CMLS, Cell. Mol. Life Sci. 55 (1999) 944–960
1420-682X/99/070944-17 $ 1.50 + 0.20/0

CMLS Cellular and Molecular Life Sciences

Review

Semenogelin I: a coagulum forming, multifunctional seminal vesicle protein

M. Robert[a] and C. Gagnon[b,*]

[a]Chugai Research Institute for Molecular Medicine, Inc., 153-2 Nagai, Niihari-mura, Ibaraki, 300-0041 (Japan)
[b]Urology Research Laboratory, Rm H6.46, Royal Victoria Hospital, 687 Pine Avenue West, Montréal, Québec, H3A 1A1 (Canada), Fax + 1 514 843 1457, e-mail: cgagnon@is.rvh.mcgill.ca

Received 21 October 1998; received after revision 15 December 1998; accepted 15 December 1998

Abstract. Human seminal plasma spontaneously coagulates after ejaculation. The major component of this coagulum is semenogelin I, a 52-kDa protein expressed exclusively in the seminal vesicles. Recently, a sperm motility inhibitor has been found to be identical to semenogelin I, suggesting that it may also be a physiological sperm motility inhibitor. The protein is rapidly cleaved after ejaculation by the chymotrypsin-like prostatic protease prostate-specific antigen, resulting in liquefaction of the semen coagulum and the progressive release of motile spermatozoa. Some of the cleavage products of Sg I may also have various biological functions. While the semenogelin I protein is unique to human and higher primates, it has recently been shown to belong to a gene family having a similar gene structure but encoding widely differing proteins. The recently elucidated characteristics of the semenogelin I gene as well as the biochemical and functional properties of the encoded protein are reviewed, and an attempt is made to integrate the various findings into a model for semen coagulation, sperm immobilization and potential other functions.

Key words. Seminal plasma; sperm motility inhibitor; prostate-specific antigen; seminal vesicle-specific antigen; inhibin-like peptides; REST gene; sperm hyaluronidase.

Introduction

Following ejaculation, human semen spontaneously coagulates into a semisolid gelatinous mass, which then liquefies within 5–20 min [1, 2]. This coagulum appears as a dense network of narrow and long fibers that are approximately 0.15 μm thick [3]. The major structural components of human semen coagulum have been described as disulfide-linked complexes of a predominant 52-kDa protein, known as semenogelin I (Sg I), and two forms of a less abundant Sg I-related protein of 71 and 76 kDa (semenogelin II, Sg II), all originating from the seminal vesicle secretions [4–6]. Recently, novel activities have been shown to be associated with Sg I, including the property to block sperm motility [7] and to activate sperm hyaluronidase [8].

The past few years have thus given rise to a more comprehensive body of information about this unique protein through experiments originally performed on different models but which eventually converged on the same protein, namely Sg I. The present review reexamines some of the original work that developed in parallel, and attempts are made to integrate the more recent findings which suggest a surprising variety in potential functions for this protein in reproduction. While in the present review emphasis is placed on Sg I, whose functional properties have been more extensively studied, some properties of the closely related Sg II will also be

* Corresponding author.

discussed, particularly as it relates to gene structure and pattern of expression where similarities suggest interesting evolutionary consequences. First, some of the original work on semen coagulation and on the seminal plasma sperm motility inhibitor (SPMI) will be reviewed, and the more recently elucidated properties and function of the proteins will then be discussed in more detail. It is first worth summarizing the original work on SPMI and Sg I in parallel to appreciate how both stories originated from the isolation of a similar proteolytic fragment of Sg I from seminal plasma.

Parallel paths converging on the same protein

Sg I, the predominant human semen coagulum protein

The foundation for most of the work on Sg I and its role in human semen coagulation rests mainly on the results of Lilja and co-workers. Their original studies looked at the effect of various protease inhibitors on the processing of larger molecular mass forms of proteins into smaller polypeptides occurring during semen liquefaction [9–11]. This led to the identification of the major coagulum components as disulfide-linked polypeptides of 52, 71 and 76 kDa [4, 5]. Most of the degradation products derived from those proteins were found to be basic polypeptides, the most basic of which was first purified and sequenced [9]. Prostatic proteases were found to be responsible for the degradation of coagulum proteins [5, 9, 10], and eventually the proteolytic activity was associated with prostate-specific antigen (PSA) [12]. These findings led to the eventual molecular cloning of the predominant 52-kDa seminal coagulum component, thereafter referred to as semenogelin I [13]. According to this sequence, several polypeptides isolated from seminal plasma [9, 14, 15] appeared to be derived from Sg I. The identity of the amino acids surrounding the deduced cleavage sites provided evidence that PSA had a chymotrypsin-like activity. Many of the earlier findings about Sg I have been previously reviewed [16]. We will therefore attempt in the present review to emphasize some of the more recent data and also to integrate the findings with those originating from work on a sperm motility inhibitor.

SPMI, a sperm motility inhibitor associated with semen coagulum

The identification and characterization of some of the properties of Sg I also originated from the study of a sperm motility inhibitor present in seminal plasma. Before the two proteins were found to be identical, the work on the latter had been initiated from studies on the control sperm motility. Using a previously developed model for the demembranation and reactivation of sea urchin spermatozoa [17], Mohri and Yanagimachi first showed that epididymal spermatozoa collected in physiological solutions were readily reactivated following demembranation [18]. On the other hand, the reinitiation of motility of ejaculated and then demembranated rabbit spermatozoa was not possible. Interestingly, the motility of spermatozoa that had been washed on a Ficoll gradient before demembranation was reinitiated following addition of Mg · adenosine triphosphate (ATP) [19, 20], suggesting that some component(s) present in seminal plasma interfered with the reinitiation of motility. The seminal plasma of various mammalian species was similarly found to contain a motility inhibitory activity [19]. Following these original experiments, a protein of 18–22 kDa associated with this activity was isolated from human seminal plasma and named seminal plasma motility inhibitor (SPMI) [21]. The finding that the purified protein was a potent dynein-ATPase inhibitor [21] suggests that this is the mode of action of SPMI on demembranated spermatozoa. On the other hand, purified SPMI was also found to interfere with the motility of intact spermatozoa [22]. However, the dose of SPMI required to completely inhibit the motility of intact spermatozoa was found to be more than 1000-fold higher than on demembranated spermatozoa, suggesting that its effect on the motility of intact spermatozoa is likely mediated through a different mechanism. The dose-dependent inhibitory activity of SPMI on intact spermatozoa caused a progressive decrease in the percentage of motile spermatozoa, the curvilinear velocity and the beat-cross frequency, without affecting the linearity. In a subsequent study, human SPMI was found to originate exclusively from the seminal vesicles and to have a 9-fold higher specific inhibitory activity in this fluid than in seminal plasma [23]. The difference turned out to be explained by the presence of a 52-kDa SPMI form in seminal plasma shortly after ejaculation which was rapidly transformed into smaller mass forms during liquefaction [24]. This was accompanied by an 80% reduction in inhibitory activity to levels that are compatible with normal sperm motility (< 250 U/ml). The phenomenon could be reconstituted in vitro by incubating secretions isolated from the seminal vesicles and prostate, thus demonstrating the prostatic origin of the processing factor. The serine protease inhibitors phenylmethyl sulfonylfluoride (PMSF) and benzamidine and the metal chelators EDTA and 1,10-phenanthroline all partially prevented the loss of inhibitory activity and processing into smaller forms [24].

The characteristics of SPMI processing after ejaculation were highly reminiscent of the proteolysis of Sg I occurring during semen liquefaction, thus suggesting the phenomena might be related. This association became apparent when all SPMI inhibitory activity and most

SPMI antigens were found to be associated with the coagulated semen fraction [25]. Moreover, semen samples that failed to liquefy spontaneously demonstrated high levels of SPMI inhibitory activity (1500 U/ml), the presence of nondegraded SPMI antigens and contained sperm having poor motility. Addition of prostatic secretions to the nonliquefied semen samples resulted in a marked increase in sperm motility with a concomitant degradation of high-mass SPMI antigens and a decrease in inhibitory activity. These findings thus provided an explanation for the reported low sperm motility in coagulated semen shortly after ejaculation, and the progressive increase in sperm motility as the protein precursor gets inactivated during semen liquefaction.

The identical glandular origin of Sg I and SPMI, their comparable molecular mass and processing by prostatic proteases displaying similar properties after ejaculation as well as the association with semen coagulum strongly suggested that these two proteins were the same molecular entity. This was eventually confirmed when the protein was purified from both seminal vesicle fluid and seminal plasma coagulum and the amino acid sequence of three different peptides, the amino acid composition and the mass were found to match very closely the values expected from the published complementary DNA (cDNA) sequence of Sg I [7].

Characterization of Sg I gene and protein

Characterization of Sg I cDNA

The characterization of the Sg I cDNA chronologically preceded the purification of the proteins and will thus be described first. The complete primary structures of Sg I and semenogelin-related proteins have been elucidated [13, 26]. An Sg I clone was originally isolated [13] by screening a cDNA expression library derived from seminal vesicle transcripts with an antibody raised against a 52-amino acid residue fragment of Sg I [9]. A full-length cDNA containing an open reading frame of 1386 nucleotides was thus isolated and characterized [13] (table 1). The resulting sequence encodes a precursor protein of 462 amino acid residues, which after cleavage of a 23-residue signal peptide yields a mature protein of 439 amino acids having an expected molecular mass of 49.6 kDa and isoelectric point of 9.6 (fig. 1). The mature protein contains a single cysteine residue at position 216 which is involved in the formation of disulfide-linked complexes [5, 13]. A cDNA for the 71-kDa Sg-related protein was similarly obtained [26]. Its characterization showed that it encodes a 559-residue protein, semenogelin II, that displays 78% identity at the amino acid level with that of the 52-kDa Sg I. Two cysteine residues are present at positions 136 and 337. The difference in size between Sg I and Sg II is related to the presence of an extended C-terminal in Sg II. The 76-kDa protein, on the other hand, appears to be a glycosylated form of the 71-kDa polypeptide Sg II [26, 27]. Sg I contains six repeats of 60 residues showing internal sequence similarity (fig. 2), whereas Sg II contains eight such repeats. The exact function of these repeats is unknown, but their structure is reminiscent of repeats found in rodent seminal vesicle proteins known to serve as substrate units in transglutaminase reactions. Sg I has been shown to be expressed exclusively in the seminal vesicles, whereas Sg II transcripts have also been detected in the epididymis [13], albeit at a much lower expression level [26].

