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AUTOCATALYTIC PROCESSING OF PROCATHEPSIN E TO CATHEPSIN E AND THEIR STRUCTURAL DIFFERENCES

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SUMMARY: The processing of human gastric procathepsin E to its mature form, cathepsin E, was studied at pH 3.5. The results revealed the autocatalytic and apparently one-step conversion of procathepsin E to cathepsin E within 10 min of incubation at 14°C under the conditions used. Analyses of the amino acid sequences of both procathepsin E and cathepsin E showed that cleavage occurred at the Met^{36}-Ile^{37} bond to produce the mature form, cathepsin E. The NH_2-terminal amino acid sequence of procathepsin E thus determined was identical with that predicted from the cDNA sequence by Azuma et al. except that the NH_2-terminal glutamine residue in the latter was converted into a pyroglutamic acid residue in the former and that the glycine residue at position 2 in the latter sequence was deleted in the former. On the other hand, the NH_2-terminal amino acid sequence of cathepsin E was identical with that reported previously by us.

Human gastric cathepsin E is an aspartic proteinase present in human gastric mucosa and different from pepsinogens A and C and cathepsin D. Previously, we purified this enzyme to apparent homogeneity and investigated its properties (1). The enzyme was shown to have a molecular weight of about 85,000 and composed of two apparently identical subunits. The properties of this enzyme was further studied by Samloff et al. (2) and by us (3). Recently Azuma et al. (4) reported the complete amino acid sequence of human gastric procathepsin E predicted from the analysis of cDNA

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clones derived from a gastric adenocarcinoma cell line. The amino acid sequence includes a 17-residue signal peptide and a 379-residue proenzyme, the boundary between these two parts being predicted from the known structures of the pre-proforms of other aspartic proteinases.

Very recently, we have reported the amino acid sequences of the NH_2-terminal region and some other parts of human gastric cathepsin E (5). Yonezawa et al. have also reported the NH_2-terminal sequence of rat gastric cathepsin E (6). Cathepsin E has been localized to the mucosal lining of the stomach, erythrocytes and also several lymphoid-associated tissues and cells including thymus, spleen, macrophages, and polymorphonuclear lymphocytes (7,8). However, its physiological function and process of activation in these tissues and cells remain obscure. Due to its intracellular localization in lymphoid-associated tissues and cells, it has been suggested that the enzyme may have a role in immune function (2,9). Therefore, further characterization of this enzyme is necessary to understand its physiological role.

In the present study, activation of procathepsin E to cathepsin E was studied using procathepsin E purified from human gastric mucosa. The results revealed the autocatalytic conversion of procathepsin E to its activated form (i.e., cathepsin E) at acidic pH, similar to the conversion of pepsinogen to pepsin. Cathepsin E was isolated from the activation mixture and its NH_2-terminal sequence and amino acid composition were analyzed together with those of procathepsin E. These analyses indicated that the cleavage occurred at the Met^{36}-Ile^{37} bond to produce cathepsin E. Further, the NH_2-terminal amino acid sequence of procathepsin E was determined for the first time on the protein level. The result agreed with the sequence predicted from the cDNA sequence analysis except that the protein had a pyroglutamic

acid residue at the NH_2-terminus and that the glycine residue at position 2 was deleted in our sequence.

MATERIALS AND METHODS

Materials. Total gastric procathepsin E was purified from the human gastric mucosa essentially as described previously (1,3) with additional chromatography on a Mono Q column. Polyvinylidene difluoride (PVDF) membrane was purchased from Millipore, pyroglutamate aminopeptidase (calf liver) from Böehringer Mannheim Biochemica, and pepstatin A from Peptide Institute (Osaka).

Activation profile of procathepsin E. Procathepsin E solution (0.16 mg/ml) in 0.1 M HCOOH/NaOH at pH 3.5 was incubated at 14°C. Aliquots were withdrawn at appropriate time intervals and mixed with a 1/3 volume of the sample buffer for SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) (0.4 M Tris-HCl buffer, pH 8.2, containing 10 % 2-mercaptoethanol, 5 % SDS, and 37 % glycerol). Aliquots of about 20 µl were subjected to electrophoresis according to Laemmli (10). The concentration of the gel was 15.0 % and the gel was stained with Coomassie Brilliant Blue R-250.

