, 57 and 83% of those in 1-month-old rats, respectively, and in 9-month-olds, only 16, 18 and 41% of those in 1-month-olds, respectively. However, the rate constants of degradation of these enzymes were not as greatly reduced on aging as compared to those of synthesis thereof. As it is demonstrated that the immunological qualities of the enzyme proteins are not altered with aging, the age-dependent impairments of the enzyme induction can be ascribed to the decreases of the rise of enzyme protein quantities due to some kind of alteration in the enzyme-forming system.

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