Comparison of PSA Binding for Prostate Cancer and BPH Using Lectin-Glycoprotein Interactions

Prostat Kanseri için Lektin-Glikoprotein Etkileşimlerini Kullanarak PSA ve BPH Bağlanmalarının Karşılaştırılması

Research Article

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ABSTRACT

The increased amount of Prostate-specific antigen (PSA) binding capacity is estimated to be associated with the initial concentration of PSA in the serum or cancer-related glycosylation patterns. The aim of this study was to determine tPSA and fPSA binding capacities of mPGMA-HDMA-Con A beads using the serum samples of patients with PCa and BPH having the same concentration of tPSA. Concanavalin A (Con A) is preferred to use as ligand due to the high sugar specificity towards mannose. The initial concentration of the Con A in the medium was changed between 0.25 and 2.5 mg/mL in order to determine the optimum amount of Con A immobilized to the beads. Both tPSA and fPSA adsorption capacity of mPGMA-HDMA-ConA beads performed using the serum sample of patient with BPH were found to be higher than that of PCa. Therefore, the reason of high PSA adsorption capacity observed in patients with BPH could be attribute to the glycosylation changes.

Key Words

Prostate cancer, prostate specific antigen, Concanavalin A, magnetic beads.

ÖZET

Prostat-spesifik antijen (PSA) bağlanma kapasitesindeki artışın başlangıçta serumda bulunan PSA derişimi ya da kansere bağlı değişen glikozilasyon paternleri ile ilişkili olduğu öngörülmektedir. Bu çalışmada, aynı tPSA derişimine sahip PCa ve BPH'lı hastaların serum örnekleri kullanılarak mPGMA-HDMA-ConA mikroküreleere tPSA ve fPSA bağlanma kapasitesinin belirlenmesi amaçlanmıştır. Concanavalin A (Con A) mannoza karşı özgüllük göstermesi nedeniyle ligand olarak kullanılması tercih edilmiştir. mPGMA mikroküreleere immobilize olan optimum Con A miktarını belirlemek amacıyla ortamdaki Con A miktarı 0.25-2.5 mg/ml arasında değiştirilmiştir. Hem tPSA hem de fPSA için mPGMA-HDMA-ConA mikrokürelerin bağlanma kapasitesinin PCa ile kıyaslandığında BPH'da daha fazla olduğu gösterilmiştir. Böylece, yüksek PSA bağlanma kapasitesinin kansere bağlı değişen glikozilasyondan kaynaklandığı doğrulanmıştır.

Anahtar Kelimeler

Prostate kanseri, prostat spesifik antijen, konkanavalin A, manyetik mikroküreler.

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INTRODUCTION

Prostate cancer (PCa) is known as adenocarcinoma of the prostate gland that is a part of the male reproductive system. PCa is the fifth most common type of cancer in the world and the second leading cause of cancer-related death in men [1,2]. Benign prostatic hyperplasia (BPH) is characterized clinically by the enlargement of prostate which occurs as part of the aging process in men. In this manner, two main disorders of the prostate affecting elderly males are PCa and BPH. Both of these diseases lead to rapid cell proliferation and similar clinical presentation is also seen among patients with BPH and PCa [3]. Prostate-specific antigen (PSA), the most frequently used biomarker for PCa, is known to be prostate specific, but not PCa specific [4]. It may be difficult to effectively discriminate PCa from BPH due to the limitations in specificity and sensitivity for screening studies [5].

PSA is a glycoprotein which is primarily secreted by the prostatic epithelial cells. Alterations in glycosylation have been indicated to be associated with disease processes. Aberrant glycan processing is a fundamental characteristic of tumorigenesis [5]. Therefore, identification of disease-specific glycoproteins and glycosylation changes has now been recognized as an attribute of potential diagnostic and therapeutic biomarkers. There are many separation tools for glycoprotein enrichment which have been employed previously. One methodology is the lectin-based detection which may be useful in targeted glycoprotein analysis for cancer biomarker discovery [6]. Lectins have the ability to bind carbohydrate residues and have been widely used for the detection of many diseases including cancer due to the lectin-glycoprotein interactions [5,6]. Consequently, a number of novel attempts have been made to develop different support materials for lectin affinity [7,8]. Among these support materials, magnetic affinity carriers with immobilized affinity ligands provide an alternative support material that eliminates the requirement for prior particle removal [9].

