

SHORT COMMUNICATION

Investigation of prostate specific antigen glycosylation

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In the present work, prostate specific antigen (PSA) glycosylation was examined by lectin affinity methods. The results obtained indicated that PSA purified from human seminal plasma contained at least one biantennary N-linked oligosaccharide chain, which was composed of the monosaccharides manose, galactose, *N*-acetylgalactosamine, fucose and sialic acid. No O-linked carbohydrate chains were detected.

Keywords: prostate specific antigen, PSA glycosylation, lectins.

Prostate specific antigen (PSA) is a kallikrein-like serine protease secreted by the prostatic glandular epithelium into the seminal plasma. This enzyme has an important physiological effect on the liquefaction of sperm coagulum. Consequently, PSA concentrations in seminal fluid are relatively high (0.5–5.0 mg/ml). In the serum of healthy men, the PSA concentration is very low, usually below 4 ng/ml, while elevated levels of serum PSA have been reported in patients with prostate pathology.

Human PSA is a single chain glycoprotein, composed of 237 amino acids and about 8 % w/w of carbohydrates. The molecular mass of this glycoprotein is about 33–34 kDa, as determined by reducing SDS-gel electrophoresis.¹ According to recent studies, PSA contains only one N-linked oligosaccharide chain attached to asparagine-45,² but the presence of three O-glycoside chains is also suspected.³ Variations in the sialic acid and/or fucose contents are considered to be the main reason for PSA microheterogeneity and for the differences of the isoelectric point values (6.8 to 7.5).² The present work represents an attempt to characterize the carbohydrate chains of prostate specific antigen by lectin affinity methods. PSA was isolated from pooled seminal plasma, obtained from volunteer donors without prostate pathology, as previously described.⁴ The molecular mass and purity of the isolated protein were determined by SDS-PAGE, using a 5 % stacking gel and a 12.5 % separating gel, as described by Laemmli.⁵ The gels were stained with Coomassie Brilliant Blue and calibrated with low molecular weight markers (Sigma).

The study of PSA glycosylation was performed with a panel of plant lectins characterised by different carbohydrate specificities. The lectins used in this work were: concanavalin A (ConA) from *Canavalia ensiformis*, pea lectin (PSL) from

Pisum sativum, phytohemagglutinin (PHA) from *Phaseolus vulgaris*, peanut lectin (PNA) from *Arachis hypoagea*, wheat germ lectin (WGA) from *Triticum vulgaris*, *Sambucus nigra* agglutinin (SNA I), *Ricinus communis* agglutinin (RCA I) and a lectin from *Artocarpus altilis* (AAA). All lectins were isolated in house. The methods used in this work were the competitive enzyme-linked lectin binding assay (ELBA) and the Western blot analysis with lectins. In the Western blot analysis, after SDS-PAGE of the PSA preparation and electroblotting onto nitrocellulose filters, the filters were incubated with lectin-HRPO conjugates for two hours at room temperature and then washed three times with TBS-Tween. Afterwards, the filters were stained with diaminobenzidine. In the ELBA, samples of isolated PSA were incubated with lectin-HRPO conjugates for two hours at room temperature in the dark. Incubation was performed in microtiter plates previously coated with glycoproteins (ovalbumin for ConA, PSL and PHA, IgA for AAA, fetuin for WGA and SNA I and asialofetuin for PNA and RCA I). Samples of PSA were added in serial dilutions starting from 2 mg/ml. After incubation, the wells were washed 3 times with PBS-Tween. Peroxidase substrate (urea-peroxide) and chromogen (3,3',5,5'-tetramethylbenzidine) were added, and the resulting color was measured at 450 nm. The applied glycoproteins were purchased from Sigma, and the other employed chemicals were of analytical grade.

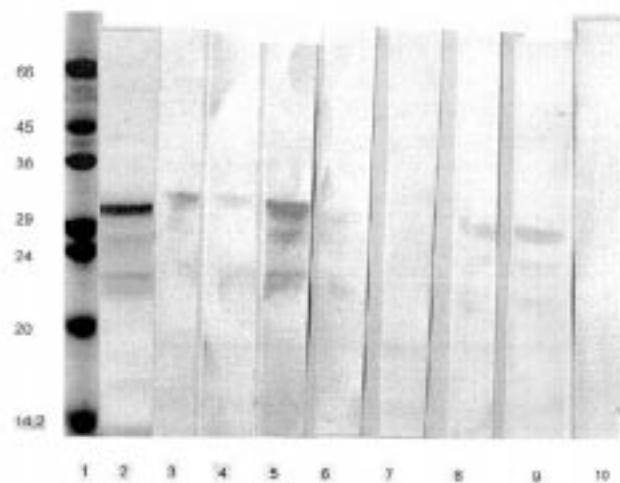


Fig. 1. 1-2: SDS-PAGE: 1: Molecular weight markers; 2: PSA preparation. 3-10: Lectin blot analysis: 3: RCA I, 4: WGA; 5: PHA; 6: PSL; 7: AAA; 8: ConA; 9: SNA I; 10: PNA.

