
Characterization and measurement of prostate-specific antigen using monoclonal antibodies

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Abstract

Monoclonal and polyclonal antibodies were produced using a pure preparation of prostate-specific antigen (PSA) as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). By SDS-PAGE, the apparent molecular weight of PSA was about 33 kD. Eighty-four monoclonal antibodies were produced, 77 of which bind PSA with an affinity higher than 10^9 M^{-1} . Use of these monoclonal antibodies to study the immunological characteristics of PSA revealed the presence of 4 epitopes. Injection of PSA into goats resulted in a production of polyclonal antibodies with high affinity. These polyclonal antibodies were purified by affinity chromatography and adsorbed on plastic tubes. By an immunometric assay, we have also demonstrated that polyclonal antibodies bind PSA at a fifth epitope that is different from those of monoclonal antibodies. Using an iodinated monoclonal antibody and polyclonal antibodies adsorbed on plastic tubes, a sensitive immunoradiometric assay could be developed, and a further increase in sensitivity could be achieved by using a mixture of 2 monoclonal antibodies. The serum PSA levels in 2,250 patients measured with this immunoradiometric assay were identical to the values determined by Tandem-R, although the present assay reached a minimum detectable value of 0.05 ng/ml compared with 0.2 ng/ml by Tandem-R.

Résumé

Des anticorps monoclonaux et polyclonaux ont été produits utilisant une préparation pure d'antigène prostatique spécifique (APS) tel que déterminé par électrophorèse sur gel de sodium dodecyl sulfate polyacrylamide (SDS-PAGE). Ainsi, le poids moléculaire de l'APS par SDS-PAGE est d'environ 33 kD. Quarante-huit (84) anticorps monoclonaux ont été produits, dont 77 lient l'APS avec une affinité plus haute que 10^9 M^{-1} . L'utilisation de ces anticorps monoclonaux pour étudier les caractéristiques immunologiques de l'APS ont démontré la présence de 4 épitopes. L'injection d'APS chez la chèvre a permis d'obtenir des anticorps polyclonaux à haute affinité. Ces anticorps polyclonaux ont été purifiés par chromatographie d'affinité et adsorbés sur des tubes de plastique. Grâce à un essai immunométrique, nous avons démontré que les anticorps polyclonaux lient l'APS sur un cinquième épitope différent de ceux mis en évidence par les anticorps monoclonaux. En utilisant un anticorps monoclonal iodé et des anticorps polyclonaux adsorbés sur les tubes, un essai immunoradiométrique très sensible a pu être développé et une augmentation additionnelle de sensibilité a pu être réalisée en utilisant un mélange de 2 anticorps monoclonaux. Les niveaux sériques d'APS mesurés chez 2,250 patients grâce à cet essai immunoradiométrique ont démontré des valeurs identiques à celles

déterminées par le test Tandem-R, bien que l'essai que nous avons développé peut atteindre une valeur minimale de détection de 0.05 ng/ml comparativement à 0.2 ng/ml pour l'essai Tandem-R.

Introduction

The name prostate-specific antigen (PSA) was given to a glycoprotein isolated from the prostate by Wang et al. in 1979 [1]. Further analysis of this molecule revealed that it is identical to both the protein known as γ -seminoprotein isolated from plasma seminal fluid by Hara et al. in 1974 [2] and the protein p30, also isolated from plasma seminal fluid by Sensabaugh in 1978 [3-5].

The molecular weight of PSA, as estimated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), is approximately 33-34 kD [1, 3]. Characterization of PSA by Lundwall and Lilja [6], Schaller et al. [7], and Riegman et al. [8] indicated that it is composed of 237 amino acids, with a molecular weight of 26,079 for the peptide moiety of the molecule. The PSA molecule contains 7-12% carbohydrates [7, 9, 10], with the presence of only 1 N-linked chain at asparagine 45 of the molecule; several isoenzymes were detected by isoelectric focusing [7, 9, 10]. Halbeek et al. [11] further confirmed this observation by carbohydrate analysis and suggested that the isoenzymes may differ in their structure by their carbohydrate composition.

The demonstration that circulating PSA levels are elevated in patients with prostate cancer has led to considerable interest in using PSA as a marker of prostate cancer [12-15]. Recognizing this important clinical application, several commercial tests for serum PSA are now available. These commercial tests use polyclonal and/or monoclonal antibodies in immunoassays or immunometric assays. Despite the wide use of PSA antibodies, however, few studies have been published on the immunological characteristics of PSA, and no description of the characteristics of the purified PSA used as standard is available [16, 17]. The present study reports the production of polyclonal and monoclonal antibodies

against PSA and demonstrates the presence of 5 major epitopes in PSA.

