

Expression of Pro Form of Prostate-specific Antigen by Mammalian Cells and Its Conversion to Mature, Active Form by Human Kallikrein 2

Abhay Kumar, Stephen D. Mikolajczyk, Amita S. Goel, Lisa S. Millar, and Mohammad S. Saedi¹

Hybritech Incorporated, San Diego, California 92196-9006

Abstract

To study the expression, biosynthesis, and processing of prostate-specific antigen (PSA) in mammalian cells, recombinant PSA was expressed in Syrian hamster tumor cell line AV12-664 (AV12-PSA). Expression of PSA was monitored by the Tandem-MP PSA assay. PSA was secreted into the medium during the logarithmic phase of cell growth at $>9 \mu\text{g/ml}$ and was stable. The PSA purified from spent medium of AV12-PSA cells did not exhibit any enzymatic activity and did not complex with the protease inhibitor, α -1-antichymotrypsin. These findings indicated that an inactive form of PSA was expressed by AV12-PSA cells. NH_2 -terminal sequencing confirmed the identity of the PSA purified from the spent medium of AV12-PSA cells to be pro-PSA. This demonstrates that PSA is expressed as pro-PSA by mammalian cells and suggests that pro-PSA may be present in biological fluids. Human kallikrein 2 (hK2), another member of the hK family, is also expressed predominantly in prostate epithelium. Although hK2 has been shown to exhibit trypsin-like activity, little is known about its natural substrates. Using purified proteins, we show that hK2 can convert pro-PSA to mature, enzymatically active PSA, thus establishing a physiological connection between hK2 and PSA. These findings imply that hK2 may be regulating PSA activity *in vivo*.

Introduction

PCa² is the most frequently diagnosed cancer in American males. PSA has been widely used as a reliable prognostic marker in the management of patients with PCa (1). PSA, a member of the hK family of serine proteases, is a $M_r \sim 34,000$ single-chain glycoprotein with one *N*-linked oligosaccharide attached to asparagine 45 (reviewed in Refs. 2-4). Molecular cloning of cDNA revealed that the mRNA of PSA codes for a 261-aa preproprotein, in which a hydrophilic signal sequence of 17 aa (prepro region) and a propeptide of 7 aa precede the mature protein of 237 aa (5). PSA mRNA expression is predominant in prostate epithelium (6) and is regulated by androgens (7). PSA has been shown to have chymotrypsin-like activity (8, 9), and its proposed primary biological role is to cleave the major gel-forming proteins, semenogelin I and II and fibronectin, in seminal fluid, resulting in enhanced sperm motility (10). More recently, PSA has been reported to cleave insulin-like growth factor-binding protein-3, resulting in increased availability of insulin-like growth factor, suggesting that PSA enzymatic activity may promote proliferation, migration, and metastasis of PCa cells (11). Although the above-mentioned studies emphasize the possible physiological substrates of PSA, the fundamental questions regarding its biosynthesis, activation, and regulation of activity remain unanswered.

Received 5/6/97; accepted 6/10/97.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¹ To whom requests for reprints should be addressed, at Hybritech Incorporated, P. O. Box 269006, San Diego, CA 92196-9006. Phone: (619) 621-3258; Fax: (619) 621-4610; E-mail: mssaedi@beckman.com.

² The abbreviations used are: PCa, prostate cancer; PSA, prostate-specific antigen; hK, human kallikrein; aa, amino acid; pPSA, pro-PSA; mAb, monoclonal antibody; ACT, α -1-antichymotrypsin; HIC, hydrophobic interaction chromatography; pNA, *p*-nitroanilide.

The hK family consists of three members designated as hK1, hK2, and hK3 (PSA; Refs. 2 and 4). hK1 is primarily produced in the kidney, pancreas, and submandibular salivary gland (12). hK2, like PSA, is produced predominantly in the prostate epithelium (13), is regulated by androgens, and shares 78% aa homology with PSA (2, 4). The attributes of hK2 as a potential PCa marker have recently been reviewed (14). PSA is a chymotrypsin-like protease, whereas hK2 is a trypsin-like protease (15), indicating that the two enzymes may have different physiological roles. Little is known about the physiological role(s) of hK2. Depertes *et al.* (16) have recently shown that fibronectin and semenogelins present in ejaculate were hydrolyzed by hK2. Although PSA and hK2 have been shown to be present in the same environment, their interaction with each other has not been studied.

