Quantification of Eight Tissue Kallikreins in the Stratum Corneum and Sweat

Journal of Investigative Dermatology (2006) **126,** 927–931. doi:10.1038/sj.jid.5700146; published online 2 February 2006

TO THE EDITOR

Human tissue kallikreins are a family of 15 trypsin or chymotrypsin-like secreted serine proteases (kallikrein protein (hK1–hK15) found in a variety of tissues (Yousef and Diamandis, 2001). In the stratum corneum (SC), we previously quantified hK5, hK6, hK7, hK8, hK10, hK11, hK13, and hK14 as candidates of desquamation-related proteases and compared the hK levels among various age groups (Komatsu *et al.*, 2005a). For sweat, hK1, hK2, and hK7 levels were quantified previously (Mann *et al.*, 1980; Mayfield *et al.*, 1989; Hibino *et al.*, 1994; Kishi *et al.*, 2004). In this study, we aimed (1) to quantitatively measure multiple hKs in the SC and sweat from normal individuals and from various body regions, (2) to determine the overall enzymatic activities of these samples, (3) to compare the results among body regions and genders, (4) to compare hK levels between the SC and sweat samples, and (5) to examine a possible correlation between the hK amounts and enzymatic activities.

The concentrations of hK5, hK6, hK8, hK10, hK11, hK13 and hK14 (trypsin-like hKs), and hK7 (chymotrypsin-like hK; Yousef and Diamandis, 2001) in normal SC and sweat of various body regions were determined (ng/mg total protein) by immunofluorometric ELISA (Tables 1a and b). Informed consent was obtained from all participants and our study was conducted according to the Declaration of Helsinki Principles. The medical ethical committee of Graduate School of Medical Science, School of Medicine, Kanazawa University approved all described studies.

In the SC, neither the mean concentration of each hK nor the total concentrations of trypsin-like hKs differed significantly between body regions (Table 1a). hK6, hK10, and hK13 in the SC were present at significantly lower concentrations in females than in males in the SC (Table 1a).

In sweat, each hK mean concentration and the total concentration of trypsin-like hKs differed widely among body regions and gender groups with significant differences (Table 1b). hK6, hK10, and hK14 were present at significantly lower concentrations in females than in males. In females, hK concentrations were consistently lowest in the axilla. For hK6, hK8, hK10, hK13, and hK14, sweat from the face contained the highest amounts of hKs (more details are given in Table 1b).

hK6 and hK8 are positively stained in both normal human axillary eccrine and apocrine sweat glands; however, hK13 was detected only in the eccrine sweat glands regardless of gender (more details are in Figure S1).

The ''trypsin-like enzymatic activity'' towards Boc-Phe-Ser-Arg-7-amino-4-methyl courmarin synthetic substrate (Suzuki *et al.*, 1996) was measured in the SC (Figure 1a) and sweat samples (Figure 1b) from each individual. The SC samples displayed no significant differences at any time point among body region and gender groups (Figure 1a). According to a standard trypsin curve (data not shown), the total trypsin-like activity per milligram of SC was approximately comparable to 675–875 pg of trypsin. In contrast, sweat trypsin-like activities differed significantly among groups (Figure 1b). The trypsin-like activity per gram of sweat was approximately comparable to that of 250–1375 pg of trypsin.

The ''chymotrypsin-like enzymatic activity'' towards MeO-Suc-Arg-Pro-Tyr-*para*-nitroanilide-HCl synthetic substrate (Suzuki *et al.*, 1996) was also measured in the SC (Figure 1c). There were no significant differences among groups in the SC samples. (Figure 1c). The *para*-nitroanilide release was insufficient to generate standard chymotrypsin curves. Sweat samples were also used for chymotrypsin-like activity measurements. However, after the samples rapidly released 3–11 nmol of *para*-nitroanilide/g sweat following 1 minute incubation at 37°C, the activity was abolished for an unclear reason (data not shown).

In the SC, not only the concentration of each hK but also the overall SC enzymatic activities were highly consistent among body regions, suggesting that the hK expression and the SC enzymatic activities may be regulated similarly throughout the SC, at least in the body regions studied.

