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INTRACELLULAR TRANSPORT AND PROCESSING OF LYSOSOMAL CATHEPSIN B

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SUMMARY: Intracellular transport and processing of lysosomal cathepsin B was investigated in the subcellular fractions of rat liver by pulse-labeling experiments with [^{35}S]methionine *in vivo*. A newly synthesized procathepsin B with a molecular weight of 39 kDa firstly appeared in the rough microsomal fraction at 10 min postinjection of label. This procathepsin B moved from the microsomal fractions to the Golgi subfractions at 30 min postinjection, and then a processed mature enzyme appeared in the lysosomal fraction at 60 min. These results suggest that the propeptide-processing of procathepsin B takes place in lysosomes in the course of intracellular transport from endoplasmic reticulum through Golgi complex to lysosomes.

Cathepsin B is a lysosomal thiol protease which is considered to play an important role in the degradation of intracellular tissue proteins in lysosomes (1). Purified cathepsin B is a glycoprotein consisting of a mixture of a 29 kDa single-chain form and its processed two-chain form enzyme with 25 kDa and 4 kDa (1-3).

It is now known that lysosomal enzymes are synthesized on membrane-bound polysomes of endoplasmic reticulum as glycosylated precursors of higher apparent molecular weights (5-8). During their synthesis and maturation, the precursors undergo multiple

ABBREVIATIONS: SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; GF-1, GF-2, and GF-3, Golgi light, intermediate, and heavy subfractions, respectively, of Ehrenreich et al. (4).

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processing steps by limited proteolysis (5-11). Recent elucidation of the determined primary structure of preprocathepsin B predicted from the cDNA cloning study has further confirmed these intracellular processing events (12).

A biosynthesis study carried out in the presence of [^{35}S]methionine by using pulse-chase experiments in cultured rat hepatocytes suggested that a glycosylated 39 kDa procathepsin B was firstly synthesized after short time of labeling and this proenzyme was subsequently converted to a 29 kDa single-chain enzyme by limited proteolysis (11). We further demonstrated the presence of latent form of procathepsin B with 39 kDa in the hepatic microsomal lumen by immunoblotting analysis (11). The latent procathepsin B was found to be converted to the enzymatically active form under acidic conditions of pH 3.0. Since the proteolytic conversion of procathepsin B to the mature enzyme was blocked with pepstatin, which is a potent inhibitor of aspartyl proteases, the result strongly suggested that the processing protease for procathepsin B might be cathepsin D, a major lysosomal aspartyl protease (11). Therefore, the propeptide-processing of procathepsin B would proceed within the lysosomes.

In this paper, we pulse-labeled living rats with [^{35}S]methionine *in vivo* and isolated cathepsin B by immunoprecipitation from subcellular fractions of rat liver to investigate the intracellular processing of cathepsin B. We present direct evidence showing that the processing of a 39 kDa procathepsin B to a 29 kDa mature cathepsin B indeed takes place in the lysosomes.

MATERIALS AND METHODS

Materials: [^{35}S]methionine (1000 Ci/mmol) and [^{14}C]methylated protein mixture (10-50 μ Ci/mg protein) were

purchased from Amersham. Protein A-Sepharose CL-4B was obtained from Pharmacia Fine Chemicals. EN^{3}HANCE was obtained from New England Nuclear Co.

Cell fractionation: The three Golgi subfractions (GF-1, GF-2, and GF-3) were prepared as described by Ehrenreich et al. (4) and the rough and smooth microsomes (13) and the crude lysosomal fraction (14) were prepared by previously described procedures.

Immunoprecipitation of cathepsin B: Male Wistar rats weighing 200-250 g were used. Rat liver lysosomal cathepsin B was purified to homogeneity essentially as described by Towatari et al. (2). Antisera against rat liver lysosomal cathepsin B was prepared by injecting the purified enzyme into rabbits. Antibody was purified by the immunoaffinity chromatography using antigen-Sepharose 4B. Aliquots of cell fractions, containing the same amounts of proteins (5 mg), were solubilized in 1 % Triton X-100/0.2 M NaCl/5 mM EDTA/50 mM Tris-Cl (pH 7.5) and centrifuged at 105,000 × g for 60 min. The supernatants were incubated with 100 μg of monospecific IgG against cathepsin B at 4°C for 14 h before the addition of protein A-Sepharose beads.

Gel electrophoresis and fluorography: SDS-PAGE was performed by the method of Laemmli (15) on 15 % gels containing 0.1 % SDS. Radioactive bands were detected by fluorography using EN^{3}HANCE on Kodak XAR-5 film. Apparent molecular weight were determined by using [^{14}C]methylated standards.

RESULTS AND DISCUSSION

In order to elucidate the kinetics of intracellular transport of cathepsin B from its site of synthesis to lysosomes, we pulse-labeled the rats with [^{35}S]methionine *in vivo* and removed the livers at various times from 10 to 180 min after [^{35}S]methionine administration. After cell fractionation into rough microsomes, smooth microsomes, three Golgi subfractions (GF-1, GF-2, and GF-3) and lysosomes, cathepsin B was isolated from these fractions by immunoprecipitation with anti-lysosomal cathepsin B antibody. The immunoprecipitate was subjected to SDS-PAGE and visualized by fluorography (Fig. 1).

