BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS **238,** 549–555 (1997)
ARTICLE NO. RC977333

# Activation of the Zymogen Form of Prostate-Specific Antigen by Human Glandular Kallikrein 2

Janita Lövgren,* Kristiina Rajakoski,† Matti Karp,† Åke Lundwall,* and Hans Lilja*
**Department of Clinical Chemistry, Lund University Hospital, S-205 02 Malmö, Sweden; and*
*†Department of Biotechnology, University of Turku, Tykistökatu 6, 20520 Turku, Finland*

Received August 12, 1997

**Prostate-specific antigen (PSA) and human glandular kallikrein 2 (hK2) are glandular kallikreins secreted by the prostate gland. Both enzymes are synthesized with a propeptide that is supposedly cleaved off in the prostate to yield the mature forms found in semen. We have purified and characterised recombinant PSA and hK2 produced in eucaryotic cells. Recombinant PSA was recovered as a zymogen and recombinant hK2 was recovered in mature form. The zymogen form of PSA had no or very low enzymatic activity. After incubation with hK2, proPSA was activated, as shown by the cleavage of the seminal gel proteins and a peptide substrate; the hK2-proPSA ratio used was similar to the enzyme-substrate ratio that prevails under phyciological conditions. Our results indicate that hK2 is responsible for the activation of proPSA, a finding that may be very important for understanding of the role of these two kallikreins in the reproductive system and in prostate cancer biology.** 

The glandular kallikreins are serine proteases encoded by a conserved multigene family with a highly varying number of members in different species (1). Glandular kallikreins are involved in processing polypeptide precursors to their bioactive forms. This includes functions such as the release of kinins from kininogens and the release of murine EGF and NGF from their respective precursors (2). Glandular kallikreins are structurally similar, but show distinct differences in substrate recognition. They are also referred to as arginine-specific esterases as they preferentially cleave their substrates at monobasic sites on the C-terminal side of an arginine (P1); and the amino acid preceding that site (P2) is generally a large hydrophobic residue. However, cleavage after lysine and at dibasic sites has also been demonstrated with peptide substrates (3). The differences in substrate specificity have been shown to be dependent on the amino acids located at positions P3 - P8 on the C-terminal side of the cleavage site indicating an extended interaction site (4).

Three glandular kallikreins are encoded by the human kallikrein gene family: tissue kallikrein (hK1), human glandular kallikrein 2 (hK2) and prostate-specific antigen (PSA or hK3) (5). PSA and hK2 are androgen regulated (6,7) and expressed at high levels in the prostate. PSA and hK2 have 80 % amino acid sequence similarity (8), display limited immunological cross-reactivity (9), and are predicted to have different substrate specificities. PSA has been shown to have restricted chymotrypsin-like activity as opposed to the trypsin like activity predicted for hK2 (8,10). The major substrates of PSA in semen are the gel proteins; semenogelin I and semenogelin II (11). Parathyroid-hormone-related peptide (PTHrP); (12), secretory leucocyte protease inhibitor (SLPI); (13), and insulin-like growth-factor-binding protein (14) have also been found to be substrates of PSA in vitro, but the physiological significance of those discoveries is presently unclear. In the prostate the hK2 mRNA levels are 2 - 10 times lower than those of PSA (6,15,16). HK2 has been isolated from seminal plasma by the use of monoclonal antibodies that do not cross-react with PSA and identified by amino terminal sequencing (17), but, as of yet, no information is available regarding the physiological function of this enzyme.

All eucaryotic endoproteases undergo cleavage of a pro region, which converts the inactive zymogen to a catalytically active mature form. The activation can occur intracellularly, i.e. in the trans-Golgi or the secretory granules, or extracellularly after secretion, and it can be either autocatalytic or dependent on the action of another enzyme. The zymogen forms of PSA (proPSA) and hK2 (prohK2) have never been purified or characterised, and the site and principles of activa-

[1] Corresponding author: Fax: +358, 23338050. E-mail: janita.lovgren@utu.fi.



