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ACTIVATION OF THE LATENT HUMAN NEUTROPHIL GELATINASE BY UREA

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The mechanism of activation of the latent human neutrophil gelatinase by urea has been studied in greater detail. After dialysis of the latent gelatinase against increasing concentrations of urea a considerable increase of its activity was observed. Moreover, the results indicate a progressive conversion of the latent 94 000 Da gelatinase into a proteolytically active fragment of 80 000 Da, which was subsequently processed to a few species of lower molecular mass inactive against gelatin. This conversion was completely inhibited by EDTA, suggesting an autocatalytic reaction. The inhibition was reversed by Zn\(^{2+}\) or Co\(^{2+}\). Thus, urea alters both the enzymatic and physical characteristics of the latent gelatinase which suggests that conformational changes may induce autoactivation of the latent enzyme.

Human neutrophils contain a number of proteinases active at neutral pH that can degrade extracellular matrix components. These enzymes include two metalloproteinases, collagenase and gelatinase, present in the cells in latent form [1, 2]. The latent neutrophil gelatinase can be activated by a variety of treatments or agents including proteinases [3-6], mercurial compounds [3, 4, 6] and urea [3, 6].

In this communication further results on the gelatinase activation by urea are described.

MATERIALS AND METHODS

Human neutrophil gelatinase was purified according to Sopata [7] and its activity was assayed as described previously [3]. One unit of gelatinase activity was defined as the amount of the enzyme which hydrolysed 1 µg of gelatin per minute at 37°C. SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was performed by the method of Laemmli [8]. Zymography was carried out in SDS-PAGE using a gelatin containing gel as described previously [9].

RESULTS AND DISCUSSION

Dialysis of the latent gelatinase against urea followed by removal of urea by dialysis against Tris buffer resulted in a considerable increase of the enzyme activity (Table 1, cf. also Fig. 2). When the urea activated gelatinase was
Table 1

Effect of different agents on gelatinase activity

The purified gelatinase was preincubated at 22°C as indicated. Trypsin activity was stopped by adding diisopropylfluorophosphate to a final concentration of 2 mM. The samples containing urea were dialysed against 0.005 M Tris/HCl buffer, pH 7.5, containing 0.2 M NaCl and 0.005 M CaCl₂, for 2 h at 22°C. After treatment the samples were incubated with gelatin at 37°C for 1 h and the resultant activity was assayed [3].

<table>
<thead>
<tr>
<th>Agent</th>
<th>Preincubation time (min)</th>
<th>Gelatinase activity (units/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>—</td>
<td>215.6</td>
</tr>
<tr>
<td>Trypsin, 100 µg/ml</td>
<td>30</td>
<td>2494.1</td>
</tr>
<tr>
<td>Urea, 8 M</td>
<td>120</td>
<td>1422.9</td>
</tr>
<tr>
<td>EDTA, 10 mM</td>
<td>30</td>
<td>—</td>
</tr>
<tr>
<td>next: urea, 8 M</td>
<td>120</td>
<td>580.2</td>
</tr>
<tr>
<td>EDTA, 10 mM</td>
<td>30</td>
<td>—</td>
</tr>
<tr>
<td>next: urea, 8 M with Zn²⁺, 0.5 mM</td>
<td>120</td>
<td>1160.4</td>
</tr>
</tbody>
</table>

analysed by SDS-PAGE it was found that the enzyme was converted into a species of lower molecular mass (Fig. 1). This process was dependent on urea concentration. Upon activation by 5 M urea the 94,000 Da protein band of the reduced latent gelatinase was partially converted into a fragment of about 80,000 Da (Fig. 1). The newly generated polypeptide degraded gelatin in a zymography gel (Fig. 2). Moreover, it is evident that the urea treated

Fig. 1. SDS-PAGE analysis of the latent gelatinase activated by urea. The latent gelatinase was dialysed against urea at 22°C for 2 h: a, protein standards; latent gelatinase: b, untreated, and after dialysis against different concentrations of urea: c, 5 M; d, 6 M; e, 7 M; f, 8 M. Before electrophoresis (SDS-PAGE) the samples were dialysed against 0.005 M Tris/HCl buffer, pH 7.5, containing 0.2 M NaCl and 0.005 M CaCl₂ for 2 h at 22°C. The protein standards were: phosphorylase b (94,000 Da), bovine serum albumin (67,000 Da), ovalbumin (43,000 Da), carbonic anhydrase (30,000 Da), and soybean trypsin inhibitor (20,000 Da).
Fig. 2. Substrate gel analysis of the latent gelatinase activated by urea. The latent gelatinase was dialysed against urea for 2 h at 22°C. Enzyme: a, untreated, and after dialysis against different concentrations of urea: b, 4 M; c, 5 M; d, 6 M; e, 7 M; f, 8 M. After treatment the samples were dialysed against Tris buffer and applied to SDS-PAGE containing 1 mg gelatin/ml. Further details as in Fig. 1.

gelatinase became active even when its molecular mass remained unchanged (Fig. 2). Analysis of the latent gelatinase after treatment with 6, 7 and 8 M urea showed that the enzyme was further processed into some additional fragments of 43000-46000 Da (Fig. 1). These smaller polypeptides were inactive towards gelatin (Fig. 2). Thus, activation of the latent gelatinase by 6-8 M urea and autolysis of the enzyme ran simultaneously. Moreover, only 8 M urea fully converted the latent gelatinase into a proteolytically active polypeptide of 80000 Da (Figs. 1, 2).

Fig. 3. Effect of different agents on activation of the latent gelatinase by urea. The latent gelatinase was dialysed against 8 M urea. a, Protein standards as in Fig. 1 plus α-lactalbumin (14000 Da); latent gelatinase: b, untreated; c, urea treated; d, EDTA (10 mM) treated prior to the urea treatment; e, EDTA (10 mM) treated prior to the urea and Zn²⁺ (0.5 mM) treatment; f, EDTA (10 mM) treated prior to the urea and Co²⁺ (5 mM) treatment. Further details as in Fig. 1.
The effect of urea on the appearance of the species of lower molecular mass was completely blocked by treating the latent gelatinase with 10 mM EDTA prior to the urea treatment (Fig. 3). The inhibition was reversed by the addition to urea of either Zn\(^{2+}\) (0.5 mM) or Co\(^{2+}\) (5 mM) (Fig. 3).

Since urea has no intrinsic ability to cleave peptide bonds it is postulated that this agent induces conformational changes in the latent gelatinase which becomes then converted to a proteolytically active form without loss of molecular mass. In turn, due to the lability of the activated conformation, the enzyme is processed to lower molecular mass species.

REFERENCES