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INTRACELLULAR TRANSPORT AND PROCESSING OF LYSOSOMAL CATHEPSIN H

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SUMMARY: Intracellular transport and processing of lysosomal cathepsin H was investigated in the subcellular fractions of rat liver by pulse-labeling experiments with [^{35}S]methionine *in vivo*. A newly synthesized procathepsin H with a molecular weight of 41 kDa first appeared in the rough microsomal fraction at 10 min postinjection of label. This procathepsin H moved from the microsomal fraction to the Golgi subfractions at 30 min. Then a processed single-chain form with 28 kDa appeared in the lysosomal fraction at 60 min and persisted as the predominant form after 180 min. These results suggest that the propeptide-processing of procathepsin H indeed takes place in lysosomes in the course of intracellular transport from endoplasmic reticulum via Golgi complex to lysosomes.

Lysosomal cysteine proteinases may well be the most active proteinases in the body (1). The precise function of the lysosomal cysteine proteinases is unclear, but they are generally thought to play an important role in intracellular protein degradation. The cystein proteinase cathepsin H acts both as an aminopeptidase and as an endopeptidase (2). The purified cathepsin H is a glycoprotein consisting of a mixture of a 28 kDa single-chain form and its processed two-chain form enzyme with 22 kDa and 6 kDa (2,3). The primary amino acid sequence (4), and

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. (5).

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carbohydrate structure (6) of cathepsin H isolated from rat liver lysosomes have been determined.

It is known that lysosomal enzymes undergo both proteolytic and glycolytic post-translational processing, giving rise to multiple biosynthetic forms detectable by pulse-chase experiments (7-10). We have recently shown from a biosynthesis study employing *in vitro* cell-free translation system that a nascent chain of cathepsin H with 36 kDa has a transient NH_2-terminal prepropeptide (11). Furthermore, pulse-chase experiments in cultured rat hepatocytes demonstrated that a 41 kDa procathepsin H was first synthesized after short time of labeling and this proenzyme was subsequently processed to the 28 kDa single-chain form by limited proteolysis (12). We have also found the presence of a latent form of procathepsin H, having a molecular weight of 41 kDa, in the hepatic microsomal lumen by immunoblotting analysis (12). Since the procathepsin H was converted to the enzymatically active form under acidic conditions, generating the mature enzyme, it seems likely therefore that removal of the propeptide portion of procathepsin H may be a step in the activation process which would be proceeding in the lysosomes.

In this paper, we pulse-labeled living rats with [^{35}S]methionine *in vivo* and isolated cathepsin H by immunoprecipitation from subcellular fractions of rat liver in order to investigate the intracellular transport and processing of cathepsin H. We report here evidence showing that the processing of a 41 kDa procathepsin H to a 28 kDa mature cathepsin H indeed takes place in the lysosomes.

MATERIALS AND METHODS

Materials: [^{35}S]Methionine (1000 Ci/mmol) and [^{14}C]methylated protein mixture (10-50 μCi/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. (5) and the rough and smooth microsomes (13) and the crude lysosomal fraction (14) were prepared by previously described procedures.

Immunoprecipitation of cathepsin H: Male Wistar rats weighing 200-250 g were used. Rat liver lysosomal cathepsin H was isolated to homogeneity essentially as described previously by Kirschke et al. (2). Antisera against rat liver lysosomal cathepsin H 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 $\times g$ for 60 min. The supernatants were incubated with 100 µg of monospecific IgG against cathepsin H at 4^{0}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 investigate the kinetics of intracellular transport of lysosomal cathepsin H from its site of synthesis to the lysosomes, we pulse-labeled the rats with [^{35}S]methionine *in vivo* and the livers were removed at various times from 10 to 180 min after the administration of [^{35}S]methionine. Livers were subfractionated into rough microsomes, smooth microsomes, three Golgi subfractions (GF-1, GF-2, and GF-3) and lysosomes. Cathepsin H was isolated from these fractions by immunoprecipitation with anti-cathepsin H antibody and the immunoprecipitate was subjected to SDS-PAGE and visualized by fluorography (Fig. 1).