Materials and methods

PSA

The PSA was obtained from Immunocorp Inc. (Montreal, Canada). The glycoprotein was purified from plasma seminal fluid as previously described by Sensabaugh [3].

SDS-PAGE

The SDS-PAGE was carried out as described by Laemmli [18]. Briefly, 1 μ g of PSA in buffer was loaded on the polyacrylamide gel (12.5% separating gel and 4% stacking gel containing 0.1% SDS). After running for 1 h, the gel was stained with Coomassie brilliant blue R-250.

Production of polyclonal and monoclonal antibodies

Twenty adult goats were injected subcutaneously at about 10 sites with an emulsion of 100 μ g of PSA in saline with complete Freund's adjuvant. The animals also received PSA in incomplete Freund's adjuvant at 1-month intervals for 3 months. The presence of PSA antibody was tested by radioimmunoassay using iodinated PSA. Goat sera having the best titers were pooled, and PSA antibodies were purified by affinity chromatography with PSA immobilized on Sepharose 4B.

Monoclonal antibodies were produced as previously reported [19], and detection of monoclonal antibodies was performed as follows. After adding 10 ng of PSA to tubes coated with purified polyclonal antibodies, medium from hybridoma cells was pipetted into the tubes. After incubation for 2 h, the tubes were decanted. Iodinated rabbit antimouse immunoglobulin G (IgG) was added, and a second incubation was carried out for 2 h, after which the tubes were decanted and counted. Characterization of epitopes present on the PSA molecule was performed as follows: 84 purified monoclonal antibodies obtained from ascitic fluid were coated on plastic tubes; of these monoclonal antibodies, 30 iodinated monoclonal antibodies were used as markers in immunoradiometric assays to detect their binding sites on the PSA molecule. Competition with unlabelled monoclonal antibodies

SDS-PAGE OF PSA

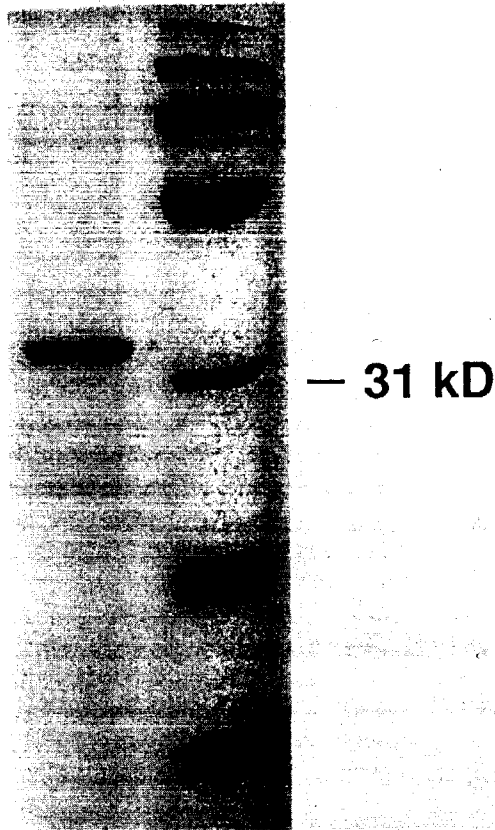


FIG. 1. SDS-polyacrylamide gel electrophoresis of PSA. Molecular weight markers were as follows: rabbit muscle phosphorylase b, 97.4 kD; bovine serum albumin, 66.2 kD; hen egg white ovalbumin, 45 kD; bovine carbonic anhydrase, 31 kD; soybean trypsin inhibitor, 21.5 kD; and hen egg white lysozyme, 14.4 kD.

was also done to further characterize the binding sites to PSA.

Finally, the immunoradiometric assay (IRMA) of PSA levels in serum was performed by adding a mixture of 250 μ l of phosphate buffer containing 25 μ l of unknown serum or standard to tubes coated with purified polyclonal antibodies. After incubation for 90 min at room temperature, the tubes were decanted and a mixture of 2 iodinated monoclonal antibodies in 250 μ l of phosphate buffer added for a second 90-min incubation. After

decanting, the tubes were washed twice with phosphate buffer and counted.

Results

As determined by SDS-PAGE, the molecular weight of the PSA antigen used in the present study was approximately 33 kD, and its purity was estimated to be greater than 95% (Fig. 1).

All 20 goats injected with PSA developed polyclonal antibodies, as detected by binding to labelled PSA. However, only 5 had serum concentrations of polyclonal antibodies high enough to bind iodinated PSA at 35% when used at a dilution higher than 50,000. The serum from these 5 goats was purified by affinity chromatography to obtain pure anti-PSA IgG (data not shown).

