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BIOCHEMICAL AND IMMUNOCHEMICAL SIMILARITY BETWEEN ERYTHROCYTE MEMBRANE ASPARTIC PROTEINASE AND CATHEPSIN E

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SUMMARY: An aspartic proteinase previously thought to be unique to erythrocyte membranes, termed "EMAP", has been shown to be closely related to cathepsin E. Enzymic comparison revealed that these two enzymes resembled each other in molecular weight, susceptibility to pepstatin and chromatographic behaviors on DEAE-Sephacel and Mono P chromatofocusing columns. They were immunoprecipitated by antiserum against human EMAP in a similar way. Immunochemical similarity between the two enzymes was also substantiated by immunoblot analysis.

In normal red cells, the aspartic proteinase is found largely in the membrane-bound form on the cytoplasmic face of the membranes (1,2). The unusual catalytic features are that the membrane-bound form is latent, as far as it is assayed with protein substrates such as hemoglobin and serum albumin, and that the soluble form is active on the protein substrates at only acidic pH values (2). This property may be of considerable importance to its physiological significance which is unknown at present. To understand the physiological significance of the enzyme, it seems important to know whether it is really unique to erythrocytes or how it is related to other intracellular aspartic proteinases, for an explanation of physiological significance of enzymes, in general, must take into account the tissue distribution or the cellular localization. Although it has been demonstrated that there are immunochemical differences between EMAP and cathepsin D [EC 3. 4. 23. 5] (3) and between cathepsin D and cathepsin E [EC 3. 4. 23. -] (4, 5),

Abbreviations: EMAP, erythrocyte membrane aspartic proteinase; SDS, sodium dodecylsulfate.

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there is little information available on the relation between EMAP and cathepsin E. In the present communication, we compare the two enzymes biochemically and immunochemically.

MATERIALS AND METHODS

Preparation. The purification of EMAP from human erythrocyte membranes were performed by the method described for rat EMAP (3). Cathepsin E, previously designated as cathepsin E-like acid proteinase, from rat spleen was purified as described before (6).

Determinations. Proteinase activities of EMAP and cathepsin E were measured at pH 3.5 using acid-denatured bovine hemoglobin as substrate as described (1). At this pH value, more than 95 % of the maximal activity for each enzyme were exhibited.

Polyacrylamide gel electrophoresis and immunoblotting. SDS-polyacrylamide gel electrophoresis was performed by the method of Laemmli (7). Gels were stained for protein using silver-stain kits from Daiichi Chemicals. For immunoblotting, proteins transferred from SDS-gels to nitrocellulose membranes were immunostained by the procedure described before (8).

RESULTS AND DISCUSSION

When the molecular weights of the purified enzymes were estimated by gel filtration on a Sephadex G-100 column (1.6 x 138 cm) equilibrated with 20 mM sodium phosphate buffer, pH 7.0, containing 0.15 M NaCl, cathepsin E had a somewhat greater molecular weight than EMAP (90,000 vs. 79000) as previously reported (3, 6). Nevertheless, SDS-polyacrylamide gel electrophoresis under reducing conditions showed that the two enzymes consisted of two identical subunits of the same size (Mr=41,000) as shown in Fig. 1, suggesting that they may be slightly different in three-dimensional configuration. When the enzymes were applied to a DEAE-Sephacel column (1.0 x 3.0 cm) equilibrated with 20 mM sodium phosphate buffer, pH 7.0, they required the same concentration of NaCl (0.3 M) for their elution. When the samples of the two enzymes were separately applied to a Mono P column (1.0 x 27 cm) packed with PBE 94 that had been equilibrated with 25 mM bis-Tris HCl (pH 6.3) and eluted with polybuffer 74 (pH 4.0), both enzymes showed the single peak of activity at the same position between pH 4.1 and 4.5.

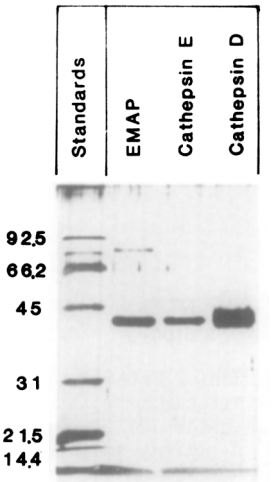

Figure 1. SDS-polyacrylamide gel electrophoresis of human EMAP, rat spleen cathepsins E and D on 10 % slab gels under reducing conditions. The gel was stained for protein by silver-stain kits.

The inhibition profiles of the two enzymes by pepstatin were also very similar. The Ki values of EMAP and cathepsin E were 5.2×10^{-9} M and 1.8×10^{-10} M, respectively. In addition, as expected from previous studies (1, 2, 6), both enzymes appear to be similar in substrate specificity, sensitivity to various protease inhibitors and amino acid composition. Taken together, the present results indicate that the two enzymes are enzymatically indistinguishable.