0006-291X/97 $25.00

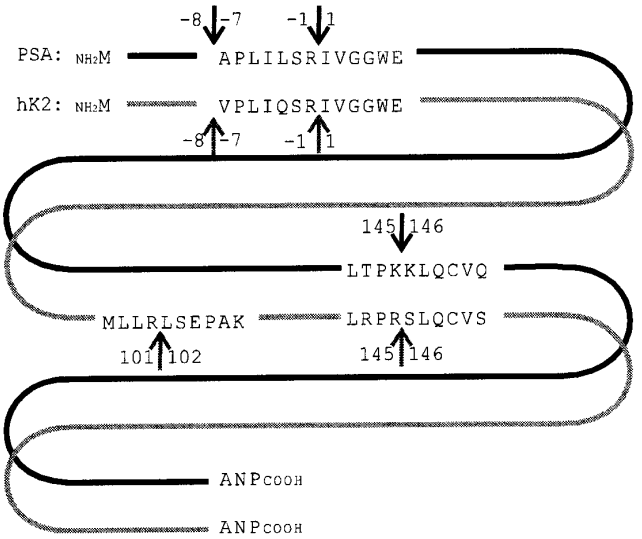


**FIG. 1.** Schematic diagram of the primary structure of preproPSA and preprohK2 showing internal cleavage sites. Removal of the propeptide by cleavage carboxy terminal of the Arginine at position -1 activates the enzymes. Additional arrows at positions 101 and 145 indicate where internal cleavages have been found.

tion are unknown as are the mechanisms that regulate the activity of these enzymes. PSA isolated from seminal plasma is in the mature form; the same is true for most of the PSA in serum, since it is complexed with alpha-1-antichymotrypsin (ACT). PSA is probably activated prior to the ejucalotary mixing of secretions from the male accessory sex glands, considering that the enzyme is active in patients with aplasia of the seminal vesicles and the deferent ducts (11). It is likely that both proPSA and prohK2 are activated by an enzyme with trypsin-like substrate specificity, since the cleavage of the activation peptide is predicted to occur at a monobasic site on the carboxyl terminal side of an arginine (Fig. 1). This kind of substrate specificity has been predicted for hK2, hence we considered that enzyme to be a candidate for the role as proPSA activator. Our goal was to investigate the activation and function of PSA and hK2 by expressing recombinant proPSA and prohK2 and subsequently using them in studies in vitro. In addition to being biologically interesting, information obtained in such experiments could help in understanding PSA as a tumour marker.

## MATERIALS AND METHODS

*Reagents.* The production and characterisation of the monoclonal anti-PSA antibody 2E9 has been described elsewhere (18). H50 was obtained from Abbott Laboratories (Abbott Park, IL, USA). A heptapeptide fluorogenic substrate, Lys-Gly-Ile-Ser-Ser-Glu-Tyr-AMC, based on a reported PSA-cleavage carboxyl-terminal of residues 129-135 in semenogelin I (Sg I), was kindly provided by Professor John T. Isaacs, Johns Hopkins Oncology Center, Baltimore, MD, USA. Affi-Gel 10 was purchased from Bio-Rad Laboratories (Richmond, CA, USA), and benzamidine hydrochloride was from Amresco (Solon, OH, USA). PSA was purified from seminal plasma as previously described (10).

*Expression of recombinant PSA and hK2.* Recombinant PSA and hK2 were produced using the semliki forest virus (SFV) expression system (19). The expression vectors used code for the prepro forms of the proteins (9). Briefly, the baby hamster kidney (BHK-21) cells used to express the proteins were cultured in MEM (Gibco BRL) supplemented with 2 mM glutamine, 100 U/ml penicillin, 100 $\mu$g/ml streptomycin, non-essential amino acids (ICN Biomedicals, Costa Mesa, CA, USA) and 5% fetal bovine serum, as described in the user manual for the SFV/Helper 2 gene expression systems (Life Technologies, Gibco BRL). After transfection, the cells were cultured in Optimem (Gibco BRL) supplemented with 0.2 % BSA, 100 U/ml penicillin, 100 $\mu$g/ml streptomycin, leupeptin 5 mg/l, chymostatin 10 mg/l, pepstatin 0.7 mg/l (ICN Biomedicals), E-64 4 mg/l and phosphoramidon 2.5 mg/l (Sigma, St.Louis, MO, USA) to inhibit proteolytic activity in culture medium during expression. The expression levels obtained after 48 hours were 300-600 $\mu$g/l PSA and 100-150 $\mu$g/l hK2.

The production of recombinant PSA and hK2 in the baculovirus expression system was done with vectors containing the same PSA and hK2 inserts as described for the SFV vectors. The recombinant baculovirus vectors were created with the Bac-to-Bac system (Gibco, Life Technologies Inc.). The proteins were produced in *Spodoptera frugiperda* (*Sf*21) or *Trichoplusia ni* (High Five) cells using cultivation/infection methods essentially as described in a manual by Summers et al. (20).

