REGULATION OF MAMMALIAN ACETYL-CoA CARBOXYLASE

Limited proteolysis mimics dephosphorylation

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1. Introduction

Acetyl-CoA carboxylase catalyses the rate-limiting step in fatty acid synthesis, and in mammals both the amount of the enzyme and its catalytic activity is regulated [1]. Short-term regulation is achieved either by variation in the levels of allosteric effectors or by reversible phosphorylation [2]. The purified rat mammary enzyme is inactivated reversibly by phosphorylation with cyclic AMP-dependent protein kinase [3] and there is evidence for the existence of other, cyclic AMP-independent protein kinases which both phosphorylate and inactivate the rabbit mammary [3] and rat liver [4] enzymes. Acetyl-CoA carboxylase purified from rabbit mammary gland in the presence of sodium fluoride was obtained in a highly phosphorylated form of low specific activity [5]. The enzyme could be reactivated and dephosphorylated using a purified protein phosphatase [5]. We now report that the same effect is obtained by limited proteolysis. Our results suggest that acetyl-CoA carboxylase contains a domain or region near one end of the polypeptide chain, which is inhibitory when the enzyme is phosphorylated, and that other purified preparations of acetyl-CoA carboxylase described in the literature may already have lost this regulatory region due to limited proteolysis.

2. Materials and methods

Rabbit mammary acetyl-CoA carboxylase was purified in the presence of 50 mM sodium fluoride as in [5]. Protein phosphatase-1 (1B fraction) was purified as in [6] and was a gift from Mr A. A. Stewart of this Department. Acetyl-CoA carboxylase was assayed as in [5] at the citrate concentration specified in the text, except that the total magnesium concentration was 2 mM less than the total concentration of ATP + citrate. Protein phosphatase-1 was assayed using phosphorylase *a* as in [6]. L-1-Tosylamide-2-phenylethyl chloromethyl ketone (TPCK)-treated trypsin was from Worthington Biochemical Corp.; soybean trypsin inhibitor (type 1-S) was from Sigma London Chemical Co. Determination of protein concentration [7], determination of alkali-labile phosphate [8], polyacrylamide gel electrophoresis [9] and sources of other biochemicals [5] were as described.

3. Results

3.1. *Activation of acetyl-CoA carboxylase by dephosphorylation or proteolysis*

As reported in [5] acetyl-CoA carboxylase prepared from lactating rabbit mammary gland in the presence of a protein phosphatase inhibitor, i.e., fluoride ion, is obtained in a form of low specific activity, which can be reactivated by treatment with protein phosphatase-1 purified from rabbit skeletal muscle (fig.1). This activation is due to dephosphorylation and not proteolysis, since it does not change the mobility of the subunit after SDS–polyacrylamide gel electrophoresis, has the same metal ion dependence as the protein phosphatase, and is sensitive to protein phosphatase inhibitor-2, a specific protein inhibitor of protein phosphatase-1 [5]. However, an identical effect can be obtained by adding traces of trypsin. Acetyl-CoA carboxylase that has not been treated

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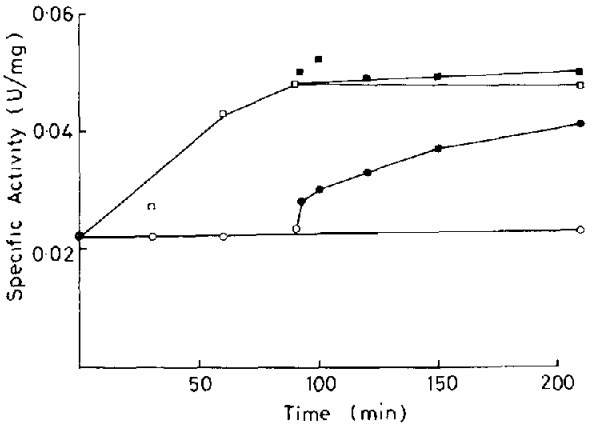

Fig.1. Activation of acetyl-CoA carboxylase by protein phosphatase-1 or trypsin. Acetyl-CoA carboxylase (0.5 mg/ml) was incubated at 30°C with or without protein phosphatase-1 (10 units/ml) in 0.05 I Tris–HCl, 1 mM EDTA, 0.1% (v/v) β-mercaptoethanol (pH 7.1). At 90 min trypsin was added to a portion of each incubation to 25 ng/ml final conc. Aliquots were removed at various times for assay of acetyl-CoA carboxylase at 1 mM citrate. Control (○); + protein phosphatase-1 (□); control + trypsin (●); + protein phosphatase-1 + trypsin (■).

