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EFFECT OF AGING ON INDUCTION OF RAT LIVER MESSENGER RNA ACTIVITY FOR MALIC ENZYME

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Rats were fasted and then refed a high carbohydrate-fat free diet, and the activities of the mRNA coding for liver malic enzyme [EC 1.1.1.40] in 6-week-old and 10-month-old male rats were determined by in vitro translation of the liver cytoplasmic poly(A)-containing RNA in a rabbit reticulocyte lysate. After refeeding the mRNA activities of the young rats were about 7-fold of those of the aged rats, and roughly parallel to the enzyme activities. This suggests that the age-dependent impairment of the enzyme induction [Iritani, N. et al. (1981) Biochim. Biophys. Acta 665, 636] can be ascribed to the decrease of mRNA activity.

When 1-, 2- and 9-month-old rats previously adapted to a commercial stock diet were fed a fat free diet, the magnitudes of induction of lipogenic enzymes were very high in 1-month-old rats and decreased with age (1). The rates of synthesis of glucose-6-phosphate dehydrogenase, malic enzyme and acetyl-CoA carboxylase in 9-month-old rats were markedly decreased to 16.1%, 18.2% and 40.8% of those in 1-month-old rats, respectively. Further, immunochemical analyses showed that the enzymes from livers of different age rats are immunologically similar to each other (Fukuda, H., Katsurada, A., Iritani., submitted).

The present study was initiated to elucidate a possible mechanism causing the reductions of lipogenic enzyme syntheses in aged rats by measuring the cellular activities of the mRNA coding for the enzymes. We present evidence that the activity of malic enzyme mRNA is increased with the enzyme activity

Abbreviation poly(A)$^+$-RNA: poly(A)-containing RNA

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by refeeding a fat free diet after fasting, and the increase of translatable mRNA level is reduced in aged rats as compared to young rats.

MATERIALS AND METHODS

Animals Male Wistar rats of 6-week-old and 10-month-old rats were fasted for 2 days and refed a high carbohydrate-fat free diet (1,2) for 3 days. The rats were killed by decapitation, and the livers were quickly removed and immediately frozen with the metal plates chilled with dry ice-acetone.

Enzyme purification and antibody preparation Malic enzyme of rat liver was purified essentially according to Hsi and Lardy (3) with a modification. After purification with DEAE-cellulose (Sigma), the enzyme was further chromatographed on an affinity column containing immobilized N^6-(6-aminohexyl)-adenosine 2',5'-bisphosphate according to Yeung and Carrico (4). The antiserum to rabbit was prepared essentially according to Marshall and Cohen (5). The antibody was purified by affinity chromatography on a protein A-Sepharose Cl-4B column (Sigma) (6).

Isolation of poly(A)$^+$-RNA Total cellular RNA was isolated from frozen liver samples by extraction with 4 M guanidium thiocyanate (Fluka purum grade, Tridon Inc.) as described by Chirgwin et al. (7). Poly(A)$^+$-RNA was separated from the total RNA extract by chromatography on oligo(dT) cellulose (Collaborative Research, Type III) (8).

Translation of malic enzyme poly(A)$^+$-RNA in reticulocyte lysate system Assays for malic enzyme mRNA activity were carried out with a nuclease-treated reticulocyte lysate system (9). The translation mixture contained 20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (Hepes)(pH 7.4), 20 μM each of 19 amino acids(all except methionine), 75 μCi [^{35}S]methionine (1000-1260 Ci/mmol; New England Nuclear), various amounts of poly(A)$^+$-RNA and nuclease-treated rabbit reticulocyte lysate in total volume of 80 μl (10). After incubation at 30°C for 60 min, the reaction was stopped by the addition of 370 μl of 20 mM Tris/HCl, pH 7.4 containing 10^{-5}M $NADP^+$, 10^{-3}M EDTA, pH 7.4 and 10 mM mercaptoethanol, and centrifuged at 105,000 x g for 60 min. Aliquots of 5 μl of the supernatant were taken for determination of radioactivity in total released proteins (Trichloroacetic acid-insoluble materials were determined according to Pelham et al. (9).).

Immunoprecipitation and electrophoresis Malic enzyme was immunoprecipitated from 400 μl of the supernatant with the antibody and formalin-fixed Staphylococcus aureus (Zymed Laboratories) (11,12) in the presence of 0.5% Nonidet P-40, 0.5% desoxycholate and 0.8 mg of rat albumin. Antigen-antibody-bacteria complexes were washed four times with 50 mM Tris/HCl (pH 7.4), 5 mM EDTA, 0.3 M NaCl, 0.5% Nonidet P-40, 0.5% desoxycholate, and 1 mg/ml ovalbumin. Five micrograms of purified malic enzyme was added to the immunoprecipitates. Then, the immunoprecipitates were dissociated and subjected to electrophoresis on 10% SDS-polyacrylamide slab gels by the method of Laemmli (13). Protein bands were located by staining with Coomassie brilliant blue R250. The radioactive bands on slab gels were located by fluorography using EN^{3}HANCE (New England Nuclear). The gels were cut into 2 mm wide slices and the radioactivity eluted from the gel slices with NCS tissue solubilizer (Amersham). Radioactivity in each sample was determined in a liquid scintillation spectrometer.

