hK5 and hK7, two serine proteinases abundant in human skin, are inhibited by LEKTI domain 6

T. Egelrud, M. Brattsand, P. Kreutzmann,* M. Walden,† K. Vitzithum,‡ U.C. Marx,‡ W.G. Forssmann† and H.J. Mägert†

Department of Public Health and Clinical Medicine, Dermatology and Venereology, University of Umeå, Umeå, Sweden
*Chemical Laboratory, University of Cambridge, Cambridge, U.K.
†IPF Pharmaceuticals GmbH, Hannover, Germany
‡Lehrstuhl Biopolymere, University of Bayreuth, Bayreuth, Germany

Correspondence
Hans-Jürgen Mägert,
Faculty 7, Anhalt University of Applied Sciences,
Köthen, Germany.
E-mail: hj-maegert@gmx.de

Accepted for publication
11 April 2005

Key words:
atopic dermatitis, desquamation, inflammation, kallikreins, Netherton syndrome, SPINK5

Conflicts of interest:
None declared.

Summary

Background Several skin diseases and atopic disorders including Netherton syndrome and atopic dermatitis have been associated with mutations and deviations of expression of SPINK5, the gene encoding the human 15-domain serine proteinase inhibitor LEKTI. The biochemical mechanisms underlying this phenomenon have not yet been fully clarified.

Objectives To identify target proteinases of LEKTI important for processes of desquamation and inflammation of the skin which will enable the development of specific drugs.

Methods The inhibitory activities of LEKTI domains 6 and 15 were tested on a number of commercially available serine proteinases and also on the purified kallikreins hK5 and hK7. In addition, recombinant hK5 was used.

Results LEKTI domain 6 is a potent inhibitor of hK5 and hK7, whereas LEKTI domain 15 exhibits inhibitory activity on plasmin. hK5 and hK7 in particular are relevant to skin disorders.

Conclusions The inhibition of hK5 and hK7 by LEKTI domain 6 indicates an important regulatory role of LEKTI in processes of skin desquamation and inflammation, which may explain the severe pathological symptoms associated with abnormalities of SPINK5 and/or its expression. Thus, LEKTI represents a potential drug for the treatment of these disorders.

Desquamation of epidermal skin layers is a natural process important for the continuous regeneration of the skin.1 In contrast, uncontrolled pathological desquamation of the skin and skin inflammation are responsible for symptoms observed in severe skin diseases such as Netherton syndrome (ichthyosis linearis circumflexa), psoriasis, atopic dermatitis and many others. Proteinases such as hK5 (SCTE) and hK7 (SCCE) play an essential role in both processes,2,3 for instance by degrading the desmosomes, structures responsible for cell–cell cohesion.4 Moreover, proteinases directly exhibit several proinflammatory effects including the proteolytic activation of certain cytokines, the attraction of leucocytes, and the induction of proinflammatory activation cascades such as the kallikrein/kinin and the complement systems.5 In 1999, we discovered a novel human 15-domain serine proteinase inhibitor LEKTI (lympho-epithelial Kazal-type-related inhibitor).6 The corresponding gene SPINK5 is expressed in lymphatic and epithelial tissues including certain layers of the skin.6–9 Mutations within SPINK5 cause the severe congenital disease Netherton syndrome.10 Symptoms of this disease include incomplete cornification of the skin and atopic manifestations such as asthma and allergic rhinitis.11 It has been shown that several gene polymorphisms are linked to atopic manifestations, including atopic dermatitis and asthma even in patients without Netherton syndrome,12–15 which indicates an important physiological role of LEKTI. In this study, we identified three LEKTI-regulated proteinases, hK5, hK7 and plasmin, at least two of which are of particular relevance for skin disorders including those mentioned above.

Materials and methods

Highly enriched fractions containing native hK5 and hK7 were prepared from extracts of plantar stratum corneum as described.3 Separation of hK5 and hK7 was obtained by gel exclusion chromatography in 0.3 mol L⁻¹ acetic acid, 0.3 mol L⁻¹ NaCl. Fractions used for experiments with LEKTI peptides were devoid of other proteolytic enzymes, as judged by casein
zymography in sodium dodecyl sulphate–polyacrylamide gel electrophoresis gels. The recombinant (r) proform of hK5 with a mutated activation site was prepared and activated with enterokinase as described.16

LEKTI domain 6 was purified from human blood filtrate as described.6 LEKTI domain 15 was produced recombinantly by means of a corresponding polymerase chain reaction-derived cDNA fragment inserted into the vector pET32a and Escherichia coli Origami (DE3) as the production strain. After purification by Co2⁺ affinity chromatography, the HIS-Tag was removed from the expression product by cleavage with PreScission protease (GE Healthcare).