To study biosynthesis and the regulation of the activation of PSA and to explore the physiological relationship between hK2 and PSA, it is imperative to stably express PSA and hK2 at high levels in mammalian cells. We have reported the expression and purification of hK2 in mammalian cells previously (17). Stable and high expression of PSA in mammalian cells has not yet been reported. In this study, we report the development of a mammalian cell line with high expression ($>9 \mu\text{g/ml}$) of PSA. We demonstrate that PSA is secreted into the spent medium by mammalian cells as enzymatically inactive and stable pPSA. pPSA was converted to enzymatically active PSA by hK2. This suggests a possible physiological relationship between hK2 and PSA and implies that hK2 could be regulating the PSA activity *in vivo*. These studies also suggest that pPSA may exist in the biological fluid and may serve as a useful marker for PCa.

Materials and Methods

Expression Vector, Cell Line, and Transfection. A 0.8-kb cDNA coding for entire pre-pPSA was cloned into the *Bcl*I site of pGT-d, under the control of the GBMT promoter, using an approach similar to the one described earlier (17), resulting in the expression vector pGTD-PSA (Fig. 1). The orientation and sequence of the insert was confirmed. AV12-664 (ATCC CRL 9595) cells, cultured in DMEM (high glucose) and 10% Fetal Clone (Hyclone Laboratories, Inc., Logan, UT), were transfected with pGTD-PSA or pGT-d using Lipofectamine (Life Technologies, Inc., Gaithersburg, MD) and single-cell clones AV12-PSA and AV12-PGTD were selected in 400 nM methotrexate (Sigma Chemical Co., St. Louis, MO).

ELISA and Western Blot Analyses. Murine mAbs HK1G 586.1 (17) and PSM 773 (18) have been described previously. PSM 773 is one of the components of Tandem-MP PSA assay (Hybritech Incorporated, San Diego, CA) and is specific for PSA. For ELISA analysis, serum-containing spent medium from AV12-PSA cells was collected on specified days. Total and uncomplexed (free) forms of PSA were measured using Tandem-MP PSA and Tandem-MP free PSA assays (both from Hybritech), respectively. Spent media from AV12 and AV12-PGTD cells were used as negative controls. For Western blot analysis, tissue culture flasks containing AV12-PSA cells were grown to approximately 60-70% confluency and washed with PBS, and serum-free HH4 medium (JRH Biosciences, Lenexa, KS) was added. Serum-free spent medium (spent medium) harvested on specified days was concentrated using a Centricon 10 (Amicon, Inc., Beverly, MA), incubated with either

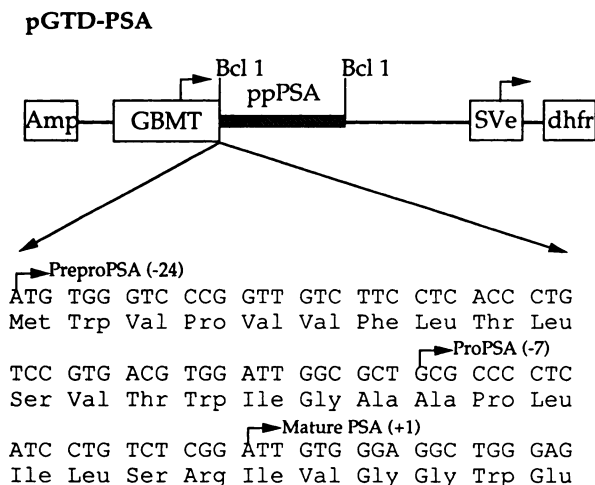


Fig. 1. Schematic representation of PSA expression vector, pGTD-PSA.

purified trypsin or purified hK2, followed by incubation with ACT, and subjected to SDS-PAGE on a 4–20% polyacrylamide gel (Novex, San Diego, CA). After electrophoresis, proteins were electroblotted onto nitrocellulose membranes. Primary antibodies (10 µg/ml) and secondary antibodies (goat antimouse IgG-horseradish peroxidase, 1:1000; Jackson Immunosearch Laboratories, Inc., West Grove, PA) were used to probe the blots. The immunoreactive signals were detected using enhanced chemiluminescence system (Amersham, Buckinghamshire, England) according to manufacturer's instructions.