*Purification and characterisation of recombinant proteins.* PSA was purified on a 2-ml Affi-Gel 10 (affinity) column containing 4 mg of immobilised monoclonal anti-PSA antibody 2E9. Cell culture supernatant was first passed through the column at a velocity of 35 ml/hour. The column was then washed with a buffer containing 50 mM Tris (pH 7.2), 0.5 M NaCl and 0.02% Na-azide and eluted with 0.2 M glycine (pH 3.0), 0.5 M NaCl and 0.02% Na-azide into tubes containing 150 $\mu$l of 2 M Tris pH 7.5 per milliliter of eluate. The eluted proteins were concentrated using Centricon-3 (Amicon, Inc., Beverly, MA, USA). All the purification steps were performed at +4 C.

HK2 was purified on a 1.5 ml Affi-Gel 10 (affinity) column containing 3 mg of the immobilised anti-PSA antibody H-50, which cross-reacts with hK2 (9). The procedure was the same as above, but the elution was done with 0.2 M glycine (pH 2.2).

For amino terminal sequencing, the recombinant proteins were further purified with reverse-phase HPLC using a C4 column equilibrated in 0.1 % trifluoro acetic acid. The elution was done with a 0 - 50 % (1 %/min.) linear aceto-nitrile gradient.

The size of the recombinant proteins was determined by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) performed in a Mini PROTEAN II system (Bio-Rad) using 12-15 % polyacrylamide gels. Thereafter, the proteins were stained with silver or Coomassie brilliant blue. Automated amino terminal sequence determinations were performed with an Applied Biosystems 470 A gas-phase sequencer.

*Measurement of enzymatic activity with a fluorogenic substrate.* A 20 mM stock solution of the substrate Lys-Gly-Ile-Ser-Ser-Glu-Tyr-AMC was prepared in DMSO immediately prior to use. The Km of 200 $\mu$M for the substrate has been determined previously using 0.2 $\mu$g/ml PSA (Prof. John Isaacs, personal communications). The kinetic measurements were carried out in microtiterplates in 200 $\mu$l of 50 mM Tris buffer (pH 7.5) containing 0.1 M NaCl and 0.2 % BSA and using 400 $\mu$M substrate and 0.5 $\mu$g/ml PSA.

*Cleavage of Sg I and Sg II with PSA and hK2.* We tested the ability of purified recombinant proPSA, mature PSA, and hK2 to cleave the two major gel proteins in freshly collected human semen,

semenogelin I (Sg I) and semenogelin II (Sg II). To accomplish this purified Sg I or Sg II (21) (4 $\mu$g/$\mu$l) in a 50 mM Tris buffer (pH 8.7) supplemented with 0.5 M NaCl, 2M Urea and 1mM EDTA was diluted 10 times with 50 mM Tris (pH 7.5) buffer containing 0.1 M NaCl and cleaved with 0.1 $\mu$g of enzyme for 2 hours at 37°C in a final volume of 10 $\mu$l. The semenogelin cleavage products were separated by SDS-PAGE in a 15 % gel under reducing conditions and subsequently stained with Coomassie blue.

*Activation of proPSA.* The conversion of proPSA into a mature, catalytically active form of PSA was studied by comparing the activity of PSA purified from seminal plasma, proPSA, and proPSA incubated with hK2 against the fluorogenic substrate Lys-Gly-Ile-Ser-Ser-Glu-Tyr-AMC and against the physiologic substrates Sg I and Sg II as described above. Activation of proPSA with hK2 was achieved by incubating 1 $\mu$g of proPSA with 0.01 and 0.1 $\mu$g of hK2 separately; this was done in 10 $\mu$l of 50 mM Tris (pH 7.5) buffer containing 0.1 M NaCl for 1-4 hours at 37°C.

## RESULTS

### *Characterisation of the Recombinant Proteins*

The purity of the affinity purified recombinant PSA was evaluated by SDS-PAGE, which showed that more than 95 % of the recovered protein was single chained and migrated as a single band, regardless of whether the sample was non-reduced or reduced and alkylated prior to analysis. The size characteristics of the recombinant PSA closely correspond to those of PSA purified from seminal plasma. However, according to the amino terminal sequence determination recombinant PSA was recovered as a zymogen, whereas the PSA in seminal plasma is in the mature form. There were two different variants of proPSA produced by BHK-21 cells: one beginning at aa -7 (Ala), and one beginning at aa -5 (Leu); the former comprising about two thirds of the amount (Table 1A). The proPSA produced by insect cells was composed of three different variants, beginning at aa -3 (Leu), aa -7 (Ala), and at aa -5 (Leu), and respectively representing about two thirds, one third and a small fraction of the recovered protein.

