Effect of Microbial and Mite Proteases on Low and High Molecular Weight Kinogens

GENERATION OF KININ AND INACTIVATION OF THIOL PROTEASE INHIBITORY ACTIVITY*

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Kinin release from guinea pig plasma high molecular weight kininogen (HMWK) induced by various microbial and mite proteases has been demonstrated previously (Molla, A., Yamamoto, T., Akaike, T., Miyoshi, S., and Maeda, H. (1989) J. Biol. Chem. 264, 10589–10594; Maruo, K., Akaike, T., Matsumura, Y., Kohmoto, S., Inada, Y., Ono, T., Arao, T., and Maeda, H. (1991) Biochim. Biophys. Acta 1074, 62–68). In this paper, we describe the effects of various microbial and mite proteases on low molecular weight kininogen (LMWK) and HMWK from human plasma. A protease from the house dust mite Dermatophagoides farinae (DF-protease) directly liberated kinin from both LMWK and HMWK to a significant degree. The $K_m$ and $k_{cat}$ values for kinin generation from LMWK were 3.24 $\mu$M, 0.61 $s^{-1}$, and $1.9 \times 10^6 M^{-1} s^{-1}$, respectively, and those for kinin generation from HMWK were 0.56 $\mu$M, 0.12 $s^{-1}$, and $2.1 \times 10^6 M^{-1} s^{-1}$, respectively; $k_{cat}/K_m$ values for DF-protease were comparable with that for glandular kallikrein. In contrast, microbial proteases showed only weak kinin-releasing activity from both human plasma kininogens. Four of ten different microbial proteases liberated kinin from LMWK, and only serratal 56-kDa protease released kinin from HMWK. Furthermore, DF-protease markedly inactivated the thiol protease inhibitory activity of LMWK and HMWK, whereas all microbial proteases (as well as the endogenous protease trypsin) did not affect this inhibitory activity of both kininogens from human plasma.

Kinis appear to play an important role in a number of pathological states, i.e. allergic (1, 2) and viral (3–5) rhinitis, bronchial asthma (6, 7), carcinoid syndrome, septic shock, inflammatory joint disease, dumping syndrome (8), and microbial infections (9–15). The ability of kininogens to inhibit thiol proteases, such as cathepsins B, H, and L and calpains, is important for the generation of kinins in vivo.

Two distinct kininogens, high molecular weight kinogen (HMWK) and low molecular weight kininogen (LMWK), are present in plasma and are single gene products containing bradykinin plus identical amino-terminal heavy chains (19, 20). Both kininogens release kinins on limited proteolysis by kallikrein-like enzymes (21, 22) and can act as a thiol protease inhibitor (16, 23, 24).

We have previously detailed the crucial roles of microbial proteases in the pathogenesis of various microbial infections (9–12, 14). For example, all bacterial proteases liberate bradykinin (BK) by activation of the Hageman factor (HF)-prekallikrein (PK) cascade, or directly from guinea pig HMWK (9–12, 14). Kinin generated by bacterial proteases in infections seems to be a pathogenic principle causing edema and pain in addition to tissue destruction and impairment of defense capability (11, 25).

We recently reported that a 30-kDa serine-type protease from the house dust mite Dermatophagoides farinae (DF-protease) could activate all steps of the kinin-generating cascade in the guinea pig, i.e. HF, PK, and HMWK (26). DF-protease also enhanced the vascular permeability reaction by activation of the kinin-generating cascade in vivo (26). These findings suggest an important role of mite protease in house dust-induced allergic diseases.

In this study, we tested the effects of various microbial and mite proteases on two functions of LMWK and HMWK from human plasma, kinin generation and thiol protease inhibition.

We found that DF-protease can liberate kinins from both kininogens and inactivate their thiol protease inhibitory activity, whereas microbial proteases are less effective.

MATERIALS AND METHODS

Reagents—LMWK and HMWK were isolated and purified from human plasma as described previously (16). DF-protease from D. farinae was purified from cultured mites according to the method of Takahashi et al. (27). The purity of DF-protease was confirmed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (Fig. 1A), by fluorographic detection of the active site serine in this enzyme (Fig. 1B), and by SDS-substrate gel electrophoresis. 2 Amino acid sequence analysis on DF-protease had been performed, and only one amino-terminal residue was found (28). Serratia pro-

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1 The abbreviations used are: HMWK, high molecular weight kinogen; LMWK, low molecular weight kininogen; HF, Hageman factor; PK, prekallikrein; BK, bradykinin; MCA, 4-methylcoumaryl-7-amide; MUGB, 4-methylumbelliferyl-p-guanidino-benzoate hydrochloride; Me, peritoneal exudate macrophage; PAGE, polyacrylamide gel electrophoresis; DFP, diisopropyl fluorophosphate; HPLC, high pressure liquid chromatography; DF, Dermatophagoides farinae.