Purification of PSA. mAb PSM 773 was coupled to AminoLink (Pierce, Rockford, IL) according to the manufacturer's instructions. Spent medium of AV12-PSA cells was harvested on specified days, concentrated, and incubated with the above resin overnight at 4°C, with mixing. The resin was then packed in a column and washed with PBS, and the PSA was eluted with 100 mM glycine-0.5 M sodium chloride, pH 2.5. The samples were immediately neutralized with 1 M Tris, pH 8.0.

Conversion of Purified pPSA to PSA by Purified hK2. Purified pPSA (4.7 µM) was incubated with 0.56 µM purified hK2 at 37°C in 100 mM Tris-2 mM EDTA, pH 8. Affinity purification of hK2 has been described previously (15, 17). The initial mixing was performed at 4°C, and an aliquot was immediately resolved by HIC. The sample was placed at 37°C, and additional aliquots were analyzed at various times, up to 2 h. HIC column specifications and buffer compositions were as follows: polypropylaspartamide column (4.6 × 250 mm), PolyLC (distributed by Western Analytical, Temecula, CA); buffer A, 1.2 M sodium sulfate-50 mM sodium phosphate, pH 6.3; and buffer B, 50 mM sodium phosphate-5% (v/v) 2-propanol, pH 7.3. Samples were prepared in 1.5 M ammonium sulfate and then injected onto the HIC column with the following gradient: 0–35% buffer B from 0 to 1 min, 30–80% buffer B from 1 to 12 min, and isocratic at 80% buffer B from 12 to 14 min, before reequilibration in buffer A. Peaks collected from the column were adsorbed to polyvinylidene difluoride membrane by using a prosorb cartridge (Applied Biosystems Instruments, Foster City, CA) and subjected to NH₂-terminal sequencing using an Applied Biosystems model 492 Procise sequencer. Enzymatic activity of peak samples was determined.

Assay for the Measurement of PSA Activity. Enzymatic activity of PSA was measured according to the published procedure (19). Briefly, PSA preparations were incubated with 1 mM pNA-derivatized peptide chromogenic substrates (methoxysuccinyl-Arg-Pro-Tyr-pNA, S2586; Pharmacia Hepar, Inc., Franklin, OH) in 200 mM Tris-5 mM EDTA, pH 8.0, at 37°C. The enzymatic activity of PSA was determined by hydrolysis of the peptide chromogenic substrates, leading to an increase in absorbance at 405 nm.

Results

To study the expression of PSA, AV12 cells were transfected with the pGTD-PSA expression vector. Cells were selected in 400 nM methotrexate for 2–3 weeks, and single-cell clones were analyzed for PSA expression using Tandem-MP PSA assay and Western blots

using mAb PSM 773. Clone AV12-PSA#8 was selected for further studies, on the basis of its high expression levels (>9 µg/ml) of a PSA-immunoreactive band at *M_r* 34,000.

To study the PSA expression pattern in mammalian cells, spent media from AV12-PSA#8 cells were collected for 6 consecutive days and analyzed using the Tandem-MP PSA assay (Fig. 2). PSA was detected in spent media at day 1, and it accumulated to >9 µg/ml by day 6. Expression of PSA was higher during the logarithmic phase of cell growth, indicating that a stable form of PSA is secreted by the cells, as opposed to being released following cell death and lysis. When the same samples were analyzed by Tandem-MP free PSA assay, similar values were obtained (data not shown), indicating that AV12-PSA#8 cells express uncomplexed or free PSA.