Gene structure of Sg I and II

The structure of the Sg I and II genes has recently been elucidated. Both genes are located on the long arm of chromosome 20 at q12–13.1 and are relatively compact, spanning only 2.7 and 3.1 kb, respectively [28]. They are arranged in tandem, 11.5 kb apart, and are similarly composed of three exons that display more than 90% sequence identity [28, 29]. These three exons encode, respectively, the signal peptide, the secreted polypeptide and the 3′ untranslated region (fig. 1). The flanking regions and introns demonstrate more than 80% iden-

Table 1. Characteristics of the semenogelin I gene and protein.

Characteristics	Properties	References
Open reading frame	1389 nucleotides	[13]
Molecular mass	predicted: 49607 measured: 49620–49958	[7, 13, 27]
Amino acid residues	signal peptide: 23 mature protein: 439 total: 462	[13]
Isolectric point	predicted: 10.1 measured: >9.5	[13]
Cysteine residue	1 (at residue 216)	[13]
Internal repeats	six repeats of 60 amino acid units	[13, 26, 27]
Exon structure	exon I: signal peptide exon II: mature protein exon III: 3′ nontranslated region	[28]
Chromosomal localization	20 q12–13.1	[28]
Tissue expression	limited to seminal vesicles	[13, 26, 34]
Similarity with Sg II	nucleic acid 89% (within exons) protein 78%	[26, 28]
Posttranslational modification	N-terminal pyrodglutamination	[13, 27, 37]
SPMI domain	85–136	[37]
MHS-5 epitope	198–223	[37]

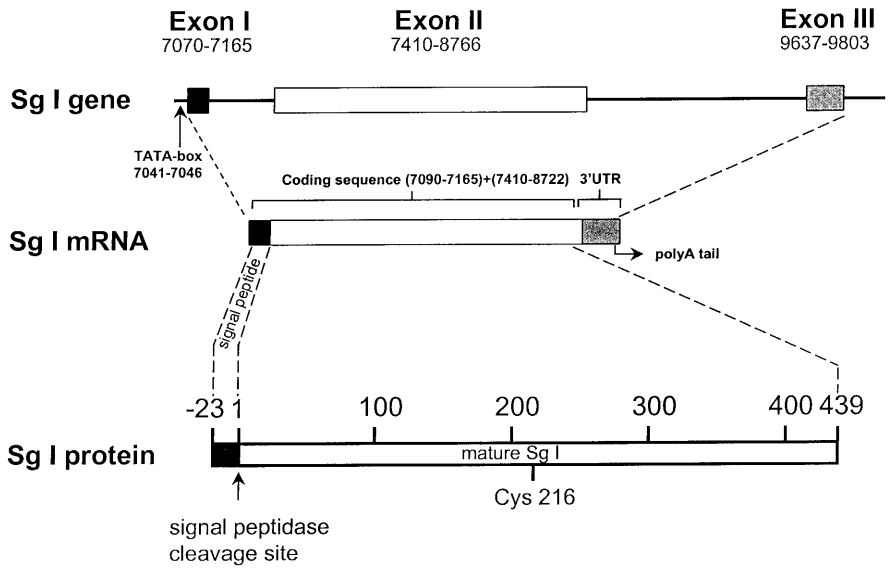


Figure 1. Schematic representation of the Sg I gene, transcript and protein. The basic structural elements of the Sg I gene showing the three-exon structure typical of the REST-gene family are shown. Nucleotides are numbered according to EMBL accession number Z47556. Dashed lines link corresponding sections of the gene, transcript and protein. The black box in Sg I protein represents the 23-residue signal peptide. The single cysteine at position 216 is marked.

tity [28]. The intergenic sequences are composed of highly repetitive DNA sequences made up of long interspersed nucleotide sequences (LINES) such as L1 repeats and Alu sequences [29]. The Sg I and II genes are believed to have originated from duplication of an 8-kb DNA fragment some 61 million years ago [29].

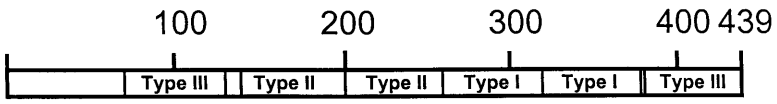
While Sg I and II proteins are highly homologous [13, 26], their primary structure shows little similarity to other abundant seminal vesicle secretory proteins responsible for semen coagulation in rodent species. However, recent studies on the structure of the genes that encode these divergent proteins have highlighted interesting similarities. All appear to be members of a novel gene family that includes both Sg I and II, the rat seminal vesicle secretory proteins SVS-II, SVS-IV, SVS-V and guinea pig seminal vesicle proteins GP1 and GP2, the major seminal clotting protein in these species [26, 30]. The common characteristics of these genes lie in their similar transcription unit made of three exons; the first one encoding the signal peptide, the second one encoding the secreted protein and the third codon encoding the 3′ untranslated region [30]. Interestingly, significant sequence similarity exists exclusively within exon I and exon III, whereas exon II is highly divergent. The results demonstrate that all these proteins are derived from a similar ancestral gene that evolved rapidly by substitution of exon 2 to give rise to different proteins [30]. These surprising findings suggest that, in spite of apparent disparity in amino acid sequence, these various polypeptides may share similarities in three-dimensional structure and/or functional domains that form the basis for intermolecular interactions involved in semen clotting or coagulation. This new family is collectively referred to as the REST (rapidly evolving substrates for transglutaminase) gene family due to the fact that most members have been found to be good substrates for transglutaminases. Members of this family have a basic isoelectric point, contain several internal repeats and an abundance of lysine and glutamine residues which serve as donor and acceptor sites in the transglutamination reaction.

Until recently, Sg I was believed to be unique to humans, since no homology to other known proteins in other species was found. However, the presence of a close homologue in rhesus monkeys was suspected by immunocytochemical study demonstrating the presence of Sg immunoreactive material in the seminal vesicle and vas deferens [31]. The Sg II gene of the rhesus monkey has recently been cloned [32]. As described above for human and rodent proteins, the transcription unit is similarly split into three exons encoding the signal peptide, the mature protein and the 3′ untranslated region. The rhesus monkey gene is about 22% larger than its human counterpart due to extension of the coding region by the presence of two additional repeats of 60 amino acid residues [32]. Interestingly, the rhesus monkey Sg II lacks cysteine residues, thus demonstrating that noncovalent interactions are respon-

sible for semen clotting or gellation and that the role of intermolecular disulfide bonds, if any in this process, is secondary in those molecules which contain cysteine residues [32]. Southern blot analysis has demonstrated that Old World monkeys such as the rhesus monkey and baboon and the New World monkey marmoset contain two different Sg genes similar to Sg I and Sg II [32]. In contrast, only one Sg-hybridizing band was found in the New World monkey cotton-top tamarin. Cloning of the gene in this primate has revealed that it contains a single Sg-related gene that is more similar to human Sg I (89% of nucleotides conserved) than to human or rhesus monkey Sg II (82%) [33]. The predicted molecular mass of this extended Sg I is 66 kDa, or 32% higher than human Sg I. However, contrary to the its human homologue, the cotton-top tamarin protein contains 14 occurrences of a consensus sequence for N-linked glycosylation. The potential for glycosylation could thus significantly increase the actual molecular mass of the protein and modify its isoelectric point estimated at 9.0 [33]. Surprisingly, a probe for semenoclotin, the major clot-forming protein in mouse, hybridized to a single restriction fragment under low stringency in the cotton-top tamarin [32]. This suggests that while this New World monkey does not contain an Sg II gene, it may contain a semenoclotin-related gene previously thought to be unique to rodents [32]. The evolutionary consequence of such a finding is that the genome of a common ancestor to early mammalians and primates likely contained both semenoclotin and Sg genes, but that murine species eventually lost Sg genes while most primates lost the semenoclotin gene. On the other hand, the cotton-top tamarin likely lost the original Sg II by substitution of a new exon in an alternative splice site, giving rise to a semenoclotin-related gene [33].

Expression profile of Sg I

Recently, a detailed localization study showed abundant semenogelin antigen expression in the cytoplasm of seminal vesicle glandular epithelial cells by immunocytochemistry using several different monoclonal and polyclonal antibodies [34]. None of the antibodies used in this study were found to be specific to either form of Sg, a finding not so surprising considering the very high



Type I repeat

```
259                                                              318
KANKISYQSSSTEERRLHYGENGVQKDVSQSSIYSQTEEKAQGKSQKQITIPSQEQEHSQ
KANKISYQSSSTEERRLHYGENGVQKDVSQRSIYSQTEKLVAGKSQIQAPNPKQEPWHGE
319                                                              378
```

Type II repeat

```
141                                                              200
ERLWVHGLSKEQTSVSGAQKGRKQGGSQSSYVLQTEELVANKQQRETKNSHQNKGHYQNV
VEVREEHSSKVQTSLCPAHQDKLQHGSKDIFSTQDELLVYNKNQHQTKNLNQDQQHGRKA
200                                                              260
```

Type III repeat

```
70                                                               129
TTKSQRHLGGSQQLLHNKQEGRDHDKSKGHFHRVVIHHKGGKAHRGTQNPSQDQGNSPSG
KGESGQSTNREQDLLSHEQKGRHQHGSHGGLDIVIIEQEDDSDRHLAQHLNNDRNPLFT
381                                                              439
```

Figure 2. Structure of repetitive amino acid units in Sg I. Upper panel: The position of three different types of repeat present in Sg I are shown along the Sg protein. Lower panel: alignment of amino acids within each type of repeat. Conserved residues are shadowed. The repeats are classified according to Lilja and Lundwall [26].

similarity in primary structure. On the other hand, the authors were able to design oligonucleotide probes specific for Sg I or Sg II, and these were used for in situ hybridization [34]. Using these specific probes, intense Sg I and Sg II transcript signals were detected in the same glandular epithelial cells shown to contain Sg antigens. Sg II transcripts were also detected in the epithelium of the cauda epididymis, whereas Sg I transcripts were absent [34]. Neither Sg I nor Sg II transcripts or antigens were found to be expressed in other parts of the epididymis (caput, corpus) or in stromal cells. Accordingly, Sg antigens were absent from testicular, caput and corpus spermatozoa but appeared on the surface of cauda epididymis spermatozoa. Localization of Sg antigens on freshly ejaculated spermatozoa showed that the protein localizes to the posterior head, midpiece and tail of spermatozoa [34], in agreement with earlier findings [13]. The testis, prostate, female genital tract and numerous tissues tested were all found to be devoid of Sg antigens in agreement with the results of localization of SPMI antigens, shown to be exclusively located in the seminal vesicles [23].

