Quantitative immunoassay for complexes of prostate-specific antigen with $\alpha_2$-macroglobulin

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We have developed two ELISAs for quantifying complexes of prostate-specific antigen (PSA) with $\alpha_2$-macroglobulin ($\alpha_2$M), using partially purified PSA:$\alpha_2$M complex as the calibrator. One ELISA was designed to evaluate PSA:$\alpha_2$M complex in fluids containing a huge excess of PSA over the amount of complex (semen-derived fluids), the other for use in fluids containing an excess of $\alpha_2$M over PSA (blood plasma). The range of the assays was 2-1000 µg/L for PSA complexed to $\alpha_2$M; the detection limit was 3 µg/L. Intra- and interassay CVs were 7-13% and 11-17%, respectively, at processed PSA concentrations of 6-500 µg/L. Seminal fluid from healthy men (n = 60) contained 5.2 ± 2.6 mg/L PSA complexed with $\alpha_2$M. Prostatic and seminal vesicle fluids contained 6.5 ± 2.9 and 0.3 ± 0.2 mg/L PSA complexed to $\alpha_2$M, respectively. When purified PSA was incubated with citrated plasma, between 45% and 65% of the added PSA was recovered as free PSA, whereas ~25% formed complexes with $\alpha_2$M, 10% complexed with $\alpha_1$-antichymotrypsin, and only 0.1-6% was complexed with protein C inhibitor. Of 30 patients with prostate disease, 20 showed detectable plasma PSA:$\alpha_2$M complexes; however, the potential diagnostic significance of this complex requires further investigation.

INDEXING TERMS: semen • seminal fluid • $\alpha_1$-antichymotrypsin • protein C inhibitor • ELISA

Prostate-specific antigen (PSA), a 32-kDa single-chain glycoprotein secreted by the epithelial cells lining the acini and ducts of the prostate gland, is present in seminal fluid at high concentrations and is detectable in the serum of men who harbor prostatic tissue [1, 2].4 This glycoprotein is a serine protease very similar to the enzymes of the glandular kallikrein family [3-5]. However, unlike other proteins of this family, PSA has chymotrypsin-like activity with strict substrate specificity [4-7]. PSA seems to be involved directly in the liquefaction of the seminal coagulum formed at ejaculation [4, 6]. Quantification of PSA has received great interest because of the important role of this marker for diagnosing prostate carcinoma and monitoring the attendant therapy [8-12]. However, concentrations of serum PSA are also increased in some patients with benign prostatic hyperplasia [13, 14], whereas a large percentage of patients with clinically localized prostate cancer have serum PSA concentrations within the normal range [15]. These factors decrease the specificity and sensitivity of the PSA assays.

In prostate cancer patients, free, noncomplexed PSA reportedly is a minor fraction in serum [16], the PSA in blood binding mainly to $\alpha_1$-antichymotrypsin ($\alpha_1$ACT) [16-19]. Indirect evidence suggests that PSA might also bind to $\alpha_2$-macroglobulin ($\alpha_2$M). Both the free form of PSA and the $\alpha_1$ACT-bound form are detectable by current immunoassays, but PSA bound to $\alpha_2$M is probably engulfed in the $\alpha_2$M structure, rendering it undetectable at present. Therefore, the total PSA measured by the available immunoassays consists of the sum of free PSA and the PSA complexed to $\alpha_1$ACT (PSA:$\alpha_1$ACT), the latter representing the major portion. Although protein C inhibitor (PCI) forms complexes with PSA in seminal plasma [20-22] and in a purified system [22], no PSA:PCI complex was detected in the plasma from prostate cancer patients with very high PSA concentrations [21].

Several groups have shown that calculation of the free PSA/total PSA ratio or the complexed PSA/total PSA ratio provides a better discrimination between benign prostate hyperplasia and prostate cancer [16-18, 23, 24]. To date, investigators have taken for granted that PSA:$\alpha_2$M complex is not detectable by immunoassays [16, 17, 19, 25]. They assumed this because, when purified PSA is added to purified $\alpha_2$M, ~50-75% of the PSA immunoreactivity (the active PSA fraction) is lost, whereas PSA added to inactivated $\alpha_2$M does not show this loss of recovery. For PSA to be detected by a sandwich ELISA, at least two epitopes in the PSA molecule must be accessible to the capture and detection anti-PSA antibodies. However, exposure of only one PSA epitope in a PSA:$\alpha_2$M complex may be