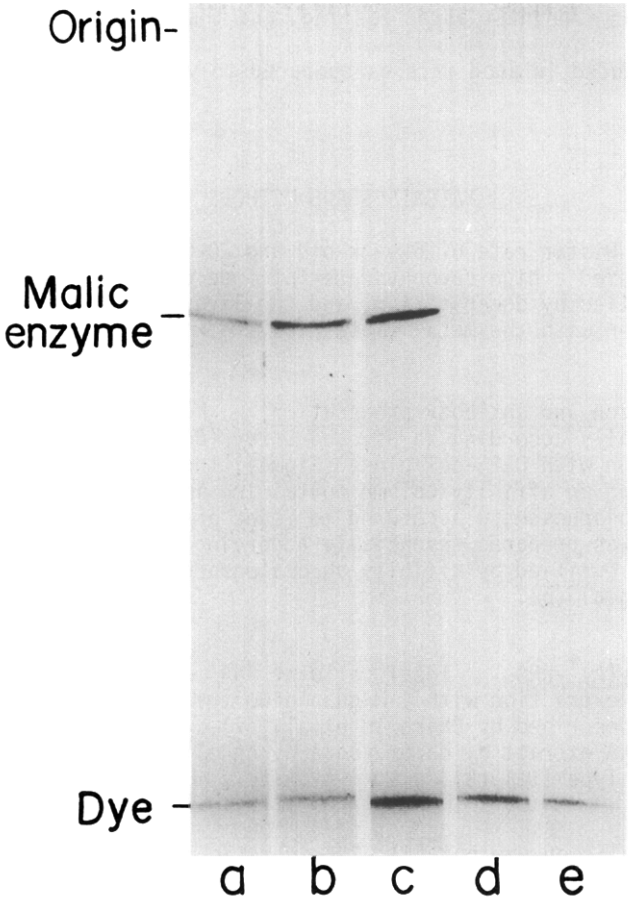

Fig. 1 Fluorogram of dodecylsulfate/polyacrylamide gel electrophoresis of the cell-free translation product. [^{35}S]Methionine was incubated with poly(A)$^+$-RNA in a rabbit reticulocyte lysate system. The methionine-labelled translation products were added 0.1 µg of purified malic enzyme and subjected to indirect immunoprecipitation with antibody. The resulting immunoprecipitates were analyzed as described in Materials and Methods. Lane (a), (b) and (c): immunoprecipitates formed with antibody to malic enzyme; 10, 20 and 30 µg/ml of poly(A)$^+$-RNA were used for (a), (b) and (c), respectively. Lane (d): normal immunoglobulin G replaced the anti-malic enzyme IgG. Lane (e): immunoprecipitate formed with antibody to malic enzyme and 10 µg malic enzyme. 20 µg/ml of poly(A)$^+$-RNA were used for (d) and (e). The mobility of authentic malic enzyme is indicated to the left of the gels.

RESULTS AND DISCUSSION

Poly(A)$^+$-RNA from rat livers was translated in the mRNA-dependent reticulocyte lysate cell-free system, in the presence of [^{35}S]methionine. The products isolated by indirect immunoprecipitation with antibody to malic enzyme were analysed by electrophoresis on a dodecylsulfate/polyacrylamide slab gel. The fluorogram is shown in Fig. 1. The radioactive band in the

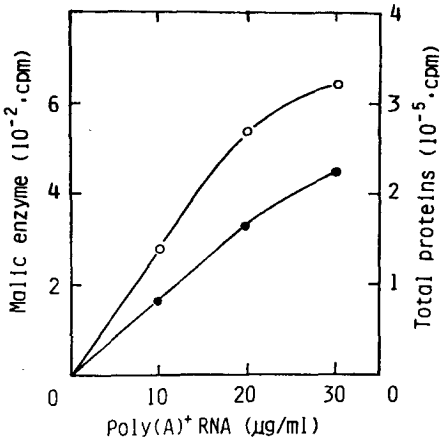

Fig. 2 Effects of various amounts of poly(A)$^+$-RNA on [^{35}S]methionine incorporation into total proteins and malic enzyme. The [^{35}S]methionine incorporations in the lane (a), (b) and (c) of Fig. 1 are shown. The indicated concentrations of Poly(A)$^+$-RNA were contained in the reaction mixture (80 μl) to protein synthesis. Total poly(A)$^+$-RNA activity (o) is expressed as the amount of [^{35}S]methionine incorporated into total protein of the reaction mixture. The activity (●) of malic enzyme poly(A)$^+$-RNA was assayed by measuring the radioactivity band of immunoprecipitate formed with antibody to malic enzyme.

