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IN VITRO BIOSYNTHESIS OF THE LYSOSOMAL CATHEPSIN H

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SUMMARY: A lysosomal thiol protease cathepsin H has been synthesized in vitro and shown to undergo co-translational segregation into the lumen of microsomal vesicles. Using cell-free synthesis, a 36 K Da cathepsin H was found to be synthesized exclusively on membrane-bound polysomes. When the microsomal membranes were present during translation, a glycosylated 41 K Da proenzyme appeared in the microsomal lumen. This proenzyme was converted to a 34 K Da protein by endoglycosidase H treatment. These results suggest that the nascent chain of cathepsin H has a transient N-terminal prepropeptide.

Cathepsin H is a lysosomal thiol protease which is considered to function as an aminopeptidase in lysosomes (1). Lysosomal cathepsin H is a glycoprotein consisting of a mixture of a single-chain form with 28 K Da and its processed two-chain form with 22 K Da and 6 K Da (2,3). The primary amino acid sequence of rat liver lysosomal cathepsin H was determined previously (4). We have recently identified from pulse-chase experiments in cultured rat hepatocytes that a 41 K Da procathepsin H was synthesized after short time of labeling and this proenzyme was subsequently converted to the single-chain form enzyme with 28 K Da post-translationally by limited proteolysis (5).

ABBREVIATIONS: ER, endoplasmic reticulum; Endo H, endoglycosidase H; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis.

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It is well established that lysosomal enzymes are synthesized on membrane-bound ribosomes and at least initially follow the secretory route (6-8). The most extensive study on intracellular biogenesis has been carried out with cathepsin D (6-8). Cathepsin D is synthesized as its preproform on polysomes associated with the ER membranes, and then by passing through the membranes it is segregated into the ER lumen. During its passage, the N-terminal prepeptide of approximately 2 K Da is cleaved off, and simultaneously the oligosaccharide moieties are added to form the glycosylated proenzyme. Recently, cDNA clones encoding preprocathepsin D has been isolated and sequenced (9). Now the validity of these initial biosynthesis event has been demonstrated by the predicted primary structure of preprocathepsin D.

In this paper, we demonstrate that the lysosomal cathepsin H is segregated intracellularly in membrane-bound polysomes and the nascent polypeptide contains a transient N-terminal signal sequence with 2 K Da which may be functionally analogous to those determining the co-translational insertion of secretory polypeptides into the ER membranes.

MATERIALS AND METHODS

Materials. [^{35}S]Methionine (1000 Ci/mmoles) and wheat germ extracts were purchased from Amersham. Protein A-Sepharose CL-4B was obtained from Pharmacia, oligo(dT)-cellulose from Collaborative Research Inc. (Waltham, MA), trypsin and chymotrypsin from Boehringer Mannheim, Endo H from Seikagaku Kogyo Co. (Tokyo, Japan).

Cell-Free Protein Synthesis. Free and membrane-bound polysomes were prepared from rat liver by the procedure of Ramsey and Steele (10). Poly A(+) RNA was prepared by chromatography on ologo(dT)-cellulose (11). A cell-free wheat germ translation system, post-translational proteolysis, immunoprecipitation, Endo H digestion, and SDS-PAGE were as described (8). Dog pancreas microsomal membranes were prepared as previously reported (12).

Labeling and Preparation of Cell Extracts. Hepatocytes isolated from male Wistar rats by the collagenase perfusion method of Seglen (13) were diluted with Eagle's minimal essential medium containing 10 % fetal calf serum and cultured at 37°C in an air 5 % CO_2 for 24 hr. The labeling of the cells and the preparation of the cell extracts were performed as described (8).

Preparation of Antisera against Cathepsin H. Cathepsin H was purified from rat liver lysosomes by a method based on that of Kirschke et al.(2). Antisera against rat liver cathepsin H was prepapred as described previously (14).

RESULTS and DISCUSSION

In vitro translation experiments with mRNA's from free and membrane-bound polysomes obtained from rat liver according to the method of Ramsey and Steele (10) were carried out in a cell-free wheat germ system to determine the subcellular site of synthesis of the lysosomal cathepsin H. The immunoprecipitated translation products from each mRNA were analyzed by SDS-PAGE and fluorography as shown in Fig. 1. A 36 K Da translation product was obtained only in the translations of the membrane-bound mRNA pool (Fig. 1, lane 1), indicating that the lysosomal cathepsin H was exclusively synthesized on the rough ER. When the dog pancreas microsomal membrane vesicles were supplemented in the cell-free system after or during translation to examine the insertion into microsomal vesicles and glycosylation of the precursor enzyme, a 41 K Da procathepsin H appeared (Fig. 2, lane 3), but this proform did not appear when the membranes were added after translation was completed (data not shown). The molecular size of in vitro synthesized procathepsin H (Fig. 2, lane 3) corresponded well with that of in vivo synthesized proenzyme which was immunoprecipitated from cultured rat hepatocytes after 30 min of pulse-labeling (Fig. 2, lane 1). The procathepsin H was resistant to the attack of the trypsin treatment, when trypsin was added after translation had been completed in the

