Proteolysis of liver acetyl coenzyme A carboxylase by cathepsin B

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Proteolysis of acetyl-CoA carboxylase was examined with cathepsin B When chicken liver acetyl-CoA carboxylase was incubated with cathepsin B at pH 6 3, the native 220-kDa polypeptide was primarily cleaved into two polypeptides of 125 and 115 kDa, and further degraded to polypeptides of 100–50 kDa

Protein degradation *Acetyl-CoA carboxylase* *Cathepsin B*

1. INTRODUCTION

Acetyl coenzyme A carboxylase (acetyl-CoA: carbon dioxide ligase (ADP-forming), EC 6.4.2.1) plays a central role in the regulation of the fatty acid biosynthesis [1,2]. The hepatic content of acetyl-CoA carboxylase depends on both the rates of synthesis and degradation of the enzyme [1,3]. Although the synthetic rate of acetyl-CoA carboxylase varies under different metabolic conditions, the degradation rate of this enzyme is hardly changed by metabolic perturbations except nutritional depletion [1,3]. Recent studies suggested that lysosomal thiol proteinases, including cathepsin B, H and L, play important roles in the degradation of endogenous proteins in the basal and nutritionally depleted states of cells [4–6]. The degradation mechanisms of individual proteins, however, have been poorly understood.

Our previous work has demonstrated that animal acetyl-CoA carboxylase is constituted of a unique multifunctional 220–230-kDa polypeptide [7,8]. It has also been shown that incubation of the native acetyl-CoA carboxylase isolated from rat liver with lysosomal extracts yields smaller polypeptides (100–130 kDa) [7]. Here, degradation of acetyl-CoA carboxylase was investigated with the most abundant thiol proteinase, cathepsin B (EC 3.4.22.1).

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2. MATERIALS AND METHODS

Acetyl-CoA carboxylase from chicken liver was purified as described previously [8]. The specific activity of the enzyme was 3.5 units/mg protein. Porcine kidney cathepsin B (type 1) was isolated according to [9]; the authentic sample was kindly donated by S. Takahashi of this institute. Specific activity of the enzyme was 4.3 units/mg protein as measured with a synthetic substrate, benzyloxycarbonyl-phenylalanyl arginine-4-methyl-7-coumarylamide. Acetyl-CoA carboxylase (50 μg) was incubated for 1 or 20 h with 5 μg of cathepsin B in a reaction mixture (0.1 ml) containing 0.1 M potassium phosphate buffer, 5 mM cysteine and 2 mM EDTA, pH 6.3. The reaction was terminated by adding 25 μl of 10 μg/ml leupeptin. Aliquots (10 μl) were removed from the reaction mixtures and treated with an equal volume of 2% SDS containing 8 M urea and 10% 2-mercaptoethanol (v/v). The mixture was heated at 100°C for 2 min and subjected to 7.5% polyacrylamide gel electrophoresis (PAGE) in the presence of 0.1% SDS as described previously [8]. The gel was stained by Coomassie brilliant blue R-250. Molecular masses of the polypeptides generated by

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cathepsin B were determined with the standards from Bio-Rad, which are myosin heavy chain, β-galactosidase, phosphorylase *b*, bovine serum albumin and ovalbumin. Protein was determined by [10] with bovine serum albumin as the standard.

3. RESULTS AND DISCUSSION

The properties of cathepsin B are not greatly different among animal species [4]. Thus, the degradation of acetyl-CoA carboxylase from chicken liver was studied with porcine kidney cathepsin B. After the incubation of native acetyl-CoA carboxylase with cathepsin B for 1 h, the native 220-kDa polypeptide almost disappeared and two polypeptides (125 and 115 kDa) were newly formed (fig.1). The prolonged incubation for 20 h resulted in the formation of additional smaller polypeptides (100–50 kDa). Therefore, the initial steps of the catheptic degradation of acetyl-CoA carboxylase seem to proceed in a sequential manner. The primary degradation products of the 125- and 115-kDa polypeptides are quite similar to the polypeptides which have been found in the enzyme preparations purified at pH 7.0–7.5 in the absence of protease inhibitors [7,8,11,12]. These polypeptides found in the animal acetyl-CoA carboxylase preparations are likely due to the proteolysis by cathepsin B, because cathepsin B exhibits a partial activity at neutral pH despite the pH optimum being 6.0–6.5, and additions of cathepsin B inhibitors, such as leupeptin and antipain [4,13] in the purification buffers markedly reduced the production of the smaller polypeptides [8].

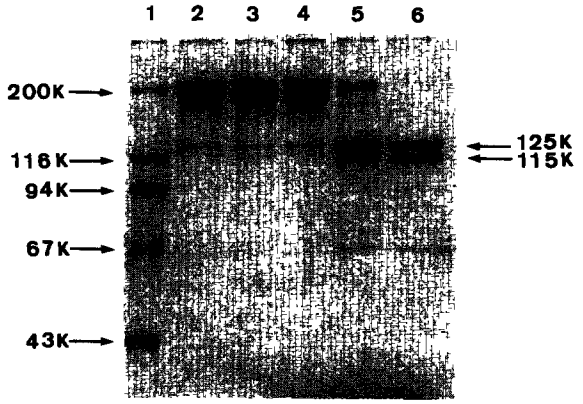

Fig.1. SDS–PAGE of acetyl-CoA carboxylase digests by cathepsin B. For experimental details see section 2. 4 μg of acetyl-CoA carboxylase was run in each lane. Lane 1, marker proteins; lane 2, acetyl-CoA carboxylase without incubation, lane 3, acetyl-CoA carboxylase alone incubated for 20 h; lanes 4–6, the enzyme treated with cathepsin B for 0, 1 and 20 h, respectively.

Accumulated indirect evidence has suggested that cathepsin B may play an important role in the general catabolism of proteins in the lysosomal system [4]. Recently it has been clearly shown that 30–45% of the degradation of endogenous proteins in cultured macrophages and fibroblasts is inhibited by a cathepsin B inhibitor [5,6]. Furthermore, it has also been reported that the breakdown of pyruvate carboxylase in 3T3-L1 cells is inhibited by leupeptin [14]. Therefore, available evidence suggests the possibility that cathepsin B has a role in the initial steps of the degradation of acetyl-CoA carboxylase in vivo.

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