immunoprecipitate formed with antibody to malic enzyme migrated with a mobility identical to the band of the polypeptide of authentic malic enzyme (stained with Coomassie blue). The intensities of radioactive bands seem to be in parallel to the amounts of poly(A)$^+$-RNA. When normal immunoglobulin G was added to the lysate instead of the antibody, no radioactive band was observed. The addition of a large excess of unlabelled malic enzyme to the translation product prior to immunoprecipitation with antibody also resulted in the disappearance of the radioactive band corresponding to malic enzyme. These results indicated that the radioactive bands represent malic enzyme synthesized in the reaction mixture. Activities of mRNA for malic enzyme in rat liver were determined by measuring the radioactivity.

As shown in Fig. 2, the amount of malic enzyme as well as total proteins synthesized in the cell-free system was proportional to the amount of poly(A)$^+$-RNA added to 20 μg/ml of the reaction mixture. Therefore, this assay can be used for quantitative purposes.

TABLE I THE EFFECT OF AGING ON CHANGES IN THE ACTIVITIES OF MALIC ENZYME AND POLY(A)$^+$-RNA CODING FOR MALIC ENZYME OF RAT LIVER DURING FASTING AND REFEEDING

	Malic enzyme activity	Protein synthesis		Relative synthesis of malic enzyme to total protein
		Total protein	Malic enzyme	
	nmol/min/mg	cpm x 10^{-5}	cpm	% x 10^{-2}
6-week-old				
Fasted	20.3 ± 1.09	5.78 ± 0.10	31 ± 5	0.54 ± 0.09
Refed	353 ± 20.4	5.22 ± 0.43	492 ± 41	9.40 ± 0.13
10-month-old				
Fasted	18.7 ± 3.87	5.66 ± 0.38	33 ± 8	0.57 ± 0.10
Refed	96.1 ± 7.85	7.55 ± 2.18	108 ± 30	1.43 ± 0.06

Poly(A)$^+$-RNA isolated from the rat livers were incubated with [^{35}S]methionine at 30°C for 60 min in a rabbit reticulocyte lysate system. The translation mixture contained 20 mM Hepes (pH 7.4), 20 µM each of 19 amino acids (all except methionine), 75 µCi of [^{35}S]methionine, 20 µg/ml of the poly(A)$^+$-RNA and nuclease-treated reticulocyte lysate in total volume of 80 µl. The methionine-labelled translation products were separated as shown in Fig. 1 and counted. Malic enzyme activity is expressed as nmol substrate utilized/min/mg of soluble protein at 37°C. Results show mean ± S.E. (n=3)

Activities of translatable malic enzyme mRNA varied in parallel with changes in enzyme activities, as shown in Table 1. The ability of added RNA to stimulate protein synthesis is similar regardless of the dietary state and the age of animals. In the fasted rats, the synthesis of malic enzyme was very low. In the refed rats, the malic enzyme syntheses were 0.094% and 0.014% of those of total proteins in 6-week-old and 10-week-old rats, respectively. Refeeding the fat free-high carbohydrate diet after fasting caused 17.4-fold and 2.5-fold increases to the corresponding fasted rats in malic enzyme mRNA activities of the young and aged rats, respectively. The activity of malic enzyme mRNA after refeeding was about 7-fold higher in the young rats than in the aged rats. The increases were roughly parallel to the enzyme activities. We have established that changes in the concentration of immunoprecipitable malic enzyme exactly parallel changes in malic enzyme activity during aging (Fukuda, H., Katsurada, A., Iritani, N., manuscript in submission). Therefore, it is suggested that the age-dependent impairment in the enzyme induction can be ascribed to the decrease in the rise in enzyme protein quantity due to the change of mRNA activity.

Correlation between enzyme synthesis and mRNA activity has been reported for lipogenic enzymes such as fatty acid synthetase, 6-phosphogluconate dehydrogenase and malic enzyme (14-19). Glucose-6-phosphate dehydrogenase is reported to be regulated partly by the mRNA activity (20). Siddiqui et al. (18) reported that translatable levels of malic enzyme mRNA paralleled changes in relative synthesis of malic enzyme in neonatal ducklings. Towle et al. (19) also reported that the induction of malic enzyme in rat liver by hormonal or dietary change is due to an increase in the activity of mRNA coding for this protein. The set of lipogenic enzymes may be coordinately regulated at the level of the mRNA activity in response to nutritional or hormonal variation. The responses seem to be reduced with aging.

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