EFFECTS OF ALLOSTERIC REGULATORS ON PROTEOLYSIS OF RAT LIVER ACETYL COENZYME A CARBOXYLASE BY LYSOSOMAL EXTRACT

Tadashi TANABE*, Kenji WADA+, Hideo OGIWARA and Shosaku NUMA‡
Department of Medical Chemistry, Kyoto University, Faculty of Medicine, Yoshida, Sakyo-ku, Kyoto 606, Japan

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1. Introduction

Acetyl coenzyme A carboxylase (acetyl-CoA: carbon-dioxide ligase (ADP-forming), EC 6.4.1.2), which catalyzes the initial step in the biqsynthesis of long-chain fatty acids, plays a critical role in the regulation of this synthetic progress [1]. The cellular content of this enzyme varies in accordance with the rate of fatty acid synthesis under different metabolic conditions [1]. It has been shown by combined immunochemical and isotopic techniques that the reduction in the amount of acetyl-CoA carboxylase in the livers of starved rats can be ascribed to both diminished synthesis and accelerated degradation of the enzyme [1]. Although evidence has been presented to indicate a possible metabolite responsible for the regulation of synthesis of acetyl-CoA carboxylase [2], no information has thus far been available concerning a metabolite which may be involved in the regulation of degradation of the enzyme.

Our previous work has demonstrated that rat liver acetyl-CoA carboxylase is composed of one kind of multifunctional polypeptide, which has mol. wt 230 000 and contains one molecule of biotin [3]. It has also been shown that incubation of the enzyme with extracts from rat liver lysosomes results in degradation of the native subunit, giving rise to polypeptides with mol. wts 100 000–130 000 [3]. The present investigation has been designed to examine the possibility that the rate of degradation of acetyl-CoA carboxylase may be modulated by allosteric regulators of the enzyme. The results presented indicate that the susceptibility of rat liver acetyl-CoA carboxylase to proteolysis by lysosomal extracts is enhanced by palmityl-CoA, an inhibitor of the enzyme, whereas it is diminished by citrate, an activator of the enzyme.

2. Materials and methods

D-[*Carbonyl*-^{14}C]biotin-labelled acetyl-CoA carboxylase from rat liver was purified through the DEAE-cellulose chromatography step as described previously [3]. The radioactivity present in this enzyme preparation was previously shown to be attributable solely to the isotope in the biotinyl prosthetic group of acetyl-CoA carboxylase [3]. Lysosomal extracts were prepared from rat liver as described previously [3]. The standard reaction mixture for proteolysis of acetyl-CoA carboxylase contained 5 μmol potassium phosphate buffer, pH 7.5, 12.5 μmol sucrose, 0.25 μmol 2-mercaptoethanol, 0.05 μmol EDTA, 35 μg [^{14}C]biotin-labelled acetyl-CoA carboxylase (57 cpm/μg protein), lysosomal extract (80 μg protein) in total vol. 50 μl. When indicated, citrate and/or palmityl-CoA were added to the reaction mixture. The solution containing all components except lysosomal extract was preincu-

* Present address: National Institute of Arthritis, Metabolism and Digestive Diseases, National Institutes of Health, Bethesda, Maryland 20014, USA

+ Present address: Medical Research Institute, Tokyo Medical and Dental University, Kanda-Surugadai, Chiyoda-ku, Tokyo 101, Japan

‡ To whom reprint requests should be addressed

bated at 37°C for 10 min and then at 25°C for 1 min. After the preincubation, the reaction was initiated by the addition of lysosomal extract. After incubation at 25°C for the specified lengths of time, a 20 μl aliquot was removed from the reaction mixture and mixed with 20 μl 1.8% sodium dodecyl sulfate containing 7.2 M urea and 1.4 M 2-mercaptoethanol. This mixture was heated at 80°C for 2 min and subjected to dodecyl sulfate–polyacrylamide gel electrophoresis as described previously [3]. The gel was sliced transversely into segments of 4 mm thickness, and each segment was assayed for radioactivity as reported previously [3]. Values given for relative content of the native subunit represent the radioactivity present in the segment(s) containing the 230 000 dalton subunit as percentage of the total radioactivity present in the gel. Palmityl-CoA was prepared according to the procedure of Young and Lynen [4] with minor modifications and was determined spectrophotometrically by measuring absorbance at 260 nm [5]. Protein was determined by the method of Lowry et al. [6] with bovine serum albumin as the standard.