In this study, the spacer arm attached Con A immobilized magnetic poly(glycidyl methacrylate) [m(PGMA-HDMA-Con A)] beads are prepared for the determination of different binding using serum samples of PCa and BPH having the same concentration of total PSA (tPSA). In addition,

MATERIALS AND METHODS

Materials

Con A, poly(vinyl pyrrolidone) (PVP, Mw: 40.000), glutaraldehyde solution (25% in water) are obtained from Sigma (St. Louis, MO, USA). Glycidyl methacrylate (GMA) and 2,2′-azobisisobutyronitrile (AIBN) are purchased from Aldrich (USA) and Acros Organics (USA), respectively. Other chemicals are obtained from Merck (Germany). All the water used in the experiments are ultrapure water which is obtained from Barnstead D3804 NANOpure® system. The blood samples of PCa and BPH patients are collected from Hacettepe University, Faculty of Medicine, Department of Urology.

Preparation of mPGMA beads

Several popular methods including co-precipitation are suggested to prepare iron oxide nanoparticles (Fe_3O_4). Therefore, superparamagnetic Fe_3O_4 nanoparticles, to be used in preparing mPGMA beads, have been synthesized with controlled co-precipitation method as described by Massart [10]. The black precipitated powder was collected by sedimentation with the help of an external magnetic field. Preparation of mPGMA beads using Fe_3O_4 nanoparticles was carried out according to the method described by Horák and Benedyk [11]. Firstly, PVP was dissolved in ethanol, and then the monomer phase was prepared by dissolving AIBN in GMA. The monomer phase and dispersion solution were mixed under stirring. Dispersion polymerization was performed in a 250 ml round-bottom flask under nitrogen atmosphere at 70 °C for 12 h (stirring rate: 550 rpm).

Con A Immobilization

Before ligand immobilization, the spacer-arm (1,6 diaminohexane-HDMA) attachment and glutaraldehyde activation were performed with the procedure described by Altintas et al. [9]. The surface of the beads was modified by using coupling agent such as glutaraldehyde. The modified mPGMA-HDMA beads were then removed from the solution, rinsed several times with water and phosphate buffer.

Con A was immobilized by covalent binding onto the activated mPGMA-HDMA beads. Unlike most other lectins, Con A is a metalloprotein and requires a transition metal ion, such as manganese and calcium ions for binding. For this purpose, 100 mM $CaCl_2$, 100 mM $MnCl_2$, and 2% (w/v) glucose were used in preparing the Con A solution. The immobilization reaction was carried out at 25°C for 18 h in a batch system. The initial concentration of the Con A in the medium was changed between 0.25 and 2.5 mg/mL in order to determine the optimum amount of Con A immobilized to the beads.

Binding studies

Before binding studies, serum samples were diluted in a ratio of 1/10 with phosphate buffer (pH 7.4). Then, high abundant proteins such as albumin and immunoglobulin G (IgG) were depleted using Cibacron blue F3GA immobilized poly(hydroxyethyl methacrylate) (PHEMA) cryogel discs [12] and HiTrap rProtein A FF column (Sigma, St. Louis, MO, USA), respectively. Ten mg of the mPGMA-HDMA-Con A beads were used for PSA binding in a batch system at 25°C (stirring rate of 100 rpm). Chemiluminescence immun assay technique was preferred to detect initial and final concentrations of tPSA and fPSA in serum. The binding capacity of the mPGMA-HDMA-Con A beads was calculated by using the Equation 1.

$$Q = [(C_i - C_f)\, V] / m \quad (1)$$

Where Q is the amount of PSA bound onto unit mass of the mPGMA-HDMA-Con A beads (ng/g), C_i and C_f are the initial and final PSA concentrations (ng/mL), respectively, V is the volume of aqueous phase (mL) and m is the amount of adsorbent used (g).

The increased amount of PSA binding capacity is thought to be associated with the initial concentration of PSA in the serum or cancer-related glycosylation patterns. For this purpose, serum samples of patients with PCa (PK1, tPSA: 12.59) and BPH (BPH3, tPSA: 12.39) having the same concentration of tPSA were used for the binding of tPSA and fPSA onto the mPGMA-HDMA-Con A beads. Therefore, the reason of high PSA adsorption capacity may be attribute to the alterations in glycosylation or the initial concentration of PSA.

RESULTS AND DISCUSSION

In clinical proteomics, serum is often used for early detection of diagnostic biomarkers. However, serum contains some high abundant proteins which mask other proteins present at low concentrations. The challenge in identifying serum biomarkers is to deplete additional abundant proteins uncovering and enriching at the same time the low-abundance ones [13]. For this purpose, a cibacron blue-dye-based method and a Protein A column system were used for albumin and IgG removal, respectively. There are many studies in the literature dealing with albumin removal in which CBF3GA immobilized support materials are used [14-16]. CBF3GA attached poly(hydroxyethyl methacrylate) cryogel was used for albumin depletion from human serum [12]. The cryogels have some advantages such as large pores, short diffusion path, low pressure drop and very short residence time during both adsorption and elution. The PHEMA could be preferable due to its excellent blood-compatibility, inertness, mechanical strength, chemical and biological stability [17,18]. Another method used for the removal of highly abundant proteins is using Protein A/G columns for the removal of the immunoglobulins. Protein A/G both have a strong affinity for the Fc portion of IgG. There are many studies showing that the removal of IgG is accomplished with Protein A column [19].

