Journal of Peptide Science
*J. Pept. Sci.* 2007; **13**: 849–855
Published online 24 September 2007 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/psc.911

JOURNAL OF PEPTIDE SCIENCE

# Structure-guided design of peptidic ligand for human prostate specific antigen

**MD IMTAIYAZ HASSAN, VIJAY KUMAR, RISHI K. SOMVANSHI, SHARMISTHA DEY, TEJ P. SINGH and SAVITA YADAV***
Department of Biophysics, All India Institute of Medical Sciences, New Delhi 110029, India

*Received 14 June 2007; Revised 2 July 2007; Accepted 3 July 2007*

**Abstract:** Prostate specific antigen (PSA) is a member of kallikrein family having serine protease-like activity and acts as a prognostic marker of prostate carcinoma. Various studies have been performed on inhibition of PSA and such targeting requires the identification of highly selective peptide inhibitors. PSA was purified from human seminal plasma by rapid and efficient methods, and binding studies for various peptides were carried out by fluorescence spectroscopy and SPR. The 'S' of PSA is predominated by hydrophobic residues, and hence many hydrophobic peptides were used to determine their binding affinity to PSA by fluorescence spectroscopy. We observed that LLFW, FFKW, and KFW binds strongly to PSA, among them LLFW showed strong binding. SPR also showed strong binding affinity of PSA toward peptides with hydrophobic and basic residues. Among the peptides used, FWYS showed dramatic increase in binding affinity ($10^{-10}$ M). The peptides analyzed for binding studies, suggests that peptide with Trp residue along with basic or hydrophobic amino acids may be useful for designing specific inhibitors for PSA. The strong affinities of designed peptides for PSA can be a valuable tool for designing therapeutic agents for prostate carcinomas.
Copyright © 2007 European Peptide Society and John Wiley & Sons, Ltd.

**Keywords:** peptide inhibitor; three-dimensional structure; prostate carcinoma; kallikrein; tumor proliferation; chymotrypsin; structure based drug design

## INTRODUCTION

PSA is one of the most important protein of human seminal plasma and possesses various important characteristics, which are essential for fertilization and normal prostate physiology [1–3]. Apart from prostate secretions, PSA content has also been detected in various tissues and fluids like urethral glands [4], endometrium, normal breast tissue [5], breast milk [6], salivary gland tissue [7], and the urine of males and females [8]. In addition to that, PSA has been also detected in the serum of women with breast, lung, or uterine cancer and in some patients with renal cancer by immunohistochemistry [9,10]. Hence, PSA is treated as a useful marker in determining and monitoring of therapeutic responses and in evaluating the prognosis of the disease [11,12]. There is a direct correlation between the volume of the cancer and the PSA level, with high PSA content being indicative of clinically advanced cancer [13,14]. There is a lot of evidence to suggest that PSA is directly responsible for prostate cancer invasion and metastasis [15]. There are two possible mechanisms to indicate the role of PSA in tumor proliferation and metastasis. PSA has been shown to activate urokinase-type plasminogen activator which is thought to be responsible for tumor invasion and metastasis, and for spreading tumor by proteolytic modulation of cell adhesion receptors [16,17]. Furthermore, PSA has been found to cleave insulin-like growth factor binding protein-3 (IGFBP-3), thereby causing release of active IGF-I which could enhance tumor growth [18]. However, PSA may also inhibit tumor growth as it has recently been shown to generate angiostatin from plasminogen [19]. These results suggest that ligands binding to PSA and modulating its enzyme activity are potentially useful for treatment of prostate cancer.

In the serum, PSA exists in a complex form with many natural inhibitors among which are most importantly, alpha-1-antichymotrypsin (ACT) [20,21], alpha-l-protease inhibitor (API) [22], protein C inhibitor (PCI) [23], alpha-2-macroglobulin (AMG) [24], and pregnancy zone protein (PZP) [25]. However, in blood and seminal fluid, significant differences occur in the amount of the PSA-inhibitor complex formation as well as in the type of protease inhibitors with which PSA interacts [26]. Many physiological inhibitors of PSA have been reported such as $Zn^{2+}$, $Hg^{2+}$, $Cd^{2+}$, spermine and spermidine [27]. The gene of PSA is known to be regulated by androgens through the action of the AR and enhancer sequences of androgen regulating elements that are present in the vicinity [28,29]. In seminal plasma, which contains a high level of PSA (1 mg/ml), it appears that the role of PSA is proteolytic cleavage of the sperm motility inhibitor, semenogelin,

Abbreviations: PSA, prostate specific antigen; Hk, Human kallikrein; SPR, surface plasmon resonance; 'S', substrate binding pocket; Amino acids are presented in one letter code.

* Correspondence to: Savita Yadav, Department of Biophysics, All India Institute of Medical Sciences, New Delhi 110029, India;
e-mail: savita11@gmail.com

Copyright © 2007 European Peptide Society and John Wiley & Sons, Ltd.

