Prostasomes are Pluripotent and Well–Organized Organelles in Human Semen

by Gunnar Ronquist

ABSTRACT

Prostasomes are submicron, membrane-surrounded organelles produced by the epithelial cells of the human prostate gland and are present in appreciable amounts in normal human semen. The prostasomes are ascribed many functional effects. They have an immunosuppressive capacity by inhibiting the lymphoproliferation and the phagocytosis of macrophages. The prostasomes also regulate the complement activation. They possess an antioxidant capacity. The prostasomes are also able to attach onto washed, prostasome-free spermatozoa and promote the forward motility of the sperm cells.

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Prostasomes es organelles submicron, membrana-rodeados producidos por las células epiteliales de la glándula humana de la próstata y está presente en cantidades apreciables en semen humano normal. Los prostasomes se atribuyen muchos efectos funcionales. Tienen una capacidad inmunosupresiva inhibiendo el lymphoproliferation y la fagocitosis de macrófagos. Los prostasomes también regulan la activación del complemento. Poseen una capacidad antioxidante. Los prostasomes pueden también asociar sobre spermatozoa lavados, prostasome-libres y promover el motility delantero de las células de la esperma.

KEY WORDS: Prostasome, prostate, semen

Prostasomes as part of prostate secretion

The prostatic secretion in most species is a slightly acidic fluid that is less viscid and proteinaceous than the seminal vesicle secretion. The acinar, epithelial cells are responsible for the secretory activity. In addition to these cells in the epithelial lining of prostatic tissue there are basal and endocrine-paracrine (APUD) cells. Two different modes of release of secretory material from glandular epithelial cells have been demonstrated in male accessory genital glands, *viz.* merocrine and apocrine secretion [1]. Merocrine secretion starts with the translation on ribosomes of rough endoplasmic reticulum where a characteristic hydrophobic N-terminal amino acid sequence is synthesized [1]. Posttranslational modifications and formation of secretory granules take place in the Golgi apparatus. Secretory granules are transferred to the apical plasma membrane, where they release their contents after fusion of granule membrane with the apical plasma membrane [1]. Subsequently, secretory proteins were identified which lack the typical N-terminal sequence, and are synthesized on free ribosomes inside the cytoplasm [2]. Apocrine secretion has been suggested as a potential release mechanism for this type of protein, *i.e.* the release of secretory material *via* apical protrusions or blebs in the absence of secretory granules [3,4]. The secretion depends not only on the synthesizing activity of the epithelial cells, but also on transudation from serum. The prostatic contribution to an average ejaculate (3.5 mL) usually is 0.5-1.0 mL [5]. The fluid is notable for its high content of monovalent and divalent cations, citric acid and many enzymes, and most of the seminal spermine and spermidine is produced by the prostate gland [5]. Besides the soluble compounds, the prostate gland secretes a particulate fraction organised in well defined organelles named prostasomes [6], first described in 1978 [7]. There is no strong support to the idea of an apocrine secretion of prostasomes. The membrane of these organelles exhibits a very high cholesterol/phospholipid ratio, 2:1, and a high amount of sphingomyelin, about 50%, [8,9] contrary to plasma membranes in general and the corresponding figures for human benign prostatic hyperplasia epithelial cells are 0.5:1, and 8%, respectively [10]. Similarly, these latter figures agree with those of human spermatozoa [11,12]. In stead, since the prostasomes appear in their intracellular context as being encased in a larger organelle, a storage vesicle [13], they may be released as small, intact organelles in the prostatic fluid (and semen) by an ordinary exocytotic event involving the membrane surrounding the storage vesicle and the plasma membrane of the prostatic secretory cell [13,37].

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The organelles are encased usually by a lipid bilayered membrane and they have a corpuscular appearance with a mean diameter of 150 nm, range 40-500 nm [14]. The prostasomes have a density of 1.03 when analyzed by continuous silica density gradient centrifugation [15] in that respect behaving as typically cellular organelles. They do not contain any cytosol but they may contain small spherical particles of approximately 15 nm in diameter [16].

A reduced amount of prostasomes in seminal plasma was observed in a patient with Klinefelter's disease who had the serum testosterone level reduced by 50% [17]. In another patient, with a well differentiated carcinoma of the prostate,

the secretion of prostasomes was reduced by 85% after 2 weeks of treatment with an antiandrogenic drug (Flutamide) [18]. These observations suggest a role for testosterone in the secretion of prostasomes.