The binding capacity is one of the most significant properties of the affinity carrier. The amount of binding varies because of the property of each carrier such as structure, functional groups, ligand loading and surface area. Different support materials including agarose, silica, polyhydroxylated polymers can be used for the immobilization of lectins. The main disadvantage of commercially available immobilized lectins on agarose-based media is challenging experimental process because of flow rate and back pressure restrictions which lead to increase analysis time and lose of sample [6].

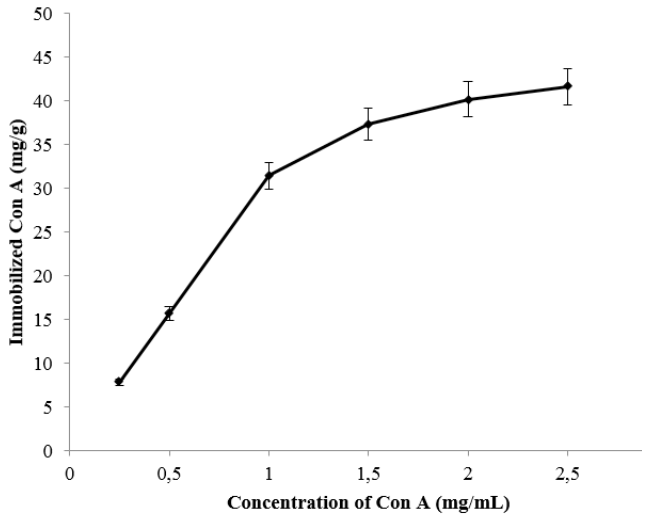

Figure 1. Effect of Con A concentration on the Con A immobilization.

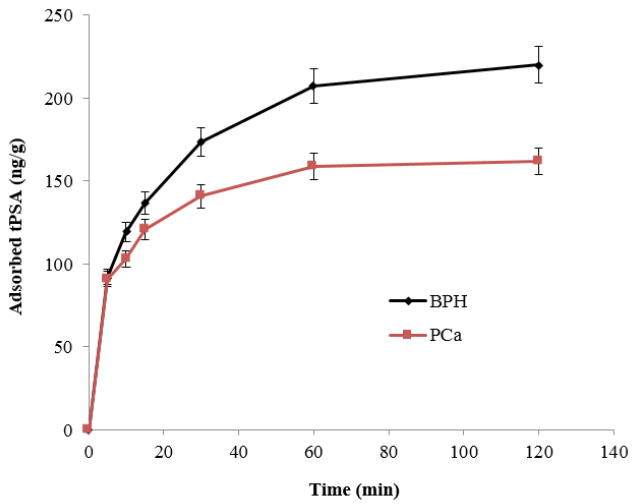

Figure 2. Effect of time on the binding of tPSA for BPH and PCa serum samples.

In recent years, the researches on serum or plasma glycoproteome in which lectin immobilized polymers used as chromatographic tools have been come into prominence for glycoprotein adsorption [2,3,6]. Affinity binding using lectins as ligands is based on the interaction between specific sugar sequences and its specific immobilized ligand [6].

In this study, Con A, the most used and well-known lectin, is preferred to use as ligand due to the high sugar specificity towards mannose [5]. Figure 1 indicates the effect of Con A concentration on the Con A immobilization.

As can be seen from Figure 1, the amount of Con A bound onto the mPGMA-HDMA beads rised

Table 1. The first- and second-order kinetic constants for PCa.

First order kinetics		Second order kinetics	
tPSA	fPSA	tPSA	fPSA
k_1 = 0.016 1/min	k_1 = 0.0184 1/min	k_2 = 0.0011 g/ng.min	k_2 = 0.0021 g/ng.min
q_{eq} = 158 ng/g	q_{eq} = 56,23 ng/g	q_{eq} = 169 ng/g	q_{eq} = 64,50 ng/g
R_2 = 0.76	R^2 = 0.75	R^2 = 0.99	R^2 = 0.99

Table 2. The first- and second-order kinetic constants for BPH.

First order kinetics		Second order kinetics	
tPSA	fPSA	tPSA	fPSA
k_1 = 0.45 1/min	k_1 = 0.45 1/min	k_2 = 0.00039 g/mg.min	k_2 = 0.0023 g/mg.min
q_{eq} = 173 ng/g	q_{eq} = 47.86 ng/g	q_{eq} = 250 ng/g	q_{eq} = 85.47 ng/g
R^2 = 0.98	R^2 = 0.88	R^2 = 0.99	R^2 = 0.99

Table 3. Overview of lectin-based researches associated with PCa and BPH.