WILEY InterScience® DISCOVER SOMETHING GREAT

which in turn results in semen liquefaction after ejaculation [30]. Various experiments have been done not only to study the inhibition of PSA either at the gene level or the protein level [31,32], but also to synthesize many inhibitors for inhibiting the activity of PSA. However, no specific results have been achieved so far [33,34].

Presently, we have synthesized many peptides using structural parameters and have subsequently determined their inhibition efficacy and the potency of PSA, which can be used to predict their effect on tumor invasion and metastasis.

## MATERIALS AND METHODS

### Human Seminal Plasma

Human semen obtained from the Department of Laboratory Medicine, All India Institute of Medical Sciences, New Delhi, was initially centrifuged at 1300 g for 15 min to separate spermatozoa. PMSF, benzamidine hydrochloride, and $ZnCl_2$ were added to inhibit most of the proteases and further centrifuged at 13 000 g to remove debris. A clear supernatant was lyophilized and stored at −20 °C.

### Purification

Lyophilized powder of human seminal plasma was dissolved in 20 mM Ammonium acetate pH 6.0, centrifuged and dialyzed against the same buffer. After extensive dialysis, the further centrifuged and resulting supernatant was subjected to cation exchanger CM-Sephadex C-50 column (2.6 × 16 cm), pre-equilibrated with 20 mM Ammonium acetate pH 6.0. After loading the sample, the column was washed with the same buffer until the absorbance at 280 nm reached the baseline to remove all unbound proteins. Elution was done using 0–0.5 M NaCl in 20 mM Ammonium acetate, pH 6.0. The eluted fractions were monitored at 280 nm and 3.0 ml fractions were collected. The fractions were further assessed by SDS-PAGE electrophoresis using 12.5% gels. The Peak b obtained at 0.2 M NaCl elution (Figure 1(A)), along with some impurities and was further purified by gel filtration chromatography on Sephadex G-75 (Figure 1(B)). Peak III of gel filtration showed a single band on SDS–PAGE (Figure 1(C)), which is corresponding to molecular mass of 27-kDa.

### *N*-Terminal Sequencing

The *N*-terminal sequence was determined, using a protein sequencer PPSQ-20 (Shimadzu Japan). The protein band was transferred after SDS–PAGE to PVDF membrane (Millipore,