The purity of affinity purified hK2 produced in the SFV and baculovirus systems was studied in a manner similar to that used to examine recombinant PSA. Both SFV-hK2 and baculo-hK2 were comprised of several bands with apparent sizes somewhat smaller than the PSA band. According to amino terminal sequence determination, SFV-hK2 was processed into the mature form with an amino terminal sequence starting at aa 1 (Ile); the major part of the protein was single chained and about one fourth of was cleaved internally between aa 145-146 (Arg-Ser); (Table 2A). Baculo-hK2 was also recovered in mature form; the major part of the protein was single chained and a part contained the same 145-146 cleavage as SFV-hK2 and an additional cleavage between aa 101-102 (Arg-Leu) (Table 2B).

### *Enzymatic Activity of the Recombinant Proteins*

The activity of purified proPSA was studied by using known PSA substrates, and all of the proPSA forms were found to lack, or show very little, enzymatic activity. For example, 4 $\mu$g of semenogelin was not cleaved during a 2-hour exposure to 0.1 $\mu$g of either SFV proPSA or baculo proPSA (Fig. 2). By comparison this amount of mature PSA cleaves the semenogelins completely. Increasing the amount of proPSA to 0.5 $\mu$g resulted in a detectable but incomplete cleavage of the semenogelins, i.e. most of the proteins remained undegraded. ProPSA (0.5 $\mu$g/ml) had no measurable effect on the fluorogenic peptide substrate Lys-Gly-Ile-Ser-Ser-Glu-Tyr-AMC during a 20-minute exposure, whereas under the same experimental conditions, a kinetic rate of 13 pmol/min was recorded for PSA purified from seminal plasma. To test the sensitivity of this assay, we made 20-min. kinetic measurements on a 0.5-0.005 $\mu$g/ml dilution series of PSA purified from seminal plasma. We found that the activity of 0.025 $\mu$g/ml of PSA could be distinguished from the background, indicating that the recombinant proPSA has less than 5 % of the activity of the mature PSA (Fig. 3).

The activity of hK2 was studied by testing its ability to cleave Sg I and Sg II. The results show that hK2 cut both of the gel proteins rapidly and with a pattern distinct from that seen upon cleavage with PSA (Fig.

**TABLE 1**

N-Terminal Amino Acid Sequence of SFV-PSA before and after Activation with hK2

| A | Sequence 1 | | Sequence 2 | |
|---|---|---|---|---|
| Sequence step | Residue | pmol | Residue | pmol |
| 1 | Ala | 56 | Leu | 34 |
| 2 | Pro | 45 | Ile | 34 |
| 3 | Leu | 61 | Leu | |
| 4 | Ile | 34 | Ser | 16 |
| 5 | Leu | 49 | Arg | 20 |
| 6 | Ser | 13 | Ile | 23 |
| 7 | Arg | 29 | Val | 46 |
| 8 | Ile | 31 | Gly | 32 |
| 9 | Val | 30 | Gly | 40 |
| 10 | Gly | 54 | Trp | 4 |

| B | Sequence 3 | |
|---|---|---|
| Sequence step | Residue | pmol |
| 1 | Ile | 32 |
| 2 | Val | 25 |
| 3 | Gly | 16 |
| 4 | Gly | 8 |
| 5 | Trp | 5 |
| 6 | Glu | 11 |

*Note.* (A) The two sequences derived from SFV PSA before activation: sequence 1 corresponds to residues −7 to 3, and sequence 2 corresponds to residues −5 to 5 in the PSA amino acid sequence. (B) The sequence which arose from SFV PSA after activation with hK2, i.e., sequence 3 corresponds to residues 1 to 6 in the PSA amino acid sequence.

**TABLE 2**

N-Terminal Amino Acid Sequence of SFV and Baculo hK2

| A Sequence step | Sequence 1 | | Sequence 2 | |
|---|---|---|---|---|
| | Residue | pmol | Residue | pmol |
| 1 | Ile | 27 | Ser | 8 |
| 2 | Val | 17 | Leu | 8 |
| 3 | Gly | 44 | Gln | 9 |
| 4 | Gly | 27 | Cys | — |
| 5 | Trp | 1 | Val | 8 |
| 6 | Glu | 14 | Ser | 6 |
| 7 | Cys | — | Leu | 6 |
| 8 | Glu | 9 | His | 1 |
| 9 | Lys | 5 | Leu | — |
| 10 | His | 2 | Leu | 14 |

| B Sequence step | Sequence 3 | | Sequence 4 | | Sequence 5 | |
|---|---|---|---|---|---|---|
| | Residue | pmol | Residue | pmol | Residue | pmol |
| 1 | Ile | 11 | Ser | 9 | Leu | 4 |
| 2 | Val | 7 | Leu | 3 | Ser | — |
| 3 | Gly | 11 | Gln | 2 | Glu | 2 |
| 4 | Gly | 4 | Cys | — | Pro | 2 |
| 5 | Trp | — | Val | 1 | Ala | 2 |
| 6 | Glu | 2 | Ser | 1 | Lys | 1 |
| 7 | Cys | — | Leu | 2 | Ile | 2 |
| 8 | Glu | 2 | | | Thr | 1 |

*Note.* (A) Sequences derived from SFV hK2: sequence 1 corresponds to residues 1–10 and sequence 2 to residues 146–155 in the hK2 amino acid sequence. (B) Sequences derived from baculo hK2: sequence 3 corresponds to residues 1–8, sequence 4 to residues 146–152, and sequence 5 to residues 101–109 in the hK2 amino acid sequence.

