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Thiophilic-interaction chromatography of enzymatically active tissue prostate-specific antigen (T-PSA) and its modulation by zinc ions☆

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Abstract

Prostate-specific antigen (PSA) is a serine protease secreted both by normal prostate glandular epithelial cells and prostate cancer cells. We explored "thiophilic-interaction chromatography" (TIC) to isolate tissue prostate-specific antigen (T-PSA) from fresh human prostate cancer tissue harvested by radical prostatectomy for the purpose to characterize T-PSA for its enzymatic activity and sensitivity to zinc ions. We have shown, for the first time, that T-PSA has strong affinity for the thiophilic gel (T-gel). The average recovery of T-PSA from T-gel is over 87%. The presence of PSA in the column eluate was confirmed by ELISA and SDS/PAGE. Western blot developed with monoclonal antibody to PSA revealed that T-PSA was predominantly in the "free" form having a molecular weight of 33 kDa. Furthermore, T-PSA was found to be enzymatically active. T-PSA was found to be less enzymatically active as compared to seminal plasma PSA. The inhibition of enzymatic activity of both f-PSA and T-PSA over a wide range of concentrations of Zn^{2+} ions (10 nM to 50 μM) was comparable. In contrast, the enzymatic activity of chymotrypsin, another serine-protease, was affected differently. At higher concentrations of Zn^{2+} (10 μM and higher) the enzymatic activity of chymotrypsin was inhibited, whereas, at lower concentrations of Zn^{2+} (5 μM and lower), the enzymatic activity was enhanced.

Keywords: Prostate cancer; Prostate-specific antigen; T-PSA; Thiophilic-interaction chromatography; Zinc

1. Introduction

Prostate-specific antigen (PSA) is a member of the tissue kallikreine family of serine proteases [1]. PSA is a 33-kDa glycoprotein with chymotrypsin like activity that is produced primarily by the human prostatic epithelium and secreted into the seminal fluid [2,3]. The only well-recognized physiological function of PSA is the digestion of the seminogelins and fibronectin present in seminal coagulum, liquefying the seminal clot shortly after ejaculation [4]. In addition, PSA is a widely utilized serum biomarker for the diagnosis and management of prostate cancer [5]. In the seminal plasma, the majority of PSA is present as free PSA (f-PSA), and is enzymatically active. Approximately, 10% of seminal plasma PSA is complexed with protein C inhibitor (PSA-PCI) [6,7]. In contrast, the majority of PSA in serum is bound to serine protease inhibitors, including α-1-antichymotrypsin (PSA-ACT), α-2-macroglobulin (PSA-A_2M), and α-1-antitrypsin (PSA-AT) [8]. f-PSA accounts for only 5–10% of total PSA in the serum [9]. The major portion of prostatic T-PSA is f-PSA, whereas, the complexed PSA forms are <2% of the total PSA [10]. The physiological relevance of T-PSA, and its value in prostate cancer progression or management, is unclear. However, available evidence suggests that PSA is down-regulated in prostate cancer tissue and, thereby, T-PSA concentrations are lower in cancerous than in non-cancerous parts of the prostate [11].

Abbreviations: PSA, Prostate-specific antigen; f-PSA, free-PSA; T-PSA, tissue-PSA; TIC, Thiophilic Interaction Chromatography; T-gel, Thiophilic-gel; ELISA, Enzyme-linked immunosorbant assay; SDS/PAGE, sodium dodecylsulfate/polyacrylamide gel electrophoresis; PSA-ACT, PSA complexed with alpha-1 antichymotrypsin; PSA-A_2M, PSA complexed with alpha-2 macroglobulin; PSA-PCI, PSA complexed with protein C inhibitor; PSA-AT, PSA complexed with anti-trypsin.

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"Thiophilic-interaction chromatography" (TIC) technique was introduced originally by Porath and his colleagues for the isolation of immunoglobulins [12]. TIC has been used successfully for characterization of human transferrin [13], Alzheimer's beta-amyloid peptides [14] and for purification of immunoglobulins from chicken sera [15]. This chromatographic step is based upon salt-promoted adsorption of proteins to the resin, where the binding of proteins to a sulfone and thio-ether-containing hetero-aliphatic ligand takes place mainly through accessible tryptophan and/or phenylalanine residues. The desorption of bound proteins is achieved when the salt concentration is reduced [12]. In our earlier studies, we have shown that PSA and PSA complexes have strong affinity for different thiophilic gels (T-gels), and that T-gel affinity can be successfully applied for the purification of PSA and PSA-complexes from biological fluids, including serum and seminal plasma [16,17]. We have shown that f-PSA purified from seminal plasma by T-gel chromatography is enzymatically active [18]. In the present study, for the first time, the use of TIC technique has been applied for the isolation and subsequent characterization of T-PSA from freshly harvested human prostate cancer tissue. TIC was selected for isolation of T-PSA because we earlier have shown that T-gel has strong affinity for all molecular forms [free and complexed forms] of PSA [16,18]. Taking this approach, we were able to show that T-PSA, isolated from human prostate tumor tissue contains only the "free" form of PSA and that this PSA is enzymatically active. The enzymatic activity of T-PSA isolated from prostate tumor tissue is lower than enzymatic activity of f-PSA isolated from seminal plasma.