In the SC, hK6 has been detected at higher amounts in males regardless of age (Komatsu *et al.*, 2005a). Higher levels of hK6 in males are also observed across body regions for both the SC and sweat, suggesting that hK6 expression might be sex hormone dependent, as suggested previously (Yousef and Diamandis, 2001; Komatsu *et al.*, 2005a).

In sweat, the total concentration of trypsin-like hKs and the trypsin-like enzymatic activity appear to be highly correlated with each other regardless of body region or gender, which might imply that hKs may contribute significantly to the total trypsin-like activity. However, further studies are necessary to elucidate the fraction of hKs that are enzymatically active in sweat.

Abbreviations: hK, kallikrein protein; SC, stratum corneum

Table 1a. Quantification of kallikreins in the SC from different body regions by ELISA

	SC (ng/mg of total protein)							
	Region							
	Forearm		Abdomen		Back		Thigh	
hK	Mean±SD	%	Mean±SD	%	Mean±SD	%	Mean±SD	%
Chymotrypsin-like hK								
hK7								
M	230±160		143±116		179±81		215±116	
F	210±123		172±72		307±142		188±64	
Trypsin-like hK								
hK8								
M	147±51	36.7	128±49	41.1	138±65	42.9	128±42	36.5
F	137±64	48.0	105±50	38.2	130±70	37.7	127±61	33.0
hK11								
M	180±72	45.0	117±55	37.6	109±61	33.9	156±47	44.5
F	106±59	37.1	123±93	44.7	142±74	41.2	217±120	56.3
hK5								
M	38±11	9.5	37±10	11.9	41±11	12.7	40±12	11.4
F	29±9.9	10.2	30±11	10.9	49±23	14.2	30±8.8	7.8
hK10								
M	25±16	6.2	19±8.6	6.1	24±17	7.5	15±7.8	4.3*
F	8.6±5.5	3.0	11±8.4	4.0	14±6.7	4.1	7.2±4.1	1.9
hK14								
M	4.9±2.3	1.2	4.0±2.5	1.3	4.1±1.8	1.3	5.4±2.5	1.5
F	3.0±1.2	1.1	2.9±1.9	1.1	5.1±3.5	1.5	2.6±1.4	0.7
hK6								
M	4.0±2.3	1.0	4.9±1.7	1.6	4.7±1.8	1.5	4.4±2.0	1.3*
F	1.5±0.82	0.5	2.4±1.5	0.9	3.7±2.8	1.1	0.91±0.37	0.2
hK13								
M	1.5±0.67	0.4	1.5±1.2	0.5	1.2±0.73	0.4	2.0±1.1	0.6*
F	0.46±0.38	0.2	0.88±0.78	0.3	0.59±0.43	0.2	0.65±0.48	0.2
Total of trypsin-like hKs								
M	400±104		311±52		322±70		351±47	
F	286±90		275±111		344±139		385±156	

F=female; hK=kallikrein protein; M=male; SC=stratum corneum.
Kallikrein concentrations are expressed in ng/mg total protein. When the concentrations are evaluated in ng/mg dry weight, as carried out in the previous study (Komatsu *et al.*, 2005a); values are similar to those reported in the previous study. The percentages represent the fraction of each trypsin-like hK to the total amount of trypsin-like hKs.
*Two-way ANOVA revealed significant differences between genders for hK6, hK10, and hK13 ($P<0.05$ or each).

Female axillary sweat contained the lowest hK levels, implying that hK secretion in the axilla might be sex dependent. hK5 levels were highest in the axilla in both genders. Facial sweat contained, in general, higher hK levels than other body regions. As hKs have been identified also in sebaceous glands (Komatsu *et al.*, 2005b), hKs originating from the sebum could contribute to the levels in the sweat samples.

It is not as yet known if axillary sweat hKs originate mainly from eccrine or apocrine sweat glands. While hK6 and hK8 were detected in both the axillary eccrine and apocrine sweat glands, hK13 was found only in the eccrine glands (Figure S1). It is therefore likely that hK13 might enter sweat from the eccrine glands only.