We have previously shown that the anti-human EMAP antibody cross-reacted not only with human EMAP but also with rat and rabbit EMAPs (3, 9). This indicates that it has a broad cross-reactivity with the enzymes from different mammalian species. Therefore, we could employ the antibody to know the immunochemical relation of human EMAP to cathepsins E and D from rat spleen. To determine whether cathepsin E in solution is quantitatively precipitated by the antiserum against human EMAP, cathepsin E was incubated at 37 °C for 10 min in 20 mM sodium phosphate buffer, pH 7.0, containing various concentrations of the antiserum and then allowed to stand at 4 °C overnight. After centrifugation, the remaining activity in the supernatant fraction was measured. As shown in Fig. 2, a progressive loss of activity was found

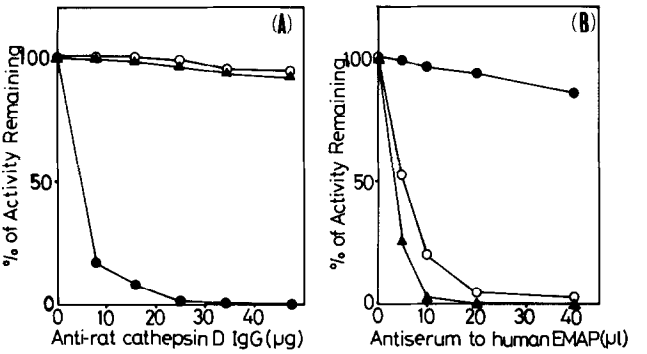

Figure 2. Immunoprecipitation of EMAP(▲), cathepsins E(○) and D(●) with the rabbit anti-rat cathepsin D antibody (A) or with the rabbit antiserum to human EMAP (B). The respective enzyme solutions containing equal amounts of activity were reacted with various concentrations of the antibody or the antiserum. After centrifugation, the remaining activity in the supernatant fraction was measured.

for cathepsin E. A similar immunoprecipitation pattern was observed for human EMAP, although the antiserum acted more efficiently than in the case of cathepsin E. In contrast, cathepsin D purified from rat spleen was hardly precipitated with the antiserum. In the experiments using the anti-rat cathepsin D antibody, both EMAP and cathepsin E were hardly precipitated. The immunochemical similarity between EMAP and cathepsin E was also substantiated by immunoblot analysis. Figure 3 illustrates the results of the immunoblot stainings using the affinity-

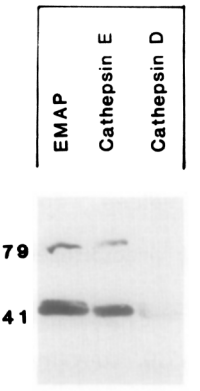

Figure 3. Immunoblot analysis of EMAP, cathepsins E and D from SDS-polyacrylamide gels. The lanes contained equal amounts of activity from each enzyme (1.4 μg EMAP, 4 μg cathepsin E and 22 μg cathepsin D). Immunodetection by the anti-human EMAP antibody after transferring of proteins to nitrocellulose membranes was performed as described (2).

purified anti-human EMAP antibody. In this experiment, equal amounts of enzyme activity, which correspond to 1.4 μg for EMAP, 4 μg for cathepsin E and 22 μg for cathepsin D, respectively, were applied to each track. After SDS-polyacrylamide gel electrophoresis under reducing conditions, the enzymes were transferred to nitrocellulose membranes from the gel. Cathepsin E showed the same immunostaining as EMAP ; a major band with Mr=41000 and a minor band with Mr=79000, indicating that the two enzymes share common antigenic structures.

The present study provides the first evidence that cathepsin E and EMAP are indistinguishable not only enzymatically but also immunochemically. This is of great interest since the two enzymes appear to be different in the tissue distribution or the cellular localization. Cathepsin E is found chiefly in polymorphonuclear cells (10) and rat spleen (4) and in small amount in macrophages and lymphocytes (10). Furthermore, of particular interest here are reports by Tarasova et al. (11) and by Muto et al. (12) who have described the homology of a non-pepsin slow-moving proteinase from gastric mucosa with EMAP and with cathepsin E, respectively. These findings suggest that EMAP may have some general function.

The monospecific antibody to human EMAP, although it hardly precipitated rat spleen cathepsin D (Fig. 2), nevertheless showed weak but detectable cross-reaction with denatured cathepsin D by immunoblotting as shown in Fig. 3. The result is in good agreement with our earlier findings that EMAP slightly cross-reacted with the affinity-purified anti-rat cathepsin D antibody, although in order to obtain a similar density of the blots the protein amount of EMAP was more than 7 times that of cathepsin D (2). The immunodetection on protein blots is not strictly quantitative, but the results suggest that EMAP and cathepsin D also have at least one common antigenic site which appears not to be exposed equally in the native proteins.

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