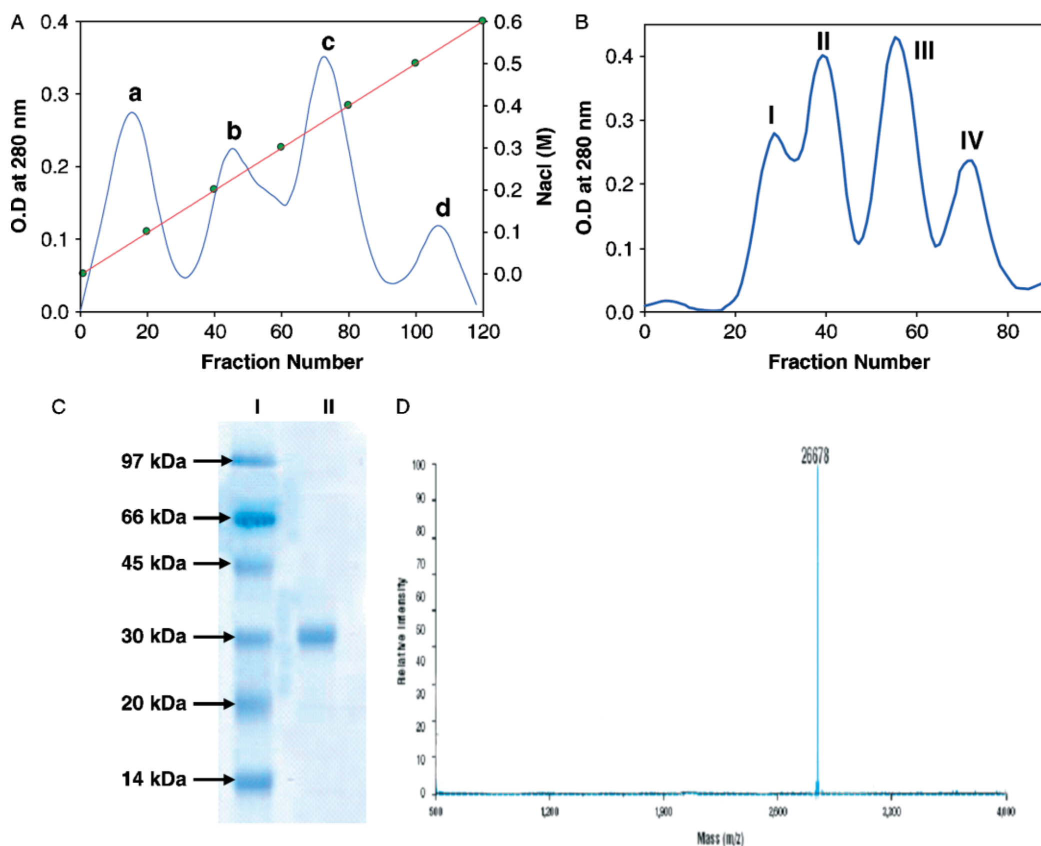


**Figure 1** (A) Elution profile of proteins bound on CM Sephadex C-50. The peaks were formed as a function of fraction number (X axis) and absorbance at 280 nm (Y axis). The second line represents the gradient of NaCl (0–0.5 M). (B) Elution profile of proteins loaded on Sephadex G-75. (C) SDS PAGE profile of PSA I; Marker (97 kDa: Phosphorylase b, 66 kDa: Bovine serum albumine, 45 kDa: Ovalbumin, 30 kDa: Carbonic anhydrase, 20 kDa: Soyabeen trypsin inhibitor and 14 kDa: Lactalbumin), **II;** Purified PSA. (D) Molecular weight determination of purified PSA after gel filtration through MALDI TOF. Peaks obtained as a function of percent abundance (Y) against $m/z$ (mass/charge) ratio (x-axis).

Copyright © 2007 European Peptide Society and John Wiley & Sons, Ltd.

DOI: 10.1002/psc

USA) using the tank method (MiniTrans-Blot Electrophoretic Transfer Cell, (Bio-Rad Laboratories, USA). The transfer was performed in electro blotting buffer consisting of 10 mM (3-2-1-1-propanesulfonic acid) in 10% methanol according to the procedure recommended by the manufacturer. Staining was performed using Coomassie Blue R 250 (Serva, Germany). The band was excised and subjected to sequencing by automated Edman degradation on a Shimadzu PPSQ-20 protein sequenator.

### MALDI-TOF Analysis

Accurate molecular mass and purity of PSA was determined by MALDI-TOF (Kratos Analytical Ltd Shimadzu group company, Japan). Lyophilized protein was dissolved in distilled water (approximately 1 mg/ml). Prior to acquisition of spectra, 100 µl of protein solution was mixed with 100 µl of 0.2% aqueous TFA. One microlitre of acidified solution was then spotted onto a stainless steel sample slide followed by 1 ml of $\alpha$-cyano-4-hydroxycinnamic acid matrix solution (10 mg/ml in 50 : 50 ethanol/water containing 0.1% TFA). Launchpad Software Version 2.4.0 (Shimadzu/Kratos, Japan) controlled data acquisition and processing. In the observed mass spectra, peak areas versus mass/electric charge ($m/z$) of mono-isotopic ions were calculated with Mascot Distiller Software Version 1.1.2.0 (Matrix Science, London, UK).

### Peptide Synthesis and Purification

The peptide was synthesized using an automated solid-phase peptide synthesizer (Rainin, USA). The resin used was Fmoc-Ser-Wang resin, and the solvent used for synthesis was dimethyl formamide (DMF). In the first step, Fmoc-Ser-Wang resin (1 g, 0.5 mM) was deprotected by 20% piperidine in DMF to form H2N-Ser-Wang resin. The uronium salt 2-(1H-benzotriazole-1-yl)- 1,1,3,3-tetramethyl uronium hexa fluorophosphates (HBTU) (455 mg, 1 mM) in the presence of base *N*-methyl morpholine (NMM; 0.4 M) activated the amino acid to form the active ester of Fmoc-Tyr-OH (551 mg, 0.5 mM). This was coupled with H2N-Ser-Wang resin to get Fmoc-Tyr-Ser-Wang resin. The above procedure was repeated for the remaining amino acids until the complete sequence Fmoc-Phe-Trp-Tyr-Ser-Wang resin was formed. The resin was cleaved from the peptide with trifluoroacetic acid (TFA). The peptide was purified by reverse phase chromatography on $C_{18}$ Rep RPC column (1.6 × 10 cm, Amersham Biosciences Upsala Sweden).

The negative-ion ESMS data was obtained on a VG Platform-II quadrupole mass spectrometer equipped with a MassLynx data system and pneumatic-nebulizer-assisted electrospray LC/MS interface. Acetonitrile–water (1 : 1 mixture) was used as carrier solvent at a flow rate of 10 µl per min and the analyte was infused through a rheodyne injector into the ESMS probe. The MS value for the peptide was $m/z$ 603 (100), which compares well with the peptide formula mass of 601.66. The synthesis of other peptides LLFW, FKW, KFW, VVFF, LFW, VVW, FFKW and KW was done by the same method.

### Fluorescence Studies of Protein–Peptide Binding

Measurements of intrinsic Trp fluorescence (excitation at 295 nm), were performed on a Perkin Elmer Life Sciences LS-50 luminescence spectrometer with a constant temperature cell holder and 1-cm path length quartz cells. The excitation and emission slits were 10 and 4, respectively. The emission spectra were collected from 300 to 400 nm at the rate of 120 nm/min. All the spectra were corrected for blank emission and emission spectra of peptide deduced from the protein sample. All samples contained 10 mM Tris-HCl pH 7.4 and 100 mM NaCl. The peptides were initially dissolved in 50% acetonitrile.