Regulation of unique Sg I tissue expression

The very restricted expression profile of Sg I as described above and the abundance of the protein in the secretions of the seminal vesicles, a gland whose secretory function is androgen-dependent, argues in favor of an androgen-regulated synthesis of this protein. Sg immunoreactivity, as determined using the antibody recognizing the basic fragment of Sg I, was previously observed on the epithelium of seminal vesicle specimens obtained from postpuberty subjects, but not on those of a newborn or 5-year-old child [31]. This is in agreement with preliminary observations from our laboratory made during the collection of seminal vesicle fluid. Seminal vesicle fluid collected from two patients who underwent radical prostatectomy and had followed a preoperative treatment with antiandrogens showed a dramatic decrease in Sg I immunoreactivity, as measured by immunoblotting, compared with those obtained from untreated patients (M. Robert and C. Gagnon, unpublished observations). Moreover, the reported Sg I gene sequence [28] contains nucleotide sequences that are closely related to a reported androgen-response element consensus [35] in both the promoter 5′ upstream sequence and in the first intron of the Sg I gene (M. Robert and C. Gagnon, unpublished observations). Together, these observations strongly suggest that the specific expression of Sg I in the seminal vesicle epithelium is androgen-dependent. On the other hand, the expression of Sg I exclusively in seminal vesicles [34] and not in other tissues that are also androgen-responsive, such as the prostate, suggests that there are additional factors that regulate this tightly restricted expression. The identification of regulatory elements within the Sg genes by focussing on the specific and unique sequences identified by Lundwall [29] and the identification of the associated regulatory DNA-binding proteins should allow elucidation of the mechanism of this unique tissue expression.

Purification and characteristics of Sg I

While the isolation of smaller Sg I polypeptides [9, 21] from seminal plasma originally led to the identification of a 52-kDa larger form in seminal vesicle and its molecular cloning, the purification of intact Sg I from isolated seminal coagulum and seminal vesicle fluid was reported only recently [7, 27]. In addition, Mandal and Bhattacharyya previously reported the purification of the predominant semen coagulum protein [36]. While the identity of the protein with Sg I was not directly demonstrated, the similarities in molecular weight, pI and chromatographic behavior strongly suggest that it is Sg I. In all cases, proper solubilization of the proteins in urea buffer, as well as reduction and alkylation of cysteine residues prior to purification, was found to be essential to ensure good recovery of the proteins and prevent their degradation [7, 27, 36]. The successful enrichment of Sg I by chromatography on heparin-Sepharose [27] is likely due to its cation exchanger properties at the alkaline pH used, as this is the type of chromatography that proved useful in the other protocols [7, 36]. However, a specific affinity of Sg I for heparin cannot be excluded. One of the major difficulties encountered during the purification of Sg I was the removal of degraded forms present in the starting material. These fragments had the tendency to coelute with the 52-kDa precursor. Their successful removal could be achieved by an additional purification step using gel filtration on Superose [27], Sephacryl [36] or high-performance reverse-phase liquid chromatography [7]. The abundance of the 52-kDa Sg I polypeptide as observed by SDS-polyacrylamide gel electrophoresis (PAGE) in samples of seminal vesicle fluid and washed semen coagulum, together with the low enrichment factor (two- to fivefold) following purification, suggest that this protein may represent more than 20% and up to 50% of the total proteins in these sources, respectively [7, 36]. In physiological solution, the purified Sg I appeared to form aggregates of large molecular mass, which were not recovered during analysis on a gel filtration column in buffer that did not contain urea [7]. This is not a surprising finding considering the coagulating properties and low solubility of Sg I, and is likely the consequence of the reported extensive noncovalent interactions existing between Sg I and Sg II polypeptides. Similar observations were also reported independently [27].

The amino acid composition of Sg I corresponds very well with that predicted from the cDNA sequence [7, 27]. Treatment of the purified protein with N-glycosidase F and specific staining using a glycoprotein detection system demonstrated the absence of any glycosylation [27]. While the site of processing by signal peptidase had been suspected from analysis of the protein sequence, the identification of a glutamine residue (in the form of a pyrrolidone carboxylate or pyroglutamine) as the N-terminal residue of the processed protein was confirmed following digestion with pyroglutamate aminopeptidase [13]. This finding thus explains the reported failure to obtain N-terminal sequence information from the purified 52-kDa Sg I protein [7, 13]. The mass of Sg I, as measured by mass spectrometry, is also in close agreement with that predicted from the cDNA sequence (table 1) [7, 27]. The slight difference of approximately 300 Da, between the two reports, is within reasonable experimental error and may reflect a variation due to the different spectrometric method used (atmospheric pressure ionization-mass spectrometry (API-MS) vs. matrix-assisted laser desorption ionization (MALDI)). The absence of a significant difference between the measured and expected mass of Sg I confirms the absence of glycosylation and rules out the possibility that the protein undergoes extensive posttranslational modification.

Sg I was shown to reversibly inhibit the motility of intact spermatozoa in a dose-dependent manner [7]. Incubation of the purified protein with prostate-specific antigen (PSA) caused degradation into smaller mass forms and a concomitant 76% decrease in its inhibitory activity, as measured on demembranated spermatozoa. Similarly, PSA treatment also considerably reduced the inhibitory activity of Sg I on intact spermatozoa. The demonstration of the immobilizing effect of purified Sg I on intact spermatozoa [7], at activity levels and protein concentrations corresponding to those measured in coagulated semen early after ejaculation [24], suggests that Sg I may be responsible for the low motility of spermatozoa in coagulated semen. The effect of Sg I on sperm motility was reversible when cells were washed and thus appeared to be mediated by association of the protein with the sperm surface. The sperm-immobilizing activity of Sg I does not appear to be exclusively related to its high isoelectric point, since cytochrome c, with a pI of 9.3, has no such activity at the same concentration [7].

The sperm-immobilizing activity of Sg I could be mapped within the N-terminal domain of the protein [37]. This segment of the protein appears highly conserved in Sg of other primates, suggesting that it may play a similar role in those species [33]. Because of its very high similarity with Sg I, Sg II also likely has sperm motility inhibitory activity. Accordingly, during purification of Sg I, sperm-immobilizing activity has also been observed in fractions enriched in Sg II [7]. It is thus tempting to speculate that Sg II, which has also been shown to be expressed in the cauda epididymis as well as on the surface of spermatozoa of the cauda epididymis [34], might be involved in sperm immobilization at this site, as recently suggested [33].

The N-terminal domain of Sg I also contains the epitope recognized by an antibody raised against a 19-kDa Sg I form present in liquefied seminal plasma [37] (table 1). On the other hand, the epitope for the MHS-5 monoclonal antibody appears to map to a more centrally located section of Sg I [37]. This antibody was originally raised against whole spermatozoa but found to recognize a sperm-coating antigen originating from the seminal vesicles [38]. The antigen recognized by MHS-5 antibody is referred to as seminal vesicle-specific antigen (SVSA) and is present on a range of polypeptides. These also undergo extensive molecular processing during semen liquefaction and have masses very similar to those from Sg I and II [38–40]. While seminal polypeptides reacting with this antibody were purified, no biochemical information was available to allow the location of SVSA within the Sg molecule [41]. However, the identification of a specific segment of Sg I that is recognized by this antibody further reinforces the suspected identity of the 58-kDa SVSA with Sg I [13, 37].

Sg I was found to be cleaved readily by PSA [37]. The effect of zinc and 1,10-phenanthroline on the in vitro degradation of Sg I by PSA and chymotrypsin led to the postulation that Sg I may be a zinc-binding protein. These findings are in good agreement with those reported for the effect of those substances on semen liquefaction and degradation of Sg I by prostatic proteases [4, 11, 24, 25]. Zinc is an important ion in many biological systems, and although it is usually present only in trace amounts in most body fluids, zinc is present in the prostate gland at the highest concentration of any organ [42] and is secreted in semen at similarly high levels. However, its function in that fluid has remained poorly understood. Interestingly, in coagulated semen, most of the seminal zinc was previously found to be tightly associated with the washed coagulated protein mass [43], and to bind specifically to various polypeptides having identical electrophoretic mobility to Sg I [44]. Moreover, as semen liquefaction progressed, zinc was shown to be associated with progressively lower mass polypeptides (< 25 kDa) of electrophoretic mobility, again very similar to those of the Sg I degradation products [44]. These results thus support the concept that Sg I may be a major zinc ligand. While this may not represent the physiological scenario, both Sg I and Sg II have recently been found to be good substrates for the fibrin cross-linking transglutaminase,

factor XIII, in vitro [45], a finding that provides functional support for the similarity of Sg I and II to the REST gene family. Transglutaminase activities have been reported in human semen, and recently a novel transglutaminase cDNA was cloned from a human prostate cDNA [46]. However, factor XIII was used for those experiments since this human prostatic transglutaminase has not yet been isolated. Interestingly, the cross-linked high molecular mass complexes of Sg were found to be resistant to PSA-mediated proteolytic digestion [45]. This may thus highlight the importance of Sg I and II conformation for proper processing by PSA, as previously suggested [37]. In addition, several Sg fragments released by PSA hydrolysis could be cross-linked by FXIIIa [45]. This may suggest that some of the proteolytic fragments are similarly cross-linked in vivo and that the phenomenon might have functional relevance to sperm function. In any case, only a small fraction of Sg I and II would be cross-linked in vivo, since no high molecular weight Sg complexes are observed in semen. However, the authors also speculated that some Sg could be cross-linked to spermatozoa and thus not be observable after removal of the cells [45].

Sg I as the main physiological substrate for PSA

The fragmentation of Sg I occurring after ejaculation has been associated mainly with the proteolytic activity of PSA [10, 12, 13, 40]. Characterization of PSA activity using nonphysiological or synthetic substrates [47, 48] and identification of the location of seminal peptides derived from Sg [13, 16] had previously associated a chymotrypsin-like activity to the enzyme in contrast to the trypsin-like specificity of the other members of the kallikrein family of proteases, to which PSA belongs. Accordingly, Sg I was recently found to be cleaved preferentially on the C-terminal side of several tyrosine and leucine residues [7, 37]. Similar findings of preferential hydrolysis by PSA at tyrosine residues were recently reported using synthetic peptide substrates [49] or substrate phage display [50]. In the former study, PSA was also found to cleave synthetic substrates at glutamine, histidine and cysteine residues, albeit at a lower efficiency but with increased specificity for PSA when compared with other proteases. In Sg I, however, the favored hydrolysis sites appear to be at the C-terminal of several tyrosine and leucine residues [13, 16, 37]. The unusual specificity of PSA appears related to the presence of a serine residue at the bottom of the substrate binding pocket instead of the aspartic acid residue found in trypsin-like enzymes [51, 52]. PSA also seems to differ from chymotrypsin in its preference for tyrosine and leucine residues, whereas chymotrypsin predominantly cleaves at the aromatic residues phenylalanine, tryptophan and tyrosine and, only to a lower extent, at leucine residues. The effect of various protease inhibitors and divalent cations on PSA-mediated cleavage of Sg I also highlights additional differences with chymotrypsin [37]. Sg I appears to be a rather specific and favored PSA substrate since PSA does not readily hydrolyze other proteins [37]. It is thus likely that PSA cleaves Sg I preferentially at peptide bonds surrounded by specific residues [47], as suggested for Sg I [16, 37]. This is further substantiated by the recent demonstration that PSA displays preferences for secondary subsites and that recognition of substrate is mediated by an extended binding site [50]. These clusters of amino acid may form specific three-dimensional structures that are preferentially recognized by the enzyme. This strict specificity would explain the apparent low activity of PSA on other substrates when compared with chymotrypsin [37, 47]. Together, these specific differences among hydrolysis site preferences and interaction with various inhibitors and ions demonstrate that PSA is a specific Sg I processing enzyme that hydrolyses and displays unique catalytic properties in spite of its close relationship to other serine proteases of the kallikrein family.