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4 Nonstandard abbreviations: PSA, prostate-specific antigen; $\alpha_2$M, $\alpha_2$-macroglobulin; $\alpha_1$ACT, $\alpha_1$-antichymotrypsin; PCI, protein C inhibitor; p-assay, polyclonal assay of total PSA; m-assay, monoclonal assay of total PSA; and APC, activated protein C.
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sufficient for binding to an anti-PSA capture antibody, even if this is not enough to permit detection of PSA by a sandwich-type ELISA. If so, we could perhaps detect the complex by using an anti-α₂M detection antibody, or vice versa (use an anti-α₂M capture antibody and an anti-PSA detection antibody to detect a PSA:α₂M complex in which only one PSA epitope in a PSA:α₂M molecule was available for binding to the anti-PSA antibody).

Here we describe quantitative ELISAs specific for PSA:α₂M complex and show the presence of substantial amounts of PSA:α₂M complex in human semen and plasma.

**Materials and Methods**

**Samples**

Semen samples were obtained from 60 voluntary donors, ages 19–46 years (mean, 28 years), by masturabation after 4–7 days of sexual abstinence. Semen was incubated at 37 °C for 3 h and then centrifuged at 10 000g for 4 min at 20 °C. Prostatic fluid and seminal vesicle secretion were collected postmortem from men at autopsy within 18 h of death, centrifuged at 13 000g for 2 min, and stored at −80 °C, if not used immediately. Single blood samples were obtained from apparently healthy donors (5 men and 35 women). Plasma was pooled from 25 apparently healthy male donors. Thirty patients with prostate disease and 20 healthy women were also included in the study. Blood was anticoagulated with 0.13 mol/L trisodium citrate (9:1, by vol) and without delay centrifuged at 2500g for 20 min at 4 °C. All samples were stored at −80 °C until assayed. The stability of the PSA:α₂M complex after several cycles of freezing and thawing and after storage at various temperatures was studied with 10 seminal samples and 10 plasma samples (from 5 men and 5 women) to which purified PSA:α₂M had been added to yield a wide range of concentrations of the complex. The procedures followed were in accordance with the Helsinki Declaration of 1975.

**Materials**

**Reagents.** PSA was purified according to the method of Sambash and Blake [26]. Traces of detectable trypsin activity were eliminated by passing the PSA preparation through an aprotinin-Sepharose column [27]. The concentration of PSA was determined by amino acid analysis, and its absorptivity was then calculated to be 1.8 L × mg⁻¹ × cm⁻¹. PSA–Sepharose was prepared by coupling purified PSA to BrCN-activated Sepharose (Pharmacia, Uppsala, Sweden), 4.0 g/L of bed, according to the manufacturer's instructions. Rabbit anti-human PSA antibody was obtained by following standard procedures as reported earlier [28]. The serum was adsorbed with 1/8 volume of plasma from healthy women and was immunopurified with use of the PSA–Sepharose column. Human α₂M and α₁ACT and rabbit anti-human α₂M (IgG fraction) were obtained from Calbiochem (San Diego, CA). Rabbit anti-human α₁ACT (IgG fraction) was purchased from Dako A/S (Glostrup, Denmark).

**Buffers.** The coating buffer in the ELISA for PSA:α₂M complex was 100 mmol/L sodium carbonate, pH 9.6, containing 0.2 g/L sodium azide. The blocking buffer consisted of 100 mmol/L Tris-HCl, pH 7.4, 140 mmol/L NaCl, 20 g/L casein, 0.2 g/L Tween 20, and 0.4 g/L sodium azide. The conjugate buffer was 10 mmol/L sodium phosphate, pH 7.4, containing 140 mmol/L NaCl, 1 g/L bovine serum albumin, 0.5 g/L Tween 20, and 0.5 g/L thimerosal, and the substrate buffer was a combination of 200 mmol/L sodium citrate and 100 mmol/L sodium phosphate, pH 5.0, containing, per liter, 0.4 mL of 30% H₂O₂.

**Purified PSA:α₂M complex.** Partially purified PSA:α₂M complex was obtained by gradual addition (within 2 h) of a total of 100 μL of purified PSA (6.1 g/L; 610 μg of PSA) to 400 μL of α₂M (1 g/L) in 0.05 mmol/L Tris-HCl, pH 7.4, containing 0.05 mol/L NaCl and 0.2 g/L sodium azide (buffer A) at 37 °C. The reaction mixture was further incubated at 37 °C for 6 h, and then filtered at 4 °C on a 2.6 × 95 cm column packed with Sephacryl S-200 (Pharmacia) in buffer A, with collection of 1-mL fractions. PSA was measured with a polyclonal antibody-based ELISA (see below), and PSA:α₂M complex was detected with a qualitative ELISA in which the polyclonal anti-PSA antibody was the capture antibody and labeled anti-α₂M antibody was the detection antibody.