3. Results

The native subunit (mol. wt 230 000) of rat liver acetyl-CoA carboxylase undergoes proteolytic degradation to varying extents during the purification of the enzyme [3]. Dodecyl sulfate–polyacrylamide gel electrophoresis of the [^{14}C]biotin-labelled acetyl-CoA carboxylase preparations used in the present study showed that more than 76% of the total radioactivity was present in the native subunit. In the experiment represented in fig.1, the labelled acetyl-CoA carboxylase was incubated with a lysosomal extract derived from rat liver in the presence or absence of citrate. The rate at which the native subunit was degraded was diminished when the enzyme was treated with citrate. After incubation for 60 min, 59% and 43% of the total radioactivity were found in the native subunit in the presence and absence of citrate, respectively. The rate of degradation of the native subunit in the absence of an allosteric regulator varied from experiment to experiment owing probably to the different proteolytic activities of the lysosomal extracts used. When incubation was carried out for 60 min with boiled lysosomal extracts or when the

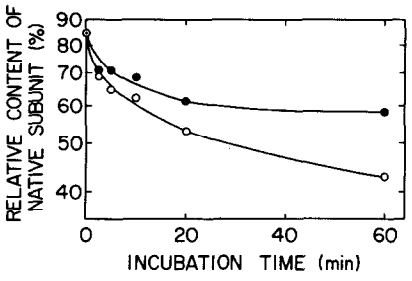

Fig.1. Time course of proteolysis of acetyl-CoA carboxylase by a lysosomal extract in the presence or absence of citrate. Incubation was performed in the standard reaction mixture with (•) or without (○) 10 mM citrate for the indicated lengths of time. For experimental details, see Materials and methods.

reaction was terminated at zero time, no decrease in the relative content of the native subunit was observed. Figure 2 exhibits the effect of palmityl-CoA on the rate of proteolysis of the enzyme by a lysosomal extract. The native subunit was degraded faster when the enzyme was treated with palmityl-CoA. After incubation for 60 min, 59% and 68% of the total radioactivity were found in the native subunit in the presence and absence of palmityl-CoA, respectively. In the experiments shown in table 1, we studied the effect of simultaneous addition of citrate and palmityl-CoA on the rate of degradation of the native subunit by a lysosomal extract. The effects of the two regulators were antagonistic.

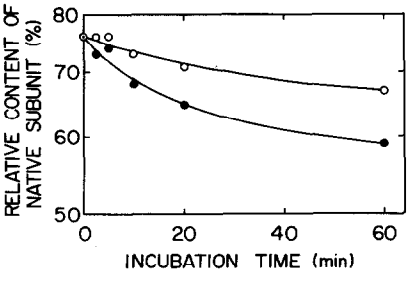

Fig.2. Time course of proteolysis of acetyl-CoA carboxylase by a lysosomal extract in the presence or absence of palmityl-CoA. Incubation was performed in the standard reaction mixture with (•) or without (○) 50 μM palmityl-CoA for the indicated lengths of time. For experimental details, see Materials and methods.

Table 1
Effect of simultaneous addition of citrate and palmityl-CoA on proteolysis of acetyl-CoA carboxylase by lysosomal extracts

Expt.	Addition or deletion	Relative content of native subunit (%)
1	None	41
	− Lysosomal extract	82
	+ Citrate (10 mM)	55
	+ Palmityl-CoA (50 μM)	31
	+ Citrate (10 mM) + palmityl-CoA (50 μM)	34
2	None	60
	− Lysosomal extract	91
	+ Citrate (10 mM)	66
	+ Palmityl-CoA (50 μM)	34
	+ Citrate (10 mM) + palmityl-CoA (50 μM)	42

Incubation was performed for 60 min in the standard reaction mixture with the addition or deletion indicated. For experimental details, see Materials and methods

4. Discussion

The present investigation indicates that the degradation of the native subunit of rat liver acetyl-CoA carboxylase by lysosomal extracts is enhanced by palmityl-CoA and diminished by citrate. The activity of hepatic acetyl-Coa carboxylase is known to be modulated by allosteric regulators, including citrate as a positive effector and long-chain acyl-CoA thioesters as negative effectors [7]. The activation and inhibition are associated with the polymerization and depolymerization of the enzyme molecule, which suggest that conformational changes of the enzyme molecule are induced by the allosteric regulators. Thus, it appears likely that the observed effects of citrate and palmityl-CoA on the susceptibility of the enzyme to proteolytic degradation are attributable to conformational changes of the enzyme molecule. Accumulating evidence supports the concept that the rate of degradation of proteins is determined by their conformation [8,9].

Studies in vivo have indicated that the half-life for the degradation of hepatic acetyl-CoA carboxylase is 2–3-fold shorter in fasted rats than in normally fed rats [1]. It has also been shown that in fasted rats the hepatic content of citrate is reduced, while that of long-chain acyl-CoA thioesters is elevated [1]. Furthermore, a correlation has been found between the rate of degradation in vivo of proteins and their susceptibility to proteolysis by lysosomal extracts [9]. These findings suggest the possibility that the degradation of hepatic acetyl-CoA carboxylase may be controlled by its allosteric regulators.

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