Amino acid sequence analysis. The NH_2-terminal amino acid sequences of procathepsin E and cathepsin E were determined by using an Applied Biosystems pulse-liquid protein sequencer model 477A/120A. The NH_2-terminal sequence of procathepsin E was analyzed before and after treatment with pyroglutamate aminopeptidase. In the latter case, procathepsin E (200 µg) was dissolved in 0.25 ml of 20 mM N-ethylmorpholine acetate buffer, pH 8.0, containing 100 mM EDTA and 10 mM DTT and mixed with 10 µg of pyroglutamate aminopeptidase. The mixture was incubated at 4°C for 18 h, followed by incubation at 25°C for 4 h. The reaction mixture was mixed with the SDS-PAGE sample buffer and subjected to electrophoresis according to Laemmli (10). Proteins in the gel were electroblotted onto PVDF membrane with 10 mM 3-[cyclohexylamino]-1-propanesulfonic acid buffer, pH 11, and the membrane was stained with Coomassie Brilliant Blue R-250. Protein bands were cut out, washed with distilled water, and subjected to the automated sequencing.

The NH_2-terminal amino acid sequence of cathepsin E was determined in the same way as above with a procathepsin E sample (200 µg) incubated at pH 3.5 and 14°C for about 3 min.

Amino acid analysis. Procathepsin E (60 µg) and an activation mixture of procathepsin E (120 µg, incubated for about 3 min at pH 3.5 and 14°C), were separately subjected to electrophoresis and electroblotting as described above. Proteins in dried PVDF bands were hydrolyzed as described (11). Amino acids were extracted from the membrane as described (12) and analyzed in an Applied Biosystems automated derivatizer-analyzer (420A/130A).

RESULTS AND DISCUSSION

Activation of Procathepsin E. The time course of activation of procathepsin E as analyzed by SDS-PAGE is shown in Fig. 1.

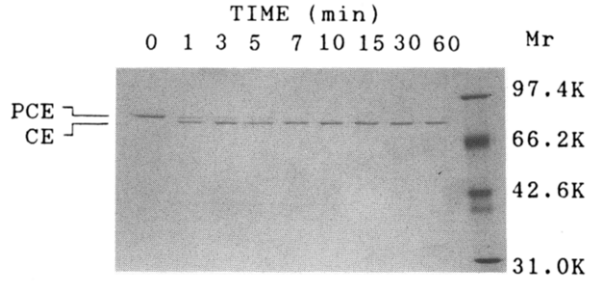

Fig. 1. Time course of activation of procathepsin E analyzed by SDS-PAGE. Procathepsin E solution (0.16 mg/ml) was incubated at pH 3.5 and 14°C. Aliquots were removed at appropriate time intervals and subjected to SDS-PAGE under non-reducing conditions. PCE, procathepsin E; CE, cathepsin E.

Apparently direct or one-step conversion of procathepsin E into cathepsin E was observed, whereas porcine procathepsin D (13) and human pepsinogens A and C (14) were shown to be activated through intermediate forms. The conversion was rapid and complete within 10 min and this convertion was inhibited completely by 0.1 mM pepstatin A (data not shown). These results indicate that procathepsin E is converted autocatalytically to its mature form, cathepsin E, at acidic pH. This fast activation rate of procathepsin E rather resembles that of human pepsinogen C (14). The molecular weights of the dimeric forms of procathepsin E and cathepsin E were estimated to be approximately 80,000 and 76,000, respectively, under non-reducing conditions (Fig. 1), and those of the monomeric forms of procathepsin E and cathepsin E produced under mild reducing conditions to be approximately 40,000 and 38,000, respectively (data not shown). These differences in molecular weight between procathepsin E and cathepsin E were consistent with the removal of the activation peptide segment from the zymogen upon conversion to its mature form, although it was difficult to determine the exact difference in molecular weight between the two proteins under the conditions used.