Thirty BALB/c mice were immunized and 19 cell fusions carried out. Fifteen of these fusions produced stable and positive clones secreting PSA antibodies. Eighty-four monoclonal antibodies were produced and 77 antibodies were found to bind PSA with an affinity higher than 10^9 M^{-1} . Table 1 presents the list of monoclonal antibodies classified by epitopes of the PSA molecule. Most of the monoclonal antibodies were produced against epitopes 1 and 4. In fact, 30 clones secreted antibodies against epitope 1, and 23 clones against epitope 4, whereas epitopes 2 and 3 were recognized by antibodies secreted by 11 and 20 clones, respectively.

Although good sensitivity for PSA detection can be obtained using a coated tube adsorbed with polyclonal antibodies and a single iodinated monoclonal antibody as tracer, our data indicate that a mixture of 2 iodinated monoclonal antibodies, namely P16 38 B3A2 and P19 733 G4 D11, increased the sensitivity of the PSA assay (Fig. 2). Finally, the PSA levels determined by IRMA and those by Hybritech Tandem-R (Hybritech) for 2,250 patients were the same (Fig. 3). However, when PSA levels were measured by IMx (Abbott), lower levels of PSA were found compared with those obtained using our system or that of Hybritech Tandem-R (data not shown). In addition, our IRMA had a minimum detectable value of 0.05 ng/ml.

Discussion

The purity of the antigen used in the present study

Table 1. Clones secreting monoclonal antibodies against different epitopes of PSA

Epitope 1	Epitope 2	Epitope 3	Epitope 4
P2 453 F2 E11	P2 265 G4 H3	P2 104 H1	P2 1108 E6 E4
P2 466 E10	P2 319 G1 F1	P2 387 D2 A11	P2 396 A6 G6
P2 485 E2 H1	P10 148 D9 F2	P2 563 A11 G4 D10	P2 396 G6 F2
P4 104 D6 G11	P16 110 D1 H4 H8	P3 62 A5 E5	P4 159 E3 B7
P4 104 G2 C2	P16 170 D3 C12	P10 128 C6 D8	P4 159 H12 A2
P4 68 E7 D3 G3	P16 449 G3 A6 B12	P10 149 B5 C3 B12	P10 1 A3 A9
P9 12 F10 F4 H4	P19 424 B1 B5	P10 256 C8 E1 B2	P10 1 F5 E12
P10 133 A4 D9 B11	P19 424 B6 F2	P19 384 D2 A3	P10 1 G6 F7
P10 487 H4 H2	P19 625 H10 B12	P19 601 A11 D9	P10 529 E4 F5
P19 384 D2 A3	P19 821 G5 E2	P22 437 F2 C11	P10 724 A6 E9
P19 601 A11 D9	P25 321 H12 E4	P22 916 H11 H4	P16 31 B9 B7
P19 733 G4 D11		P23 78 G1 C10	P16 33 G3 B6
P21 9 G5 E6		P25 18 B10 F2	P16 38 B3 A2
P21 269 B1 E8		P25 48 C2 H10	P16 230 A4 E12
P21 304 G12		P25 158 C10 A1	P21 37 G10 A1
P21 820 D4 F8		P25 297 A5	P21 293 G4
P22 437 A6 B9		P27 496 G9 F11	P21 314 F4 H2
P22 437 F2 C11 G9		P27 577 F5 H3	P21 496 B3 H8
P23 7 C2 A1		P31 408 B4 F2	P23 49 B5 C5
P23 106 E10 H7		P31 273 H2 A7	P24 32 F4 D4
P23 110 F2 C4			P25 831 F7 D12
P23 165 B8			P31 4 D2 F12
P25 277 A5 A12 F3			P31 408 B4 F2
P25 289 E8 C8			
P25 317 F7 C1			
P25 406 G3 F4			
P27 385 B1 H6 H8			
P27 432 C7 B12 F1			
P31 1 E11 A5			
P31 89 A2 A8			

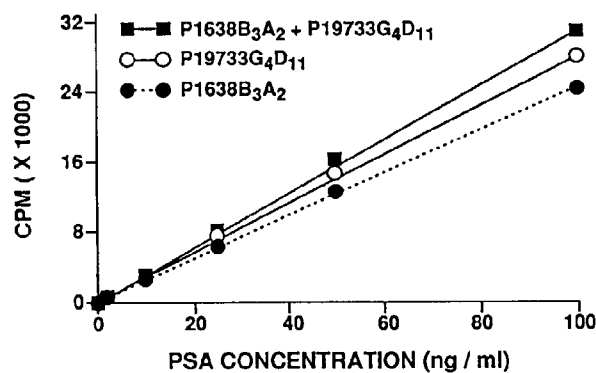


FIG. 2. Immunoradiometric assay of PSA with 2 high-affinity monoclonal antibodies used alone or in combination. Tubes were coated with purified goat PSA polyclonal antibodies.

for the production of PSA antibodies was tested by SDS-PAGE. This technique clearly demonstrates a homogeneous PSA preparation. Furthermore, the same standard was analyzed by ion spray mass spectrometer, and we observed a single chromatographic peak with a molecular weight of 28,430 Da (manuscript in preparation, Bélanger et al.).