2 SDS-substrate polyacrylamide gel electrophoresis embedded with casein also indicated that there was only one proteolytic zone corresponding to the band of DF-protease in SDS-PAGE (data not shown).
Microbial and Mite Protease Effect on Kinogens

**RESULTS**

*Generation of Kinins from LMWK and HMWK by Various Microbial and Mite Proteases—As shown in Table I, Df-protease efficiently released kinin from LMWK and HMWK. Kinin was generated from LMWK or HMWK in a stoichiometric manner by Df-protease at a molar ratio of 0.2 or 0.4; 294 nM kinin was liberated from 300 nM LMWK, and 168 nM kinin was released from 170 nM HMWK. Although six microbial proteases did not cause release of kinin from LMWK, four proteases (Serratia, Aspergillus, and Streptomyces proteases, and subtilisin) generated a small amount of kinin. Kinin was not liberated from HMWK by the microbial proteases except for serratial 56-kDa protease. The kinetic parameters of kinin generation by Df-protease were $K_m = 5.24 \mu M$, $k_{cat} = 0.61 s^{-1}$, and $k_{cat}/K_m = 1.88 \times 10^6 M^{-1} s^{-1}$ (LMWK) and $K_m = 0.56 \mu M$, $k_{cat} = 0.12 s^{-1}$, and $k_{cat}/K_m = 2.14 \times 10^6 M^{-1} s^{-1}$ (HMWK). The kinetic parameters of kinin release from LMWK by glandular kallikrein were $K_m = 0.48 \mu M$, $k_{cat}$
TABLE I

<table>
<thead>
<tr>
<th>Protease</th>
<th>From LMWK</th>
<th>From HMWK</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glandular kallikrein</td>
<td>288</td>
<td>ND*</td>
</tr>
<tr>
<td>Df-protease</td>
<td>294</td>
<td>168</td>
</tr>
<tr>
<td>Serratia 56-kDa protease</td>
<td>13.2</td>
<td>14.4</td>
</tr>
<tr>
<td>Aspergillus protease</td>
<td>32.4</td>
<td>&lt;10&lt;sup&gt;θ&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pseudomonas elastase</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Pseudomonas alkaline protease</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Candida protease</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Vibrio vulnificus protease</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Streptomyces protease</td>
<td>28.5</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Subtilisin</td>
<td>28.5</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Thermolysin</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Staphylococcus V8 protease</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
</tbody>
</table>

*Not determined.
<sup>θ</sup> Obtained values were below 10 ng/ml, which was the detection limit of this method.

FIG. 2. Kinin generation from LMWK or HMWK by Df-protease. LMWK (A) or HMWK (B) was incubated with Df-protease (●) or bovine pancreatic trypsin (○) at various molar ratios for 15 min at 37 °C in 0.02 m Tris-HCl buffer (pH 7.4) containing 0.15 m NaCl, 0.1% bovine serum albumin, and 0.02% NaN₃. After deproteination, the kinin content was measured by enzyme immunoassay as described under “Materials and Methods.”

= 0.08 s<sup>−1</sup> and k<sub>cat</sub>/K<sub>m</sub> = 1.67 × 10<sup>5</sup> M<sup>−1</sup> s<sup>−1</sup>. The kinetic parameters (k<sub>cat</sub>/K<sub>m</sub>) of kinin generation from both kinogens by Df-protease were comparable with that of glandular kallikrein, an endogenous specific enzyme for LMWK.

Furthermore, we tested the efficacy of Df-protease for kinin liberation from LMWK and HMWK. Df-protease was incubated with an excess amount of LMWK or HMWK at various molar ratios for 15 min at 37 °C. Kinin release from both kinogens became apparent (20% release) at a molar ratio of about 0.0033 (Df-protease/kinigen). Maximal release of kinin was observed at a molar ratio of 0.033 (Fig. 2A) in the reaction system of Df-protease/LMWK and at a molar ratio of 0.1 (Fig. 2B) in the system with HMWK. Kinin was released from both kinogens by Df-protease in a time-dependent manner (Fig. 3). In addition, Df-protease liberated kinin more efficiently than did trypsin (Figs. 2 and 3).