Lectins	Outcome	References
Canavalia ensiformis (Con A)	Canavalia ensiformis (Con A) Con A-serum PSA interaction in PCa was significantly lower than that of BPH using carbohydrate precipitation method.	[20]
Ulex europaeus (UEA-1)	Serum fPSA in patients with PCa showed increased fucosylation compared with that of BPH.	[21]
Maackia amurensis lectin (MAL)	α2,3-linked sialic acid was increased in serum PSA in prostate cancer patients in comparison with BPH and controls	[22]
Aleuria aurantia agglutinin (AAA)	No remarkable difference was observed in bound fraction of serum PSA between BPH and PCa patients	[23]
Trichosanthes japonica agglutinin-II (TJA-II)	TJA-II-bound serum PSA content and TJA-II binding ratio (bound PSA/unbound PSA) could be discriminative between PCa and BPH.	[23]

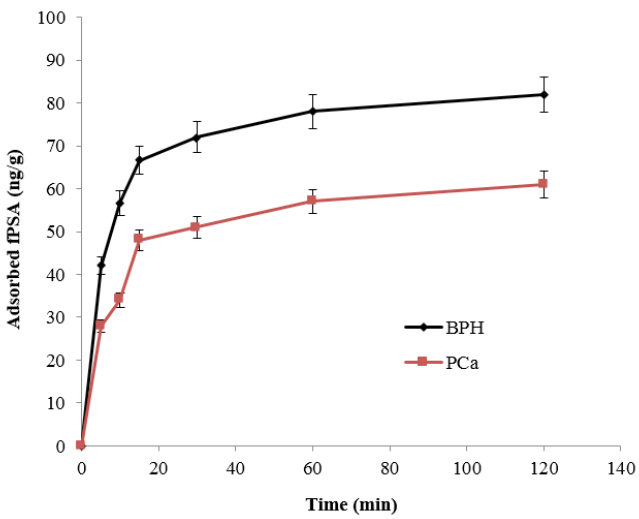

Figure 3. Effect of time on the binding of fPSA for BPH and PCa serum samples.

up by increasing the concentration of Con A, and then almost reached a plateau. In addition, it can be seen that the attachment of spacer-arm resulted in high Con A immobilization and glutaraldehyde activation corresponds to a larger number of activated binding sites on the surface of the support material. Therefore, as expected, higher amounts of Con A were coupled on the activated beads with higher number of activated sites. The number of functional groups on the support material and the size of the ligand molecules affect saturation capacity. Nevertheless, the important point in the performance of ligands immobilized on the surface of support materials is the ligand motility after immobilization rather than the total amount of ligands suitable for binding. The effective usage of active regions on ligand would be reduced by tightly immobilization of ligand to the surface with multiple interactions. When these parameters were taken into consideration, it can be assumed that 1.5 mg/ml Con A degree is optimal, and may be useful in further experiments.

The adsorbents having high surface area are known to have a high binding capacity with rapid adsorption kinetics. The preparation of support materials with high adsorption capacity is required to facilitate the adsorption separation [9]. However, geometric limitations (i.e. steric hindrance) prevents the binding of more PSA to the immobilized Con A molecule. For this purpose, the HDMA was attached onto the surface of mPGMA beads to prevent the undesirable side interaction between large Con A molecules and support material. The immobilization of Con A via glutaraldehyde coupling onto spacer-arm attached beads resulted in a higher PSA adsorption. tPSA and fPSA adsorption capacity of mPGMA-HDMA-Con A beads firstly increased rapidly, and then reached almost a plateau value after 60 min. The first- and second-order kinetic constants for PCa and BPH were shown in Tables 1 and 2, respectively. Both tPSA and fPSA adsorption capacities of mPGMA-HDMA-ConA beads performed using the serum sample of patient with BPH were found to be higher than that of PCa (Figures 2 and 3).

Lectins have found considerable applications in literature associated with several diseases including cancer. Table 3 explains some of the researches that have performed with different lectins to distinguish PCa and BPH.

The need for the improvement of blood-based test that has the ability to differentiate PCa and BPH grows louder and louder. Alterations in glycosylation patterns of proteins are found in approximately half of all serum proteins. Besides being one of the most common posttranslational modifications of proteins, glycosylation has been shown to be associated with several malignancies including PCa [21]. Aberrant protein glycosylation reflects the alterations of glycan structures which may affect many biological activities such as protein-protein interactions, protein folding and trafficking, immune recognition, cell signalling and migration. The glycan structures of glycoproteins, presenting binding specificity to lectin ligands, have been recognized as an attribute of discovering potential cancer biomarkers.

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