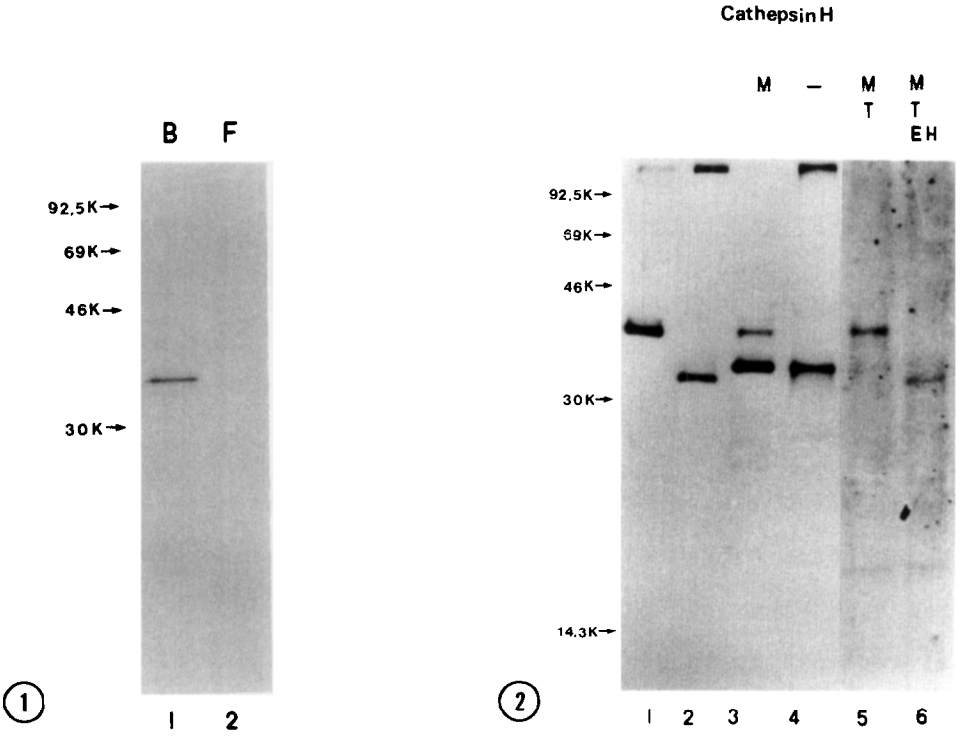

Figure 1. In vitro biosynthesis of cathepsin H. Messenger RNA samples isolated from free (lane 2) or membrane-bound polysomes (lane 1) prepared from rat liver were used to program wheat germ cell-free translation systems. Samples (0.2 ml) containing 10^7 cpm of incorporated [^{35}S]methionine were used for immunoprecipitation of the in vitro synthesized polypeptides with specific antibody against cathepsin H. The immunoprecipitates were analyzed by 15 % SDS-PAGE followed by fluorography (3 weeks) of the dried slab gel. The numbers to the left indicate the migration of the molecular weight standards.

Figure 2. Co-translational and processing of cathepsin H by dog pancreas microsomal membranes. Messenger RNA samples isolated from membrane-bound polysomes prepared from rat liver were translated in wheat germ cell-free systems. The in vitro products were compared with the polypeptides labeled in vivo in cultured rat hepatocytes which were incubated for 30 min with [^{35}S]methionine. All samples were purified by immunoprecipitation with specific antibody against cathepsin H and analyzed by 15 % SDS-PAGE followed by fluorography of the dried slab gel. Lane 1, the in vivo-labeled product isolated from cultured rat hepatocytes; lane 2, as lane 1, except the labeled product was treated with Endo H; lane 3, the in vitro translation product synthesized in the presence of microsomal membranes (4 A_{260}/ml); lane 4, the in vitro translation product synthesized in the absence of microsomal membranes; lane 5, as lane 3, except the labeled product was incubated (3 h, 4°C) post-translationally with a mixture of trypsin and chymotrypsin (final concentration of each protease, 3 mg/ml); lane 6, as lane 5, except the labeled product was treated with Endo H. The numbers to the left indicate the migration of the molecular weight standards.

presence of membranes (Fig. 2, lane 5). This result indicates that the co-translationally modified lysosomal procathepsin H was completely segregated into the microsomal lumen.

The proenzyme synthesized in vitro showed complete sensitivity to Endo H treatment and the proenzyme was converted to the 34 K Da protein (Fig. 2, lane 6). This 34 K Da unglycosylated form (Fig. 2, lane 6) comigrated with the Endo H-treated procathepsin H which was immunoprecipitated from cultured rat hepatocytes (Fig. 2, lane 2). The difference of the electrophoretic mobility on SDS-PAGE between the procathepsin H (41 K Da) and the product of Endo H-treated proenzyme (34 K Da) suggests that the procathepsin H is glycosylated with 7 K Da high-mannose type oligosaccharides. The amino acid sequence of the propeptide of procathepsin H has not been defined until now. However, it is reasonable to speculate that the propeptide region of procathepsin H may be N-glycosylated with 2 high-mannose type oligosaccharide chains , because it is known from the primary amino acid sequence of lysosomal cathepsin H that one potential N-glycosylation site is included within the molecule (4). Recently, cDNA clones encoding human and mouse preprocathepsin B , lysosomal thiol protease, have been isolated and sequenced (15). The predicted complete structure of preprocathepsin B demonstrated that a potential N-glycosylation site was also included in the propeptide sequence.

From the results presented in this paper, it is concluded that the lysosomal cathepsin H is segregated into the ER lumen as soon as it has been synthesized, whereby removal of a 2 K Da signal peptide and glycosylation take place at an initial stage of biosynthesis. This biosynthetic mechanism may be in analogy to the cases of other lysosomal enzymes reported previously (6-8).

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