The serine protease inhibitor protein C inhibitor (PCI) has been recently shown to bind to both PSA and Sg II in vitro and in vivo [53, 54] and to inhibit the PSA-mediated degradation of Sg I and Sg II [54]. Interestingly, in the latter study, divalent cations and particularly Zn^{2+} (0.01–0.1 mM) were found to significantly reduce binding of Sg II to both PCI and PSA [54]. These interactions have so far only been demonstrated clearly with Sg II. Caution must thus be exercised, since such an interaction could be mediated by the carbohydrate residues that appear to be present on the 76-kDa form of Sg II but absent from Sg I. However, if the interaction is mediated by the polypeptide backbone, then it is highly probable that Sg I, PSA, PCI and seminal zinc may similarly interact to coordinate Sg I and II processing.

While PSA appears to be the main Sg I processing enzyme after ejaculation, the possibility that additional proteases may also participate in the phenomenon cannot be excluded [24]. The human kallikrein hK2, which is closely related to PSA [55, 56], and the recently characterized prostasin [57], both of which are prostatic trypsin-like proteases that correspond well with the prostatic proteolytic activity inhibited by benzamidine [11, 24], are also proteases that may play a more marginal role. The former possibility is especially likely considering the recent demonstration that hK2 can degrade Sg I and II in vitro at the carboxyl side of arginine residues [58]. However, while these enzymes could contribute to cleave Sg I at alternative sites, their effect is likely to be marginal, since PSA generates Sg I fragments that are identical in size to those observed in

liquefied semen [7, 10, 37, 40, 59]. In addition, various Sg I fragments purified from liquefied seminal plasma were shown to have terminal leucine or tyrosine residues corresponding to cleavage by PSA [14, 15, 60].

Functions of Sg I

The major function of Sg I in the reproductive process has so far been associated with the formation of the seminal coagulum [5, 16]. The finding that Sg I displays sperm immobilizing activity in vitro suggests that it may also be a physiological motility inhibitor at least shortly after ejaculation [7]. While these two functions may be intimately linked, their physiological role in the human reproductive process and the mechanism of action has not been addressed extensively and remains of a rather speculative nature.

Sg I and semen coagulation and liquefaction

The phenomenon of semen coagulation occurs in most mammals, but the mechanisms appear to vary widely [61]. The semen of dogs and cats does not coagulate, and interestingly these mammals lack seminal vesicles, the main source of coagulating substrates in most mammalian species [61]. In some species, the formation of a vaginal plug is believed to be essential to prevent the outflow of semen from the female's vagina. In rodents, it is also thought to prevent secondary inseminations of spermatozoa by nondominant males [62]. Such a plug may act as a reservoir and contribute to the gradual release of spermatozoa in species where the female does not possess a long cervix and cervical mucus, and where semen is deposited directly into the uterus. Similarly, the semisolid and adherent properties of the human semen coagulum may also contribute to some extent to prevent outflow of spermatozoa from the female's reproductive tract, until they are released progressively during liquefaction. Whether the phenomenon of semen coagulation in humans is simply a remnant of our evolution from a common earlier ancestor is not clearly established. As described above, Sg I and Sg II have been found to belong to the REST gene family whose several members are known transglutaminase substrates [30].

While the primary structure and several biochemical properties of Sg I and Sg II have been elucidated, the precise mechanism accounting for the formation of the gelatinous network of proteins found in the coagulum has not yet been convincingly demonstrated. However, the integration of results derived from various studies provides some insights into some likely events. First, the mechanism responsible for human semen coagulation is different from the one involved in blood coagulation, since factor XII, fibrinogen and prothrombin are all absent from seminal plasma [61]. In addition, while in rodents transglutaminases from the coagulating gland cross-link protein substrates from the seminal vesicle to form the so-called copulatory plug [63], no such high molecular weight complexes of Sg I and II resulting from covalent cross-linking of those proteins have been observed when coagulum components are analyzed by SDS-PAGE [5, 7, 25]. On the other hand, Sg I and II, the predominant components of the coagulum, are known to form disulfide-linked high molecular weight complexes [5, 6, 12]. However, dithiothreitol does not cause apparent macroscopic changes on the coagulum structure [5], suggesting that these disulfide interactions do not constitute the main force holding coagulum proteins together. Disulfide bridges thus likely play a supporting and indirect role in the coagulation and liquefaction of human semen, since semen liquefaction is enhanced in the presence of the reducing agent β-mercaptoethanol [6]. This effect is therefore likely mediated by a change in the three-dimensional structure of Sg I and II induced by reduction of disulfides, which in turn increases their sensitivity to proteolytic degradation. The importance of noncovalent interactions in maintaining the coagulum structure is highlighted by the fact that denaturing agents such as guanidine-HCl (3 M) and urea (4–8 M) readily solubilize semen coagulum [5, 7, 27, 36].

The exact mechanism by which noncovalent interactions between Sg I and other components trigger semen coagulation thus remains speculative. Fibronectin originates from seminal vesicle fluid and, like Sg I, is enclosed in the coagulum structure and gets progressively fragmented and released into the fluid phase of semen during semen liquefaction [12]. At ejaculation, prostatic components such as zinc may induce a polymerization reaction involving Sg I, Sg II and fibronectin by inducing conformational changes in these proteins, thus triggering coagulation [12]. The authors speculated that this might arise by inducing an opening of fibronectin dimers and consequent exposure of structures that have affinity for Sg. Alternately, one model [64] proposed that interactions between sialoglycoproteins and metal ions are responsible for coagulum formation [65, 66]. According to this model, the initial stage of liquefaction would occur by reduction of those metal ions by L-ascorbic acid. However, Sg I does not appear to be glycosylated, thus raising doubts about such a mechanism as the main pathway. It could nonetheless be a contributing factor, since Sg I and Sg II may form heterodimers [13], and the high molecular mass (76 kDa) form of Sg II has been shown to be glycosylated [27]. Finally, another model proposed that coagulation occurs through reticulation of proteins by formation of stable links between fructose and monomer of vesicular

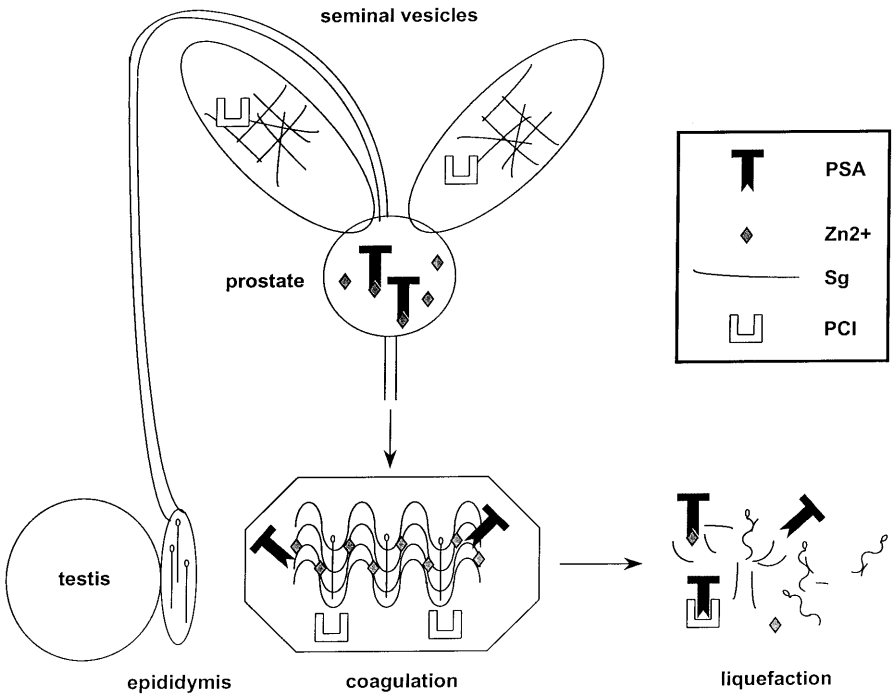


Figure 3. Model of semen coagulation and liquefaction based on Sg I properties. A simplified diagram of some of the components of the male reproductive tract is shown. Sg I and PCI are present exclusively in the seminal vesicles, whereas PSA and zinc ions originate from the prostate. At ejaculation, these components mix, and a conformational change in the Sg I, Sg II and fibronectin is triggered by prostatic components (most likely Zn^{2+}), causing the Sg molecules to interact with each other by a yet unknown mechanism, resulting in aggregation and semen coagulation. Spermatozoa are thus immobilized in the coagulum likely through the immobilizing activity of Sg I. Chelation of zinc ions in the coagulum activates PSA, which gradually degrades Sg I and II, causing the release of smaller fragments and reversing the sperm motility inhibition. This in turn releases zinc and PCI in solution, which bind to and inactivate PSA to prevent further undesirable proteolysis. Fibronectin, which also appears to participate in the coagulum formation, is left out for clarity and because its interaction with the other components remains unclear.

proteins, such as Sg, in the formation of semen coagulum [67].

Semen liquefaction, on the other hand, is associated with the proteolytic degradation of Sg I and II, the predominant coagulum components, since the disappearance of the gel structure parallels fragmentation of these proteins [10, 12, 24, 25, 39, 68, 69]. Fragmentation of Sg I and II probably reduces the extent of noncovalent interactions between these proteins and fibronectin, thus causing liquefaction, potentially by allowing movement of interacting surfaces that were limited in the uncleaved backbone. This is reflected in the fact that intact Sg molecules in seminal vesicle fluid or seminal coagulum are insoluble due to the creation of a vast network of protein aggregates, whereas Sg fragments of liquefied semen are soluble. Originally, a collagenase-like activity [64], seminin and plasminogen activator [70] were associated with the liquefying activity. However, it is now clear that PSA plays the major role in degrading the structural components of the coagulum, Sg I and Sg II [4, 10, 12, 37, 40, 69, 71]. In those studies, purified PSA was shown to cleave the predominant coagulum proteins in a manner essentially identical to their degradation during semen liquefaction and in parallel to the progressive release of motile spermatozoa. The predominant role of PSA in Sg I degradation and semen liquefaction is further substantiated by the observation that various polypeptides isolated from seminal plasma have terminal amino acid residues corresponding to cleavage of Sg I at leucine or tyrosine residues, sites associated with the chymotrypsin-like proteolytic activity of PSA [13–15, 37, 60, 72].