### SPR-Binding Studies of Peptides-PSA-Binding

The binding studies were carried out using the BIAcore 2000 apparatus (Pharmacia BIAcore AB, Upsala, Sweden). The BIAcore apparatus is a biosensor-based system for real-time specific interaction analysis [35]. The sensor chips CM5, surfactant P20, the amine coupling kit containing *N*-hydroxysuccinimide (NHS), *N*-methyl-N′-3 (diethylamino-propyl) carbadiimide (EDC), ethanolamine hydrochloride were used. The running buffer used was 10 mM Sodium acetate (pH 5.5), 0.005% surfactant P20. The immobilization of human PSA was done at a flow rate of 10 µl/min at 25 °C. The dextran on the chip was equilibrated with running buffer and carbomethylated matrix was activated with an EDC/NHS mixture. 210 µl of PSA (50 µg/ml) in 10 mM sodium acetate (pH 5.5) was injected and un-reacted groups were blocked by injection of ethanolamine (pH 8.5). The SPR signal for immobilized PSA was used for the calculation of binding affinity of different peptides using software BIAcore 3.0 Biaevaluation.

## RESULTS AND DISCUSSION

The acidic and basic fractions were separated using CM sephadex C-50 at pH 6.0 and eluted with a linear gradient of NaCl (0.0–0.5 M). Peak B obtained at 0.2 M NaCl (Figure 1(A)) along with some impurities, was further purified by gel filtration chromatography on Sephadex G-75 (Figure 1(B)). Peak II of gel filtration showed a single band on SDS PAGE (Figure 1(C)), which corresponds to a molecular mass of 27 kDa. On *N*-terminal sequencing, the first 15 residues were Ile-Val-Gly-Gly-Trp-Glu-Cys-Glu-Lys-His-Ser-Gln-Pro-Trp-Gln, upon TrEMBL/BLAST; it was identified as PSA (accession number P07288). The purity of protein was further assessed by MALDI-TOF; it showed a single peak at 26 678 Da (Figure 1(D)). We have for the first time purified PSA in a two-step procedure by using simplified chromatography techniques like Ion exchange and gel filtration. Recently PSA was purified in a two-step process, based on anti-PSA. Mab's carried on binding and elution experiments of PSA antigen in 96-well plates [36]. In order to optimize the purification procedure, they test several washing and elution conditions (chaotropic agents, high ionic strength solution, and extreme pH). The other method developed for PSA purification is a simple two-step procedure, based on principles of hydrophobic charge-induction chromatography and molecular size chromatography to provide a pure free-PSA (f-PSA) preparation that is free from all

Copyright © 2007 European Peptide Society and John Wiley & Sons, Ltd.

DOI: 10.1002/psc

other known PSA complexes as well as hK2 [37]. Both the recently documented methods for PSA purification are cost effective but time consuming as compared to what has been described here. The other disadvantage of using a solution of high ionic strength is that it provides a harsh environment to the protein, causes extensive denaturation, and consequently creates problems during crystallization and folding studies. Overall, we report a simple, quick, and nonexpensive procedure to obtain free-PSA from human seminal plasma at high purity levels with high yield.

On the basis of a unique biology of prostate cancer, it was initially proposed that PSA could be valuable target therapies, selectively to metstatic prostate cancer sites within the patient; such targeting required the identification of a highly selective peptide for PSA. Our constructed model for human PSA was taken into account for designing a selective peptidic inhibitor [38]. The structural feature of loop $Trp^{215} - Ala^{220}$ adopts a conformation that alters the structure of the specificity pocket considerably and their side chains are mainly responsible for substrate accessibility [39]. Binding of peptide containing Trp, may have pushed $Trp^{215}$ into the interior of the pocket and caused it to be held in this position by the hydrogen bond $N_1$ Trp215–O Glu219. The conformational changes may have to occur in order to allow the binding of substrates or inhibitors to PSA. The substrate accessibility is determined mainly by the side chain residues of PSA, which has maximum emission at 345 nm with an excitation at 295 nm. The binding studies of various peptides with PSA have shown the shift in maximum emission, and changes in the emission intensity on titration with ligands are indicative of the binding/stacking of ligands against a Trp-residue [40,41]. The drastic change in fluorescence intensity upon binding with all the hydrophobic peptides was observed (Figure 2). In all cases, there was a measurable blue shift observed in the maximum emission, upon PSA-peptide target complex formation as compared with the maximum fluorescence emission peak from the PSA. The extent of the blue shift and change in fluorescence intensity are directly related to the interaction between the peptides and PSA (Table 1). The peptide LLFW showed maximum change in fluorescence intensity, and hence has maximum affinity. The addition of two aromatic residues and one basic (FFKW) residue retains the same binding affinity. On decreasing the size of the peptides (three residues), a decrease in binding affinity was observed. The order of binding like KFW > VFW > LFW, indicating the role of Trp and Lys is the most significant residue for PSA-binding as is evident from the structure; the substrate-binding groove is occupied predominantly by hydrophobic residues. It is interesting to note that a four amino-acid long peptide devoid of Trp showed least binding (Table 1 and Figure 2), and it further supports the strong binding of hydrophobic peptides to PSA. In contrast, when we performed binding assays with albumin and lactoferrin these peptides showed poor binding.