**Procedures**

**ELISAs for PSA:α₂M complex.** The ELISAs for PSA:α₂M complex were similar to those described for PSA:α₁ACT [24] and PSA:PCI [21] complexes. We developed two variants of the ELISA (A and B). Method A was designed to evaluate PSA:α₂M complex in fluids containing a huge excess of PSA over the amount of complex (semen-derived fluids), method B for fluids containing an excess of α₂M over PSA (blood plasma). In both methods, all room temperature incubations with test samples, conjugate antibody, and substrate were done in a vibratory shaker (IKA MTS-4; Ika-Works, Wilmington, NC) at 500/min.

In method A, specific rabbit anti-α₂M (IgG fraction) diluted to 50 mg/L in coating buffer was used to coat microtiter plates overnight at 4 °C. Thereafter, the wells were washed five times with 220 μL of blocking buffer and blocked with 300 μL of blocking buffer for at least 1 h at 37 °C. We then transferred into each well in duplicate 50 μL of samples diluted to convenient concentrations with blocking buffer and incubated the plates at room temperature for 2 h. After washing the plates three times with blocking buffer and three times with conjugate buffer, we incubated them for a further 1 h at room temperature with peroxidase-labeled immunopurified anti-PSA antibody (diluted in conjugate buffer), 50 μL/well. Thereafter, the plates were washed five times with conjugate buffer and incubated with 50 μL (per well) of a 400 mg/L solution of o-phenylenediamine in substrate buffer for 60 min at room temperature. The reaction was stopped by the addition to each well of 35 μL of 4 mol/L H₂SO₄. The absorbance of each well was read at 492 nm in an ELISA microplate autoreader (EAR 400; SLT Labinstruments, Grödig, Austria). A calibration curve was run in each microplate, constructed with several dilutions of partially purified PSA:α₂M complex (from 800 to 3.125 μg/L PSA in the complex), so as to correlate the increase in absorbance at 492 nm with the concentration of PSA complexed to α₂M. Purified PSA...
or \( \alpha_2M \) at concentrations of 1–1000 mg/L and normal plasma were used as negative controls.

In method B, the capture and detection antibodies were interchanged, and undiluted female plasma was added as an additional negative control.

The detection limit of the PSA: \( \alpha_2M \) assays was defined as the concentration of complex that gave an absorbance equal to that of the assay buffer + 2 SD (calculated from 15 replicates).

**In vitro formation of PSA complex in plasma.** To study the distribution of PSA between its plasma inhibitors in vitro, we incubated 50 \( \mu L \) of purified PSA (0, 0.1, 0.2, 1, 2, 10, 40, and 200 mg/L) in buffer (50 mmol/L Tris-HCl, 140 mmol/L NaCl, 0.2 g/L sodium azide, and 1 g/L bovine serum albumin), pH 7.4, with 50 \( \mu L \) of pooled normal human plasma or buffer at 37 °C for 4 h. Thereafter, we stored the samples at −80 °C in small aliquots until used. Thawed samples were analyzed for PCI activity, total PSA, and PSA complexed with \( \alpha_1ACT \), \( \alpha_2M \), and PCI.

**Other methods:** \( \alpha_1ACT \) and \( \alpha_2M \) were assayed by homemade, specific sandwich ELISAs, developed according to standard procedures [28]. The ELISA for total PSA (p-assay) utilized the polyclonal antibody indicated above as the capture and detection antibody and was calibrated with purified PSA [24]. Some samples were also measured with a commercial PSA assay (Tandem-E PSA; Hybritech, San Diego, CA) based on two monoclonal antibodies (m-assay). PSA: \( \alpha_1ACT \) complex was assayed by ELISA as described earlier [24]. PCI activity was assayed as previously reported [20, 29]. Briefly, purified activated protein C (APC) was added to a test plasma in the presence of heparin, and the amount of APC:PCI complex formed, as measured by a specific ELISA, was used to calculate the concentration of functional PCI in the plasma.