4). This was expected due to the trypsin-like substrate specificity of hK2, as compared to the chymotrypsin-like specificity of PSA.

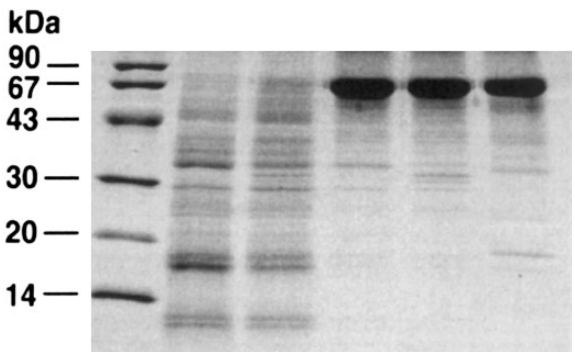


**FIG. 2.** Activation of proPSA by hK2 analysed as the ability to cleave of Sg II. SFV proPSA and baculo proPSA were activated by incubation with baculo hK2. Enzymatic activity of the zymogen forms and the activated forms was studied by cutting Sg II (4 $\mu$g) with 0.1 $\mu$g of the respective proteins. Lane 1, molecular-mass-marker proteins; lane 2, Sg II cleaved with activated SFV PSA; lane 3, Sg II cleaved with activated baculo PSA; lane 4, Sg II cleaved with SFV proPSA; lane 5, Sg II cleaved with baculo proPSA; lane 6, Sg II cleaved with 0.01 $\mu$g baculo hK2.

*Activation of the PSA Zymogen with hK2*

Incubation of the zymogen form of PSA (proPSA) with baculovirus hK2 converted PSA to a highly active form. This was demonstrated by comparing the ability of proPSA, hK2-activated proPSA, and PSA purified from seminal plasma to cleave the semenogelins and the fluorochrome substrate Lys-Gly-Ile-Ser-Ser-Glu-Tyr-AMC. The amounts of hK2 used to activate PSA were, respectively, 10 and 100 times smaller than the amounts of proPSA that were present. These values are similar to physiological proportions, i.e. the amounts of the enzymes we found in homogenized prostate and seminal fluid (unpublished results). As negative controls, we incubated the substrate alone, and with the same amount of hK2 used in the activation of proPSA. Activated proPSA cut Sg II in a manner similar to cleavage caused by PSA purified from seminal plasma (Fig. 2). The amount of hK2 present in this experiment resulted in a minute, but detectable, cleavage of Sg II.

The activation of the PSA zymogen was even more clearly illustrated by comparing the ability of proPSA, hK2-activated proPSA and PSA purified from semen to cleave the fluorochrome peptide substrate. Activity was revealed in kinetic plots obtained with 0.1 $\mu$g of