Representative Sensorgrams show the peptide binding to PSA (1/1). 100 µl of peptide, dissolved in 140 mM NaCl, 20 mM Sodium acetate pH 5.5, 1 mM EDTA, were injected at 30 µl/min for 3.3 min over the PSA coupled to a Sensor Chip L1 at 25 °C. Peptides were applied in the following order: (A) 0.005, (B) 0.05 and (C) 0.5 µg/ml. The SPR experiments were run in triplicate to avoid ambiguity. The analysis of data was carried out using BIAevaluation software (Pharmacia BIAcore) [42]. Before passing the next peptide on immobilized PSA, regeneration of the immobilized protein was performed with 10 mM Glycine–HCl buffer, pH 2.5. For the selection of peptides, again a constructed model was

**Table 1** Change in fluorescence intensity, while binding of peptides with PSA. $\Delta f$: change in fluorescence intensity (fluorescence intensity of PSA + peptide complex subtracted from fluorescence intensity of PSA alone)

| Peptide code | Peptide | Blue shift (nm) | $\Delta f$ |
|---|---|---|---|
| 1 | KFW | 12 | 690 |
| 2 | VVW | 5 | 640 |
| 3 | LFW | 10 | 585 |
| 4 | FFKW | 5 | 700 |
| 5 | LLFW | 5 | 710 |
| 6 | VVFF | 2 | 270 |

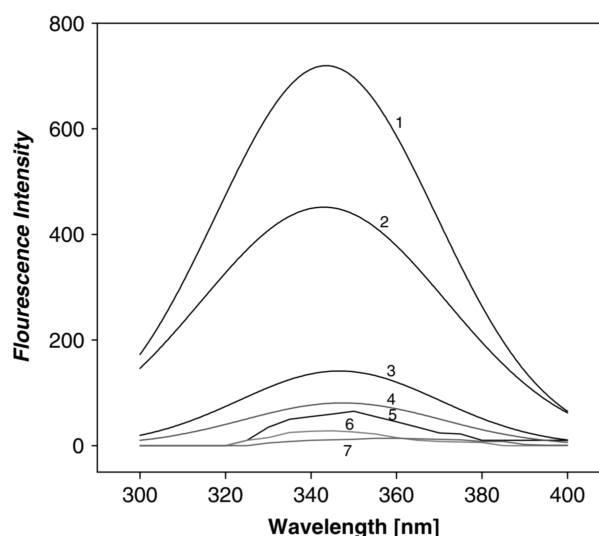


**Figure 2** Emission spectrum from the steady state tryptophan fluorescence of the PSA-peptide complex. Protein and peptide concentrations were 15 and 10 µM respectively. PSA alone (curve 1); PSA + VVFF (curve 2); PSA + LFW (curve 3); PSA + VVW (curve 4); PSA + KFW (curve 5); PSA + LLFW (curve 6); PSA + FFKW (curve 7).

Copyright © 2007 European Peptide Society and John Wiley & Sons, Ltd.

DOI: 10.1002/psc

taken into account. In a complex structure of bovine pancreatic trypsin inhibitor (PTI), Ala and Lys occupy the 'S1' and 'S2' sub sites of porcine tissue kallikrein, as shown in the crystal structure of the complex formed by them [43,44]. Present model of human PSA shares the same structural features and may suggest that 'S1' site preferentially accepts peptide with short side chains while 'S 2' preferentially accepts residues with basic side chains. Keeping this in mind, the peptides were designed and tested for their binding affinity for PSA. Instead of Ala, Trp was used, as an outcome of fluorescence studies (Figure 3). The peptide KW has binding constant of the order of $10^{-9}$. In the subsequent experiment, Phe, resulted in 10 times decrease in the binding affinity (Table 'as replaced by Lys. In the next experiment, Ser was introduced, in addition to hydrophobic Phe (FYS); further decrease in the binding constant was observed. However, the introduction of Trp showed the highest binding of the order of $10^{-10}$ M (Table 2), and it further suggested the importance of Trp in constructing an efficient ligand for PSA. It is interesting to note that all the peptides have greater affinity for PSA than $Zn^{2+}$ ($ZnCl_2$), a well-known inhibitor of PSA.

It is evident from the structures, that the specificities of tissue kallikrein seems to be as good a determinant as that of 'S2', which requires hydrophobic, and preferentially aromatic, amino acids [45]. In human PSA, the hydrophobic crevice between $Tyr^{99}$ and $Trp^{215}$ is also available and required for substrate specificity. All the data from fluorescence and SPR binding studies suggests that a peptide containing Trp with basic residues may act as a potent inhibitor for PSA. However further work is needed to characterize different peptides from the peptide library and design a specific peptide with high-affinity and selectivity, which may act as a therapeutic agent for prostate and breast carcinomas. These findings suggest that peptides with Trp residues, function as a potential chemotherapeutic agent for controlling the growth of human prostate carcinoma cells.

