The semenogelins: proteins with functions beyond reproduction?

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Visions and Reflections

The semenogelins—proteins with functions beyond reproduction?

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Short title: The semenogelins

ABSTRACT

The coagulum proteins of human semen, semenogelins I and II, are secreted in abundance by the seminal vesicles. Their function in reproduction is poorly understood as they are rapidly degraded in ejaculated semen. However, more recent results indicate that it is time to put the semenogelins in a broader physiological perspective that goes beyond reproduction and fertility.

Key words: semenogelin, zinc, fertility, seminal vesicle, kallikrein, reproduction

In the 1980s, semenogelin I and semenogelin II were—as their names imply—recognized as the major structural and gel-forming proteins in human semen [1-4]. Studies conducted at that time also showed that the semenogelins are secreted mainly by the seminal vesicles, and they were identified as the major substrates for the kallikrein-like protease called prostate-specific antigen (PSA) [5-7]. Semenogelin I has a molecular mass of 50 kDa, and semenogelin II is a 63-kDa protein that also exists in a glycosylated form [8]. The semenogelins exhibit 78% similarity in their primary structure, and they are composed of repetitive units [9]. The genes that encode these proteins are located 11.5 kbp apart on the long arm of chromosome 20 [10]. The two gene products belong to a family of proteins that are encoded by three exons, the first of which gives rise to the signal peptide, the second encodes the secreted protein, and the third contains the 3' untranslated nucleotides. The first and third exons are conserved between family members, whereas the second is not. Thus, the expressed proteins in this family show wide variation in primary structure. Accordingly, it seems that they have evolved rapidly [11, 12], which would explain why semenogelins I and II are apparently found solely in primates, and, even within that taxonomic group, there are obvious interspecies differences in the molecules [13, 14]. Rats and mice also express seminal vesicle proteins that belong to the same family and show high sequence similarity in the products encoded by exons one and three, whereas the translated middle exon exhibits little similarity to the primate counterpart [12].

It is common with a history of rapid evolution for proteins involved in reproduction [15]. Naturally, as major constituents of semen, the semenogelins are closely associated with reproduction, and a large proportion of our knowledge on these proteins consists of results and hypotheses related to fertility [16]. A correlation has been found between the degree of female promiscuity within a species and the evolution of the two genes encoding

semenogelins I and II, and thus it has been suggested that post-copulatory sperm competition drives the evolution of these genes [13, 17, 18]. In addition to having coagulum-forming functions and being susceptible to cleavage by kallikrein-like peptidases, the semenogelins are assumed to activate sperm hyaluronidase [19], affect sperm motility [20], possess anti-microbial activity [21], serve as substrates for transglutaminase [22], and have amyloid properties [23]. In short, it is suggested these two proteins participate in several steps that affect the ability of a spermatozoon to reach and fuse with an ovum. In the human population, differences in the two semenogelin genes are poorly mapped, and the only studies of genetic anomalies that have been performed have concerned individuals carrying a common variant semenogelin I allele encoding a truncated protein. No homozygotes for that allele have been found, and heterozygosity does not seem to affect fertility [24, 25].

Studies have shown that semenogelins I and II are expressed in tissues unrelated to fertility , *e.g.* breast, salvary glands, trachea, kidney and intestine [26] and in lung cancer cell lines [27], which indicates that these proteins are involved in functions beyond reproduction. Notably, it was subsequently discovered that the semenogelins represent the major binders of the zinc ions that are found in abundance in semen. [28], and, even more recently, it was also reported that the semenogelins are expressed in retina, a tissue known to contain high levels of zinc [29]. The high capacity of the semenogelins for extracellular binding of Zn^{2+} is a function that may have implications throughout the body, and hence it has been proposed that they play a physiological role as modulators of zinc-dependent serine proteases [28]. PSA is the major protease in seminal plasma. It is a member of the tissue kallikrein family and has a unique specificity [30]. The proteolytic activity of PSA is inhibited by zinc ions [30], and experiments *in vitro* have shown that adding semenogelins to a zinc-rich environment such as semen can decrease the amount of free zinc to a level low enough to block the inhibition and

thereby enable activation of PSA [28]. However, since the semenogelins are continually broken down into smaller fragments, it seems likely that the number of zinc-binding sites gradually decreases, which would in turn increase the number of zinc ions available for binding to PSA and eventually more or less inhibit the protease. This is an example of a self-limited system in which PSA is maintained in an active state by its substrates [28]. Human kallikrein related peptidases 2 and 5 (KLK2 and KLK5) are other proteins in same family as PSA. Interestingly, their activity is also inhibited by zinc [31, 32], suggesting that these peptidases may interact with Zn^{2+} and the semenogelins in a similar way as PSA. Furthermore, the semenogelins are co localized with PSA, KLK2 and KLK5 in several tissues outside the reproductive organs, such as breast,salivary gland, trachea and the intestine [26, 33-35]

The two semenogelin genes are surrounded by a locus containing 14 genes that are structurally similar in that they exhibit conservation of the first exon encoding the signal peptide and the last exon containing the 3' untranslated nucleotides. The locus produces small proteins that have been suggested to inhibit serine proteases and that have been found to contain a characteristic structural motif, which was first discovered in whey acidic protein and is now known to consist of four conserved disulfide bridges. The locus includes the genes encoding elafin and secretory leukocyte protease inhibitor [36, 37], and both those genes have been observed to show homology to exons one and three of the semenogelin genes [38]. The expression patterns of the small serine protease inhibitors are not restricted to the reproductive organs, and it has been suggested that these molecules play a role in the defense against a wide spectrum of microbes. Antimicrobial activity has also been proposed for the semenogelins [21]. Furthermore, the protein eppin, which is encoded in the whey-acidic-protein-type locus, has been identified in complex with semenogelin I both in seminal plasma

and on the surface of spermatozoa [39]. Thus it seems that the proteins encoded in this locus are evolutionary and physiologically connected with the semenogelins.

It is plausible that semenogelins I and II, like many other proteins, have several functions. Some of the effects that have already been assigned to the semenogelins and the role of these proteins in semen may be both essential for and specific to reproduction. Nonetheless, the semenogelins are expressed in non-reproductive organs, they have a high capacity for binding zinc, and they may modulate zinc-dependent proteases also outside the reproduction area. It seems that we need to take a wider view of these proteins. Therefore, if we assume that a positive selection pressure underlies the rapid evolution of the semenogelin genes, the mentioned findings might motivate going beyond reproductive biology to search for the factor that provokes such selection. One such function might be as extracellular zinc regulators.

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