By integrating the various properties of semen coagulum and Sg I and II described above, it is possible to propose a mechanism for semen coagulation and liquefaction (fig. 3). Prior to ejaculation, Sg I and Sg II present in the lumen of seminal vesicles may be in a random, unordered arrangement and protected from proteolysis by PCI also originating from the same gland. In prostatic secretions, zinc present at concentrations up to 7 mM [42] is likely to inhibit most of PSA activity [37, 48, 73]. As mentioned previously, Sg I is

likely a zinc-binding protein. At ejaculation, Sg I might chelate most of the free zinc contributed by prostatic secretions. Chelation of such a high concentration of zinc is not impossible considering that Sg I may be present in semen at concentrations as high as 0.4–0.6 mM (20–30 mg/ml) and that each molecule may bind several zinc atoms. Zinc binding might induce structural changes in the Sg I and II molecules that would trigger noncovalent interactions with fibronectin and aggregation of the proteins, culminating in the formation of the insoluble protein complex of the coagulum and immobilization of spermatozoa. The resulting sudden decrease in free zinc concentration would cause PSA activation and release the interaction of Sg I and II with PCI, allowing PSA to cleave the coagulum proteins and causing the gradual release of free PCI into the soluble liquefied phase [54] and gradual Sg I proteolysis. As Sg I would become progressively hydrolyzed into smaller polypeptides, interactions between the proteins would be loosened, causing semen liquefaction. Zinc would be released due to structural changes around the metal coordinating residues of the protein caused by the cleavage of the polypeptide backbone, and sperm motility would increase gradually. In agreement with this hypothesis is the finding that several histidine residues that are the likely metal-binding residues in Sg I were found in the proximity of various PSA cleavage sites [37]. Finally, the resulting increase in free zinc would again inactivate PSA, thus preventing further undesirable proteolysis of semen components and structural damage to spermatozoa or surrounding tissues. Soluble PCI might also reform a complex with PSA, although only a small proportion of total PSA is likely inactivated by PCI [53]. While this model remains speculative in nature, it provides a mechanistic link between various properties of Sg I and the observations on the role of divalent metal ions in the phenomenon of semen coagulation-liquefaction [4, 24, 25, 37, 44, 74], and supports a physiological function for seminal zinc.

Sg I as a physiological sperm motility inhibitor?

As described previously, a fragment of Sg I was originally purified from seminal plasma as a factor that could inhibit the motility of demembranated spermatozoa likely acting as a dynein-ATPase inhibitor [21]. However, both degraded fragments of Sg I and the purified intact protein were also found to inhibit intact sperm motility in vitro [7, 22]. This effect, together with the observed change in inhibitory activity as Sg I gets degraded during semen liquefaction and the ensuing increase in sperm motility, suggests that Sg I may be a physiological sperm motility inhibitor [7, 24, 25]. The exact mechanism by which Sg I may exert its sperm-immobilizing activity on intact spermatozoa is still unknown. Nonetheless, it is possible to draw some inferences as to the nature of this activity. Obviously, physical entrapment of spermatozoa within the coagulum structure may represent one direct form of immobilization by restricting movement of the sperm flagellum. However, such a mechanism is unlikely to explain the complete flagellum beating arrest observed in experiments using soluble purified Sg I [7]. Moreover, the soluble 19-kDa Sg I fragment isolated from liquefied seminal plasma was also shown to inhibit intact sperm motility [22], while it had obviously lost the ability to form a coagulated mass. There are thus additional effects that likely come into play in the mechanism of sperm immobilization.

As previously mentioned, immunolocalization studies using various anti-Sg monoclonal and polyclonal antibodies as well as the MHS-5 demonstrated the presence of Sg antigens on the postacrosomal sheath of the head, midregion and tail of human spermatozoa [13, 34, 38]. The localization of Sg antigens to sperm structures involved in motility fits well with the sperm motility inhibitory activity that has been associated with Sg I [7]. While a more detailed microscopic study has not been performed, the findings that the motility of Sg I-immobilized spermatozoa can be recovered by washing the cells and that prostatic extracellular proteases inactivate Sg I and allow recovery of sperm motility [7] argue in favor of a surface-mediated mode of action. Sg I thus likely binds to the sperm surface in these regions and affects motility through a mechanism yet to be elucidated. However, the integration of various studies allows speculation about a possible mechanism. Receptor-binding proteins are usually present in trace amounts in biological fluids, and subtle differences in their concentrations trigger biological responses. As such, the mere abundance of Sg I in seminal plasma would tend to argue against such a receptor-mediated mechanism. On the other hand, the gradual decrease in sperm-immobilizing activity of the 52-kDa Sg I protein as it is cleaved suggests that a specific domain of the precursor molecule may be involved in maximizing the interaction between the active domain and its target site on spermatozoa, assuming that those two activities (sperm binding and immobilization) are located on separate domains. These observations share interesting similarities with previous findings concerning inhibin-like peptides. Originally, three different inhibin-like peptides were isolated from seminal plasma during a search for factors that could suppress pituitary follicle-stimulating hormone (FSH) secretion in vitro. Their complete amino acid sequences were elucidated and interestingly, all turned out to have primary structures corresponding to fragments of Sg I derived between residues 45 and 136 [13, 14]; α-inhibin-92, -52 and -31 correspond to residues 45–136, 85–136 and 85–115,

respectively (fig. 4). Moreover, Sg I fragments identical to α-inhibin-92 and -52 were obtained following PSA-mediated degradation of SPMI precursor and correspond well to the presumptive active (sperm-immobilizing) domain of Sg I [37]. Perhaps not so coincidentally, α-inhibin-92 and α-inhibin-52 were found to be 40- and 3.4-fold more active, respectively, than α-inhibin-31 in inhibiting FSH release in vitro [14]. The observed decrease in the activity of inhibin-like peptides as they are fragmented into smaller peptides is thus highly reminiscent of the drop of Sg I-immobilizing activity that occurs during its processing by PSA [7]. The affinity of larger SPMI and inhibin-like peptides for their respective target appears to be greater than that of smaller fragments and may thus be regulated by progressive proteolysis. While the mechanism of action of these peptides on the pituitary has not been clearly established, direct binding of the peptides to human pituitary membranes has been demonstrated [75]. Moreover, cross-linking experiments using labeled α-inhibin-92 and pituitary membrane revealed the presence of a specific 90-kDa complex [75]. It is therefore tempting to speculate that Sg I could similarly bind to a specific acceptor protein present on spermatozoa. Whether the pituitary receptor of inhibin-like peptides bears any similarity to the target site of Sg I on human spermatozoa remains to be established. A similar study attempting to identify such an Sg I ligand on the sperm membrane might yield clues to better define its action on spermatozoa. Such an Sg I 'receptor' on the sperm membrane might modulate its suggested immobilizing activity. Subsequent recovery of motility during liquefaction would then occur due to a decrease in affinity for the receptor following degradation by PSA in vivo, or by removal of the protein by washing the cells in vitro as we have observed [7].

Alternately, it cannot be excluded that Sg I might bind to membrane components other than proteins. Membrane phospholipids could also be a binding site for Sg I, as has been shown for a family of bovine seminal plasma proteins [76] and porcine seminal proteins called spermadhesins [77, 78]. Interestingly, one member of the latter family (AQN-3) has recently been shown to be almost identical to a component of boar SPMI [79]. While there is no obvious sequence homology between Sg I and AQN-3, the fact that they are both sperm motility inhibitors having similar properties [7, 21, 80] suggests some possible structural and functional homology. Interaction of Sg I with membrane phospholipids might cause destabilization of the bilayer and subsequent entry of extracellular components, such as ions, that might inhibit sperm motility in vivo. The composi-

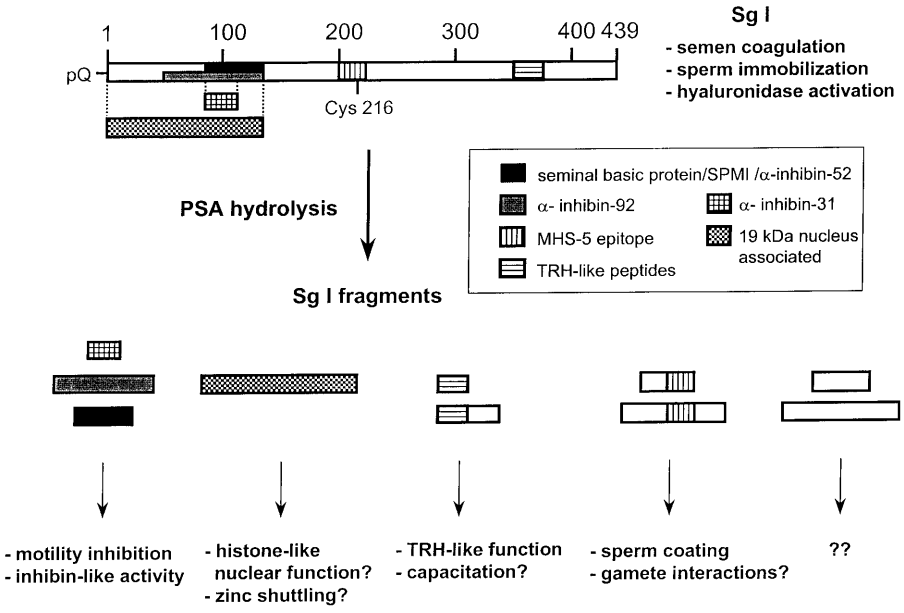


Figure 4. Functional map of Sg I and polypeptides released by hydrolysis. The whole Sg I mature protein is represented: pQ indicates the pyroglutamyl residue at the N-terminal of the mature protein. Segments of Sg I that have been associated with specific biological activity or reactivity with antibodies are highlighted with patterned boxes (see text). During semen liquefaction, PSA cleaves Sg I at the C-terminal of several leucine and tyrosine residues, releasing numerous polypeptides that may have various biological activities as stated. The peptides released are shown as a model only. Details of actual cleavage and derived peptides were previously described [16, 37].

tion of the phospholipid bilayer of the sperm plasma membrane is known to vary in different areas of the sperm surface. Preferential binding of Sg I to phospholipids that are predominantly present on the sperm tail and midpiece could provide an attractive explanation for the reported binding of Sg I to those areas of the sperm surface [34]. While the hydropathy index of the boar SPMI/AQN-3 shows a rather hydrophilic character, there are segments of the protein that could potentially form small hydrophobic domains that could interact with the sperm membrane, thus causing its destabilization and resulting in changes in motility [79]. A similar phenomenon in Sg I might also result in reversible interactions with the sperm membrane and effects on sperm motility. Interestingly, a synthetic peptide derived from an antimicrobial protein was found to have a reversible and dose-dependent sperm immobilizing activity in vitro [81]. Its effect was found to be specific, since five other cationic antimicrobial peptides did not similarly inhibit sperm motility. In addition, the peptide did not cause detergent-type membrane disruption but rather caused functional membrane changes. These characteristics are similar to those of Sg I or its fragments, and it is tempting to speculate that they may act similarly.

