INCREASE IN ACETYL COENZYME A CARBOXYLASE-SYNTHESIZING POLYSOMES IN THE LIVERS OF GENETICALLY OBESE MICE

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Received 3 May 1976

1. Introduction

Genetically obese hyperglycemic mice (C57BL/6J-*ob*), carrying a single recessive mutant gene for obesity [1], exhibit an increased level of hepatic lipogenesis [2]. In the livers of these mice, the level of activity of acetyl coenzyme A carboxylase (acetyl-CoA:carbondioxide ligase (ADP-forming), EC 6.4.1.2), which plays a critical role in the regulation of long-chain fatty acid biosynthesis [3], is markedly elevated [4]. Our previous studies with immunochemical and other methods have shown that this rise in the level of hepatic acetyl-CoA carboxylase activity can be ascribed to an increase in the quantity of the enzyme protein [5]. In addition, it has been demonstrated with combined immunochemical and isotopic techniques that the increase in the acetyl-CoA carboxylase content of the liver is due mainly to accelerated synthesis of the enzyme, and in a minor degree, to diminished degradation of the enzyme [5].

Recently, we have been able to identify specific polysomes involved in the synthesis of acetyl-CoA carboxylase by the binding of ^{125}I-labeled antiacetyl-CoA carboxylase to rat liver polysomes [6]. Using this binding technique, we have shown that the relative content of acetyl-CoA carboxylase-synthesizing polysomes in the liver correlates well with the rate of hepatic synthesis of the enzyme in rats subjected to different dietary conditions as well as in alloxan-diabetic rats with or without insulin treatment [6]. The present investigation has demonstrated that the relative content of acetyl-CoA carboxylase-synthesizing polysomes in the liver is higher in obese mice than in non-obese mice. This increase in the amount of the specific polysomes can nearly account for the higher rate of hepatic synthesis of acetyl-CoA carboxylase in obese mice.

2. Materials and methods

Male obese hyperglycemic mice (C57BL/6J-*ob*) and their male non-obese littermates were obtained from the Laboratory Animal Centre, Wakayama Medical College, Wakayama, Japan. Animals were fed a balanced diet (Oriental Yeast, Tokyo, Japan) ad libitum. The mice used were 9–12 weeks of age. ^{125}I-labeled antibody binding experiments were carried out according to the principle described by Schimke's group [7–9]. Rabbit antibody against homogenous rat liver acetyl-CoA carboxylase was utilized, since this antibody crossreacts with the mouse liver enzyme [5]. All the materials and methods used, including preparation of antiacetyl-CoA carboxylase and of control γ-globulin from nonimmunized rabbits, iodination of antiacetyl-CoA carboxylase and isolation of liver polysomes, were as described in our previous paper [6] unless otherwise specified.

3. Results

In the experiment shown in fig.1, polysomes isolated from the livers of obese mice were incubated with ^{125}I-labeled antiacetyl-CoA carboxylase, and the binding of the labeled antibody to the polysomes was examined by sucrose gradient centrifugation.

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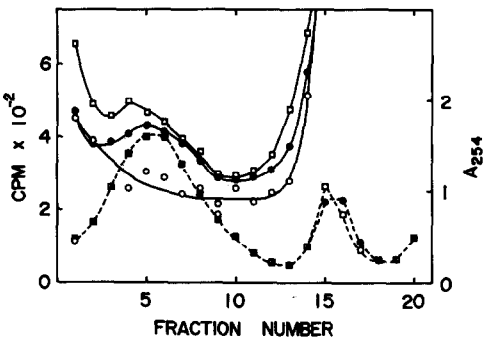

Fig.1. Specific binding of ^{125}I-antiacetyl-CoA carboxylase to mouse liver polysomes. Polysomes isolated from the livers of obese mice were used. The conditions of incubation were as follows: (a) 15 A_{254} units of polysomes were incubated at 0°C for 60 min with 18.5 μg of ^{125}I-antiacetyl-CoA carboxylase (specific radioactivity, 4.1 × 10^4 cpm/μg) in 1.2 ml (total volume) of polysome buffer (50 mM Tris-HCl pH 7.7, 25 mM NaCl, 5 mM $MgCl_2$ and 0.5 mg/ml sodium heparin) (•); (b) 15 A_{254} units of polysomes were incubated first with 0.27 mg of unlabeled antiacetyl-CoA carboxylase at 0°C for 60 min and then with ^{125}I-antibody as under (a) (○); (c) 15 A_{254} units of polysomes were incubated first with 0.27 mg of unlabeled control γ-globulin at 0°C for 60 min and then with ^{125}I-antibody as under (a) (□). After the incubation, each mixture was layered over 11 ml of a linear sucrose gradient in polysome buffer (15 to 50%, w/v). The tubes were centrifuged at 2°C and 41 000 rev/min for 85 min in a Spinco SW-41 rotor. Fractions (0.6 ml) were collected from the bottom, and radioactivity (———) and absorbance at 254 nm (– – – – –) were determined. Centrifugation from right to left.