## CONCLUSIONS

The binding studies were performed in order to investigate the inhibitory activities of peptides for PSA.

**Table 2** Binding affinity of PSA for different peptides $K_D$: dissociation constant (M)

| S. no | Peptide | $K_D$ (M) |
|---|---|---|
| 1 | KW | $9.41 \times 10^{-9}$ (±2%) |
| 2 | FW | $6.47 \times 10^{-8}$ (±2.8%) |
| 3 | FYS | $1.04 \times 10^{-7}$ (±2.2%) |
| 4 | FWYS | $8.31 \times 10^{-10}$ (±3.8%) |
| 5 | $ZnCl_2$ | $1.06 \times 10^{-7}$ (±1.7%) |

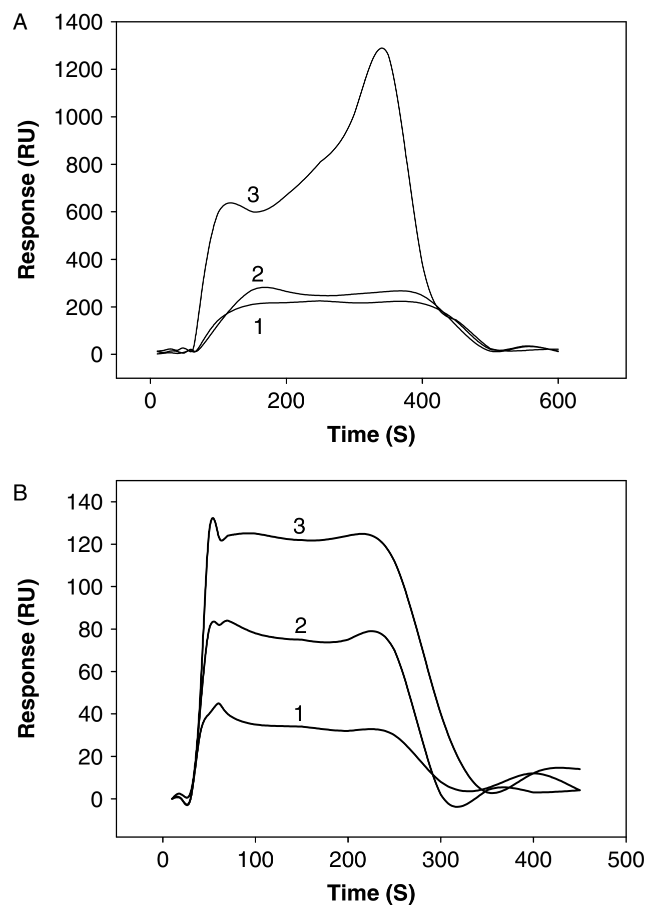


**Figure 3** SPR sensogram data obtained for the interaction of peptides with PSA. (A) Peptide KW, (B) Peptide FWYS. **Curve 1:** 0.005 ug/ml, **Curve 2:** 0.05 ug/ml, and **Curve 3:** 0.5 ug/ml. The change in surface response (*R*) over time (*t*) is plotted, RU = response unit.

Among hydrophobic peptides, FFKW is most effective as compared to other peptides. The SPR binding experiment also gave efficient binding of FWYS and KW of the order of $10^{-10}$ M. These data indicate that PSA inhibitors are a promising molecular target for prostate cancer therapy. Our peptide binding studies of PSA opens a promising channel for structure-based drug designing for prostate and breast carcinomas. Strong binding of peptides to PSA encourages us to believe these peptides can be taken into account. Further work is needed to achieve clinically acceptable peptides, verify their selectivity, and extend the method to biological objectives.

## Acknowledgements

This work was supported by grants from the All India Institute of Medical Sciences (A.I.I.M.S), New Delhi, India. Hassan M.I and Kumar V thank the Council of Scientific and Industrial Research (CSIR) for the grant of fellowship. We also thank Dr Sarman Singh (Additional Professor, Department of Laboratory

Copyright © 2007 European Peptide Society and John Wiley & Sons, Ltd.

Medicine, AIIMS) for providing the human seminal samples. The assistance of Mr W.E. John, (Department of English, Delhi University) in the form of language editing is gratefully acknowledged.