The possibility of a large protein readily penetrating into spermatozoa and reaching the dynein ATPase and acting similarly as on demembranated spermatozoa appears unlikely. However, the isolation and identification, in human sperm nuclei, of a 19-kDa polypeptide that appears to be a proteolytic product derived from the N-terminal domain of Sg [82] may suggest that at least some fragments of the SPMI precursor may also enter the cell. In addition, we have observed that both detergent soluble and insoluble fractions of spermatozoa washed twice on Percoll gradients to maximize the removal of seminal plasma proteins and proteins loosely associated with the sperm plasma membrane contained polypeptides recognized by the SPMI antiserum (M. Robert and C. Gagnon, unpublished observations). Interestingly, forms of mass greater than 20 kDa were observed in the insoluble fraction, supporting the notion that Sg I may have penetrated the cells and was thus partially protected from PSA hydrolysis occurring in seminal plasma. Thus, specific mechanisms that allow the penetration or translocation of seminal proteins through sperm membranes may exist.

While all the above observations do not yet allow us to draw conclusions about the exact target site of Sg I on spermatozoa, they do permit the elaboration of a potential model of action. By integrating these various observations, it is possible to postulate that under normal circumstances, Sg I binds to an as yet uncharacterized acceptor site on the flagellum of spermatozoa and immobilizes the cell. As the 52-kDa molecule gets degraded by PSA, its affinity for its acceptor site on spermatozoa is reduced by degradation of a 'docking domain', and the molecule is gradually released or remains bound in a degraded state that is no longer affecting the motility of normal spermatozoa. Note that such a mechanism would explain the progressive activation of sperm motility during semen liquefaction in terms of progressive inactivation of the inhibitory activity of Sg I on sperm motility rather than by activation of motility by Sg I fragments or other seminal components. However, the participation of such activating factors cannot be ruled out.

Alternately, in a subset of infertility cases where a large proportion of spermatozoa remain immobilized in spite of apparent normal semen liquefaction and Sg I processing into low mass fragments, a different mechanism more similar to the effect of Sg on demembranated spermatozoa might be involved. In such ejaculates, a large proportion of spermatozoa may have membrane defects that allow Sg I fragments to reach the intracellular space and directly interact with the axonemal dynein arms and cause irreversible arrest of motility and associated infertility. Interestingly, an earlier study [83] demonstrated that a motility inhibitor was present in cytosolic extracts prepared from spermatozoa displaying low motility, whereas extracts from normal spermatozoa did not contain such an inhibitor. Unfortunately, the lack of biochemical information on this immobilizing factor does not allow us to conclude whether Sg I was responsible for this activity.

The rationale for temporary sperm immobilization after ejaculation remains elusive. However, temporary sperm immobilization by Sg might serve as a mechanism to prevent energy exhaustion at times when sperm motility is not required [33]. In addition, the bulk of seminal plasma is removed by passage of spermatozoa through the viscous cervical mucus. We may therefore speculate that sperm immobilization by Sg I in the first few minutes post-ejaculation might have beneficial effects by providing sufficient time for seminal factors, affecting sperm function, to interact with spermatozoa before crossing the cervical mucus. Moreover, temporary immobilization of spermatozoa by Sg I and their physical protection from immune cells by entrapment within the coagulum may also provide the necessary time for soluble seminal factors to elicit immunosuppressive reactions in the female reproductive tract before the progressive release of spermatozoa.

Other functions for Sg I and its proteolytic fragments?

As elaborated above, the main functions of Sg I appear to be related to the coagulation and liquefaction of semen and the concomitant early immobilization and subsequent progressive release of motile spermatozoa in

Table 2. Established and potential functions of Sg I and/or of its fragments.

Function	References
Semen coagulation	[5, 12, 13]
Sperm motility inhibitor	[7, 37]
Sperm hyaluronidase activation	[8]
Inhibin-like activity	[14, 95]
Thyrotropin releasing hormone-like peptide	[72, 85]
Activation of sperm capacitation	[85]
Role in the sperm nucleus	[82]
Zinc shuttling	[87, 88]

the female reproductive tract. In addition, the purified predominant seminal coagulum proteins which appear identical to Sg I and II have recently been shown to be potent activators of sperm hyaluronidase activity [8]. This suggests the potential participation of those proteins in the degradation of the egg's envelope at fertilization and sperm penetration. Liquefied seminal plasma was found to retain the activity, suggesting that cleaved forms of Sg I are also active. Thus, the possibility that some of the numerous polypeptides released by PSA proteolysis of Sg I may have various biological activities is attractive. This hypothesis is supported by various findings described below and by the fact that PSA is a member of the kallikrein family of proteases, known for their growth factors and vasoactive polypeptide-processing activities [84]. Possible functions for such bioactive peptides released by Sg I hydrolysis include the inhibin-like activity associated seminal plasma polypeptides that appear to be derived from Sg residues 45 to 136 [13, 14] (table 2 and fig. 4). However, while binding of those peptides to specific acceptor sites on human pituitary membranes has been demonstrated [75], the targets and physiological effect of such inhibin-like activity on the reproductive tissues remain to be established. In addition, extended forms of thyrotropin-releasing hormone (TRH)-like peptides have been isolated from human seminal plasma [72]. These peptides correspond to sequences occurring between residues 350 and 374 of Sg I and are thus likely derived therefrom. The N-terminal of one of these peptides occurs at a position along the Sg I sequence that is consistent with cleavage by PSA at a leucine residue. The authors of this study noted that the peptides likely possess biological activity, since they observed the presence of an amide bond at the C-terminal, a feature believed to be essential for activity [72]. In addition, one such peptide was recently shown to increase the level of sperm capacitation [85]. However, the presence of a tryptophan residue adjacent to the C-terminal end of the peptides in Sg I instead of the usual glycine residue raises the possibility that they are generated from a precursor that is closely related to but different from Sg I [72, 85]. Obviously, the exact physiological role of such bioactive peptides as inhibin-like peptides and TRH-like peptides in human semen remains to be established. However, it is possible that they bind to acceptor sites on spermatozoa and modulate functions such as motility, hyperactivation, capacitation, egg-binding and so on. Alternatively, receptor sites might be within the female reproductive tract and trigger immunosuppressive reactions or muscle contraction to facilitate sperm transport along the reproductive tract, as observed for a kinin-like substance released by proteolysis of seminal vesicle proteins [86]. In addition, the isolation of a 19-kDa polypeptide in association with the human sperm nuclei and which appears to be derived from Sg I [82] may suggest yet an additional role for the protein within spermatozoa. This particular polypeptide had previously been believed to be a sperm-specific histone. It is also possible that Sg I and/or such a peptide fragment, through zinc-binding properties, may be responsible for the observed exchange of zinc between seminal plasma and spermatozoa [87, 88]. These polypeptides could serve as molecular shuttles in the exchange of zinc ions between seminal plasma and sperm nuclei, a phenomenon believed to be important form the integrity of sperm DNA [87, 88]. Obviously, a more detailed assessment of the physiological relevance of all these activities in seminal plasma will require further investigation. Nonetheless, these observations underline the fact that Sg I is an important seminal protein that plays a central role in the formation of semen coagulum and probably also in regulating sperm motility shortly after ejaculation. At the same time, it may serve as a precursor molecule which gives rise to multiple bioactive polypeptides having various functions.

Applications and benefits derived from Sg I

The unique properties, structure and pattern of expression of the Sg I gene and protein suggest various potential applications, some of which are already being harnessed. The MHS-5 antibody, which recognizes a specific Sg I epitope, is available commercially as an immunoassay kit for the detection of semen in forensic specimens. Its value rests principally on its high specificity for semen, its stability and its presence in semen from normal and vasectomized men [38, 89, 90]. In addition, the same antibody has also been used as a specific and reliable tool for the diagnostic of agenesis of the seminal vesicles and vas deferens [91], due to its specific reactivity with secretions of the seminal vesicles. One interesting and potentially promising application derived from Sg I and its derived proteolytic fragment has been recently proposed by Denmeade and co-workers [49] for the treatment of prostatic carcinoma. The

strategy is based on harnessing the high specificity and affinity of peptides derived from the Sg sequence for PSA. Peptide substrates that have been optimized for specificity and affinity for PSA might be used as carriers for the targeting of cytotoxic prodrugs. Ideally, these would be cleaved specifically in situ, releasing active drug exclusively in PSA-secreting prostatic tissue as well as metastatic prostate cancer cells while sparing non-prostatic normal tissues. Preliminary in vitro tests recently demonstrated that the cytotoxic prodrug doxorubicin coupled to the carboxyl terminal of one such peptide could be specifically activated by PSA released from lymph node carcinoma of the prostate (LNCaP) cells in culture [92], resulting in cell death. On the other hand, TSU cells which do not secrete PSA were unaffected by 10-fold higher doses of the prodrug. While this magic bullet strategy appears promising, it remains to be seen whether location-specific hydrolysis and cytotoxicity will be observed in vivo. In addition, the usefulness of the unique characteristics of the Sg genes and related genes of the REST family in the unraveling of evolutionary mysteries related to male accessory sex gland evolution and function is already being harnessed in various studies [30, 33, 93, 94]. Finally, the high specificity and unique tissue distribution of Sg I may make it an attractive candidate for further studies on gene expression, particularly as it relates to the male accessory reproductive glands. It may allow the characterization of novel gene regulatory mechanisms in these androgen-dependent tissues and the identification of specific transcription factors at play in glands whose functions remains widely unknown and unexplored.