Some biochemical features of prostasomes

Neuroendocrine components. Besides a high content of sphingomyelin and a high cholesterol/phospholipid ratio [8,9], the membrane architecture of prostasomes is otherwise also complex, and 2-dimensional gel-electrophoresis of prostasomes has revealed about 80 different protein entities [19,20]. The presence of neuroendocrine markers as chromogranin B, neuropeptide Y, and vasoactive intestinal polypeptide in about equimolar amounts has been demonstrated by radioimmunoassay measurement and immunoelectron microscopy of human prostasomes [21]. Chromogranin A has been found in about 2% of that amount [21]. It has also been shown that prostasomes express a newly described common secretory granule protein, *viz.* granulophysin [22]. This molecule has a similar structure as the neuroprotein synaptophysin [23], which has been used as a marker for endocrine, neuroendocrine, and neuronal tissue [24]. In neurones, synaptophysin is located in the small synaptic vesicles that contain the classic neurotransmittors, while the chromogranin family of proteins is generally associated with the large dense core vesicles that contain neuropeptides [24,25]. From that point of view it is possible that prostasomes consist of a mixture of both kinds of vesicles, which also would fit with the very wide range in organellar size. However, another possibility could be that prostasomes are a new kind of vesicles sharing properties common to both types of vesicles.

Tissue factor. Tissue factor is a plasma membrane-associated glycoprotein that serves as a receptor and essential cofactor for factors VII and VIIa of the coagulation cascade [26]. The complete protein molecule consists of 263 amino acid residues, has a derived molecular weight of 29593, and contains three potential N-linked carbohydrate chains on its extracellular domain. As a potent initiator of coagulation, tissue factor has critical functions in hemostasis and thrombogenesis [27]. In addition, tissue factor is involved in the functional exertion of the cellular immune response and in the pathogenesis of certain infections [28]. Seminal plasma contains procoagulant activity, and it was recently found by immunoelectron microscopy that tissue factor antigen was located on the surfaces of prostasomes, conforming with the idea that prostasomes harbour all of the tissue factor of seminal plasma [29]. Tissue factor possesses other functions, independent of triggering blood clotting [27], which may contribute to some of the known functional activities of the prostasomes. Specifically, prostasomes bind to spermatozoa [14] and protect them from inflammatory reactions developed in the female genital tract [30,31]. Tissue factor could play a role in this protective defence of spermatozoa. Although no information is available about the effects of various cytokines on regulation of properties of prostasomes, cytokines might modulate tissue factor availability as they do for inflammatory cells [29].

Dipeptidylpeptidase IV/CD26. Dipeptidylpeptidase IV (DPP IV) is a membrane – associated serine protease cleaving dipeptides from the N-terminal end of proteins and peptides with a primary specificity for proline. Otherwise, peptide bonds containing a proline residue are resistant to a wide variety of peptidases and proteases. DPP IV is omnipresent in mammalian tissues, specifically on epithelial cells, endothelial cells and lymphocytes. On human T-lymphocytes, DPP IV is assigned to the CD26 cluster of activation antigen [32]. DPP IV/CD26 takes part in interleukin-2 secretion, T-cell proliferation and recruitment of cytotoxic T-lymphocytes [32]. Signalling by CD26 has an absolute requirement for the expression of the T-cell receptor CD3 complex [33,34]. An extremely high specific activity of DPP IV was noted in prostasomes when comparing several different tissues and cells [35]. Subsequently, DPP IV/CD26 was identified as an abundantly occurring antigen on the prostasomal membrane by monoclonal antibodies against prostasomes [36]. Interestingly, DPP IV/CD26 may play a role in HIV infection and apoptosis [34]. The HIV-1 Tat (trans-activating) protein binds and inhibits the activity of DPP IV [38], and the HIV-1 envelope glycoprotein gp120 also interacts with DPP IV [39]. In this way, prostasomes may bind HIV viruses via prostasome membrane-bound DPP IV. In this context it is worthy of note that prostasomes also contain complement inhibitors such as CD46 [40], CD55, and CD59 [41]. Complement inhibitors may be present in semen to protect the spermatozoa, but it has been suggested that they may also protect pathogens [42]. The interaction between complement inhibitors and virus has been investigated in model systems. The HIV virus, when incubated with CD55 and CD59, acquires these inhibitors in its membrane, which increases its resistance to attack by complement [43]. Since these complement inhibitors do exist on the prostasome membrane surface, the same working mechanism may be valid in the presence of prostasomes. Hence, we may here discern a new principle, by which prostasomes render HIV virus an advantage of survival in human semen.

Functional characteristics of prostasomes

Sperm motility. Buffer washings of normozoospermic spermatozoa result in a gradual loss of their forward motility [6,44]. The perturbation that is brought about by the buffer treatment is not of an irreversible nature since the spermatozoa can be functionally restored rather momentaneously by the addition of prostasomes [6,45]. Furthermore, the spermatozoa were metabolically capable of exploiting the energy potential of hexoses as evidenced by the corroborative action by any of fructose, glucose or mannose on prostasome-promotive effect on sperm forward motility [45]. The motility pattern evoked by prostasomes is similar to that of albumin although prostasomes are

superior to albumin in every respect when compared on an equal protein basis [44,45]. It should be kept in mind that prostasomes being organelles surrounded by a typical biologic membrane, only expose a fraction of their total protein content to the external milieu. Since the membrane is rich in lipids with an unusually high cholesterol/ phospholipid ratio [8], a direct comparison is not feasible on a protein basis between prostasomes and albumin. Prostasomes, however, are more efficient, since they render a higher proportion of forwardly motile spermatozoa with a higher amplitude of lateral head displacement , both parameters being positively correlated to the fertilizing potential of spermatozoa [46].