Within 10 min after the injection of [^{35}S]methionine, a newly synthesized procathepsin H with a molecular weight of 41 kDa appeared first in the rough microsomal fraction (Fig. 1A, lane 1). The radiolabeled proenzyme was also seen in the smooth

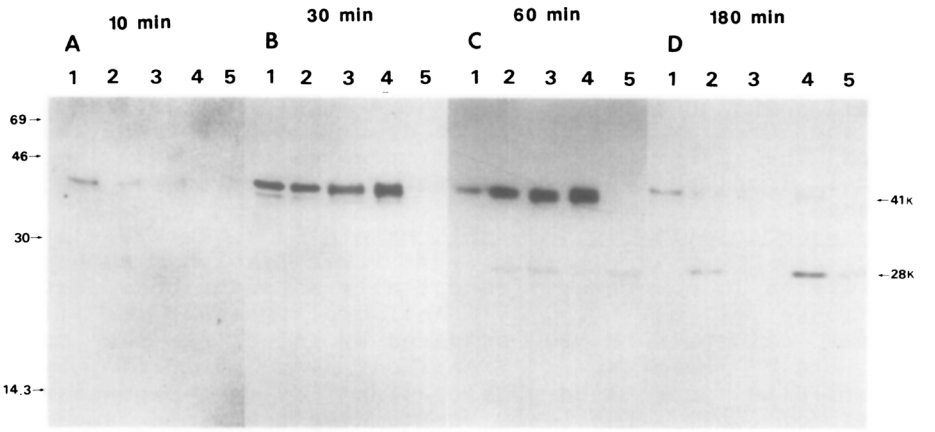

Figure 1. Appearance of biosynthetically labeled cathepsin H in subcellular fractions. Fractions were isolated from livers of rats sacrificed at selected time intervals after an intravenous administration of [^{35}S]methionine (250 μCi/100 g body weight). Shown are fluorographs of SDS-PAGE electrophoretograms of polypeptides immunoprecipitated with specific antibody against cathepsin H. 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 two different cathepsin H polypeptides are indicated at the right. The numbers to the left indicate the masses (in kilodaltons) of the molecular weight standards; bovine serum albumin (69,000), ovalbumin (46,000), carbonic anhydrase (30,000), and lysozyme (14,400).

microsomal fraction with lower intensity (Fig. 1A, lane 2) as compared with that in the rough microsomal fraction.

At 30 min postinjection, procathepsin H moved from the microsomal fractions to the Golgi subfractions (Fig. 1B). The radiolabeled procathepsin H with 41 kDa did not show an apparent molecular weight change based on electrophoretic mobility in the microsomal and Golgi subfractions (Fig. 1B, lanes 1-4). It is known that GF-3 is the heavy Golgi subfraction containing mainly cis-Golgi elements, while GF-1 is the light Golgi subfraction containing mainly trans-Golgi elements (5). These results therefore suggest that the processing of propeptide of procathepsin H proceeds neither in the microsomes nor in the Golgi complex.

In further pulse-labeling experiments at 60 min postinjection, the processed single-chain form of cathepsin H

with 28 kDa appeared in the lysosomal fraction (Fig. 1C, lane 5), but the proform enzyme was not detected in the lysosomal fraction. This observation indicates that the propeptide of the procathepsin H is rapidly cleaved within the lysosomes. The mature form of cathepsin H with 28 kDa also appeared in the microsomal and Golgi subfractions at 60 min (Fig. 1C, lanes 2-4). The presence of mature enzyme in condsiderable amounts in the microsomal and Golgi subfractions could be ascribed to lysosomal fraction contamination.

At 180 min postinjection, the 28 kDa form of cathepsin H still remained unprocessed in the lysosomal fraction (Fig. 1D, lane 5), suggesting that the proteolytic conversion of the single-chain form to the two-chain form does not occur in the lysosomes until 180 min after the administration of label. The presence of 28 kDa mature enzyme in the microsomal and Golgi subfractions could be due to the contamination by the lysosomal fraction (Fig. 1D, lanes 2-4).

The in vivo pulse-labeling studies with [^{35}S]methionine of the intracellular transport and processing of lysosomal cathepsin B have recently shown that a newly synthesized procathepsin B with a molecular weight of 39 kDa moved from the endoplasmic reticulum to the Golgi complex between 10 and 30 min postinjection of the label, and additional 30 min was required for it to be transported to the lysosomes (16). Upon delivery to the lysosomes, the propeptide of procathepsin B was cleaved rapidly, generating the single-chain enzyme and the following processing of the single-chain form to the two-chain form took place concomitantly in the lysosomes between 60 and 180 min postinjection (16). Thus our kinetic experiments, carried out in vivo on the rat liver, regarding the intracellular transport and processing of lysosomal cathepsin H demonstrate

that procathepsin H is transported from the endoplasmic reticulum via the Golgi complex to the lysosomes in which the propeptide portion of proenzyme is concomitantly cleaved off, yielding the single-chain form. The subsequent conversion of the single-chain cathepsin H to the two-chain enzyme would be considered to occur in the lysosomes with rather slower kinetics as compared with the case of cathepsin B. Although the functional significance of cleaving the single-chain cathepsins B and H to the two-chain enzymes is unknown, it appears that cathepsins B and H may undergo distinct post-translational processing kinetics in the lysosomes.

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