**Results**

Figure 1 shows the elution profile of the incubation mixture of PSA and \( \alpha_2M \). Fractions containing PSA: \( \alpha_2M \) complex (fractions 200–225, 26 mL) were concentrated in PM 30 membranes (Amicon, Lexington, MA) to 1 mL (pool 1); the \( \alpha_2M \) content was 360 mg/L. Fractions with PSA immunoreactivity (fractions 280–350, 70 mL) were also pooled (pool 2) to recover all free PSA from the incubation mixture. This pool contained 8.36 mg/L PSA (585 \( \mu g \) of PSA), which indicates that ~25 \( \mu g \) of PSA was complexed to \( \alpha_2M \). Hence, the concentration of PSA in complex with \( \alpha_2M \) in pool 1 was calculated to be 25 mg/L. However, the concentration of PSA in the pool as measured with the p-assay was 0.115 mg/L, which confirmed previous reports that PSA complexed to \( \alpha_2M \) loses almost all of its immunoreactivity\(\rightarrow 99\%\), in our hands. These results demonstrate the difficulty of obtaining well-characterized, purified PSA: \( \alpha_2M \) complexes by simply incubating a known amount of PSA with excess \( \alpha_2M \): All PSA preparations contain ~30% of inactive PSA that will not react with \( \alpha_2M \), and the molecular masses of free \( \alpha_2M \) and PSA: \( \alpha_2M \) complex do not differ enough for separation by gel filtration. Consequently, preparations of PSA: \( \alpha_2M \) complex are contaminated with variable (unknown) amounts of free \( \alpha_2M \), so the amount of \( \alpha_2M \) in the complex cannot be used for calculating the amount of PSA: \( \alpha_2M \) complex. By using an excess of PSA as indicated above, we can separate free PSA from complexed PSA, measure the amount of PSA that remains in the free form, and indirectly calculate the amount of PSA complexed to \( \alpha_2M \). To assess the validity of this approach, we treated an aliquot of the preparation of PSA: \( \alpha_2M \) obtained as described above (25 mg/L PSA complexed to \( \alpha_2M \)) with diithreitol and iodoacet acid to expose most of the PSA antigen determinants [30]. PSA in the reduced and S-carboxymethylated complex measured with the PSA p-assay showed a concentration of 19 mg/L (mean of three separate experiments)—close to the concentration of 25 mg/L calculated indirectly by gel filtration. Although we expect that not all of the PSA: \( \alpha_2M \) complex in the various preparations and test samples will have the same stoichiometry, this approach seems to be reasonable, and the amount of PSA: \( \alpha_2M \) so calculated is a good (if imprecise) approximation to the actual amount of PSA complexed to \( \alpha_2M \).

Another approach is to add a known amount of PSA to purified \( \alpha_2M \) and calculate the loss of PSA immunoreactivity in comparison with that for a control (the same amount of PSA added to buffer instead of to \( \alpha_2M \)). The reduction in PSA immunoreactivity may be taken as the amount of PSA complexed to \( \alpha_2M \). We performed five experiments with different proportions of PSA and \( \alpha_2M \) and found that the amount of PSA complexed to \( \alpha_2M \) calculated in this way matched the amount measured with the ELISA for PSA: \( \alpha_2M \) calibrated with the partially purified PSA: \( \alpha_2M \) preparation.

Figure 2 shows calibration curves for the two PSA: \( \alpha_2M \) complex assays. The calibration curves of purified PSA: \( \alpha_2M \) complex diluted in blocking buffer were linear within the range of 3–800 \( \mu g/L \) concentrations of PSA complexed to \( \alpha_2M \) under the assay conditions used. No differences were seen when the dilutions of the complex were made in control plasma (Fig. 2, right; method B). When we added purified PSA: \( \alpha_2M \) complex to
semenal fluid from a single individual that had been incubated for 3 h at 37 °C and that initially contained 402 μg/L PSA complexed to α₂M, the plot of absorbance against serial dilutions of the mixture was parallel to the calibration curve of purified PSA:α₂M complex diluted in blocking buffer (Fig. 2, left; method A), and ~110% of the complex added was recovered in the assay. In all cases, the background signal for buffer and negative controls was 0.0005 ΔA/min or less.

The detection limit of the assays was 3 μg/L PSA complexed to α₂M. This corresponds to a biological detection limit in plasma samples of 6 μg/L, because optimal results are obtained when test plasma is diluted at least twofold. The intra- and interassay CVs for PSA complexed to α₂M were 7–14% and 11–19%, respectively, at complexed PSA concentrations of 6–500 μg/L (n = 8).

