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Biosyntheses and processing of lysosomal cysteine proteinases in rat macrophages

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The intracellular processing and release of three lysosomal cysteine proteinases, cathepsin B, H and L, by rat peritoneal macrophages were investigated by pulse-chase experiments. Newly synthesized procathepsins B (39 kDa), H(41 kDa) and L (39 kDa) after 15 min labeling were processed to the mature, single-chain enzymes within 1 h. The single-chain forms of cathepsin B, H and L were further processed to two-chain forms at different rates: conversion of cathepsin L to the two-chain form was rapid, whereas the conversions cathepsin B and H took at least 6 h. Macrophages released 30% of the procathepsins B and L, and 10% of the procathepsin H.

Cathepsin B; Cathepsin H; Cathepsin L; Intracellular processing; (Rat macrophage)

1. INTRODUCTION

Cathepsins B, H and L are major lysosomal cysteine proteinases that are important in the degradation of intracellular proteins and exogenous proteins taken up by lysosomes [1,2]. Purified cathepsins B, H and L are all glycoproteins consisting of mixtures of single chain forms and their processed two chain forms [3-6]. In recent studies on cloning of the cDNA for cathepsins B [7], H [8] and L [9], the amino acid sequences of the preproenzymes have been determined, and biosynthetic studies in two laboratories [10-12] have shown that cathepsins B and H are synthesized as larger precursor forms, procathepsins B and H, which in the glycosylated state have molecular masses of 39 kDa and 41 kDa, respectively. However there have been no comparative studies on the biosyntheses and processing of the three cathepsins B, H and L.

Macrophages contain high amounts of lysosomal cysteine proteinases [13], which may be released from the cells in the process of phagocytosis. In the present paper we report differences in the intracellular processing and release of the three cathepsins by rat peritoneal macrophages.

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Abbreviation: SDS-PAGE, SDS-polyacrylamide gel electrophoresis

2. MATERIALS AND METHODS

[^{35}S]Methionine (1 mCi/mmol), radioactive ^{14}C-methylated rainbow standard proteins and Amplifier were purchased from Amersham. Formaldehyde-fixed *Staphylococcus aureus* (IgG sorb) was from the Enzyme Center, Franklin, MA, USA.

Macrophages were obtained from Wistar rats of 8-10 weeks old, 3 days after intraperitoneal injection of 20 ml of 6% sodium caseinate, and were cultured as described [14]. The cells were incubated overnight in methionine-free Eagle's MEM containing 10% (v/v) heat inactivated calf serum. The cells were labeled with 100 μCi of [^{35}S]methionine (1 mCi/mmol) in 1.0 ml of Eagle's MEM containing no serum, and the labeling medium was replaced by RPMI 1640 containing 2% calf serum at the beginning of the chase period. After labeling, the medium was concentrated in a Centricon Y-10. The labeled macrophages

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were rinsed twice with 3 ml of phosphate buffered saline (PBS) and lysed in 0.5 ml PBS containing 0.2% Triton X-100. The cell extract and medium were subjected to freezing-thawing twice and centrifuged at $105\,000 \times g$ for 1 h. Cathepsins B, H and L were precipitated from the extracts with monospecific antisera against the respective enzymes. Antisera against rat cathepsins B, H [13] and L [6] were prepared as described. The immunoprecipitates were collected, washed and solubilized, and the radioactive polypeptides were analyzed by 10–20% gradient SDS-PAGE followed by fluorography using Amplifier on Kodak XAR-5 film. For sequential precipitation of several lysosomal cathepsins, the supernatant obtained after each immuno-precipitation step was supplemented with another antiserum and processed as described above.

3. RESULTS AND DISCUSSION

The biosyntheses of cathepsins B, H and L were studied by pulse-chase experiments. Rat macrophages were pulsed with 100 μCi/1.0 ml of [^{35}S]methionine for 15 min, and then chased with fresh medium containing normal methionine and 2% calf serum. At the times indicated in fig.1, cathepsin B was immunoprecipitated from both the cell lysate and the medium. The earliest form of cathepsin B synthesized was a 39 kDa precursor polypeptide that was processed first to a single-chain form of 29 kDa within 1 h and then to the two-chain form of the enzyme over a period of 21 h (fig.1a, left). Only the 25 kDa heavy chain of the two-chain form of cathepsin B was detected, since the 4 kDa light chain migrated with the dye front in this polyacrylamide gel system. The clear sequential appearances of the three major forms (39 kDa, 29 kDa and 25 kDa) of cathepsin B suggested a precursor-product relationship of the three forms. After 0.5 h of chase, the 39 kDa polypeptide was found in the culture medium and its amount increased with time (fig. 1a, right). About 30% of the cathepsin B containing [^{35}S]methionine was secreted by the macrophages. With other preparations of macrophages, mature forms of the enzyme (single and two-chain forms) were secreted into the media as well as the 39 kDa polypeptide (not shown). The results of pulse-chase analysis of cathepsin H are shown in fig.1b. Cathepsin H was initially synthesized as a 41 kDa precursor polypeptide that was processed to a 28 kDa single-chain form within 1 h of chase and finally to a 23 kDa two-chain form after 6 h of chase (fig.1b, left). Although the mode of intracellular processing of cathepsin H is similar to that of cathepsin B,