HPLC Analysis of Kinins Generated from LMWK and HMWK by Df-protease—Kinin generation from LMWK and HMWK by Df-protease was also examined by using reverse-phase HPLC. As demonstrated in Fig. 4, BK was produced by Df-protease from both LMWK and HMWK after a 30-min incubation. The identity of BK in the eluate of the HPLC analysis was confirmed by using an enzyme immunoassay as described above. BK production from LMWK by Df-protease is in clear contrast to the proteolytic functioning of glandular kallikrein against LMWK; in this case, the sole product is kallidin (Lys-BK) (data not shown). (38)

Effects of Df-protease on Antiprotease Activity of LMWK or HMWK—The papain inhibitory activity of LMWK and HMWK was investigated after their treatment with proteases. Df-protease inactivated papain inhibitory activity in both LMWK and HMWK in a dose-dependent manner within 15 or 60 min (Fig. 5). The decrease in papain inhibitory activity became apparent (>10%) at the molar ratio of 0.3 or higher, and, at the molar ratio of 1.0, the activity of LMWK and HMWK diminished to 10 and 20%, respectively, after 60 min of incubation. Furthermore, this inhibitory activity diminished in a time-dependent manner after incubation of kinogens with Df-protease (Fig. 6, A and B). However, trypsin and all 10 microbial proteases tested did not affect the papain inhibitory activity of LMWK or HMWK (data not shown). We also suggest that kinin-releasing domains of both kinogens (Fig. 3) were more susceptible to the proteolytic action of Df-protease than were thiol protease inhibitory domains (Fig. 6).

Df-protease inactivated the anticathepsin activity of
that all 11 microbial proteases tested generated appreciable plasma and secretions in vertebrates, by limited proteolysis plasma LMWK and HMWK, which are kinin precursors in the guinea pig. Our previous reports showed amounts of kinin from guinea pig plasma HMWK (9, 11, 12, 14). Therefore, there may be a species difference in susceptibility of kininogens to various microbial proteases. Similar results for HF were reported by Yamamoto et al. (39) and others (40), which were attributed to the different amino acid sequences around the cleavage site in the molecules of HF from human and guinea pig.

It has been well recognized that house dust (containing Dermatophagoides) induces bronchial asthma, allergic rhinitis, and atopic dermatitis (41, 42). Stewart et al. (43) reported that mite extracts and house dust contained proteolytic activities belonging to serine and cysteine proteases, and the proteolytic activities in house dust were an important feature contributing to mite-related allergic diseases (43). In fact, our environmental studies revealed that house dust collected from homes of healthy volunteers contained a serine-type protease, which possessed the same substrate specificities as DF-protease and was immunologically identical to DF-protease.

More importantly, the protease in the house dust also efficiently generated BK from LMWK and HMWK. Kinins were identified in nasal and bronchial secretions of atopic subjects and may contribute to the symptoms of allergic and viral rhinitis and of asthma (1–7). We therefore speculate that inhalation of house dust mite protease, such as DF-protease, can induce kinin generation and exacerbate inflammatory reactions in some pathological conditions.

Both LMWK and HMWK play an important role as thiol protease inhibitors (16, 17, 23, 24) in addition to that of a kinin precursor (21, 22). Kininogens can inhibit thiol proteases, and this ability has an important role in some inflammatory conditions (16–18). We found that the thiol protease inhibitory activity of LMWK and HMWK against papain or cathepsins from Mφs was decreased by DF-protease treatment. Thus, thiol proteases such as cathepsins and calpains, which are released in inflammatory tissues, may remain uninhibited in the presence of DF-protease. In our previous study, DF-protease showed proteolytic action against the HF-PK-dependent kinin-generating cascade even in the presence of serine-protease inhibitors (serpins) and α2-macroglobulin in plasma (26). Therefore, tissue damage may be increased not only by direct action of DF-protease but also by proteolytic activity of thiol proteases.

In addition, our recent investigation indicates that DF-protease can strongly activate the complement system in human plasma, resulting in histamine release from mast cells and chemotaxis of polymorphonuclear cells. Hence, house dust mite protease, such as DF-protease, may thus contribute to the pathogenesis of house dust–induced allergic diseases.

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