At least in the mouse and goat, PSA is a highly antigenic molecule. Polyclonal goat antibodies with a high titer could be easily obtained by injection of PSA, as also observed with rabbits. Furthermore, stable clones that secrete monoclonal antibodies to PSA with high specificity and high affinity constant were also produced. Most interesting is the observation that the antibodies produced bind PSA at several distinct sites. In fact, using monoclonal anti-

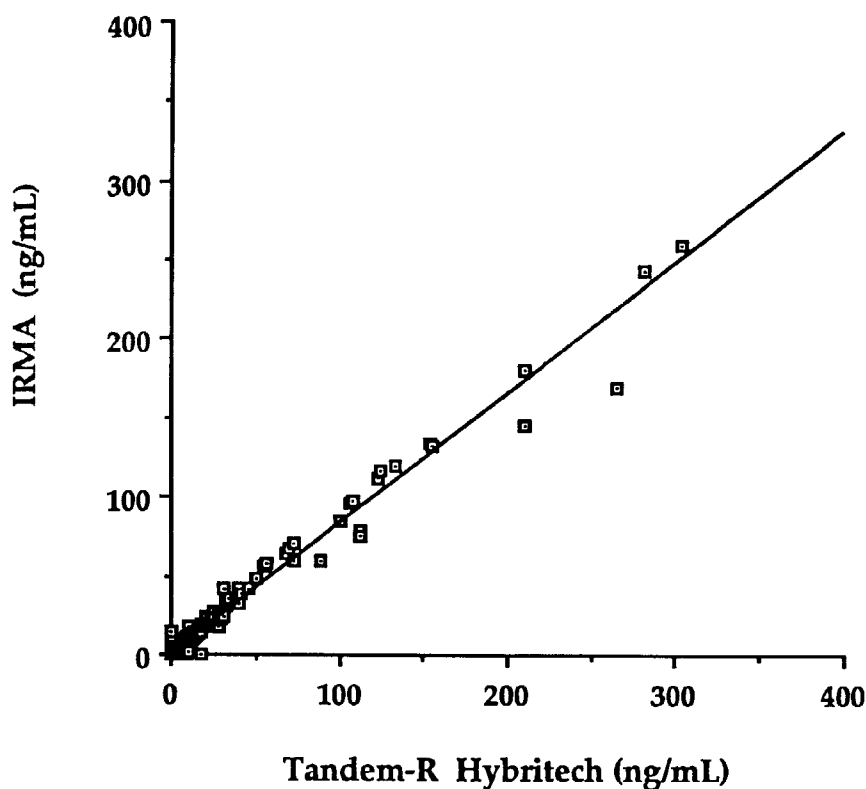


FIG. 3. Values of serum PSA measured in 2250 patients by an immunoradiometric assay (IRMA) using 2 monoclonal antibodies and purified polyclonal antibodies adsorbed on tubes and Hybritech Tandem-R assay.

bodies, we have demonstrated the presence of 4 major epitopes, 2 of which are responsible for the production of the largest number of monoclonal antibodies. Furthermore, polyclonal antibodies recognized a fifth epitope that is different from the 4 detected by monoclonal antibodies. Competition of several monoclonal antibodies with polyclonal antibodies by immunometric assay, however, suggested that the binding site of polyclonal antibodies is probably localized in the region of epitopes 2 and 3. The difference in the recognition of PSA by polyclonal and monoclonal antibodies is probably due to the species used for the production of antibodies. It is also important to note that a previous study using monoclonal antibodies reported the presence of 2 epitopes in PSA but, to our knowledge, no further immunological characterization was done [16]. In the present study, the production of polyclonal antibodies and a large number of

monoclonal antibodies has clearly established the presence of 5 epitopes in PSA.

Finally, a sensitive and specific IRMA was developed using 2 monoclonal antibodies and polyclonal antibodies coated on plastic tubes. The present data show that serum PSA levels determined by both IRMA and the Tandem-R assay were identical. However, PSA levels in the range of 0–3 ng/ml, as determined by these 2 methods, were lower when measured by IMx, probably because of the existence of PSA-binding proteins in the serum, which may interfere with the immunological detection of PSA by IMx [17, 20].

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