The amounts of hK8 and hK11 were similar in magnitude in the SC. However, in sweat, hK8 concentrations were significantly higher than the other trypsin-like hKs, suggesting that hK8 expression could be more

Table 1b. Quantification of kallikreins in sweat from different body regions by ELISA

	Sweat (ng/mg of total protein)					
	Region					
	Face		Axilla		Abdomen	
hK	Mean ± SD	%	Mean ± SD	%	Mean ± SD	%
Chymotrypsin-like hK						
hK7	345 ± 228		393 ± 306		434 ± 308	
M						
F	219 ± 165		165 ± 155		262 ± 227	
Trypsin-like hK						
hK8						
M	334 ± 207	58.0	255 ± 112	52.3	234 ± 92	61.0[##]
F	379 ± 230	69.0	62 ± 49*[1]	65.7	227 ± 115	67.6
hK11						
M	134 ± 64	23.4	147 ± 71	30.1	100 ± 76	26.2
F	100 ± 81	18.2	12 ± 5.4*[2]	12.7	75 ± 31	22.4
hK5						
M	8.8 ± 2.3	1.5	45 ± 27*[3]	9.2	18 ± 12	4.8
F	17 ± 11	3.1	14 ± 13	14.3	22 ± 20	6.5
hK10						
M	41 ± 18*[4]	7.2	21 ± 18	4.3	13 ± 10	3.4[#]
F	31 ± 23*[5]	5.7	4.0 ± 3.4	4.2	5.7 ± 3.1	1.7
hK6						
M	32 ± 15*[5]	5.6	7.8 ± 4.0	1.6	10 ± 5.5	2.7[#]
F	12 ± 1.7	2.3	1.4 ± 1.3	1.4	3.9 ± 1.5	1.2
hK13						
M	18 ± 21*[4]	3.2	7.0 ± 5.2	1.4	3.6 ± 4.2	0.9
F	7.3 ± 7.2	1.3	0.90 ± 0.88	1.0	1.1 ± 0.92	0.3
hK14						
M	6.1 ± 3.4*[4]	1.1	4.8 ± 2.3	1.0	4.1 ± 2.6	1.1[#]
F	1.9 ± 1.4	0.4	0.74 ± 0.71	0.8	1.0 ± 0.45	0.3
Total of trypsin-like hKs						
M	575 ± 183		488 ± 157		383 ± 166	
F	549 ± 221		95 ± 49*[6]		336 ± 94	

F=female; hK=kallikrein protein; M=male; SC=stratum corneum.
Kallikrein concentrations are in ng/mg total protein. The percentages represent the fraction of each trypsin-like hK within the total amount of trypsin-like hKs.
[#]Two-way ANOVA displayed significant differences between genders for hK6, hK10, and hK14 ($P<0.05$ for each). The *post hoc* test (Bonferroni method to reflect multiple comparisons) showed significant differences ($P<0.05$ for each) as follows: *[1], F-axilla versus F-face for hK8; *[2]F-axilla versus M-axilla for hK11; *[3]M-axilla versus M-face and F axilla for hK5; *[4]M-face versus M-abdomen for hK6, hK13, and hK14; *[5]face versus axilla and abdomen for F-hK10 and M-hK6; *[6]F-axilla versus F-face, F-abdomen, and M-axilla for the total concentration of trypsin-like hKs.
[##]One-way ANOVA revealed significantly higher hK8 levels compared with the other trypsin-like hKs ($P<0.05$ for each), except in the axilla of females.

crucial to sweat production. In general, the relative proportions of each hK in sweat were similar to those in the SC, suggesting that hK expression in sweat and the SC could be regulated via a shared mechanism. Sweat might be a fluid facilitating transport of hKs towards the skin surface.

In both the SC and sweat, on the basis of immunoreactive amounts, hK8 and hK11 are the major trypsin-like hKs; hK5 and hK10 are intermediate hKs; and hK6, hK13, and hK14 are the minor hKs. The intermediate and minor hKs tended to show gender differences in both the sweat and SC, suggesting a sex hormone-dependent regulation. hK7 is the major chymotrypsin-like hK.