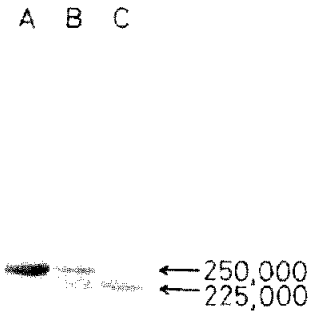

Fig.2. Limited proteolysis of acetyl-CoA carboxylase. Acetyl-CoA carboxylase (0.5 mg/ml) was incubated at 30°C with trypsin (25 ng/ml) in 0.05 I Tris–HCl, 1 mM EDTA, 0.1% (v/v) β-mercaptoethanol (pH 7.1). Aliquots were removed after incubation for 0 min (A); 15 min (B); and 120 min (C) and were added to a 10-fold excess of boiling SDS sample buffer [9]. The photograph shows the Coomassie blue-stained polypeptides after electrophoresis in SDS–4% polyacrylamide gels.

with protein phosphatase is activated by incubation with 0.005% (w/w) trypsin, whereas acetyl-CoA carboxylase that had been dephosphorylated is not affected (fig.1). The effects of protein phosphatase or trypsin treatment are very similar and are not additive.

3.2. *Effect of trypsin on the subunit structure of acetyl-CoA carboxylase*

Treatment of acetyl-CoA carboxylase with 0.005% (w/w) trypsin results in quantitative conversion of the M_r 250 000 subunit into a fragment of M_r 225 000, with an apparent intermediate of M_r 235 000 (fig.2). The M_r 235 000 and M_r 225 000 components correspond to the minor components present in the purified preparation which we had suspected to be the result of partial proteolysis during the enzyme preparation [9]. We have been unable to detect the small fragments that are released by the proteolysis and presume that they are very quickly degraded into small peptides which are not detectable by the electrophoretic technique used.

The activation of the phosphorylated enzyme by trypsin (fig.1) correlates with the conversion of the M_r 250 000 subunit to the M_r 225 000 fragment. If the enzyme is dephosphorylated using protein phosphatase-1 before trypsin treatment, the cleavage occurs at the same rate, but is not accompanied by any activation (fig.1). Upon prolonged digestion with trypsin the M_r 225 000 fragment is further cleaved into sub-fragments of ~120 000 and ~110 000 M_r (not shown). This prolonged digestion produces some loss of enzyme activity. Both the primary (M_r 250 000 225 000) and secondary (M_r 225 000–120 000 and 110 000) cleavages are inhibited by the presence of 10 mM citrate in the medium (not shown).

3.3. *Effect of dephosphorylation or proteolysis on phosphate content*

Acetyl-CoA carboxylase prepared in the presence of fluoride contained 6.0 ± 0.2 alkali-labile phosphate molecules/M_r 250 000 subunit. The enzyme was treated with protein phosphatase-1 or trypsin as described for fig.3 and the reaction was stopped by adding trichloroacetic acid to 5% (w/v) after incubation for 2 h. Alkali-labile phosphate was

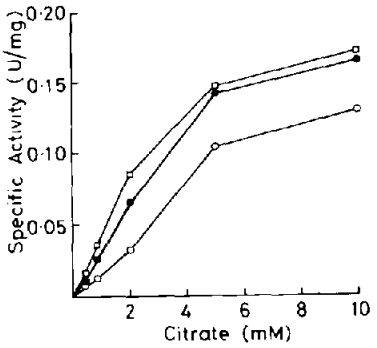

Fig.3. Citrate dependence of control, dephosphorylated, and trypsin-treated forms of acetyl-CoA carboxylase. Acetyl-CoA carboxylase (0.5 mg/ml) was incubated for 2 h at 30°C in 0.05 I Tris–HCl, 1 mM EDTA, 0.1% (v/v) β-mercaptoethanol (pH 7.1) with or without protein phosphatase-1 (10 units/ml) or trypsin (25 ng/ml). Tryptic activity was stopped by adding trypsin inhibitor to 0.25 μg/ml final conc. Each form of the enzyme was then assayed at the citrate concentration shown as described in section 2. Control (○); + protein phosphatase-1 (□); + trypsin (●).