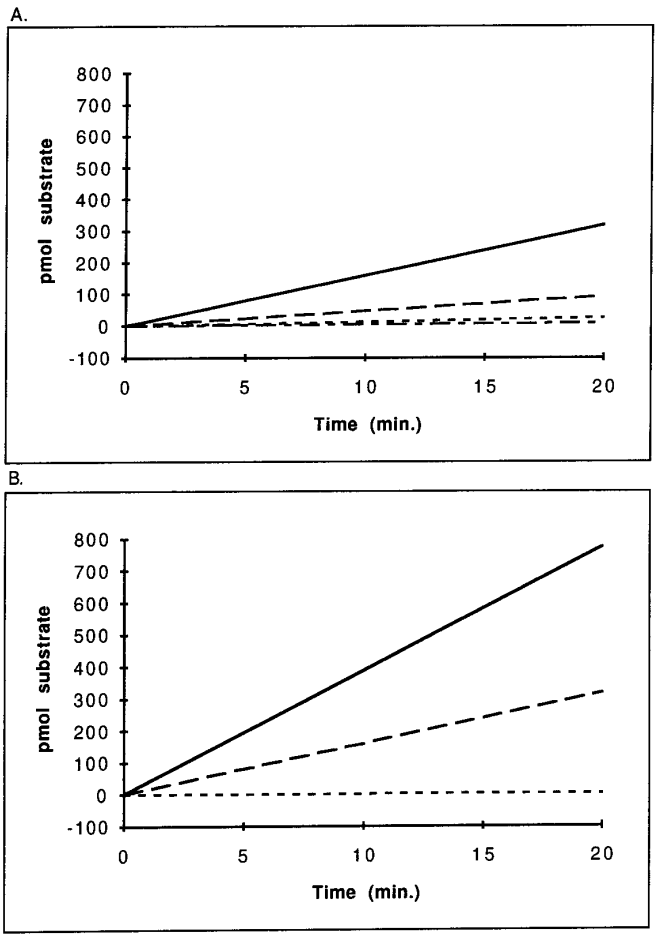


**FIG. 3.** Rate of hydrolysis of a fluorogenic peptide substrate by PSA purified from seminal plasma, SFV proPSA, and activated SFV PSA. A. The sensitivity of the enzyme activity measurement was tested by cleaving the peptide substrate Lys-Gly-Ile-Ser-Ser-Glu-Tyr-AMC (400 $\mu$M) with different amounts of PSA purified from seminal plasma: 500 ng/ml (—); 250 ng/ml (– –); 50 ng/ml (---); 25 ng/ml (– -). B. Rate of hydrolysis of the fluorogenic peptide substrate (400 $\mu$M) by 500 ng/ml of PSA purified from seminal plasma (—), SFV proPSA (---), and activated SFV PSA (– –). The measurement interval was 30 seconds.

the purified proteins. A rate of 38 pmol/min was noted for hK2-activated proPSA, whereas PSA purified from seminal plasma, which is known to be partially inactivated by internal cleavages (10), exhibited a considerably lower rate 13 pmol/min. ProPSA showed no detectable activity (Fig. 3) and the amount of hK2 used to activate proPSA did not have detectable activity against the peptide substrate.

Activation of proPSA was further verified by amino terminal sequencing SFV PSA after activation with hK2. The results show that over 80 % of proPSA had been converted to the mature form, beginning with aa 1 (Ile); no internal peptide bond cleavages were detected (Table 1B).

## DISCUSSION

We examined the activation of the prostate produced glandular kallikreins PSA and hK2 by first expressing the prepro forms of both proteins in the SFV and baculovirus expression systems and then using the purified proteins in studies in vitro. Purification and characterisation of the recombinant proteins showed that PSA produced by both hamster and insect cells was recovered in a zymogen form whereas hK2 was recovered exclusively in a mature form. The length of the propeptide in the zymogen form of PSA varied from 3 to 7 amino acids, but all of the variants had no or very little enzymatic activity. The mature recombinant hK2 was enzymatically active. It rapidly cleaved the gel forming proteins of semen (semenogelin I and II) resulting in fragments clearly different from those found after cleavage by PSA. This was expected due to the different substrate specificities of these two enzymes. The hK2 recovered after purification was partially internally cleaved between amino acids 145-146 (Arg-Ser) and 101-102 (Arg-Leu). Both of the cleavages in hK2 are on the carboxyl-terminal side of an arginine, indicating that they were caused by an enzyme with trypsin-like substrate specificity, i.e. possibly by hK2 itself. The same type of cleavage between amino acids 145-146 (Lys-Lys); (Fig. 1) has been found in 10-25% of the PSA purified from seminal plasma (10); this cleavage renders PSA inactive, hence it may also inactivate hK2.

The sequence of the pro peptides of both PSA and hK2 indicate that they are activated by an enzyme cleaving on the carboxyl side of an arginine, which is the kind of substrate specificity hK2 is predicted to have. Our results show that PSA is expressed as an inactive zymogen that requires activation by another protease and that hK2 is capable of inducing this acti-

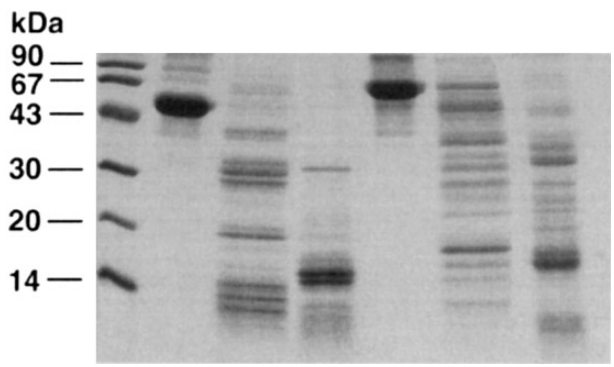


**FIG. 4.** Enzymatic activity of baculo hK2. The gel-forming proteins of semen Sg I and Sg II (4 $\mu$g) were cut with baculo hK2 (0.5 $\mu$g) and with PSA purified from seminal plasma (0.5 $\mu$g). Lane 1, molecular-mass-marker proteins; lane 2, Sg I; lane 3, Sg I cleaved with baculo hK2; lane 4, Sg I cleaved with PSA; lane 5, Sg II; lane 6, Sg II cleaved with baculo hK2; lane 7, Sg II cleaved with PSA.

