Abstract

Proteinase inhibitors are important negative regulators of proteinase action in vivo and are thus involved in several pathophysiological processes. Starting with the isolation of two new peptides from human blood filtrate, we succeeded in cloning a cDNA encoding the precursor protein for a novel 15-domain Kazal-type-related serine proteinase inhibitor. Two of the 15 domains almost exactly match the Kazal-type pattern, whereas the other 13 domains exhibit only four instead of six cysteine residues. Since the corresponding gene is expressed in several lympho-epithelial tissues, we termed this inhibitor lympho-epithelial Kazal-type-related inhibitor (LEKTI). For three of the 15 LEKTI domains, we demonstrated a significant trypsin-inhibiting activity. Recent results of another group show a relation between mutations within the LEKTI gene and the severe congenital disorder Netherton syndrome. In this review article, we give an overview of the already known data on the structure, processing, gene expression, and pathophysiological role of LEKTI. © 2002 Elsevier Science Ltd. All rights reserved.

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1. Introduction

We have used human blood filtrate (hemofiltrate) obtained from a nephrological center for many years as a natural source for the systematic isolation of novel peptides, preferentially those which exhibit a therapeutical potential. Following this strategy, in 1995 we purified two hitherto unknown peptides which, according to their molecular weight, were designated as HF6478 and HF7665. The cDNA cloning and analysis revealed that both peptides are represented within a 1064-amino-acid precursor protein exhibiting a total of 15 related domains. Because of its relation to Kazal-type serine proteinase inhibitors and the expression pattern of the corresponding gene, we termed the entire protein lympho-epithelial Kazal-type-related inhibitor (LEKTI) [1]. However, Chavanas et al. who are analyzing LEKTI at the genomic level termed the gene serine protease inhibitor, Kazal-type 5 (SPINK5) [2]. In terms of the high number of domains, the disulfide bondage pattern which is novel for serine proteinase inhibitors, and its relation to the severe congenital disease Netherton syndrome, LEKTI represents an interesting molecule whose biological role still remains to be analyzed in detail.

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Fig. 1. Amino-acid sequence of human LEC2 as derived from the cDNA sequence. Conserved regions are boxed. A typical secretory signal peptide printed in green and the classic KK sites being absent in the purified peptides are underlined. Cystine residues are printed in red. Domains 1, 5, and 6 have been purified from blood filtrate and are printed in blue. Domains 2 and 3 almost exactly match the pattern of Kazal-type inhibitors.
2. Structure

By means of several successive RT-PCR steps, we amplified and cloned a 3530 bp cDNA from vaginal epithelium (GenBank/EMBL accession no. AJ228139) which encodes a 1064-amino-acid precursor protein including HF6478 and HF7665. Sequence comparisons with the Prosite database revealed that two typical Kazal-type serine proteinase inhibitor domains (for review see [3]) are represented within the protein sequence. Moreover, the precursor protein contains 11 additional domains closely related to HF6478 (1st domain) and HF7665 (6th domain) in both their amino-acid sequences and the four cysteine patterns (Fig. 1).

Comparing the 15 LEKTI domains, we found that their cysteine patterns are identical but that the cysteines 3 and 6 of the Kazal-type domains are missing in the 13 non-Kazal-type domains. However, biochemical and mass spectrometric analysis of HF7665 (6th domain) revealed a 1–4/2–3 disulfide pattern of the remaining four cysteines which corresponds to the 1–5/2–4/3–6 disulfide pattern of the six cysteines of the Kazal-type domain [1].

3. Synthesis and degradation

For determination of the LEKTI gene expression pattern, we performed real-time quantitative RT-PCR, a state-of-the-art technique for the precise analysis of expression levels, with 15 different human tissues. The highest level of LEKTI gene expression was found in oral mucosa followed by the tonsils, parathyroid gland, thymus, and trachea (unpublished). Hybridization with a commercially available dot blot of mouse RNA revealed the strongest signals from prostate and epididymis. In descending order of intensity, we also obtained signals from uterus and embryo (17 days), eye and thyroid gland, and thymus and embryo (15 days, unpublished). In agreement with this data, Manfred Reinecke of the University of Zurich recently detected a strong LEKTI-specific immunoreactivity in human prostate (personal communication).