It is known that, Zn^{2+} concentrations that is abundantly present in prostate gland, varies significantly in normal and malignant human prostate tissue [19,20]. Zinc is also known to have a significant influence upon enzymatic activity of serine proteases that includes PSA [21,22]. The f-PSA isolated from seminal plasma and T-PSA isolated from prostate tissue facilitated studying the effect of zinc, and other essential trace elements, on the enzymatic activity of PSA. For comparative purposes, chymotrypsin, another serine protease, was used as a control. The substrate used for determination of enzymatic activity of PSA is highly specific for this serine protease [23].

2. Experimental

2.1. Seminal plasma

Based upon a protocol approved by the Institutional Review Board at Roswell Park Cancer Institute, large pools of leftover seminal fluid were obtained from the Infertility and IVF Medical Associates of Western New York after they were finished with all of their needs. The seminal fluid was spun at 10,000 × *g* for 20 min to remove cellular debris and the supernatant dialyzed overnight against 15 mM sodium phosphate buffer containing 0.15 M sodium chloride, pH 7.0. After dialysis, the seminal plasma was spun again at 10,000 × *g* for 20 min and the supernatant containing the PSA was frozen in small aliquots at −70 °C until used. None of the samples were frozen and thawed more than once.

2.2. Tissue samples

De-identified human prostate cancer tissue samples were obtained from the Tissue Procurement Core Resource at Roswell Park Cancer Institute under approval of the Institutional Review Board. Small pieces of tissue were dissected immediately after removal of the prostate and immediately stored in liquid nitrogen until analysis.

2.3. Preparation of tissue extracts

Fifty milligrams of frozen prostate cancer tissue samples were cut into small pieces and homogenized in a Wheaton glass homogenizer in 500 μl of extraction buffer that contained 0.05 M Tris–HCl, pH 7.4, 0.001 M EDTA, 0.001 M dithiothreitol, and 0.01 M NaCl. The homogenized tissue samples were sonicated three times, with 5 s of sonication and 30 s cooling between each sonication. The sonicated tissue extract was centrifuged at 100,000 × *g* for 30 min. The supernatant was removed and stored at −80 °C until analysis. The pellet was suspended in 500 μl of 0.05 M phosphate buffer, pH 7.4, 2 M NaCl, 0.002 M EDTA until analyzed for DNA contents.

2.4. DNA determination

DNA concentrations in the pellets of the prostate cancer tissue homogenates were determined using bis-benzimide, commonly known as Hoechst 33258 dye (H33258, Calbiochem, San Diego), according to the procedure described by Labarca and Paigen [24]. Tissue pellets were resuspended in 0.05 M phosphate buffer that contained 2.0 M sodium chloride and 2.0 mM EDTA, pH 7.4. Calf thymus DNA was used as a standard. Briefly, 5.0 μl of the dye (10 μg/ml) was added to 490 μl of 0.05 M phosphate buffer, 2.0 M NaCl, pH 7.4 and 5.0 μl of appropriately diluted tissue pellet test solution. Fluorescence was measured with a PerkinElmer LS-45 Luminescence Spectrometer (excitation 356 nm and emission 458 nm). Calf thymus DNA at concentration range 0.1–1.0 μg was used to prepare a standard curve.

2.5. Chromatographic ligands and other chemicals

Fractogel TA 650 (s) (T-gel) was purchased from EM Separation Science (Gibbstown, NJ, USA). Ultrogel AcA-54 was purchased from BioSepra, SA, Division of Ciphergen Biosystems (Fremont, CA, USA). Precast gradient gels and molecular weight standards for SDS/PAGE gel electrophoresis were obtained from Bio-Rad Labs (Hercules, CA, USA). Chemiluminescence reagents (ECL) were acquired from NEN Life Science Products (Boston, MA). α-chymotrypsin and BTEE were purchased from Sigma Chemical Company (St. Louis, MO, USA). All other chemicals and reagents used were of analytical grade, or of the highest purity available.