For testing the inhibitory activities of LEKTI domains 6 and 15, lyophilized preparations were dissolved in water to a final concentration of 100 μmol L⁻¹. Incubations were carried out in flat-bottomed 96-well culture plates. Each incubation mixture contained: 5 μL of inhibitor, appropriately diluted in water, 5 μL of proteinase (final concentrations for hK5 and hK7 2.5 μg mL⁻¹, respectively), rhK5 5 μg mL⁻¹ (≈140 nmol L⁻¹), bovine pancreatic trypsin (T-8253; Sigma) 25 ng mL⁻¹ (≈1·1 nmol L⁻¹) and 40 μL of the chromogenic substrate [H-D-Ile-Pro-Arg-pNA.HCl (S-2586; Chromogenix) for trypsin and hK5; MeO-Suc-Arg-Pro-Tyr-pNA.HCl (S-2288; Chromogenix) for trypsin and hK7; S-2834; Chromogenix) for hK7] prepared by mixing one volume of 6·2 mmol L⁻¹ in water, with one volume of 0·2 mol L⁻¹ Tris–HCl pH 8·0, 0·2 mol L⁻¹ NaCl (final concentrations of substrate 2·5 mmol L⁻¹; Tris–HCl 80 mmol L⁻¹; NaCl 80 mmol L⁻¹). The plates were incubated at 37 °C, and absorbance at 405 nm measured in an enzyme-linked immunoassorbent assay reader after 1·2 h (absorbance corrected for blank values 0±1–0·3). Inhibition tests of human plasmin [527621; Calbiochem; 470 ng mL⁻¹ (≈6·0 nmol L⁻¹)] were performed in a similar manner to those carried out by Kreutzmann et al.17 by using N-p-tosyl-Gly-Pro-Lys 4-nitroanilide (T-6140; Sigma) as substrate. Inhibitory activities on the other commercially available serine proteinases were tested as described.6

Results

To clarify the regulatory mechanisms of LEKTI and to evaluate its future use as a drug for the treatment of skin diseases, we tested LEKTI domains 6 and 15 for their inhibitory activity on a selected number of commercially available serine proteinases (trypsin, chymotrypsin, leucocyte elastase, thrombin, granzyme B, trypase, plasmin, urokinase and chymase). LEKTI domain 6 almost exactly represents the consensus sequence of LEKTI domains 3–14, and contains two disulphide bonds. In contrast, LEKTI domain 15 contains three disulphide bonds, which is characteristic of the typical Kazal-type inhibitors. Moreover, it shares partial sequence identity with LDTI, a leech-derived trypase inhibitor.18

As has been found in earlier experiments, LEKTI domain 6 showed an inhibitory effect on trypsin.6,17 (Fig. 1C). A similar activity was also detectable for LEKTI domain 15 (Fig. 1A). In addition, we found a significant inhibitory effect of LEKTI domain 15 on plasmin, a serine proteinase responsible for fibrinolysis (Fig. 1B). In contrast, plasmin inhibition by LEKTI domain 6 was not detected (data not shown).

Because the described inhibition of trypsin by LEKTI domain 6 and of plasmin by LEKTI domain 15 does not explain the pathophysiological relevance of SPINK5 mutations for Netherton syndrome, we searched for potential LEKTI target proteinases that fulfill important biological functions in the skin. Among these, two members of the kallikrein family of serine proteinases, hK5 and hK7, appeared to be promising candidates. The corresponding genes are expressed in human epidermis and both proteinases have been reported to
participate in the degradation of proteins of the corneodesmosomes, structures responsible for cell–cell cohesion in the stratum corneum.\textsuperscript{1,3,4,19–21}

Our most striking result is that LEKTI domain 6 strongly inhibits hK5 and hK7 with an even higher efficacy than for inhibition of trypsin (Fig. 1C). Thus, hK5 and hK7 represent at least two of the main LEKTI target proteinases. In contrast, hK5 and hK7 are not inhibited by the LEKTI domain 15 (Fig. 1A). With the exception of a weak elastase inhibition observed for LEKTI domain 15 (data not shown), none of the above-mentioned serine proteinases other than trypsin, hK5, hK7 and plasmin was inhibited by LEKTI domains 6 or 15 (Table 1).

