Native and Acid-denatured l-Lactate Dehydrogenase Are Different in the Lysosomal Proteinases Involving in Their Overall Degradation

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When native and acid-denatured lactate dehydrogenase (LDH) were incubated with total lysosomal enzymes in vitro, amino acids from their degradation were produced at various acidic pH. The pH profile in the overall degradation of native LDH was markedly different from that of acid-denatured LDH. Disappearance of the 35-kDa subunit of native LDH was markedly suppressed by a low level of cystatin α as well as by a general cysteine proteinase inhibitor, N-(l-3-trans-carboxyoxirane-2-carbonyl)-l-leucine-3-methylbutylamide (E-64-c). On the other hand, the degradation of acid-denatured LDH was only slightly suppressed by these inhibitors. It was concluded that at least a part of the proteinases involved in the overall degradation of native LDH is different from the proteinases involved in the degradation of acid-denatured form and a role of a cystatin α-sensitive cysteine proteinase is critical in the lysosomal degradation of native LDH, but not in that of acid-denatured form.

Key words: lysosomes; cysteine proteinases; cathepsins; cystatin; l-lactate dehydrogenase

Various species of cellular proteins, whether native or denatured, are thought to be ultimately sequestered into lysosomes, because sequestration of cytoplasmic proteins into lysosomes by autophagy is nonspecific.1,2 Many species of proteinases are present in the lysosomes and degrade various proteins completely. However, substrate specificities of lysosomal proteinases for protein substrates are not completely understood. Lysosomal cysteine proteinases are believed to be important in the lysosomal degradation of various cellular proteins because the lysosomal degradation of various cellular proteins is markedly suppressed by cysteine proteinase inhibitors.3-6 Various cytosolic enzymes including l-lactate dehydrogenase (LDH) accumulate in the lysosomes after exposure to leupeptin in vivo and retain their enzymatic activities in the lysosomes.1,3 An interesting feature of the lysosomal degradation of LDH is that the degradation of its native form is markedly suppressed by a low level of cystatin α in vitro.7 Whether a role of this cystatin α-sensitive cysteine proteinase is also critical in the lysosomal degradation of acid-denatured LDH has not been known.

When native and acid-denatured LDH were incubated at various pH with disrupted lysosomes prepared from rat liver in vitro, they were effectively degraded. Figure 1 shows that the pH profile in the overall degradation of native LDH by total lysosomal enzymes was different from that in the overall degradation of the acid-denatured form. The rate of the degradation of native LDH was higher at pH 4.0 but much lower above pH 5.0 than that of acid-denatured LDH.

SDS-PAGE analysis shows that 35-kDa subunits of native and acid-denatured LDH both largely disappeared during incubation with total lysosomal enzymes (lanes 2 in Fig. 2A and 2B). Disappearance of the 35-kDa subunit of native-LDH was markedly suppressed by a low level of cystatin α as well as E-64-c (lane 3 and 4 in Fig. 2A). On the other hand, the degradation of acid-denatured LDH was only slightly suppressed by these inhibitors (lanes 3 and 4 in Fig. 2B). Pepstatin, an inhibitor of acid proteinases including cathepsin D had little or no effect on the degradation of both native and acid-denatured LDH (data not shown). These results indicate that at least a part of the proteinases involved in the overall degradation of native LDH are different from the proteinases involved in the degradation of acid-denatured LDH and the role of a cystatin α-sensitive cysteine proteinase is critical in the lysosomal degradation of native LDH but is not in that of acid-denatured LDH. This cystatin α-sensitive cysteine proteinase seems to be distinct from cathepsins B, H, L, and J (C), because it is separated from cathepsin J (C) by Sephacryl S-200 gel-filtration column chromatography and from cathepsin H by DEAE-Sephadex A-50 anion-exchange column chromatography,7 and neither of cathepsins B and L inactivates LDH in vitro.8 Whether or not it is distinct from other reported lysosomal cysteine proteinases remains unknown. As cathepsin S is reported to be inhibited by low levels of cystatins,9 it may be active in the

Abbreviations: LDH, l-lactate dehydrogenase (EC 1.1.1.27); FITC, fluorescein isothiocyanate; E-64-c, N-(l-3-trans-carboxyoxirane-2-carbonyl)-l-leucine-3-methylbutylamide
lyosomal degradation of native LDH, although its expression in the liver is much less than those of cathepsins B and L.\textsuperscript{(10)}

Conformational structures of the substrate proteins are thought to be an important factor in their degradation. The high activities of degradation of acid-denatured LDH at broad ranges of pH may be due to the actions of various lysosomal proteinases with various optimal pH. The high activities of degradation of native LDH at around pH 4.0 are probably not only due to the action of a specific proteinase with optimal pH around pH 4.0 but also to the conformational structure of LDH being disrupted by acidic pH.

Fig. 1. pH Profiles of Overall Degradation of Native LDH and Its Acid-denatured form by Total lysosomal Enzymes.

LDH was purified from the cytosol fraction of rat liver by the method of Scopes.\textsuperscript{(11)} Acid-denatured LDH was prepared by incubating purified LDH (10 mg/ml) supplemented with 100 mM sodium acetate, pH 4.0, for 2 hours at 37°C. Lysosomes were prepared as described elsewhere.\textsuperscript{(11)} The incubation mixtures (100 μl each) containing disrupted lysosomes (an equivalent of 50 mM arylsulfatase), 10 mM 2-mercaptoethanol, 1 mM EDTA, 100 mM sodium acetate adjusted to various acid pH (4.0–6.0) and substrate protein (100 μg each) of native LDH or acid denatured LDH were incubated for 0 or 30 min at 37°C. The production of amino acids by the degradation of native LDH (○) or acid-denatured LDH (△) was measured by the method of FITC labeling and chromatography.\textsuperscript{(11)} The values for the fractions containing FITC-labeled amino acids are means for two experiments and were corrected for the value at 0 min and plotted.

Fig. 2. Effects of Cysteine Proteinase Inhibitors on the Lysosomal Degradation of Native LDH and Its Acid-denatured form in Vitro.

The incubation mixtures (100 μl each) containing materials as described in Fig. 1 were incubated at pH 5.0, for 0 or 60 min at 37°C in the absence or presence of a proteinase inhibitor, then treated with an equal volume of SDS buffer as described by Laemmli.\textsuperscript{(11)} The samples (10 μl each) were resolved by SDS-PAGE on 10% gels. The degradations of native LDH and acid-denatured LDH are shown in panels A and B, respectively. The samples were obtained from preparations incubated for 0 min (lanes 1) or 60 min in the absence of proteinase inhibitor (lanes 2) and in the presence of 0.5 μg cystatin α (lanes 3) or 1.0 μg E-64-c (lanes 4). Cystatin α was purified from a recombinant strain of E. coli\textsuperscript{(12)} and E-64-c was obtained from Peptide Institute (Osaka). The arrowheads is the position of the 35-kDa subunit band of LDH. Molecular mass markers (lanes 5) were myosin (200 kDa), β-galactosidase (116.2 kDa), rabbit muscle phosphorylase b (97.4 kDa), bovine serum albumin (66.2 kDa), hen egg white albumin (45.0 kDa), bovine carbonic anhydrase (31.0 kDa), and soybean trypsin inhibitor (21.5 kDa).

References