NH_2-Terminal amino acid sequences and amino acid compositions of procathepsin E and cathepsin E. When procathepsin E was analyzed

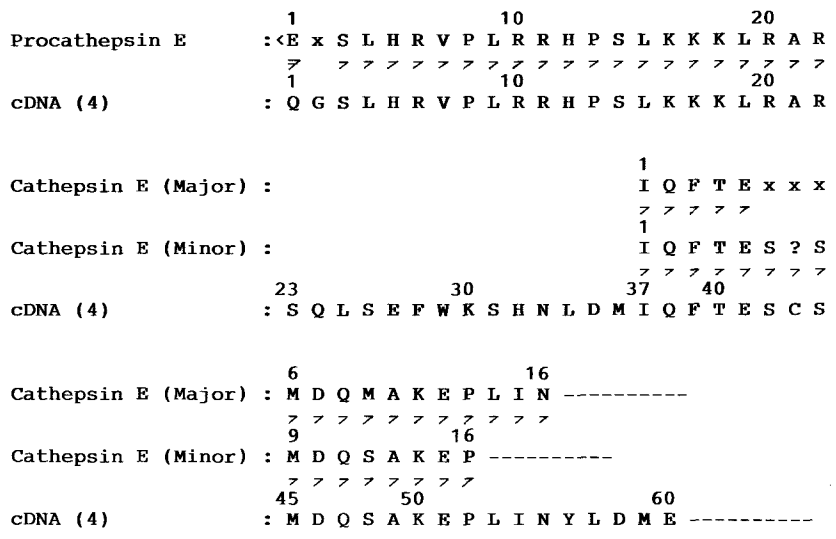

Fig. 2. Comparison of the NH_2-terminal amino acid sequences of human gastric procathepsin E and cathepsin E with the corresponding sequence of the procathepsin E predicted from its cDNA sequence (4). <E, pyroglutamic acid residue; x, deletion; ⇂, released by the action of pyroglutamate aminopeptidase; →, each cycle of Edman degradation.

directly by the protein sequencer, no phenylthiohydantoin (PTH)-amino acids could be detected in a significant amount as reported previously (5), indicating that the NH_2-terminus of the protein may be blocked. This is consistent with the putative NH_2-terminal glutamine residue of procathepsin E (4), which may be easily converted into a pyroglutamic acid residue. Therefore, in the present study, procathepsin E was first treated with pyroglutamate aminopeptidase, then purified by SDS-PAGE and electroblotting and sequenced. Thus we could obtain the NH_2-terminal 20-residue amino acid sequence of procathepsin E as shown in Fig. 2. This result is also consistent with the occurrence of a glutamine residue at the NH_2-terminus of procathepsin E, as suggested by Azuma et al. (4). However, after the pyroglutamate aminopeptidase treatment, the amino acid sequence was found to start from the third amino acid residue, Ser^3, of the sequence predicted from the cDNA sequence (4). This suggests the deletion of the second amino acid residue, Gly^2, in

the protein sequence. This is probably due to the occurrence of isozymogens, as observed previously for cathepsin E (5).

Previously, we determined the NH_2-terminal 39-residue sequence of cathepsin E using the enzyme obtained by treatment of procathepsin E at pH 4.0 for about 3 h followed by affinity purification on pepstatin-Sepharose. On the other hand, when a cathepsin E sample similarly prepared was stored at pH 8.0 and $0^{\circ}C$ for about three months and then sequenced, the NH_2-terminal sequence was found to start mostly from Ser^{42} of procathepsin E (4). This suggested the occurrence of autodigestion of cathepsin E during storage. To minimize such a possibility of autodigestion, if any, cathepsin E was obtained by incubating procathepsin E for a shorter period (3 min) at pH 3.5 and $14^{\circ}C$, then sequenced in the present study. One major and one minor sequences of each 16 amino acid residues could be determined, which were present in an approximate molar ratio of 3 : 2 as shown in Fig. 2. This result is essentially the same as that obtained previously (5), thus confirming Ile^{37} of procathepsin E to be the NH_2-terminal amino acid residue of cathepsin E.

The amino acid analysis of procathepsin E yielded a result comparable to that obtained previously (1), whereas the contents of basic amino acids, especially arginine and lysine residues, in cathepsin E were notably lower than those in procathepsin E (data not shown). These differences are consistent with the relatively higher content of arginine and lysine residues in the activation peptide segment of the procathepsin E sequence predicted from the cDNA sequence (4).

Cathepsin E is now known to be an intracellular aspartic proteinase. It is present not only in stomach mucosa, but also in other lymphoid-associated tissues and cells (7,8). Moreover, the existence of membrane-associated forms have been reported in

erythrocytes (15) and neutrophils (8). The mechanism of processing of procathepsin E to its mature form in these different tissues and cellular compartments at intracellular neutral pH is not clearly understood at present. Studies are in progress in our laboratory to clarify this point.

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