Conclusion

Overall, the properties of Sg I and II and their degradation products are gradually becoming clearer. Sg I appears to be mainly involved in the coagulation of semen and the concomitant immobilization of spermatozoa observed in semen shortly after ejaculation. Proteolytic processing by PSA causes semen liquefaction and a progressive increase in sperm motility. Abnormal processing of Sg I by PSA may result in low sperm motility and infertility. Recent findings on Sg I gene and protein structure, associated biochemical characteristics and molecular processing are paving the way for further functional studies on the role of this protein and its degradation peptides. Such investigations are necessary to better understand the various functions of what appears to be a complex and multifunctional protein. The fact that multiple and very different activities can be associated with the same precursor molecule and its degradation products is also of significant biological interest. An attempt to integrate these functions and to uncover the nature of the interactions and mechanism of action of these polypeptides during the reproductive process would constitute a logical follow-up to previous studies. Since the primary structure of the Sg gene and protein is well established, mutation and deletion studies followed by expression in a suitable experimental system could be attempted to answer these interrogations. Such studies will possibly enable better understanding of the modulation of sperm function by seminal plasma components, a biological fluid that had been traditionally neglected and considered to be simply the carrier of spermatozoa. The recent findings about Sg I alone suggest that it is much more than that. Sustained developments in this field may consequently lead to novel approaches to the diagnosis and treatment of infertility, elucidation of gene expression regulation in male accessory sex glands as well as the development of pioneering therapies for the treatment of prostate cancer.

Acknowledgments. The authors are grateful to Dr. E. de Lamirande and D. White for sustained help and advice and to Lina Ordonselli for secretarial assistance. The support of the Medical Research Council of Canada to C.G. is gratefully acknowledged.

1 Tauber P. F. and Zaneveld L. J. (1981) Coagulation and Liquefaction of Human Semen. In: Biochemical Andrology, pp. 153–166, Hafez E. S. E. (ed.), Mosby, St. Louis
2 Amelar R. D. (1962) Coagulation, liquefaction and viscosity of human semen. J. Urol. **87:** 187–190
3 Zaneveld L. J., Tauber P. F., Port C., Propping D. and Schumacher G. F. (1974) Scanning electron microscopy of the human, guinea-pig and rhesus monkey seminal coagulum. J. Reprod. Fertil. **40:** 223–225
4 Lilja H. and Laurell C. B. (1984) Liquefaction of coagulated human semen. Scand. J. Clin. Lab. Invest. **44:** 447–452
5 Lilja H. and Laurell C. B. (1985) The predominant protein in human seminal coagulate. Scand. J. Clin. Lab. Invest. **45:** 635–641
6 Chaistitvanich N. and Boonsaeng V. (1983) Molecular structure of human seminal coagulum: the role of disulfide bonds. Andrologia **15:** 446–451
7 Robert M. and Gagnon C. (1996) Purification and characterization of the active precursor of a human sperm motility inhibitor secreted by the seminal vesicles: identity with semenogelin. Biol. Reprod. **55:** 813–821
8 Mandal A. and Bhattacharyya A. K. (1995) Sperm hyaluronidase activation by purified predominant and major basic human seminal coagulum proteins. Hum. Reprod. **10:** 1745–1750
9 Lilja H., Laurell C. B. and Jeppsson J. O. (1984) Characterization of the predominant basic protein in human seminal plasma, one cleavage product of the major seminal vesicle protein. Scand. J. Clin. Lab. Invest. **44:** 439–446
10 Lilja H. (1985) A kallikrein-like serine protease in prostatic fluid cleaves the predominant seminal vesicle protein. J. Clin. Invest. **76:** 1899–1903
11 Lilja H. and Weiber H. (1984) Synthetic protease inhibitors and post-ejaculatory degradation of human semen proteins. Scand. J. Clin. Lab. Invest. **44:** 433–438
12 Lilja H., Oldbring J., Rannevik G. and Laurell C. B. (1987) Seminal vesicle-secreted proteins and their reactions during gelation and liquefaction of human semen. J. Clin. Invest. **80:** 281–285

13 Lilja H., Abrahamsson P. A. and Lundwall A. (1989) Semenogelin, the predominant protein in human semen. Primary structure and identification of closely related proteins in the male accessory sex glands and on the spermatozoa. J. Biol. Chem. **264:** 1894–1900

14 Li C. H., Hammonds R. G. Jr., Ramasharma K. and Chung D. (1985) Human seminal alpha inhibins: isolation, characterization and structure. Proc. Natl. Acad. Sci. USA **82:** 4041–4044

15 Schneider K., Kausler W., Tripier D., Jouvenal K. and Spiteller G. (1989) Isolation and structure determination of two peptides occurring in human seminal plasma. Biol. Chem. Hoppe Seyler **370:** 353–356

16 Lilja H. (1990) Cell biology of semenogelin. Andrologia **22:** 132–141

17 Lindemann C. B. and Gibbons I. R. (1975) Adenosine triphosphate-induced motility and sliding of filaments in mammalian sperm extracted with Triton X-100. J. Cell Biol. **65:** 147–162

18 Mohri H. and Yanagimachi R. (1980) Characteristics of motor apparatus in testicular, epididymal and ejaculated spermatozoa. A study using demembranated sperm models. Exp. Cell Res. **127:** 191–196

19 de Lamirande E., Bardin C. W. and Gagnon C. (1983) Aprotinin and a seminal plasma factor inhibit the motility of demembranated reactivated rabbit spermatozoa. Biol. Reprod. **28:** 788–796

20 de Lamirande E. and Gagnon C. (1983) Aprotinin and a seminal plasma factor provide two new tools to study the regulation of sperm motility. J. Submicrosc. Cytol. **15:** 83–87

21 Iwamoto T. and Gagnon C. (1988) Purification and characterization of a sperm motility inhibitor in human seminal plasma. J. Androl. **9:** 377–383

22 Iwamoto T. and Gagnon C. (1988) A human seminal plasma protein blocks the motility of human spermatozoa. J. Urol. **140:** 1045–1048

23 Luterman M., Iwamoto T. and Gagnon C. (1991) Origin of the human seminal plasma motility inhibitor within the reproductive tract. Int. J. Androl. **14:** 91–98

24 Robert M. and Gagnon C. (1994) Sperm motility inhibitor from human seminal plasma: presence of a precursor molecule in seminal vesicle fluid and its molecular processing after ejaculation. Int. J. Androl. **17:** 232–240

25 Robert M. and Gagnon C. (1995) Sperm motility inhibitor from human seminal plasma: association with semen coagulum. Hum. Reprod. **10:** 2192–2197

26 Lilja H. and Lundwall A. (1992) Molecular cloning of epididymal and seminal vesicular transcripts encoding a semenogelin-related protein. Proc. Natl. Acad. Sci. USA **89:** 4559–4563

27 Malm J., Hellman J., Magnusson H., Laurell C. B. and Lilja H. (1996) Isolation and characterization of the major gel proteins in human semen, semenogelin I and semenogelin II. Eur. J. Biochem. **238:** 48–53

28 Ulvsback M., Lazure C., Lilja H., Spurr N. K., Rao V. V., Loffler C. et al. (1992) Gene structure of semenogelin I and II. The predominant proteins in human semen are encoded by two homologous genes on chromosome 20. J. Biol. Chem. **267:** 18080–18084

29 Lundwall A. (1996) The structure of the semenogelin gene locus – nucleotide sequence of the intergenic and the flanking DNA. Eur. J. Biochem. **235:** 466–470

30 Lundwall A. and Lazure C. (1995) A novel gene family encoding proteins with highly differing structure because of a rapidly evolving exon. FEBS Lett. **374:** 53–56

31 Aumuller G., Seitz J., Lilja H., Abrahamsson P. A., von der Kammer H. and Scheit K. H. (1990) Species- and organ-specificity of secretory proteins derived from human prostate and seminal vesicles. Prostate **17:** 31–40

32 Ulvsback M. and Lundwall A. (1997) Cloning of the semenogelin II gene of the rhesus monkey. Duplications of 360 bp extend the coding region in man, rhesus monkey and baboon. Eur. J. Biochem. **245:** 25–31

33 Lundwall A. (1998) The cotton-top tamarin carries an extended semenogelin I gene but no semenogelin II gene. Eur. J. Biochem. **255:** 45–51

34 Bjartell A., Malm J., Moller C., Gunnarsson M., Lundwell A. and Lilja H. (1996) Distribution and tissue expression of semenogelin I and II in man as demonstrated by in situ hybridization and immunocytochemistry. J. Androl. **17:** 17–26

35 Luke M. C. and Coffey D. S. (1994) Human androgen receptor binding to the androgen response element of prostate specific antigen. J. Androl. **15:** 41–51

36 Mandal A. and Bhattacharyya A. K. (1994) Isolation of the predominant coagulum protein of human semen before liquefaction. Hum. Reprod. **9:** 320–324

37 Robert M., Gibbs B. F., Jacobson E. and Gagnon C. (1997) Characterization of prostate-specific antigen proteolytic activity on its major physiological substrate, the sperm motility inhibitor precursor/semenogelin I. Biochemistry **36:** 3811–3819

38 Herr J. C., Summers T. A., McGee R. S., Sutherland W. M., Sigman M. and Evans R. J. (1986) Characterization of a monoclonal antibody to a conserved epitope on human seminal vesicle-specific peptides: a novel probe/marker system for semen identification. Biol. Reprod. **35:** 773–784

39 McGee R. S. and Herr J. C. (1987) Human seminal vesicle-specific antigen during semen liquefaction. Biol. Reprod. **37:** 431–439

40 McGee R. S. and Herr J. C. (1988) Human seminal vesicle-specific antigen is a substrate for prostate–specific antigen (or P-30). Biol. Reprod. **39:** 499–510

41 Herr J. C., Conklin D. J. and McGee R. S. (1989) Purification of low molecular weight forms of seminal vesicle specific antigen by immunoaffinity chromatography on bound monoclonal antibody MHS-5. J. Reprod. Immunol. **16:** 99–113

42 Lynch M. J., Masters J., Pryor J. P., Lindon J. C., Spraul M., Foxall P. J. et al. (1994) Ultra high field NMR spectroscopic studies on human seminal fluid, seminal vesicle and prostatic secretions. J. Pharm. Biomed. Anal. **12:** 5–19

43 Mandal A. and Bhattacharyya A. K. (1990) Biochemical composition of washed human seminal coagulum in comparison to sperm-free semen from the same donors. J. Reprod. Fertil. **88:** 113–118

44 Frenette G., Tremblay R. R. and Dube J. Y. (1989) Zinc binding to major human seminal coagulum proteins. Arch. Androl. **23:** 155–163

45 Peter A., Lilja H., Lundwall A. and Malm J. (1998) Semenogelin I and semenogelin II, the major gel-forming proteins in human semen, are substrates for transglutaminase. Eur. J. Biochem. **252:** 216–221

46 Grant F. J., Taylor D. A., Sheppard P. O., Mathewes S. L., Lint W., Vanaja E. et al. (1994) Molecular cloning and characterization of a novel transglutaminase cDNA from a human prostate cDNA library. Biochem. Biophys. Res. Commun. **203:** 1117–1123

47 Akiyama K., Nakamura T., Iwanaga S. and Hara M. (1987) The chymotrypsin-like activity of human prostate–specific antigen, gamma-seminoprotein. FEBS Lett. **225:** 168–172