Within 10 min after injection of [^{35}S]methionine, a newly synthesized procathepsin B with a molecular weight of 39 kDa appeared first in the rough microsomal fraction and also, almost simultaneously, in the smooth microsomal fraction where it exhibited a slightly slower electrophoretic mobility as compared with that in the rough microsomal fraction (Fig. 1A). A lower intensity of radiolabeled enzyme was seen in the smooth

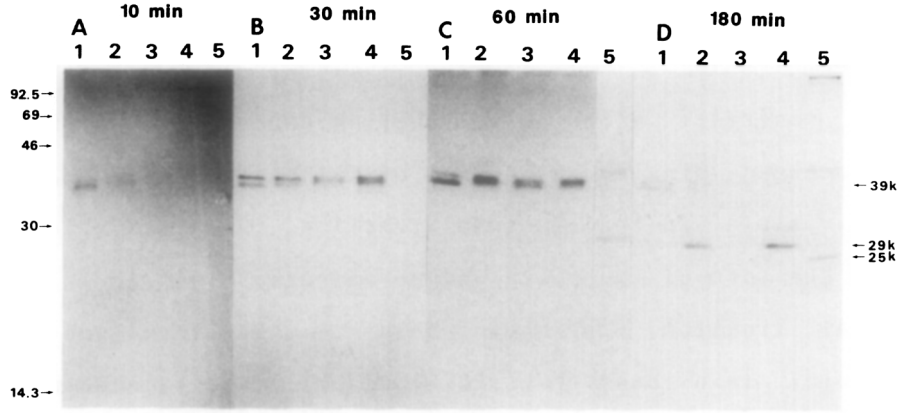

Figure 1. Appearance of biosynthetically labeled cathepsin B in subcellular fractions. Fractions were isolated from livers of rats sacrificed at selected time intervals after an intravenous administration of [^{35}S]methionine (250 μCi/100 g body weight). Shown are fluorographs of SDS-PAGE electrophoretograms of polypeptides immunoprecipitated with specific antibody against cathepsin B. Fluorograms are: (A), 10 min postinjection of the label; (B), 30 min postinjection of the label; (C), 60 min postinjection of the label; (D), 180 min postinjection of the label. Lane 1, rough microsomes; lane 2, smooth microsomes; lane 3, GF-3; lane 4, GF-1 + GF-2; lane 5, lysosomes. The molecular masses (K=1000) of the three different cathepsin B polypeptides are indicated at the right. The numbers to the left indicate the masses (in kilodaltons) of the molecular weight standards; phosphorylase b (92,500), bovine serum albumin (69,000), ovalbumin (46,000), carbonic anhydrase (30, 000), and lysozyme (14,400).

microsomal fraction (Fig. 1A, lane 2). The proenzyme derived from the rough microsomal fraction was sensitive to endoglycosidase H treatment (data not shown), suggesting that the proenzyme is core glycosylated.

At 30 min postinjection, the procathepsin B moved to the Golgi subfractions (Fig. 1B). In the rough and smooth microsomal fractions and the three Golgi subfractions, the only radiolabeled band in the anti-cathepsin B immunoprecipitate appeared to be a 39 kDa procathepsin B and this proenzyme form did not show an apparent molecular weight change in the electrophoretic mobility (Fig. 1B). GF-3 is the heavy Golgi subfraction containing mainly cis-Golgi elements such as cisternae, while GF-1 is the light Golgi subfraction which is composed of trans-Golgi elements, such as secretory vesicles (4). These results, therefore, suggest that the propeptide-processing

of procathepsin B takes place neither in the microsomes nor in the Golgi complex.

By contrast, after pulse-labeling experiment for 60 min, the processed single-chain form of cathepsin B with 29 kDa became visible in the lysosomal fraction (Fig. 1C, lane 5). The 25 kDa form of two-chain enzyme was also discernible in the lysosomal fraction, although this 25 kDa form was hardly detectable, faint band (Fig. 1C, lane 5). The two-chain enzyme with 25 kDa and 4 kDa is considered to be the processed form of the single-chain 29 kDa protein (1-3). Hence, the proteolytic conversion of the single-chain enzyme to the two-chain enzyme appears to proceed following delivery to the lysosomes. The proenzyme form was not observed in the lysosomal fraction, confirming that the propeptide of procathepsin B is indeed cleaved within the lysosomes. On the other hand, in the microsomal and Golgi subfractions, the 39 kDa proenzyme form still remained as a major one.

At 180 min postinjection, the processed two-chain form of cathepsin B was found not only in the lysosomal fraction, but also in the microsomal and Golgi subfractions (Fig. 1D). The presence of the processed two-chain enzyme in considerable amounts in the microsomal and Golgi subfractions could be ascribed to lysosomal fraction contamination. The ratio of intensity of the 25 kDa form to that of the 29 kDa form increased in the lysosomal fraction, indicating that the proteolytic conversion of the 29 kDa form to the 25 kDa form is occurring in the lysosomes (Fig. 1D, lane 5).

Taken together, we conclude from these results that a 39 kDa procathepsin B initially detected in the rough microsomal fraction at the earliest interval, 10 min after [^{35}S]methionine administration, moves from the endoplasmic reticulum to the

Golgi complex within 30 min, and is subsequently transported to the lysosomes between 30 and 60 min postinjection. When the procathepsin B arrives in the lysosomes, the propeptide of proenzyme is rapidly cleaved, yielding the single-chain enzyme. Further processing of the single-chain enzyme to the two-chain enzyme also takes place in the lysosomes. The findings reported here validate the preliminary results recently reported from our laboratory (11): lysosomal thiol proteases, cathepsins B, H, and L, are first synthesized as larger molecules enzymatically inactive proforms in the endoplasmic reticulum, and these proenzymes are then transformed into the active forms in the lysosomes.

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