To determine the identity of the protein that is secreted on day 1, the spent media from AV12-PSA#8 cells were collected and concentrated. The PSA in the media was purified by affinity chromatography using PSM 773, a PSA-specific mAb. NH₂-terminal sequencing of the purified protein revealed the sequence: APLILSRIVGG. This sequence corresponds with the sequence predicted for the NH₂ terminus of pPSA that starts with Ala⁻⁷. Another species, starting at Leu⁻⁵, was also identified at comparable levels. Similar results were obtained when the same purification protocol was used to purify PSA from day 7 spent media of AV12-PSA#8 cells. This result indicates that PSA is secreted as pPSA by AV12-PSA#8 cells beginning on day 1 and that pPSA is stable in the spent media, even after 7 days.

To demonstrate that pPSA expressed by AV12-PSA#8 is enzymatically inactive and can be converted to enzymatically active PSA by mild trypsin treatment, a spectrophotometric assay was used, using the commercially available chromogenic substrate, methoxysuccinyl-Arg-Pro-Tyr-pNA (S-2586). A 10-fold increase in activity (from 2.38 × 10⁻⁴ to 2.27 × 10⁻³ activity units/min) was observed when spent media from AV12-PSA#8 (containing the equivalent to 14 µg of PSA, as measured by Tandem-MP PSA assay) were treated with trypsin (0.28 µg) for 60 min at 37°C. Spent media from AV12-PGTD cells (AV12 cells transfected by empty pGT-d vector) that were similarly treated with trypsin showed no detectable activity, indicating that no endogenous PSA-like proteases are secreted by AV12 cells. The positive control, PSA (14 µg), purified from seminal plasma and similarly treated with trypsin, exhibited an activity of 9.49 × 10⁻³ activity units/min.

It has previously been shown that PSA complexes with ACT in

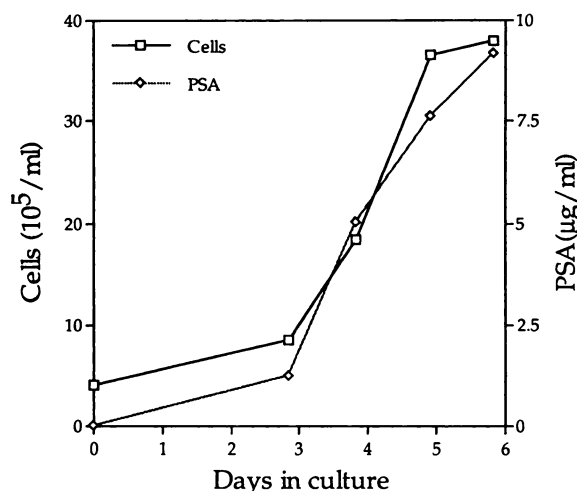


Fig. 2. Expression of PSA by AV12-PSA#8 cells. Serum-containing spent media of AV12-PSA#8 cells were harvested each day for 6 consecutive days. PSA concentration was measured using the Tandem-MP PSA assay. Viable cells were counted each day using trypan blue dye.