The role of hKs in skin structure and function has been increasingly the focus of research efforts. The distribution of hKs in the SC and stratum granulosum (Ekholm *et al.*, 2000; Ko-

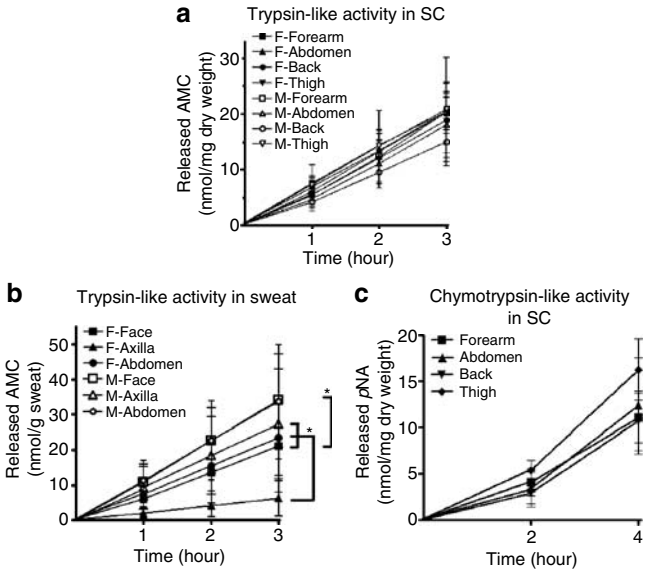

Figure 1. The stratum corneum trypsin- and chymotrypsin-like enzymatic activities among body regions. The SC (**a**) and sweat (**b**) trypsin-like activities, and the SC chymotrypsin-like enzymatic activities (**c**) (mean ± SD) are indicated by the released 7-amino-4-methyl courmarin (AMC) or *para*-nitroanilide (*p*NA), respectively, from synthetic substrates. (**a** and **b**) Subjects are divided into groups on the basis of body region and further subdivided by gender. (**c**) Subjects are divided into groups on the basis of region for a subset of subjects ($n = 8$ for each SC group, male to female ratio = 1). In (**c**), measurements were taken at 2 and 4 hours. *A significant difference ($P < 0.05$) between specified age groups at 2 and 3 hours.

matsu *et al.*, 2005b), the transportation of hKs via lamellar granules to the intercellular space (Sondell *et al.*, 1995; Ishida-Yamamoto *et al.*, 2004, 2005), and the degradation of desmosomes and/or corneodesmosomes by hK5 and hK7 (Simon *et al.*, 2001; Caubet *et al.*, 2004) together imply that hK functions in the skin are intimately related to desquamation. The involvement of hK8 in skin differentiation has also been suggested (Kirihara *et al.*, 2003). hKs may represent an enzymatic cascade pathway (Yousef and Diamandis, 2002), given the evidence that hK5 is able to activate hK7 and hK14 (Caubet *et al.*, 2004; Brattsand *et al.*, 2005).

In summary, numerous hKs in normal SC and sweat from various body regions were quantitatively measured. The concentrations of the hKs varied widely, but were correlated between sweat and SC. These results further implicate hKs as an important skin protease group with a large diversity in both quantity and activity. The data presented here may help understand skin barrier function, especially from the viewpoint of serine protease activity in the skin tissue. Previous studies implicated mainly hK5 and hK7 as major players in desquamation. The present data pinpoint to hK8 and hK11, as well as to hK6, hK10, hK13, and hK14 as important kallikreins in the skin physiology.

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SUPPLEMENTARY MATERIAL

Materials and Methods.

Figure S1. Immunohistochemical localization of hK6, hK8, and hK13 in apocrine and eccrine sweat glands in normal human axillary skin Scale bars indicate 100 μm. Arrows and arrowheads indicate secretory segments of apocrine and eccrine sweat glands, respectively. Positive staining for hK6 and hK8, but not hK13, was observed in the eccrine and apocrine sweat glands in normal human axillary skin. hK6 and hK8 were diffusely detected, not only in the inner lumen side but also the outer side of the apocrine secretory segments. Their intensity was comparable to that seen in eccrine secretory segments. On the other hand, hK13 was found in eccrine but not in apocrine glands. No apparent differences were observed for hK staining between male and females.

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