vation. The hK2 levels in the prostate are 1-3 % of the PSA levels (unpublished results). Such amounts are sufficient to cause a complete conversion of proPSA to active PSA, according to our present data obtained by aminoterminal sequencing of hK2-activated proPSA. There was no decrease in the enzymatic activity of PSA and no detectable internal cleavages after prolonged incubations with hK2. The kinetic measurements showed that hK2-activated PSA exhibited clearly higher enzymatic activity per $\mu$g of protein than PSA purified from seminal plasma did; this was probably due to internal cleavages found in the latter enzyme (10). As both PSA and hK2 are secreted into the lumen of the prostatic ducts and it has previously been concluded that the activation of PSA presumably occurs in the lumen of the prostatic ducts, since PSA present in prostatic fluid has the capacity to incorporate DFP (an irreversible inhibitor of serine proteases); (11) and PSA escaping into the extracellular fluids readily forms complexes with alpha1-antichymotrypsin, which is also an indication of an active protease (18) we consider hK2 to be the physiological activator of PSA.

The propeptides of hK2 and PSA are highly similar (Fig. 1), thus it is not unlikely that the maturation process of hK2 involves autoactivation. Autoactivation has been reported for all groups of proteases, including serine proteases such as the coagulation factors VII and XII (22,23), the subtilisin-like prohormone convertase PC2 (24), and plasma kallikrein (25). The activation of hK2 could occur after secretion in the prostatic ducts, since Kumar et al. (26) have shown that the expressed prohK2 is slowly activated in the spent medium of AV12, PC3, and DU 145 cells. This is also in accordance with our finding that preprohK2 expressed in both insect cells and hamster cells is, after purification, recovered exclusively in a mature form.

It has been speculated that hK2 could also be a valuable tumour marker for prostate cancer. Elevated serum hK2 levels have been observed in some individuals, but so far no correlation with any particular disease has been found (27). As the activator of PSA, hK2 could affect the serum PSA levels indirectly. There is a small fraction of free PSA in serum, the major immunodetectable part being complexed to the protease inhibitor ACT whereas an unknown part maybe complexed to alpha-2-macroglobulin (18). The free PSA could be in the zymogen form, or it could be internally cleaved and inactive. It has been shown that prostate cancer patients have lower free PSA to total PSA ratios than patients with benign prostatic hyperplasia (28,29). An increase in the proportion of active PSA leaking into the blood circulation in cancer would result in relatively higher amounts of PSA-ACT in serum and lower free PSA to total PSA ratios, the effect being more pronounced as the clearance of the PSA-ACT complexes is slower than clearance of the smaller free molecules (30,31). Changes in hK2 expression, failed control of hK2 activity or expression of an inactive protease could change the form in which PSA leaks into blood circulation. The role of hK2 in monitoring prostate cancer becomes thus an increasingly interesting question.

## ACKNOWLEDGMENTS

A part of this work has been previously communicated (32). The work was supported by grants from the Biomed 2 program, Area 4.1.7. (contract number BMH4-CT96-0453), the Swedish Medical Research Council (project numbers 7903, 8660), the Swedish Cancer Society (project number 3555), the Faculty of Medicine at Lund University, the Research Fund and the Cancer Research Fund at the University Hospital, the Crafoord Foundation, the Gunnar, Arvid, and Elisabeth Nilsson Foundation, and the Fundacion Frederico S. A.