48 Watt K. W., Lee P. J., M'Timkulu T., Chan W. P. and Loor R. (1986) Human prostate-specific antigen: structural and functional similarity with serine proteases. Proc. Natl. Acad. Sci. USA **83:** 3166–3170

49 Denmeade S. R., Lou W., Lovgren J., Malm J., Lilja H. and Isaacs J. T. (1997) Specific and efficient peptide substrates for assaying the proteolytic activity of prostate-specific antigen. Cancer Res. **57:** 4924–4930

50 Coombs G. S., Bergstrom R. C., Pellequer J. L., Baker S. I., Navre M., Smith M. M. et al. (1998) Substrate specificity of prostate-specific antigen (PSA). Chem. Biol. **5:** 475–488

51 Lundwall A. and Lilja H. (1987) Molecular cloning of human prostate specific antigen cDNA. FEBS Lett. **214:** 317–322

52 Vihinen M. (1994) Modeling of prostate specific antigen and human glandular kallikrein structures. Biochem. Biophys. Res. Commun. **204:** 1251–1256

53 Christensson A. and Lilja H. (1994) Complex formation between protein C inhibitor and prostate-specific antigen in vitro and in human semen. Eur. J. Biochem. **220:** 45–53

54 Kise H., Nishioka J., Kawamura J. and Suzuki K. (1996) Characterization of semenogelin II and its molecular interaction with prostate-specific antigen and protein C inhibitor. Eur. J. Biochem. **238:** 88–96

55 Paradis G., Tremblay R. R. and Dube J. Y. (1989) Looking for human glandular kallikrein-1 in the prostate. Prostate **15:** 343–353
56 Deperthes D., Chapdelaine P., Tremblay R. R., Brunet C., Berton J., Hebert J. et al. (1995) Isolation of prostatic kallikrein hK2, also known as hGK-1, in human seminal plasma. Biochim. Biophys. Acta **1245:** 311–316
57 Yu J. X., Chao L. and Chao J. (1994) Prostasin is a novel human serine proteinase from seminal fluid. Purification, tissue distribution, and localization in prostate gland. J. Biol. Chem. **269:** 18843–18848
58 Deperthes D., Frenette G., Brillard-Bourdet M., Bourgeois L., Gauthier F., Tremblay R. R. et al. (1996) Potential involvement of kallikrein hK2 in the hydrolysis of the human seminal vesicle proteins after ejaculation. J. Androl. **17:** 659–665
59 Lilja H. and Jeppsson J. O. (1985) Amino acid sequence of the predominant basic protein in human seminal plasma. FEBS Lett. **182:** 181–184
60 Kausler W. and Spiteller G. (1992) Analysis of peptides of human seminal plasma by mass spectrometry. Biol. Mass Spectrom. **21:** 567–575
61 Mann T. and Lutwak-Mann C. (1981) Male Reproductive Function and Semen. Themes and Trends in Physiology, Biochemistry and Investigative Andrology, Springer, Berlin
62 Aumuller G. and Seitz J. (1990) Protein secretion and secretory processes in male accessory sex glands. Int. Rev. Cytol. **121:** 127–231
63 Williams-Ashman H. G. (1984) Transglutaminases and the clotting of mammalian seminal fluids. Mol. Cell. Biochem. **58:** 51–61
64 Lukac J. and Koren E. (1979) Mechanism of liquefaction of the human ejaculate. II. Role of collagenase-like peptidase and seminal proteinase. J. Reprod. Fertil. **56:** 501–506
65 Polak B. and Daunter B. (1989) Seminal plasma biochemistry. IV: Enzymes involved in the liquefaction of human seminal plasma. Int. J. Androl. **12:** 187–194
66 Polak B. and Daunter B. (1990) Seminal plasma biochemistry. III: Characterization of coagulum components. Eur. J. Obstet. Gynecol. Reprod. Biol. **35:** 223–234
67 Montagnon D., Valtat B., Vignon F. and Koll-Back M. H. (1990) Secretory proteins of human seminal vesicles and their relationship to lipids and sugars. Andrologia **22** (Suppl. 1)**:** 193–205
68 Boonsaeng V. (1986) Molecular structure of human seminal coagulum: the role of proteolysis. Andrologia **18:** 252–258
69 Lee C., Keefer M., Zhao Z. W., Kroes R., Berg L., Liu X. X. et al. (1989) Demonstration of the role of prostate-specific antigen in semen liquefaction by two-dimensional electrophoresis. J. Androl. **10:** 432–438
70 Hisazumi H. (1970) Studies on the fibrinolytic activity in human semen studied by gel filtration. Invest. Urol. **7:** 410–421
71 Flickinger C. J., Herr J. C., McGee R. S., Sigman M., Evans R. J., Sutherland W. M. et al. (1990) Dynamics of a human seminal vesicle specific protein. Andrologia **22:** 142–154
72 Khan Z. and Smyth D. G. (1993) Isolation and identification of N-terminally extended forms of 5-oxoprolylglutamylprolinamide (Glp-Glu-Pro-NH2), a thyrotropin-releasing-hormone (TRH)-like peptide present in human semen. Eur. J. Biochem. **212:** 35–40
73 Schaller J., Akiyama K., Tsuda R., Hara M., Marti T. and Rickli E. E. (1987) Isolation, characterization and amino-acid sequence of gamma-seminoprotein, a glycoprotein from human seminal plasma. Eur. J. Biochem. **170:** 111–120
74 Daunter B., Hill R., Hennessey J. and Mackay E. V. (1981) Seminal plasma biochemistry. I. Preliminary report: a possible mechanism for the liquefaction of human seminal plasma and its relationship to spermatozoal motility. Andrologia **13:** 131–141
75 Ramasharma K. and Li C. H. (1987) Characteristics of binding of human seminal alpha-inhibin-92 to human pituitary membranes. Proc. Natl. Acad. Sci. USA **84:** 3595–3598
76 Desnoyers L. and Manjunath P. (1992) Major proteins of bovine seminal plasma exhibit novel interactions with phospholipid. J. Biol. Chem. **267:** 10149–10155
77 Dostalova Z., Calvete J. J. and Topfer-Petersen E. (1995) Interaction of non-aggregated boar AWN-1 and AQN-3 with phospholipid matrices. A model for coating of spermadhesins to the sperm surface. Biol. Chem. Hoppe Seyler **376:** 237–242
78 Ensslin M., Calvete J. J., Thole H. H., Sierralta W. D., Adermann K., Sanz L. et al. (1995) Identification by affinity chromatography of boar sperm membrane-associated proteins bound to immobilized porcine zona pellucida. Mapping of the phosphorylethanolamine-binding region of spermadhesin AWN. Biol. Chem. Hoppe Seyler **376:** 733–738
79 Iwamoto T., Hiroaki H., Furuichi Y., Wada K., Satoh M., Osada T. et al. (1995) Cloning of boar SPMI gene which is expressed specifically in seminal vesicle and codes for a sperm motility inhibitor protein. FEBS Lett. **368:** 420–424
80 Iwamoto T., Tsang A., Luterman M., Dickson J., de Lamirande E., Okuno M. et al. (1992) Purification and characterization of a sperm motility-dynein ATPase inhibitor from boar seminal plasma. Mol. Reprod. Dev. **31:** 55–62
81 D'Cruz O. J., Pereira H. A. and Haas G. G. Jr. (1995) Sperm immobilizing activity of a synthetic bioactive peptide 20–44 of 37-kDa cationic antimicrobial protein (CAP37) of human neutrophils. J. Androl. **16:** 432–440
82 Zalensky A. O., Yau P., Breneman J. W. and Bradbury E. M. (1993) The abundant 19-kilodalton protein associated with human sperm nuclei that is related to seminal plasma alpha-inhibins. Mol. Reprod. Dev. **36:** 164–173
83 de Lamirande E., Sherins R. J. and Gagnon C. (1986) The presence of a motility inhibitor within spermatozoa may explain the poor sperm motility of some infertile men. J. Androl. **7:** 215–219
84 Clements J. A. (1989) The glandular kallikrein family of enzymes: tissue-specific expression and hormonal regulation. Endocr. Rev. **10:** 393–419
85 Huber A. E., Fraser H., del Rio-Garcia J., Kreil G. and Smyth D. G. (1998) Molecular cloning in the marmoset shows that semenogelin is not the precursor of the TRH-like peptide pGlu-Glu-Pro amide. Biochim. Biophys. Acta **1387:** 143–152
86 Fichtner J., Graves H. C., Thatcher K., Yemoto C. and Shortliffe L. M. (1996) Prostate specific antigen releases a kinin-like substance on proteolysis of seminal vesicle fluid that stimulates smooth muscle contraction. J. Urol. **155:** 738–742
87 Kjellberg S., Bjorndahl L. and Kvist U. (1992) Sperm chromatin stability and zinc binding properties in semen from men in barren unions. Int. J. Androl. **15:** 103–113
88 Kvist U., Kjellberg S., Bjorndahl L., Soufir J. C. and Arver S. (1990) Seminal fluid from men with agenesis of the Wolffian ducts: zinc-binding properties and effects on sperm chromatin stability. Int. J. Androl. **13:** 245–252
89 Herr J. C. and Woodward M. P. (1987) An enzyme-linked immunosorbent assay (ELISA) for human semen identification based on a biotinylated monoclonal antibody to a seminal vesicle-specific antigen. J. Forensic Sci. **32:** 346–356
90 Keil W., Bachus J. and Troger H. D. (1996) Evaluation of MHS-5 in detecting seminal fluid in vaginal swabs. Int. J. Legal Med. **108:** 186–190
91 Calderon I., Barak M., Abramovici H., Gruener N., Yavez H., Paz G. et al. (1994) The use of a seminal vesicle specific protein (MHS-5 antigen) for diagnosis of agenesis of vas deferens and seminal vesicles in azoospermic men. J. Androl. **15:** 603–607
92 Denmeade S. R., Nagy A., Gao J., Lilja H., Schally A. V. and Isaacs J. T. (1998) Enzymatic activation of a doxorubicin-peptide prodrug by prostate-specific antigen. Cancer Res. **58:** 2537–2540
93 Hagstrom J. E., Fautsch M. P., Perdok M., Vrabel A. and Wieben E. D. (1996) Exons lost and found. Unusual evolution of a seminal vesicle transglutaminase substrate. J. Biol. Chem. **271:** 21114–21119
94 Lundwall A. (1996) The cloning of a rapidly evolving seminal-vesicle-transcribed gene encoding the major clot-forming protein of mouse semen. Eur. J. Biochem. **235:** 424–430
95 Ramasharma K., Sairam M. R., Seidah N. G., Chretien M., Manjunath P., Schiller P. W. et al. (1984) Isolation, structure and synthesis of a human seminal plasma peptide with inhibin-like activity. Science **223:** 1199–1202