SCRIPPS NEWS

VOLUME 11 NUMBER 1 SRPING 1997

A publication of SCRIPPS LABORATORIES, INC.

IN THIS ISSUE

The Prostate Specific Antigen - α_2-Macroglobulin Complex:

A SIGNIFICANT FORM OF TOTAL SERUM PSA

The complex formed in serum between PSA and α2-macroglobulin goes undetected by current immunoassays

Previously, we have reported on prostate specific antigen (PSA), the complexes it forms with the serum enzyme inhibitor α_1-antichymotrypsin (ACT), and how these PSA forms relate to the diagnosis of prostate cancer.[1,2] In short, it is believed that PSA in human serum primarily exists as either unbound, free PSA (fPSA) or as a complex with ACT (PSA-ACT). The total amount of PSA in serum (tPSA), generally defined as fPSA + PSA-ACT, is often elevated in prostate cancer and in benign prostatic hyperplasia (BPH); however, the relative amount of fPSA compared to tPSA (the fPSA:tPSA ratio) is often greater in BPH than in prostate cancer.[1,2,3] In fact, the last two years have seen several reports confirm that fPSA:tPSA determinations are useful in distinguishing BPH from prostate cancer.[3,4,5,6,7] Recent evidence indicates, however, that measuring fPSA and PSA-ACT may not accurately reflect the true tPSA levels in prostate cancer or BPH patients. It now appears that a significant proportion of serum PSA is being overlooked, that which is bound to another serum enzyme inhibitor, α_2-macroglobulin (A2M).

PSA-A2M represents a significant form of serum PSA

In 1990, Christensson et al.[8] added purified, ^{125}I-labeled PSA to human blood plasma, incubated for 30 minutes at 37°C, then subjected the mixture to gel filtration chromatography. Three resultant peaks contained most of the radioactivity and indicatedthat approximately 40% of the PSA was present as PSA-A2M, ~20% as PSA-ACT, and ~20% remained unbound as fPSA. In addition, because A2M acts by encapsulating its target protease, formation of the PSA-A2M complex was associated with a loss of PSA immunoreactivity. Since then, other studies have confirmed significant (as high as 60%) *in vitro* formation of PSA-A2M complexes, reporting a similar obstruction of immunologically-identifiable PSA epitopes.[9,10,11,12] In one *in vivo* study of prostate cancer patient sera, Western blot analysis revealed that 35-59% of the PSA was present as PSA-A2M.[13] These studies indicate that PSA-A2M represents a significant form of PSA in serum. Furthermore, the loss of PSA immunoreactivity is noteworthy in that it suggests that the presently available commercial immunoassays for PSA do not detect PSA-A2M.[11]

Not only does PSA-A2M appear to a be a significant form of serum PSA, but evidence indicates that A2M binds PSA more aggressively and more rapidly than does ACT. In a series of experiments designed to simulate *in vivo* conditions, Leinonen et al.[14] added PSA to separate A2M and ACT solutions, to an A2M/ACT mixture, and to female normal human serum. When added to separate A2M and ACT solutions, PSA reacted much more rapidly with A2M. Immediately after the addition of PSA, 35% of the PSA had reacted with A2M in the A2M solution, while only 11% had

reacted with ACT in the ACT solution. After 2 hours of incubation at 37°C, 50% of the PSA was bound in the A2M solution, and 30% in the ACT solution. Maximal binding of PSA occurred at 48 hours in both solutions: 85% of the PSA had reacted in the A2M solution, 75% in the ACT solution. When added to an A2M/ACT mixture, 66% of the PSA was present as PSA-A2M, 17% as PSA-ACT, and 17% as fPSA. These figures were approximately the same when PSA was added to female normal human serum. In a related study, Chen et al.,[12] added purified PSA to female human serum and studied the resultant serum forms of PSA using a commercially-available immunoassay and Western blot. After incubation, 60% of the PSA immunoreactivity was lost using the immunoassay. Western blot revealed that most of this loss was due to the formation of PSA-A2M. The 60 and 66% PSA-A2M formation reported here is considerably greater than the 40% reported by Christensson et al. above, but differences in the quantitative methods used may account for these discrepant results.