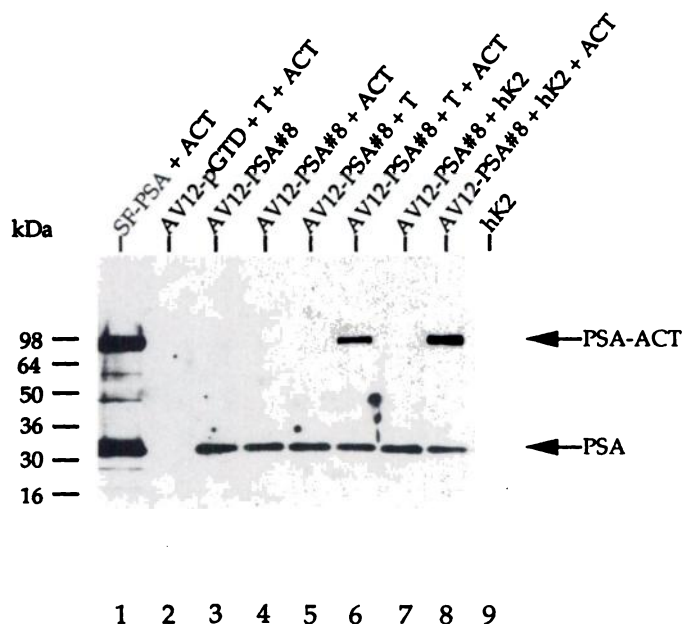


Fig. 3. Trypsin- and hK2-treated AV12-PSA#8 spent medium forms a complex with ACT. The day 7 spent media of AV12-PSA#8 cells and AV12-PGTD cells were appropriately concentrated. Concentrated spent medium (containing 10 $\mu\text{g/ml}$ of PSA, as measured by the Tandem-MP PSA assay) was mixed with 0.2 $\mu\text{g/ml}$ purified trypsin or 10 $\mu\text{g/ml}$ purified hK2 in a final reaction volume of 0.2 ml. After incubation for 60 min at 37°C, the reactions were quenched by adding 0.5 $\mu\text{g/ml}$ aprotinin. One-half of each sample was incubated with ACT (50 $\mu\text{g/ml}$) for an additional 4 h. The reactions were stopped with SDS-PAGE sample buffer and β -mercaptoethanol and electrophoresed on a 4–20% gradient polyacrylamide gel. Proteins were electroblotted onto a nitrocellulose filter and probed with PSA-specific mAb PSM 773. *Left*, molecular weight markers. SF-PSA, PSA purified from seminal fluid; AV12-PSA#8, spent medium from AV12-PSA#8 cells; AV12-pGTD, spent medium from AV12-pGTD cells; T, trypsin.

serum. We inferred that enzymatically inactive pPSA would not complex with ACT. To test whether the trypsin converted PSA would complex with ACT, ACT was added to the spent media of AV12-PSA#8 cells following preincubation with trypsin. After 4 h, the reaction was stopped by the addition of SDS-PAGE sample buffer and β -mercaptoethanol and boiling. The samples were then electrophoresed, electroblotted, and probed with PSM 773 mAb (Fig. 3). A M_r 94,000 complex comigrating with PSA-ACT (Lane 1) was detected in trypsin-treated spent media of AV12-PSA#8 cells (Lane 6). In contrast, PSA-ACT complex was not observed when untreated AV12-PSA#8 spent media were incubated with ACT (Lane 4). No immunoreactive band was observed when trypsin-preincubated AV12-PGTD day 7 spent media were incubated with ACT (Lane 2). These results confirmed that pPSA in the day 7 spent media of AV12-PSA#8 cells was enzymatically inactive and did not complex with ACT. These results also indicated that, upon mild trypsin treatment, this pPSA was converted to an enzymatically active form that could covalently complex with ACT. It appears that Arg⁻¹ of pPSA is the major site for trypsin reactivity because no PSA degradation products were detected by Western blot using PF1D 215 mAb (data not shown). PF1D 215 mAb has previously been shown to detect PSA degradation products present in seminal fluid (20).

hK2, another member of hK family, is predominantly expressed in the prostate epithelium, the site where PSA is also abundantly expressed. hK2 exhibits arginine-restricted trypsin-like activity (15). To test whether pPSA can be converted to PSA by hK2, purified hK2 was added to the spent media of AV12-PSA#8 cells. After 60 min of incubation at 37°C, ACT was added to these samples. After another 4 h of incubation at 37°C, the samples were electrophoresed, electroblotted, and probed with PSM773 (Fig. 3). A M_r ~94,000 band comigrating with purified PSA-ACT (Lane 1) was detected in hK2-

treated spent media (Lane 8). Although hK2 and PSA share ~78% aa similarity, hK2 was not immunodetected by PSM 773 mAb (Lane 9), confirming the PSA specificity of this mAb. These results indicate that hK2 can convert enzymatically inactive pPSA to an active form of PSA, which can form a complex with ACT.