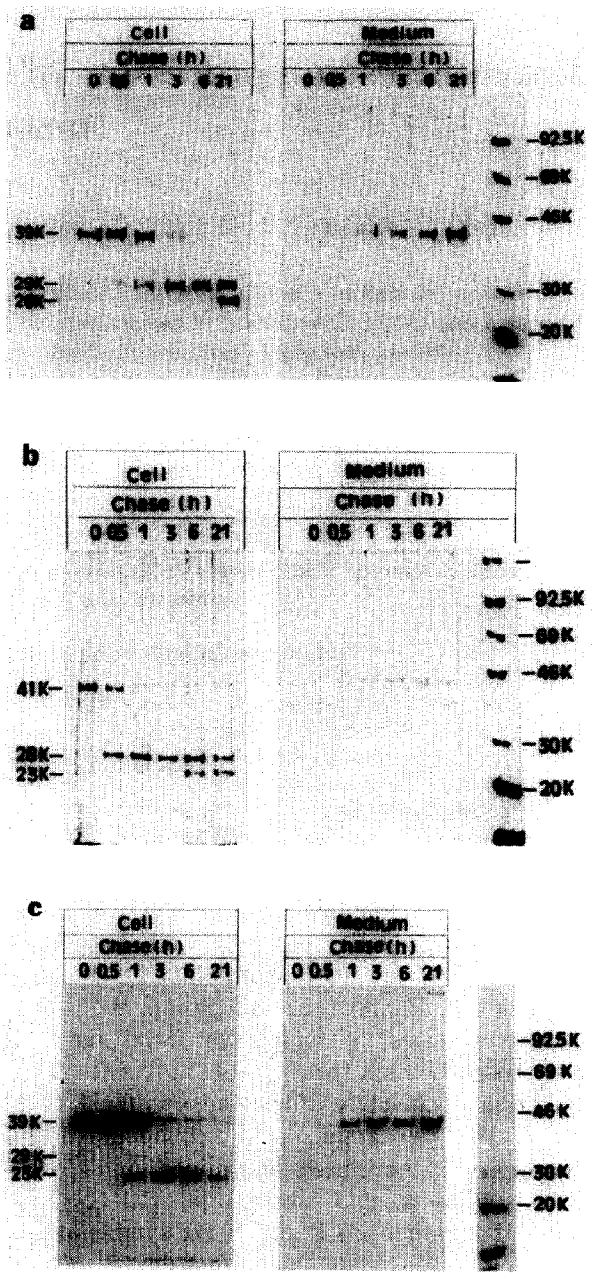

Fig.1. Biosynthesis, processing and release of cathepsin B, H and L. Rat peritoneal macrophages were pulse-labeled for 15 min with [^{35}S]methionine and then incubated in chase medium. At the indicated times, the cells (left) and media (right) were collected. The cells were lysed in PBS containing 0.2% Triton X-100 and centrifuged and the supernatants were incubated with 10 μg of anti-cathepsin B (a), H (b) or L (c) mono-specific antibody. After concentration, the media were incubated sequentially with anti-cathepsin B, H and L antibody. The immunoprecipitates were washed, resuspended in gel sample buffer, and subjected to SDS-PAGE followed by fluorography.

release of the 41 kDa precursor of cathepsin H into the culture medium was much less than that of cathepsin B precursor (fig.1b, right): about 10% of the cathepsin H containing [^{35}S]methionine was released by macrophages.

Cathepsin L was also synthesized as a 39 kDa precursor polypeptide that was processed to a 29 kDa single-chain form within 30 min of chase. The latter was rapidly converted to a 23 kDa two-chain form within 1 h (fig.1c, left). Thus, the single-chain form of cathepsin L was only found transiently as a faint band. The intensity of the heavy-chain form (23 kDa) of cathepsin L decreased with time after 6 h of chase, possibly due to its degradation. Release of the label incorporated in the 39 kDa precursor cathepsin L into the culture medium was observed after 1 h of chase and increased with time (fig.1c, right). About 30% of cathepsin L containing [^{35}S]methionine was released by macrophages.

The present study showed that precursors to three macrophage lysosome cysteine proteinases, cathepsin B, H and L are processed proteolytically to mature single-chain forms at similar rates after their synthesis, but that there is a large difference between the rates of processing of the single-chain forms of cathepsin L and cathepsin B and H to the two-chain forms (fig. 1a–c). In the steady state, the single-chain forms of cathepsin B and H were detected by Western blotting in several tissues, such as the kidney, spleen, lung and brain (unpublished). However, only traces of the single-chain form of cathepsin L were found in the many tissues examined, supporting the finding in pulse-chase experiments that the single-chain form of cathepsin L is very unstable in cells. The processing of the single-chain forms of the three enzymes to the two-chain forms in lysosomes were markedly inhibited by a specific cysteine proteinase inhibitor, E-64 [15].

As shown in fig.1, large amounts of procathepsins B, H and L are rapidly released from macrophages. These results suggest that newly synthesized cathepsins B, H and L follow one of two intracellular pathways. One pathway ends in the rapid secretion of about 30% of the procathepsins B and L and about 10% of the procathepsin H, while the other pathway ends with the formation of mature enzymes that are deposited in lysosomes. It seemed important to examine whether the three procathepsins released are phosphorylated. The phosphorylated form of the mannosyl residue of lysosomal enzymes serves as a recognition marker that mediates binding of the enzymes to specific receptors and their transport into lysosomes [16–18]. If the procathepsins released are phosphorylated, they may be unusual lysosomal proteins, mannose 6-phosphate-containing secretory proteins, like the major excreted protein (MEP, 19). In mice, MEP is suggested to be procathepsin L [8,20,21]. Thus procathepsin L secreted into the culture medium of macrophages is probably phosphorylated. The extensive release of cathepsins by sodium-caseinate-elicited macrophages is possibly related with their extracellular proteolytic function in inflammation.

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