The recovery in the complex assay (method A) after addition of 10–400 μg/L PSA complexed with α₂M to seminal plasma ranged from 97% to 117%, whereas the recovery in blood plasma (method B) ranged from 89% to 108%. This overestimation of the PSA:α₂M added to seminal fluid may represent the complex formed between the PSA in the seminal fluid and some molecules of α₂M in the purified complex that still have free reactive centers.

The stability of the PSA:α₂M complex was studied in seminal fluid and in blood plasma samples to which purified PSA:α₂M had been added to give 6–500 μg/L PSA complexed to α₂M. The recovery was 85–107% after 4 days at 4 °C, 65–93% after 4 days at room temperature, and 88–110% after freezing and thawing the samples four times.

The ELISA for PSA:α₂M complex (method A) was used to detect and quantify complexes in seminal fluid and in prostatic and seminal vesicle fluids. Table 1 summarizes the results obtained. Significant amounts of PSA:α₂M complex were detected in all the fluids analyzed, but seminal plasma and prostatic fluid had higher PSA:α₂M complex concentrations than did seminal vesicle fluid. The same was true of the PSA:α₁ACT complex. As reported earlier, the majority of the PCI was of seminal vesicle origin [20], but prostatic fluid also contained minor amounts, which is in accord with a report showing that prostatic tissue produces PCI [31]. Note that both seminal vesicle and prostatic fluid contain substantial amounts of α₂M and α₁ACT.

To study the distribution of PSA between its main plasma inhibitors α₁ACT, α₂M, and PCI, we incubated several amounts of purified PSA with plasma for 4 h at 37 °C and then measured the total PSA and PSA-inhibitor complexes. The results are summarized in Table 1. Between 18% and 30% of the total PSA added was recovered as PSA:α₂M complex, as assayed by method B, whereas 10–13% was complexed with α₁ACT and 0.1–6% with PCI. PCI activity decreased with increasing amounts of PSA added, and ~4 mg/L PSA reduced PCI activity by 50%.

Using method B, we also measured the concentrations of PSA:α₂M in plasma from 30 patients with prostate disease, whose total PSA concentrations ranged from 1.6 to 12 900 μg/L (median, 10.5 μg/L). Twenty of these patients had detectable concentrations of PSA complexed to α₂M ranging from 10 to

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**Table 1. Concentrations of several analytes found in seminal fluid and in prostatic and seminal vesicle fluids.**

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Seminal plasma (n = 60)</th>
<th>Prostatic fluid (n = 3)</th>
<th>Seminal vesicle fluid (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCI</td>
<td>163 ± 32</td>
<td>16 ± 6</td>
<td>209 ± 63</td>
</tr>
<tr>
<td>PSA</td>
<td>2100 ± 400</td>
<td>1840 ± 687</td>
<td>5.4 ± 2.4</td>
</tr>
<tr>
<td>α₁ACT</td>
<td>175 ± 62</td>
<td>306 ± 110</td>
<td>160 ± 55</td>
</tr>
<tr>
<td>α₂M</td>
<td>41 ± 10</td>
<td>53 ± 15</td>
<td>10 ± 3</td>
</tr>
<tr>
<td>PSA:PCI*</td>
<td>21.1 ± 6.4</td>
<td>3 ± 2</td>
<td>4 ± 3</td>
</tr>
<tr>
<td>PSA:α₂ACT*</td>
<td>1.6 ± 0.9</td>
<td>2.5 ± 1.9</td>
<td>0.1 ± 0.1</td>
</tr>
<tr>
<td>PSA:α₂M*</td>
<td>2.5 ± 2.6</td>
<td>6.5 ± 2.9</td>
<td>0.3 ± 0.2</td>
</tr>
</tbody>
</table>

* Complex concentration expressed as mg/L PSA complexed to the corresponding inhibitor.
1050 μg/L (median, 25 μg/L). However, there was no correlation between the concentrations of total PSA and those of PSA complexed to αM. For example, two patients with 12 900 and 5320 μg/L total PSA had <6 and 20 μg/L PSA complexed to αM, respectively, whereas two patients with 1.9 and 14 μg/L total PSA had 56 and 240 μg/L PSA complexed to αM, respectively. None of the 20 plasma samples from healthy women had detectable PSA:αM complexes.