To confirm the above results, affinity-purified preparations of hK2 (15, 17) and pPSA were mixed and the conversion of pPSA to mature PSA was analyzed by HIC (Fig. 4) and aa sequencing. Despite their similarities, hK2, pPSA, and mature PSA have distinctly different retention times. Fig. 4A shows the mixture of hK2 and pPSA at time 0, and Fig. 4B shows the same mixture after a 2-h incubation at 37°C. The retention times for hK2 (peak 1) and pPSA (peak 2) were identical to that of purified hK2 and pPSA, respectively, when injected separately (data not shown). The peak of hK2-converted PSA (peak 3) eluted at the same position as mature PSA purified from seminal fluid (data not shown). Fig. 4B shows that, after an incubation of 2 h at 37°C with purified hK2, the pPSA peak was almost entirely converted to mature PSA. Intermediate conversion of pPSA to mature PSA was observed when samples were analyzed at 20 min and 60 min of incubation (data not shown). The conversion from the pPSA to mature PSA was confirmed by aa sequencing. This analysis showed that peak 2 is composed of pPSA starting at Ala⁻⁷ and that peak 3 is entirely composed of mature PSA starting at Ile¹. Additionally, the hK2-converted mature PSA (peak 3) had enzymatic activity of 58 nmol/min/mg on S-2586 substrate. This value was comparable to the enzymatic activity of PSA purified from seminal fluid. In contrast, the pPSA sample, prior to hK2 treatment (peak 2), contained <5% of the

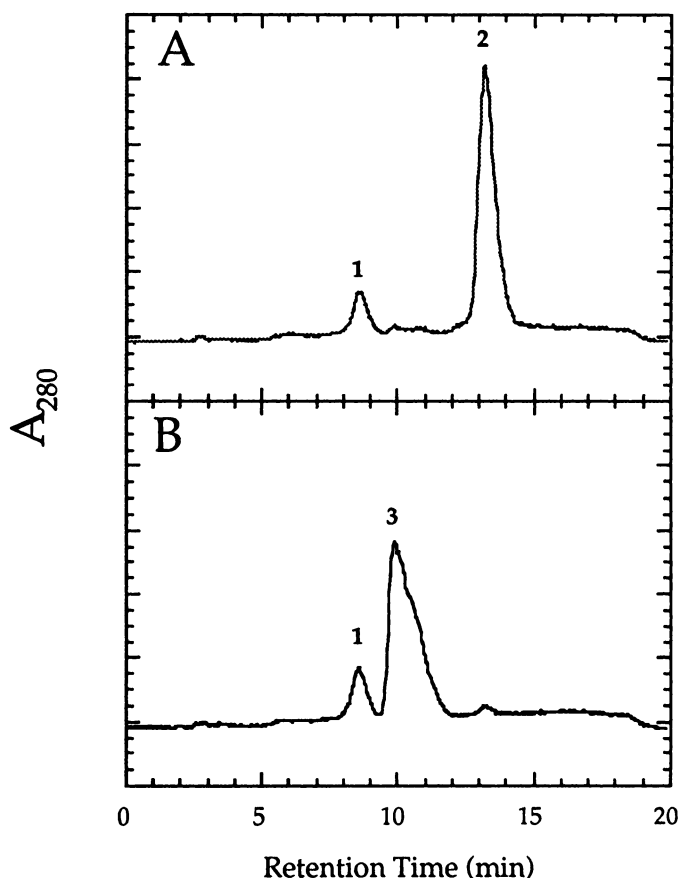


Fig. 4. HIC chromatography of hK2 incubated with purified pPSA. Purified hK2 was mixed with purified pPSA and injected immediately (A) or incubated for 2 h at 37°C before injection (B). Peak 1, hK2; peak 2, pPSA; peak 3, mature PSA, as confirmed by NH₂-terminal sequencing.

PSA enzymatic activity. Control sample of purified pPSA incubated without hK2 showed no conversion to PSA (data not shown).