## REFERENCES

1. Clements, J. A. (1989) *Endocrine Reviews* **10**(4), 393–419.
2. Drinkwater, C. C., Evans, B. A., and Richards, R. I. (1988) *TIBS* **13,** 169–172.
3. Blaber, M., Isacson, P. J., Masters, J. C., Burnier, J. P., and Bradshaw, R. A. (1989) *Biochemistry* **28,** 7813–7819.
4. Brillard-Bourdet, M., Moreau, T., and Gauthier, F. (1995) *Biochimica et Biophysica Acta* **1246,** 47–52.
5. Clements, J. A. (1992) *Molecular and Cellular Endocrinology* **99,** C1–C6.
6. Young, C. Y.-F., Andrews, P. E., Montgomery, B. T., and Tindall, D. J. (1992) *Biochemistry* **31,** 818–824.
7. Wolf, D. A., Schulz, P., and Fittler, F. (1992) *Molecular Endocrinology* **6**(5), 753–762.
8. Schedlich, L. J., Bennetts, B. H., and Morris, B. J. (1987) *DNA* **6**(5), 429–437.
9. Lövgren, J., Piironen, T., Övermo, C., Dowell, B., Karp, M., Pettersson, K., Lilja, H., and Lundwall, Å. (1995) *Biochem. and Biophys. Res. Comm.* **213**(3), 888–895.
10. Christensson, A., Laurell, C.-B., and Lilja, H. (1990) *Eur. J. Biochem.* **194,** 755–763.
11. Lilja, H. (1985) *J. Clin. Invest.* **76,** 1899–1903.
12. Iwamura, M., Hellman, J., Cockett, A., Lilja, H., and Gershagen, S. (1996) *Urology* **48**(2), 317–325.
13. Ohlsson, K., Bjartell, A., and Lilja, H. (1995) *J. Andrology* **16**(1), 64–73.
14. Cohen, P., Graves, H. C. B., Pheel, D. M., Kamarei, M., Giudice, L. C., and Rosenfeld, R. G. (1992) *Journal of Clinical Endocrinology and Metabolism* **75**(4), 1046–1053.
15. Chapdeline, P., Paradis, G., Tremblay, R. R., and Dubé, J. Y. (1988) *FEBS Letters* **236**(1), 205–208.
16. Henttu, P., Lukkarinen, O., and Vihko, P. (1990) *Int. J. Cancer* **45,** 654–660.
17. Deperthes, D., Chapdelaine, P., Tremblay, R., R., Brunet, C., Berton, J., Hébert, J., Lazure, C., and Dubé, J., Y. (1995) *Biochimica et Biophysica Acta* **1245,** 311–316.
18. Lilja, H., Christensson, A., Dahlén, U., Matikainen, M.-T., Nilsson, O., Pettersson, K., and Lövgren, T. (1991) *Clin. Chem.* **37**(9), 1619–1625.
19. Liljeström, P., and Garoff, H. (1991) *Bio/Technology* **9,** 1356–1361.
20. Summers, M. D., and Smith, G. E. (1987) *A Manual of Methods*

*for Baculovirus Vectors and Insect Cell Culture Procedures,* Texas Agricultural Experiment Station Bulletin No. 1555, Texas A&M University.

21. Malm, J., Hellman, J., Magnusson, H., Laurell, C.-B., and Lilja, H. (1996) *Eur. J. Biochem.* **238**(1), 48–53.
22. Yamamoto, M., Nakagaki, T., and Kisiel, W. (1992) *J. Biol. Chem.* **267**(27), 19089–19094.
23. Bernardo, M. M., Day, D. E., Olson, S. T., and Shore, J. D. (1993) *J. Biol. Chem.* **268**(17), 12468–12476.
24. Matthews, G., Shennan, K. I. J., Seal, A. J., Taylor, N. A., Colman, A., and Docherty, K. (1994) *J. Biol. Chem.* **269**(Jan 7), 588–592.
25. Tans, G., Rosing, J., Berrettini, M., Lämmle, B., and Griffin, J. H. (1987) *J. Biol. Chem.* **262**(23), 11308–11314.
26. Kumar, A., Goel, A. S., Hill, T. M., Mikolajczyk, S. D., Millar, L. S., Kuus-Reichel, K., and Saedi, M. S. (1996) *Cancer Research* **56,** 5397–5402.
27. Piironen, T., Lövgren, J., Karp, M., Eerola, R., Lundwall, Å., Dowell, B., Lövgren, T., Lilja, H., and Pettersson, K. (1996) *Clin. Chem.* **42**(7), 1034–1041.
28. Christensson, A., Björk, T., Nilsson, Dahlén, U., Matikainen, M.-T., Cockett, A. T. K., Abrahamsson, P.-A., and Lilja, H. (1993) *The Journal of Urology* **150,** 100–105.
29. Stenman, U.-H., Leinonen, J., Alfthan, H., Rannikko, S., Tuhkanen, K., and Alfthan, O. (1991) *Cancer Research* **51**(January 1), 222–226.
30. Björk, T., Abrahamsson, P-A., and Lilja, H. (1995) *J. Urology* **153**(295A), abstract 265.
31. Richardson, T. D., Wojno, K. J., Liang, L. W., Giacherio, D. A., England, B. G., Henricks, W. H., Schork, A., and Oesterling, J. E. (1996) *Urology* **48**(6A), 40–44.
32. Lövgren, J., Rajakoski, K., Karp, M., Lundwall, Å., and Lilja, H. (1997) *J. Urology* **157**(Supplement), Abstract 23.