The molecular mass of the isolated protein, determined by SDS-PAGE, was 33 kDa (Fig. 1). The concentration of total proteins was determined by the Lowry method,⁶ and the concentration of PSA was measured by IRMA-PSA INEP assay. Based on these results, a 95 % purity of the PSA preparation was estimated. SDS-PAGE also revealed the presence of three weak protein bands, corresponding to molecular masses of 22, 23 and 28 kDa, not originating from PSA (as demon-

strated by immunochemical methods, our unpublished data). Western blot analysis with lectin-HRPO conjugates was applied to show whether PSA, and/or the contaminants reacted with lectins. The obtained results indicated strong interaction of PSA with the lectins ConA, PHA, WGA, SNA I, RCA I and PSL. Some of the protein bands corresponding to the contaminating materials were also weakly stained. These lectins are characterized by a binding affinity for N-type oligosaccharide chains. The PNA and AAA lectins, which are predominantly specific for O-linked carbohydrate chains, did not react with the PSA preparation in this system. These results suggested that the contaminants present in the PSA preparation, comprising less than 5 % of the total proteins, did not influence significantly the examination of PSA interactions with lectins. Therefore, the isolated PSA was used for further examination by more sensitive lectin methods.

In the other part of this work, ELBA was used for quantification of PSA interaction with plant lectins. The affinity of the lectins for PSA binding was expressed as the concentration of PSA resulting in a 50 % inhibition of lectin binding to an immobilized glycoprotein. The obtained results (Table I) indicated that PSA was bound by the applied lectins in the following order of decreasing affinity: ConA>SNA I>PHA>RCA I>WGA>AAA>PSL>PNA.

TABLE I. Concentration of PSA resulting in 50 % inhibition of lectin binding to immobilized glycoproteins

Glycoprotein - lectin system	PSA/(mg/ml)
Ovalbumin - ConA	65
Fetuin - SNA I	70
Ovalbumin - PHA	120
Asialofetuin - RCA I	125
Fetuin - WGA	150
sIgA - AAA	235
Ovalbumin - PSL	490
Asialofetuin - PNA	>2000

Concanavalin A demonstrated the strongest affinity for the PSA isolated from the pooled seminal plasma. This is in accordance with the presence of biantennary complex oligosaccharide chains with a trimannosile core. SNA I, a lectin specific for terminal sialic acid, showed almost the same affinity for PSA. Both PHA and RCA I bound PSA to some extent, indicating that part of the PSA oligosaccharide chains are terminated by galactose. These results suggest that the PSA carbohydrate chains contained terminal NeuAca2-6Gal structure, partially desialinised.

Lectin WGA possesses a strong binding affinity for GalNAcb1-4GalNAcb1-4GalNAc and Manb1-4GalNAcb1-4GalNAc, so its interaction with PSA indicated the presence of some of these structures. On the other hand, the binding of *Pisum sativum* lectin suggested the presence of Fuca1-6GlcNAc-Asn residues in the core

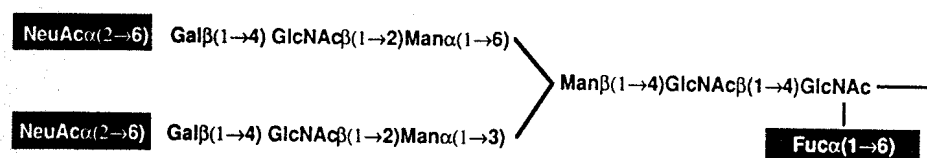


Fig. 2. Carbohydrate structure of PSA, Belanger *et al.* 1995.²

of the PSA carbohydrate chain. This binding is relatively weak, due to the absence of fucose in a number of PSA molecules. The presence or absence of fucose could be the second possibility for the microheterogeneity of PSA.

These results confirmed that the PSA contained at least one N-linked carbohydrate chain. Peanut lectin, which binds terminal galactose (particularly Gal β 1-3GlcNAc and less Gal β 1-4Glc structure) in O-type oligosaccharide chains, did not react with PSA, neither in ELBA nor in the lectin blot analysis. The possibility that PNA binding was disabled by the presence of terminal sialic acid was tested by using PSA desialinised by neuraminidase treatment. In this assay the binding of PNA was not changed. This result suggests that the examined PSA population did not contain O-type oligosaccharides. The interaction of lectin AAA with PSA in the lectin blot analysis was negligible and it was very low in the ELBA. As AAA mostly binds terminal galactose in O-linked oligosaccharide chains, this provides additional evidence that O-type oligosaccharides were not attached to the examined PSA species. The weak AAA-PSA binding observed in the sensitive ELBA system may originate from the weak affinity of AAA for terminal galactose in N-type glycans.

The present results indicate that the glycoside structure of PSA could be an N-type biantennary complex, which contains the monosaccharides manose, galactose, *N*-acetylgalactosamine, fucose and sialic acid. There was no evidence for the presence of O-linked oligosaccharide chains. The proposed carbohydrate structure is in agreement with the data obtained by Belanger *et al.*² (Fig. 2). The applied lectin based methods, which are simple compared to chemical analysis of glycoproteins, could be useful for the examination of individual samples of PSA, or even for the discrimination of different prostatic diseases.

ИЗВОД

ИЗУЧАВАЊЕ ГЛИКОЗИЛАЦИЈЕ ПРОСТАТА СПЕЦИФИЧНОГ АНТИГЕНА

ВЕСНА МАРИНОВИЋ и МАРГИТА ЧУПЕРЛОВИЋ

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У овом раду испитана је гликозилација простата специфичног антигена (PSA) лектинским афинитетним методама. Добијени резултати указали су да PSA пречишћен из хумане семене плазме садржи најмање један N-гликозидно везани биантенарни олигосахаридни ланац, кога чине моносахариди маноза, галактоза, N-ацетилгалактозамин, фукоза и сијалинска киселина. Присуство О-гликозидно везаних угљенохидратних ланаца није утврђено.

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