Discussion

Like other serine proteases, PSA is translated as an inactive pre-PSA precursor. After passage through the secretory pathway, the signal peptide is cleaved, yielding the pro form of the protein. Here, we describe the development of a stable mammalian cell line (AV12-PSA#8) with high expression ($>9 \mu\text{g/ml}$) of PSA. The following four lines of evidence presented here unequivocally demonstrate that PSA is secreted as pPSA from mammalian cells: spent media of AV12-PSA#8 cells had no PSA-like enzymatic activity; the PSA form in the spent media did not complex with ACT, as demonstrated by Western blot analysis; trypsin treatment of the spent media resulted in mature PSA that was enzymatically active and formed a complex with ACT; and purification and sequence analysis of the PSA form present in the spent media confirmed its identity as pPSA. PSA exists in many forms in seminal fluid and in serum (reviewed in Ref. 2). These forms include complexes of PSA with protease inhibitors, such as ACT and protein C inhibitor. PSA also exists as a PSA- α 2-macroglobulin complex and as free PSA. Our data suggest that pPSA may exist in biological fluids, implying that some of the free PSA in the serum and seminal fluid may be pPSA. This suggests that pPSA may be a useful marker for prostatic diseases, a hypothesis currently under investigation.

Thus far, the protease(s) responsible for cleaving the propeptide from pPSA to form the active molecule have not been identified. hK2, another member of hK family, exhibits arginine-restricted, trypsin-like activity (15), and like PSA, it is predominantly expressed in the prostate epithelium (13). hK2 is also secreted as prohK2 by mammalian cells (17), which is converted to enzymatically active mature hK2 extracellularly. hK2 has autocatalytic activity because it can cleave prohK2 at Arg^{-1} , releasing the propeptide (21). Here, our data clearly show that mature hK2 clips the propeptide from the pPSA, converting it to enzymatically active mature PSA (Fig. 4), which can then complex with ACT (Fig. 3). This result suggests a possible physiological regulatory relationship between hK2 and PSA. Studies are in progress to discover if hK2 can also cleave mature PSA, further regulating PSA activity and complex formation with protease inhibitors. Similarly, PSA may also be regulating hK2 activity by clipping hK2 at chymotrypsin-sensitive sites. The reagents described here plus the hK2 expressed in mammalian cells (17) should facilitate these experiments.

The above results collectively indicate that PSA is expressed as pro form in mammalian cells and can be converted to the enzymatically active mature form extracellularly by hK2. These results also suggest that pPSA may be present in biological fluids and, therefore, could be a useful marker for prostatic diseases. The cell lines described here constitute a valuable source of pPSA to be used as an immunogen and to study the biological roles of pPSA and PSA in detail.

Acknowledgments

We thank Drs. Robert Wolfert, Harry Rittenhouse, and K. Kuus-Reichel for critically reviewing the manuscript.