determined on the trichloroacetic acid-insoluble pellets and values of 5.2 ± 0.1 and 4.9 ± 0.3 phosphate molecules/subunit were found after protein phosphatase and trypsin treatment, respectively.

3.4. *Effect of citrate concentration on enzyme activity*

When assayed in Tris–HCl buffer, the preparation of acetyl-CoA carboxylase exhibited a sigmoidal dependence on the concentration of the allosteric activator, citrate (fig.3). Treatment with either protein phosphatase-1 or trypsin produced similar effects on this citrate dependence. The major effect was a downward shift in the concentration of citrate required for half-maximal activation, so that the effect of dephosphorylation or proteolysis on the activity of the enzyme was greatest at low citrate concentration.

4. Discussion

These data suggest that rabbit mammary acetyl-CoA carboxylase contains a regulatory domain or region, of M_r ~25 000, located at one end of the polypeptide chain. This region increases the dependence of enzyme activity on citrate concentration, but only when the enzyme is in the highly phosphorylated form. This inhibitory effect is reversed either by partial dephosphorylation, or by limited proteolysis, which removes the regulatory region. Since trypsin treatment or protein phosphatase treatment reduce the alkali-labile phosphate content to similar extents, the phosphate removed by protein phosphatase-1 treatment is most likely to be present on the trypsin-sensitive regulatory region. Trypsin treatment presumably digests this region into small peptides that are soluble in trichloroacetic acid and hence are not detected by our procedure for determination of alkali-labile phosphate.

These data shed new light on a variety of observations. In 1967 it was reported that crude preparations of rat liver acetyl-CoA carboxylase were activated by trypsin [10]. It has also been observed that incubation of rat liver extracts at 37°C produces a 'heat activation' of acetyl-CoA carboxylase [11]. This heat activation could be the result of either dephosphorylation or proteolysis, or both. The effects we observe are different from those in [12] where treatment of a preparation of rat liver acetyl-CoA carboxylase with much higher concentrations of trypsin produced a form of the enzyme that was not dependent on citrate for activity. The effect of this treatment on the subunit structure was not reported. Numa's group has more recently reported that different purified preparations of rat liver acetyl-CoA carboxylase contained varying proportions of polypeptides of M_r 230 000, 124 000 and 118 000. The M_r 230 000 component could be converted into the M_r 124 000 and 118 000 components using either trypsin, chymotrypsin or rat liver lysosomal extract [13]. This proteolytic modification was retarded in the presence of the allosteric activator, citrate [14]. Comparison with our data suggests that these workers were observing the secondary cleavage that occurs upon prolonged treatment with trypsin which is also retarded by citrate in our experiments. It is therefore possible that the M_r 230 000 polypeptide obtained in [13] is not the intact enzyme and has already lost the regulatory region. This conclusion may also be valid for other preparations of acetyl-CoA carboxylase which contain mixtures of polypeptides of M_r ~230 000 and 120 000 [15,16].

Effects of limited proteolysis on the activity of enzymes that are regulated by reversible phosphorylation have been described. However in most cases, limited proteolysis mimics, at least qualitatively, the effect of phosphorylation. Thus limited proteolysis, or phosphorylation, decreases the K_a for Ca^{2+} of skeletal muscle phosphorylase kinase [17], increases the dependence of skeletal muscle glycogen synthase on glucose 6-phosphate [18], and increases the app. K_m for phosphoenolpyruvate of liver pyruvate

kinase [19]. These data represent the opposite case where limited proteolysis mimics dephosphorylation.

While this manuscript was in preparation, it was reported that the radioactivity in ^{32}P-labelled acetyl-CoA carboxylase purified from [^{32}P]phosphate-treated isolated hepatocytes was rapidly released as small peptides on incubation with trypsin [20].

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