Discussion

The presented data show a significant inhibition of hK5 and hK7 by LEKTI domain 6. rhK5 seems to be less efficiently inhibited than native hK5, which may be due to two different reasons: firstly, the preparation of native hK5 may have contained other proteinases active towards the substrate S-2288 but not seen on zymography gels. Secondly, rhK5 produced in insect cells may differ from native hK5 by the pattern of glycosylation, which could affect interactions with the inhibitor.

hK5 and hK7 belong to the 15 members comprising the kallikrein group of serine proteinases, the genes of which are located within a cluster on human chromosome 19q13.4.19 Both exhibit a comparable expression pattern and stand in similar physiological contexts in so far as they degrade proteins such as desmoglein and corneodesmosin which belong to the corneodesmosomes.\textsuperscript{1,4,20,21} Furthermore, trypsin-like enzymes of the skin such as hK5 proteolytically activate the hK7 proenzyme\textsuperscript{16,21} and possibly also phospholipase A2, which leads to the synthesis of inflammation-mediating prostaglandins and premature lamellar body secretion.\textsuperscript{22,23} As a hypothesis, the latter effect may be mediated via activation of the proteinase-activated receptor 2 by the trypsin-like hK5 followed by G-protein-dependent activation of phospholipase A2 enzymes. hK7, on the other hand, may be capable of converting the precursor protein of interleukin-1\textbeta, a proinflammatory cytokine, into its active form.\textsuperscript{24} Increased expression of hK7 has been found in the skin of patients with atopic dermatitis and psoriasis.\textsuperscript{25} Thus, both proteinases may be of great importance in skin desquamation as well as inflammation (Fig. 2).

<table>
<thead>
<tr>
<th>Proteinase</th>
<th>hTrypsin</th>
<th>hChymotrypsin</th>
<th>hElastase</th>
<th>hThrombin</th>
<th>hGranzyme B</th>
<th>hβ-Trypsin</th>
<th>hPlasmin</th>
<th>hUrokinase</th>
<th>hChymase</th>
<th>hK5</th>
<th>hK7</th>
</tr>
</thead>
<tbody>
<tr>
<td>LD 6</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>rLD 15</td>
<td>+</td>
<td>–</td>
<td>±</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

+, significant inhibition; ±, weak inhibition; –, no inhibition; r, recombinant; b, bovine; h, human.

![Fig 2. Hypothetical regulatory role of LEKTI in the process of skin desquamation and inflammation. Using different pathways, hK5, hK7 and probably other kallikreins are involved in processes of skin desquamation and inflammation which may be pathological if negative regulation is impaired. Native LEKTI domain 6 is capable of inhibiting hK5 and hK7, and thus (probably together with other LEKTI domains) might be of high relevance for the control of these processes. PAR-2, proteinase-activated receptor 2.](image-url)
hK5 and hK7 are likely to fulfill important roles in skin desquamation and inflammation (see above). Furthermore, as is the case for almost all kallikreins, the corresponding genes are expressed with that encoding LEKTI (SPINK5) in epidermal cells.2,24 Thus, the inhibition of hK5 and hK7 by LEKTI domain 6 indicates an important regulatory role of LEKTI within the skin, which is supported by the fact that LEKTI domain 6 represents the consensus sequence of most of the LEKTI domains. The inhibition of other kallikreins, for instance the trypsin-like hK14, by certain LEKTI domains appears to be probable and has to be investigated in future studies.

As demonstrated by our group for domain 15, Mitsudo et al. recently described the inhibition of plasmin by the entire LEKTI protein.27 At present, the physiological significance of this activity is unclear but a future medical use of LEKTI domain 15 as an inhibitor of fibrinolysis in the treatment of burns and transplantation surgery is conceivable.

In summary, the inhibition of the kallikreins hK5 and hK7 by LEKTI domain 6, a typical representative of most of the 15 LEKTI domains, at least partially explains some of the symptoms occurring in skin diseases caused by certain SPINKs mutations (Fig. 2). Recent investigations show an increased proteolytic activity in the skin of mice,28 which confirms the assumed important regulatory role of LEKTI. As other members of the kallikrein family of serine proteinases may also participate in pathological processes in the skin, further investigations are required to determine the entire inhibitory range of LEKTI and to evaluate its therapeutic potential.

References