References

- Catalona, W. J., Smith, D. S., Ratliff, T. L., Dodds, K. M., Coplen, D. E., Yuan, J. J., Tetros, J. A., and Andriole, G. L. Measurement of prostate specific antigen in serum as a screening test for prostate cancer. *N. Engl. J. Med.*, **324**: 1156–1161, 1991.
- McCormack, R. T., Rittenhouse, H. G., Finlay, J. A., Sokoloff, R. L., Wang, T. J., Wolfert, R. L., Lilja, H., and Oesterling, J. E. Molecular forms of prostate-specific antigen and the human kallikrein gene family: a new era. *Urology*, **45**: 729–744, 1995.
- Malm, J., and Lilja, H. Biochemistry of prostate specific antigen, PSA. *Scand. J. Clin. Lab. Invest.*, **221** (Suppl. 5): 15–22, 1995.
- Young, C. Y. F., Andrews, P. E., and Tindall, D. J. Expression and androgenic regulation of human prostate-specific kallikreins. *J. Androl.*, **16**: 97–99, 1995.
- Lundwall, A., and Lilja, H. Molecular cloning of human prostate-specific antigen cDNA. *FEBS Lett.*, **214**: 317–322, 1987.
- Qui, S., Young, C. Y. F., Bilhartz, D. L., Prescott, J. L., Farrow, G. M., He, W. W., and Tindall, D. J. *In situ* hybridization of prostate-specific antigen mRNA in human prostate. *J. Urol.*, **144**: 1550–1556, 1990.
- Young, C. Y. F., Montgomery, B. T., Andrews, P. E., Qui, S., Bilhartz, D. L., and Tindall, D. J. Hormonal regulation of prostate-specific antigen mRNA in a human prostatic adenocarcinoma cell line LNCaP. *Cancer Res.*, **51**: 3748–3752, 1991.
- Watt, K. W., Lee, P.-J., M'TimKulu, T., Chan, W.-P., and Loor, R. Human prostate-specific antigen: structural and functional similarity with serine proteases. *Proc. Natl. Acad. Sci. USA*, **83**: 3166–3170, 1986.
- Ban, Y., Wang, M. C., Watt, K. W. K., Loor, R., and Chu, T. M. The proteolytic activity of human prostate-specific antigen. *Biochem. Biophys. Res. Commun.*, **123**: 482–488, 1984.
- Lilja, H. A kallikrein-like serine protease in prostatic fluid cleaves the predominant seminal vesicle protein. *J. Clin. Invest.*, **76**: 1899–1903, 1985.
- Cohen, P., Graves, H. C. B., Peehl, D. M., Kamarei, M., Giudice, L. C., and Rosenfeld, R. G. Prostate-specific antigen is an insulin-like growth factor binding protein-3 protease found in seminal plasma. *J. Clin. Endocrinol. & Metab.*, **75**: 1046–1053, 1992.
- Fukushima, D., Kitamura, N., and Nakanishi, S. Nucleotide sequence of cloned cDNA for human pancreatic kallikrein. *Biochemistry*, **24**: 8037–8043, 1985.
- Morris, B. J. *hGK-1*: a kallikrein gene expressed in human prostate. *Clin. Exp. Pharm. Phys.*, **16**: 345–351, 1989.
- Young, C. Y. F., Seay, T., Hogen, K., Charlesworth, M. C., Roche, P. C., Klee, G. G., and Tindall, D. J. Prostate-specific human glandular kallikrein (hK2) as a novel marker for prostate cancer. *Prostate Suppl.*, **7**: 17–24, 1996.
- Mikolajczyk, S. D., Millar, L. S., Kumar, A., and Saedi, M. S. Human glandular kallikrein (hK2) shows arginine-restricted specificity and forms complexes with plasma protease inhibitors. *Prostate*, **33**: in press, 1997.
- Deperthes, D., Frenette, G., Brillard-Bourdet, M., Bourgois, L., Gauthier, F., Tremblay, R., and Dube, J. Y. Potential involvement of kallikrein hK2 in the hydrolysis of the human seminal vesicle proteins after ejaculation. *J. Androl.*, **17**: 659–665, 1996.
- Kumar, A., Goel, A. S., Hill, T. M., Mikolajczyk, S. D., Millar, L. S., Kuus-Reichel, K., and Saedi, M. S. Expression of human glandular kallikrein, hK2, in mammalian cells. *Cancer Res.*, **56**: 5397–5402, 1996.
- Wang, T. J., Finaly, J. F., Mikolajczyk, S., Rittenhouse, H. G., and Wolfert, R. L. Analysis of cross-reactivity of anti-PSA monoclonal antibodies with recombinant human glandular kallikrein. *J. Urol.*, **155**: 695A, 1996.
- Christensson, A., Laurell, C.-B., and Lilja, H. Enzymatic activity of prostate-specific antigen and its reactions with extracellular serine proteinase inhibitors. *Eur. J. Biochem.*, **194**: 755–763, 1990.
- Wang, T. J., Linton, H. J., Sokoloff, R. L., Grauer, L. S., Rittenhouse, H. G., and Wolfert, R. L. Antibody specificities for PSA and PSA fragments by SDS-PAGE Western blot analysis. *Tumor Biol.*, in press, 1997.
- Mikolajczyk, S. D., Millar, L. S., Marker, K. M., Grauer, L. S., Goel, A. S., Cass, M. M. J., Kumar, A., and Saedi, M. S. Ala 217 is important for the catalytic function and autoactivation of prostate-specific human kallikrein 2, hK2. *Eur. J. Biochem.*, **246**: 440–446, 1997.