So far, we do not know very much about the degradation of the entire LEKTI protein. A typical N-terminally located secretory signal peptide deduced from the cDNA sequence is, as expected, not included in the first LEKTI domain which we isolated from blood filtrate. Since up to now we have isolated a total of 3 of the 15 LEKTI domains (domains 1, 5, and 6), a post-translational cleavage of the LEKTI precursor protein is probable. Furthermore, Ahmed et al. recently described the isolation of a 30 kDa protein from human epidermal keratinocytes whose N-terminus is identical to that of the predicted LEKTI domain 8 [4]. Several potential dibasic cleavage sites (KR) are positioned between predicted domains (see Fig. 1). Thus, it is conceivable that, by post-translational removal of these motifs, the LEKTI precursor protein is cleaved into functional domains, thereby providing a high number of inhibitors from only one precursor molecule.

4. Biological function

Using domain 6, which was available in sufficient amounts from human blood filtrate (concentration > 100 pM), the inhibitory properties of LEKTI were first analyzed. Testing the serine proteinases thrombin, factor Xa, tissue plasminogen activator, urokinase, plasmin, tissue kallikrein, plasma kallikrein, tryptase, trypsin, chymotrypsin, and leukocyte elastase, we obtained a significant but temporary inhibitory effect on trypsin with an apparent IC50 value of approximately 150 nM. Recently, trypsin-inhibiting activities were also found for the native LEKTI domain 5 (HF7072) and the recombinant LEKTI domain 15 (unpublished). Interestingly, domain 15 shares significant sequence identity with the leech-derived tryptase inhibitor (LDTI) [5], particularly in the P1-site surrounding region. Thus, a tryptase-inhibiting function of LEKTI is conceivable. Preliminary results from tryptase-inhibition tests using the particular recombinant domain 15 were negative. However, it cannot yet be excluded that an extended portion of the entire LEKTI protein is required for tryptase inhibition. From the expression pattern of the LEKTI gene, we assume that the biological function of LEKTI is not mainly the inhibition of trypsin. Its expression in mucous epithelia and associated glands as well as in lymphoid organs indicates a possible role in the regulation of T-lymphocyte differentiation, for instance...
by inhibition of apoptosis-inducing granzymes and/or antimicrobial protection by inhibition of proteinases essential for certain microorganisms. Thus, the main target proteinases of LEKTI still remain to be determined.

5. Possible medical and industrial applications

Using the radiation hybrid mapping technique, we localized the LEKTI gene on human chromosome 5q31-32 [1]. Chavanas et al. [6] later determined a correlation of this locus to the severe congenital dermatological disease Netherton syndrome by means of linkage analysis and homozygosity mapping. Indeed, the same group found that several mutations within the LEKTI gene (which they term SPINK5) are capable of causing Netherton syndrome [2]. Symptoms of this disease include defective cornification, atopic manifestations such as asthma, and a predisposition for bacterial infections (for review see [7]), verifying some of the assumptions mentioned. In addition, Cookson and co-workers found that certain coding polymorphisms within the LEKTI gene show associations with atopy, asthma and eczema in children without Netherton syndrome [8,9]. Since tryptase is a well-known asthma mediator (for review see [10]), a tryptase-inhibiting activity of LEKTI still has to be taken into consideration. A relation between LEKTI and failure to keratinize may be due to the action of proteinase-activated receptor 2 (PAR-2), agonists of which are capable of inhibiting growth and differentiation of keratinocytes [11]. Tryptase represents the PAR-2-specific agonist which again indicates a tryptase-inhibiting potential of LEKTI. However, this assumption still remains to be verified at the biochemical level, preferentially by use of the entire LEKTI protein. In summary, a future medical application of LEKTI is conceivable for the treatment of skin diseases, asthma, and microbial infections.

References