A broad peak of radioactivity was observed in the heavier polysome region. Evidence indicating that this radioactivity peak represented the specific binding of ^{125}I-labeled antibody to polysomes was provided by the following data. When polysomes were incubated with a large amount of unlabeled antiacetyl-CoA carboxylase prior to incubation with ^{125}I-labeled antibody, the radioactivity peak in the polysome region disappeared. In contrast, prior incubation of polysomes with unlabeled control γ-globulin failed to prevent the binding of the labeled antibody. Even after prior incubation of polysomes with an excess of unlabeled antiacetyl-CoA carboxylase, some radioactivity (230 cpm per fraction) remained in the polysome region. This residual radioactivity was regarded as the background radioactivity, and it was subtracted from the radioactivity in the polysome region when the extent of binding of ^{125}I-antiacetyl-CoA carboxylase to polysomes was estimated (see below).

An experiment was next conducted to compare the extents of binding ^{125}I-antiacetyl-CoA carboxylase to polysomes isolated from the livers of obese and non-obese mice. The results presented in fig.2 showed that, after subtraction of the background radioactivity, the amount of the labeled antibody bound to liver polysomes was approx. 2-fold higher in obese mice than in non-obese mice. Since the scale of incubation for ^{125}I-labeled antibody binding was doubled in this experiment, twice as much radioactivity (460 cpm per fraction) was subtracted as the background radioactivity. The yields of ribosomes (including monosomes) from the livers of obese and non-obese mice were essentially the same, being 36 and 34 A_{254} units/g liver, respectively. These results indicate that the relative content of acetyl-CoA carboxylase-synthesizing polysomes/g liver is increased approx. 2-fold in obese mice as compared with non-obese mice.

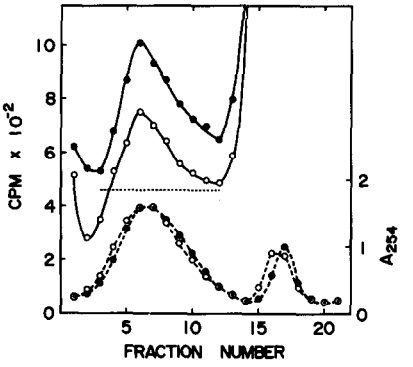

Fig.2. Comparison of the amounts of ^{125}I-antiacetyl-CoA carboxylase bound to liver polysomes from obese and non-obese mice. ^{125}I-Antiacetyl-CoA carboxylase (37 μg; specific radioactivity, 4.1 × 10^4 cpm/μg) was incubated at 0°C for 60 min with 30 A_{254} units of polysomes isolated from the livers of obese (•) and non-obese (○) mice in 2.5 ml (total volume) of polysome buffer. After the incubation, each mixture was layered over 28 ml of a linear sucrose gradient in polysome buffer (15 to 50%, w/v). The tubes were centrifuged at 2°C and 25 000 rev/min for 4 h in a Spinco SW-25.1 rotor. Fractions (1.4 ml) were collected from the bottom and assayed for radioactivity (———) and for absorbance at 254 nm (– – – – –). (. . . . .) Indicates the background radioactivity. Centrifugation from right to left.

4. Discussion

Our previous studies have demonstrated that the rate of acetyl-CoA carboxylase synthesis per liver is 7.7-fold higher in obese mice than in non-obese mice [5]. In terms of the rate of enzyme synthesis/g of liver, this increase is 2.7-fold, because the livers of obese mice weigh more than those of non-obese mice [5]. Thus, the higher rate of hepatic synthesis of acetyl-CoA carboxylase in obese mice can be nearly accounted for by the observed increase in the relative content of polysomes synthesizing this enzyme in the liver. This suggests that the accelerated synthesis of acetyl-CoA carboxylase in the livers of obese mice can be ascribed to an elevated hepatic content of specific mRNA. The possibility that the increase in the amount of acetyl-CoA carboxylase-synthesizing polysomes may result from enhanced translational processes seems unlikely, since polysomes isolated from the livers of obese and non-obese mice exhibited essentially identical sucrose gradient profiles with respect to both position of radioactivity peak and absorbance at 254 nm, as shown in fig.2.

Acknowledgements

We are indebted to Dr M. Nishimura for providing us with obese mice and their non-obese littermates. We thank also Dr S. C. Hubbard for critically reading the manuscript. A portion of the work with ^{125}I was conducted in the Radioisotope Laboratory, Kyoto University Faculty of Medicine. This investigation was supported in part by research grants from the Ministry of Education of Japan, the Mitsubishi Foundation, the Foundation for the Promotion of Research on Medical Resources, the Japanese Foundation for Metabolism and Diseases, and the Japanese Medical Association.

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