## REFERENCES


1. Watt KW, Lee PJ, M'Timkulu T, Chan WP, Loor R. Human prostate-specific antigen: structural and functional similarity with serine proteases. *Proc. Natl. Acad. Sci. U.S.A.* 1986; **83**: 3166–3170.
2. Digby M, Zhang XY, Richards RI. Human prostate specific antigen (PSA) gene: structure and linkage to the kallikrein-like gene, hGK-1. *Nucleic Acids Res.* 1989; **17**: 2137.
3. Lilja H, Abrahamsson PA, Lundwall A. Semenogelin, the predominant protein in human semen. Primary structure and identification of closely related proteins in the male accessory sex glands and on the spermatozoa. *J. Biol. Chem.* 1989; **264**: 1894–1900.
4. Satoh S, Ujiie T, Kubo T, Ohhori T, Iwasaki T, Satodate R, Numasato S. Prostatic epithelial polyp of the prostatic urethra. *Eur. Urol.* 1989; **16**: 92–96.
5. Malatesta M, Mannello F, Sebastiani M, Bianchi G, Gazzanelli G. Prostate-specific antigen found in type I breast cyst fluids is a secretory product of the apocrine cells lining breast gross cysts. *Breast Cancer Res. Treat.* 1999; **57**: 157–163.
6. Diamandis EP, Nadkarni S, Bhaumik B, *et al.* Immunofluorometric assay of pepsinogen C and preliminary clinical applications. *Clin. Chem.* 1997; **43**: 1365–1371.
7. Maeda K, Sueishi K. A monoclonal antibody that defines basal cells of stratified epithelia in various human and rabbit tissues. *Histochemistry* 1989; **92**: 319–324.
8. Shibata K, Kajihara J, Kato K, Hirano K. Purification and characterization of prostate specific antigen from human urine. *Biochim. Biophys. Acta* 1997; **1336**: 425–433.
9. Zaviacic M, Ablin RJ. The female prostate and prostate-specific antigen. Immunohistochemical localization, implications of this prostate marker in women and reasons for using the term "prostate" in the human female. *Histol. Histopathol.* 2000; **15**: 131–142.
10. Kato H, Kobayashi S, Islam AM, Nishizawa O. Female para-urethral adenocarcinoma: histological and immunohistochemical study. *Int. J. Urol.* 2005; **12**: 117–119.
11. Bilhartz DL, Tindall DJ, Oesterling JE. Prostate-specific antigen and prostatic acid phosphatase: biomolecular and physiologic characteristics. *Urology* 1991; **38**: 95–102.
12. Lilja H. Structure, function, and regulation of the enzyme activity of prostate-specific antigen. *World J. Urol.* 1993; **11**: 188–191.
13. Pijoan JM. Diagnostic methodology for the biochemical recurrence of prostate cancer after radiotherapy. *Arch. Esp. Urol.* 2006; **59**: 1053–1062.
14. Grossklaus DJ, Smith JA Jr, Shappell SB, Coffey CS, Chang SS, Cookson MS. The free/total prostate-specific antigen ratio (%fPSA) is the best predictor of tumor involvement in the radical prostatectomy specimen among men with an elevated PSA. *Urol. Oncol.* 2002; **7**: 195–198.
15. Babaian RJ, Fritsche HA, Evans RB. Prostate-specific antigen and prostate gland volume: correlation and clinical application. *J. Clin. Lab. Anal.* 1990; **4**: 135–137.
16. Steuber T, Vickers A, Haese A, *et al.* Free PSA isoforms and intact and cleaved forms of urokinase plasminogen activator receptor in serum improve selection of patients for prostate cancer biopsy. *Int. J. Cancer* 2007; **120**: 1499–1504.
17. McCabe NP, Angwafo FF III, Zaher A, Selman SH, Kouinche A, Jankun J. Expression of soluble urokinase plasminogen activator receptor may be related to outcome in prostate cancer patients. *Oncol. Rep.* 2000; **7**: 879–882.
18. Cohen P, Peehl DM, Graves HC, Rosenfeld RG. Biological effects of prostate specific antigen as an insulin-like growth factor binding protein-3 protease. *J. Endocrinol.* 1994; **142**: 407–415.
19. Yoshida E, Ohmura S, Sugiki M, Maruyama M, Mihara H. Prostate-specific antigen activates single-chain urokinase-type plasminogen activator. *Int. J. Cancer* 1995; **63**: 863–865.
20. Lilja H, Christensson A, Dahlen U, *et al.* Prostate-specific antigen in serum occurs predominantly in complex with alpha 1-antichymotrypsin. *Clin. Chem.* 1991; **37**: 1618–1625.
21. Lilja H, Cockett AT, Abrahamsson PA. Prostate specific antigen predominantly forms a complex with alpha 1-antichymotrypsin in blood. Implications for procedures to measure prostate specific antigen in serum. *Cancer* 1992; **70**: 230–234.
22. Zhang WM, Finne P, Leinonen J, Stenman UH. Characterization and determination of the complex between prostate-specific antigen and alpha 1-protease inhibitor in benign and malignant prostatic diseases. *Scand. J. Clin. Lab. Invest. Suppl.* 2000; **233**: 51–58.
23. Espana F, Navarro S, Medina P, Zorio E, Estelles A. The role of protein C inhibitor in human reproduction. *Semin. Thromb. Hemost.* 2007; **33**: 41–45.
24. Otto A, Bar J, Birkenmeier G. Prostate-specific antigen forms complexes with human alpha 2-macroglobulin and binds to the alpha 2-macroglobulin receptor/LDL receptor-related protein. *J. Urol.* 1998; **159**: 297–303.
25. Finne P, Zhang WM, Auvinen A, *et al.* Use of the complex between prostate specific antigen and alpha 1-protease inhibitor for screening prostate cancer. *J. Urol.* 2000; **164**: 1956–1960.
26. Qian Y, Sensibar JA, Zelner DJ, *et al.* Two-dimensional gel electrophoresis detects prostate-specific antigen-alpha1-antichymotrypsin complex in serum but not in prostatic fluid. *Clin. Chem.* 1997; **43**: 352–359.
27. Malm J, Hellman J, Hogg P, Lilja H. Enzymatic action of prostate-specific antigen (PSA or hK3): substrate specificity and regulation by Zn(2+), a tight-binding inhibitor. *Prostate* 2000; **45**: 132–139.
28. Bhuiyan MM, Li Y, Banerjee S, *et al.* Down-regulation of androgen receptor by 3,3′-diindolylmethane contributes to inhibition of cell proliferation and induction of apoptosis in both hormone-sensitive LNCaP and insensitive C4-2B prostate cancer cells. *Cancer Res.* 2006; **66**: 10064–10072.
29. Agoulnik IU, Vaid A, Nakka M, *et al.* Androgens modulate expression of transcription intermediary factor 2, an androgen receptor coactivator whose expression level correlates with early biochemical recurrence in prostate cancer. *Cancer Res.* 2006; **66**: 10594–10602.
30. de Lamirande E. Semenogelin, the main protein of the human semen coagulum, regulates sperm function. *Semin. Thromb. Hemost.* 2007; **33**: 60–68.
31. Dehm SM, Tindall DJ. Ligand-independent androgen receptor activity is activation function-2-independent and resistant to antiandrogens in androgen refractory prostate cancer cells. *J. Biol. Chem.* 2006; **281**: 27882–27893.
32. Quayle SN, Mawji NR, Wang J, Sadar MD. Androgen receptor decoy molecules block the growth of prostate cancer. *Proc. Natl. Acad. Sci. U.S.A.* 2007; **104**: 1331–1336.
33. Tahmatzopoulos A, Gudegast C, Stockle M, *et al.* Proteasome inhibitors: induction of apoptosis as new therapeutic option in prostate cancer. *Aktuelle Urol.* 2004; **35**: 491–496.
34. Leinonen J, Wu P, Koivunen E, Narvanen A, Stenman UH. Development of novel peptide ligands modulating the enzyme activity of prostate-specific antigen. *Scand. J. Clin. Lab. Invest. Suppl.* 2000; **233**: 59–64.
35. Szabo A, Stolz L, Granzow R. Surface plasmon resonance and its use in biomolecular interaction analysis (BIA). *Curr. Opin. Struct. Biol.* 1995; **5**: 699–705.
36. Acevedo B, Perera Y, Torres E, Penton D, Ayala M, Gavilondo J. Fast and novel purification method to obtain the prostate specific antigen (PSA) from human seminal plasma. *Prostate* 2006; **66**: 1029–1036.