Discussion

When purified PSA is added to blood plasma, most of the active PSA complexes to αM and α1ACT, and the immunoreactivity of the PSA complexed to αM is lost [27]. However, Christenson et al. [27] were able to trace the PSA bound to αM by means of gel filtration, using 125I-labeled PSA and immunoblotting, and found that 40% of the PSA was complexed to αM, 20% complexed to α1ACT, and 20% was free. Therefore, some have speculated that patients with prostate disease and detectable serum PSA have circulating PSA:αM complexes. However, no assay for quantifying PSA:αM complex has been reported, despite its potential clinical significance. Here, we present an ELISA specific for PSA:αM complexes that is able to detect as little as 3 μg/L PSA in complex with αM. Using this assay (method A), we have identified and quantified PSA:αM complexes in seminal plasma and in seminal vesicle and prostatic fluids. Our results show that seminal plasma contains substantial amounts of PSA:αM complex, although the major portion of PSA is complexed to PCI, probably because of the higher concentration of PCI in semen. Our results also show that both α1ACT and α2M are present in prostatic and seminal vesicle fluids.

Using a variant (method B) of the initial PSA:αM assay, we studied the in vitro formation of PSA:inhibitor complexes after the incubation of purified PSA with plasma. After incubation at 37 °C for 4 h, αM appeared to be the major PSA inhibitor, in agreement with a previous report [27]. Thus, at a final PSA concentration of 50 μg/L, ~30% of the PSA was complexed to αM, 11% to α1ACT, and 6% to PCI. At 5 mg/L PSA or higher, 20% was complexed to αM, 10% to α1ACT, and 0.15% to PCI. According to the molar concentration of each inhibitor in plasma, PCI seems to have a higher second-order rate constant (k2), and αM and α1ACT should have similar apparent constants for the inhibition of PSA. However, the results obtained in seminal plasma do not bear out this interpretation, there being three times more PSA:αM complexes than PSA:α1ACT complexes despite the higher molar concentration of α1ACT (2.5 μmol/L) than of αM (0.03 μmol/L). We have no explanation for this discrepancy, perhaps in the presence of an excess of PSA, such as occurs in semen, α1ACT is used as a substrate and is inactivated by PSA without subsequent formation of a complex.

Our results also indicate that PCI may act as a substrate for PSA, especially at a high PSA:PCI molar ratio. Thus, addition of 20 mg/L PSA to plasma resulted in >80% of the PCI activity being lost, with only 0.1% of plasma PCI complexed to PSA. Also, in seminal plasma incubated for 3 h at 37 °C, >99% of PCI activity was destroyed but only 20–40% of PCI was complexed to PSA [20].

Immunoblotting studies suggest that some patients may have large amounts of PSA complexed to αM. Because the fraction of PSA bound to αM is not detectable by current immunoassays for total PSA, the complexed PSA/total PSA ratio is calculated from values for total PSA concentrations that may not be the actual value of PSA in serum. In fact, <1% of the amount of PSA complexed to αM is detected in the ELISA for total PSA, so the actual amount of total PSA is underestimated. Thus, some patients actually have much lower ratios of complexed PSA (PSA:αM)/total PSA than the values previously reported. In contrast, if we use the total complexed PSA (PSA:α1ACT + PSA:α2M)/total PSA ratio, patients with high concentrations of PSA:α2M complex will show higher ratios.

As a preliminary study to confirm this possibility, we measured the amount of PSA complexed to αM in plasma from 30 patients with prostate disease (total PSA range, 1.6–12 900 μg/L by p-assay) and showed that 20 of them had detectable amounts of PSA:αM complex, whereas none of the 20 healthy women studied had detectable circulating complex. Earlier studies showed the usefulness of measuring the ratio of free PSA or the ratio of α1ACT–complexed PSA to total PSA to improve the clinical specificity of prostate cancer detection [16–18, 23, 24]. The presence of circulating PSA:αM complexes in some patients raises the possibility that determination of the ratio of PSA:αM to total PSA or free PSA or PSA:α1ACT, or of other ratios involving these components, may be useful in discriminating prostate cancer from benign prostatic hyperplasia. Unlike methods based on immunoblotting techniques, our ELISA for PSA:αM complex seems to be sensitive to ≥6 μg/L PSA complexed to αM in plasma. However, it is possible that this detection limit should be increased to permit the measurement of lower circulating complex concentrations for routine usage. Such investigations are underway.

In conclusion, the results presented here demonstrate the feasibility of an ELISA for quantifying PSA:αM complexes in
patients with prostatic disease. Whether the use of the PSA:α1M (or PSA:α1M + PSA:α1ACT)/total PSA ratio in serum will improve the early detection of prostate cancer requires further investigation.

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