Active vs. inactive PSA

Of note in Leinonen's study are the reactivities of the various PSA isoenzymes with A2M and ACT (Table 1). The authors identified and evaluated seven PSA isoenzymes. Two isoenzymes were enzymatically active (PSA-A and PSA-B) and readily, but not completely, reacted with both ACT and A2M. The authors suggested that some of this active PSA did not bind to A2M or ACT either due to residual inactive PSA present in the active PSA fractions, or as the result of a partially-reversible, equilibrium reaction:

with the reaction favoring the right side of the equation. The other five isoenzymes (PSA-C, PSA-D', PSA-D, PSA-E, and PSA-F) contained internal cleavages that left them with very little or no enzymatic activity. These five forms reacted poorly or not at all with ACT, but reacted significantly with A2M. The reason for this is unknown, but the authors proposed that either some residual active PSA was present in the inactive PSA fractions, or that some of the enzymatically less-active PSA may be nicked in such a way that allows it to react with A2M, but not with ACT. Other, similar reports have noted that approximately 30% of PSA is nicked and has reduced enzymatic activity.[8,15] This suggests that a substantial amount of PSA present *in vivo* may bind only to A2M and, thus, supports the theory that a significant proportion of serum PSA is present as PSA-A2M.

PSA-A2M levels in serum samples

Early reports indicated that PSA in serum is predominantly present as PSA-ACT.[16,17,18] Despite the high percentages of PSA-A2M reported above, both *in vitro* and *in vivo*, this may still be true and may be explained by the high turnover of the PSA-A2M complex. At present, the half-life of PSA-A2M is unknown, but the half-lives for other A2M complexes in serum have been reported as low as 1-3 minutes.[14] If PSA-A2M is cleared from the body with similar swiftness, it is very likely that at any given moment, the PSA-A2M concentration will be less than the PSA-ACT concentration. Due to the aggressiveness of the PSA-A2M interaction described above, however, Leinonen et al. suggested that PSA-A2M levels may increase significantly after the clotting that occurs with the handling of patient sera samples.[14] Clotting of the sample not only prevents the clearance of the PSA-A2M complex, but may also inhibit the steric dissociation of the PSA-A2M complex that would normally occur *in vivo*. As such, PSA-A2M levels would increase, surpass the PSA-ACT levels, and contribute significantly to the total amount of PSA-enzyme inhibitor complex found in prostate cancer or BPH serum samples.

ELISA for PSA-A2M

The clinical detection of PSA-A2M is inherently complex due to the encapsulation of PSA by A2M and the subsequent loss of PSA immunoreactive epitopes. Current methods for the determination of PSA-A2M include immunoblot and Western blot analyses. Although accurate, these methods are time-consuming and relatively expensive, deterring their wide-spread use in a clinical setting. Recently, however, an ELISA has been described for PSA-A2M. España et al.[19] developed an ELISA using rabbit anti-PSA as the capture antibody and rabbit anti-A2M as the trapping or labeling antibody. The authors hypothesized that if merely one PSA epitope is available in the PSA-A2M complex, ELISA development is possible. Their system detects as little as 3 µg/L PSA complexed to A2M in solution, which equates to approximately 6 µg/L in serum. Although the sensitivity of the described ELISA must improve by nearly 10-fold to be clinically useful, the results described indicate that it is possible to design a sensitive and specific ELISA for PSA-A2M.

Closing remarks

At present, the clinical significance of the PSA-A2M complex *in vivo* is unknown. It's been hypothesized that individuals with elevated levels of A2M, such as in cases of nephrotic syndrome, may artificially reduce the tPSA levels as determined by current methods.[20] Furthermore, due to the apparent changes in PSA-A2M concentrations that occur as a result of the handling of patient samples, tPSA

Table 1. Proportion of various forms of PSA bound by A2M or ACT (from Leinonen et al.[14])

		Percent PSA bound						
		ACTIVE PSA		INACTIVE PSA				
Enzyme Inhibitor	Incubation Duration	PSA-A	PSA-B	PSA-C	PSA-D'	PSA-D	PSA-E	PSA-F
A2M	2 hr	79	83	44	0	0	0	0
	24 hr	84	87	78	36	48	37	9
ACT	2 hr	51	34	0	0	0	0	0
	24 hr	60	53	16	0	0	0	0

levels may be under-reported in nearly all suspected prostate cancer patients. Both of these conditions would result in an increased fPSA:tPSA ratio and, thus, direct the physician away from a diagnosis of prostate cancer. At present, the consequences of these circumstances are unknown. Enough evidence exists, however, to suggest that PSA-A2M constitutes a sizable proportion of serum tPSA and to recommend further study to elucidate the significance of PSA-A2M as it relates to tPSA determinations and the differentiation of prostate cancer from BPH.

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