Copyright © 2007 European Peptide Society and John Wiley & Sons, Ltd.

DOI: 10.1002/psc

37. Bindukumar B, Kawinski E, Cherrin C, *et al.* Two step procedure for purification of enzymatically active prostate-specific antigen from seminal plasma. *J. Chromatogr. B, Analyt. Technol. Biomed. Life Sci.* 2004; **813**: 113–120.
38. Hassan MI, Kumar V, Singh TP, Yadav S. Structural model of human PSA: A target for prostate cancer therapy. *Chem Biol Drug Des.* 2007; **70**(3): 261–267.
39. Villoutreix BO, Getzoff ED, Griffin JH. A structural model for the prostate disease marker, human prostate-specific antigen. *Protein Sci.* 1994; **3**: 2033–2044.
40. Wolfe C, Cladera J, O'Shea P. Amino acid sequences which promote and prevent the binding and membrane insertion of surface-active peptides: comparison of melittin and promelittin. *Mol. Membr. Biol.* 1998; **15**: 221–227.
41. Golding C, Senior S, O'Shea P. Interaction of a signal peptide with phospholipid vesicles: the kinetics of binding, insertion and structural changes. *Biochem. Soc. Trans.* 1995; **23**: 554S.
42. Nieba L, Krebber A, Pluckthun A. Competition BIAcore for measuring true affinities: large differences from values determined from binding kinetics. *Anal. Biochem.* 1996; **234**: 155–165.
43. Chen Z, Bode W. Refined 2.5 A X-ray crystal structure of the complex formed by porcine kallikrein A and the bovine pancreatic trypsin inhibitor. Crystallization, Patterson search, structure determination, refinement, structure and comparison with its components and with the bovine trypsin-pancreatic trypsin inhibitor complex. *J. Mol. Biol.* 1983; **164**: 283–311.
44. Bode W, Chen Z. The X-ray structures of porcine pancreatic kallikrein and of its complex with bovine pancreatic trypsin inhibitor. *Adv. Exp. Med. Biol.* 1983; **156**: 289–308.
45. Del Nery E, Chagas JR, Juliano MA, Juliano L, Prado ES. Comparison of human and porcine tissue kallikrein substrate specificities. *Immunopharmacology* 1999; **45**: 151–157.

Copyright © 2007 European Peptide Society and John Wiley & Sons, Ltd.


DOI: 10.1002/psc