The mechanism by which prostasomes initiate forward motility in buffer-washed spermatozoa is not known. However, prostasomes interact with spermatozoa [14] and may affect several membrane properties, including membrane permeability to Ca^{2+} and H^{+}. Another possibility might be an effect via cyclic AMP of vasoactive intestinal polypeptide, being a constituent of prostasomes [21], on spermatozoa. Results of previous research implicate intracellular cyclic AMP to be related to induction of sperm motility [47,48]. Analogously, a higher recovery of motile spermatozoa is obtained after swim-up if albumin in the ordinary standard balanced salt solution is exchanged for prostasomes or if this solution containing albumin is supplemented with prostasomes [46]. Prostasomes also increase the number of hyperactivated spermatozoa [46], which is thought to be important for penetration and fertilization [49]. It is suggested that swim-up media containing prostasomes might also improve the recovery of hyperactivated motile spermatozoa from semen samples with a reduced number of motile spermatozoa. An increase in the number of post-thaw motile spermatozoa by prostasome inclusion in swim-up medium is also evident [50], and this adds credence to the view that prostasomes could be of benefit in increasing the fertilization rate achieved in assisted reproductive technologies.

Immunosuppressive activity of prostasomes. The prostasomes have been identified as inhibitors in lymphoproliferation assays [31,51]. This activity accounts for a significant proportion of the immunosuppressive activity of human seminal plasma [30]. Since the prostasomes have the ability to adhere to spermatozoa [14], there is the probability that the immunosuppressive effect associated with the prostasomes can be carried up the female genital tract with the spermatozoa [30]. Pure preparations of prostasomes inhibit mitogen-induced lymphoproliferation in a dose-dependent manner with a concentration of prostasomes equivalent to 40% of that seen in seminal plasma giving 69% suppression of thymidine incorporation [30]. A direct effect of prostasomes on macrophage function is also apparent [31].

Prostasomes bind rapidly to the leukocyte cell membrane followed by internalization of adsorbed material. Interaction of prostasomes with neutrophils and monocytes inhibits their ability to phagocytose latex particles [31]. Similarly, endocytosis by the cells of prostasomes may suppress their ability to generate oxygen radicals [31]. Hence, phagocytosing cells will ingest prostasomes and consequently will become more or less inactivated. This would promote the survival of spermatozoa in the female genital tract.

Prostasomes contain in their membrane the MAC (membrane attack complex) inhibitory protein CD59 [41]. There are reasons to believe that CD59 is carried on the surface of the prostasomes in a GPI (glycosylphosphatidylinositol) anchor and that spermatozoa may acquire CD59 molecules as a result of interaction with the prostasomes [41]. Accordingly, prostasomes may represent a pool of CD59 from which protein lost from spermatozoa, possibly as a result of normal membrane turnover or of low level C attack, may be replenished, thus ensuring that the sperm cells will advance in the female reproductive tract being guarded against the membrane attack complex.

Antioxidant capacity. Reactive oxygen species (ROS) are a major cause of idiopathic male infertility. An abnormally high production of ROS has been shown in 40% of semen samples from infertile individuals, while very few were found in samples from fertile donors [52]. Human spermatozoa are very sensitive to oxidative stress, resulting in peroxidative damage. This sensitivity is due to the high content of unsaturated fatty acids in their plasma membranes and their small cytoplasmic volume, which limits their scavenging potential [53]. Controversy still exists regarding the origin of ROS in semen, but leukocytes infiltrating the semen, particularly the polymorphonuclear neutrophils seem to be the major source of ROS generation [54]. It has been shown that prostasomes can interact with neutrophils and reduce their capacity to produce superoxide anion after stimulation [31]. Hence, it was apparent that prostasomes could play a role as an antioxidant factor. This theme was further elaborated by Saez et al. [54]. These authors noticed that prostasomes had the ability to reduce ROS production by sperm preparations containing polymorphonuclear neutrophils. Their conclusion was that the inhibitory effect of prostasomes on ROS production probably involved an interaction between prostasomes and polymorphonuclear neutrophils, since prostasomes do not directly act as ROS scavengers. They therefore launched what they called *"the membrane-based hypothesis"* regarding the mechanism involved in the inhibition of ROS production by polymorphonuclear neutrophils in semen. The hypothesis was based upon an observed rigidification of the plasma membrane of the neutrophils that occurred when the neutrophils were incubated with prostasomes. This rigidification could either be explained by a fusion process between prostasomes and neutrophils or by an exchange of cholesterol from prostasomes to neutrophils [54].

Prostasomes in other species

Prostasome-like vesicles have been described in ram [55],

dog [56], and stallion [57,58]. In addition, a bovine organelle production and secretion in seminal vesicles have been described with no corresponding formation in the prostate gland of the bull and these particles have been denoted vesiculosomes [59]. Hence, there is some evidence that an interplay between prostasomes or prostasome-like vesicles and spermatozoa may be a common phenomenon in reproduction.

Concluding remarks

Prostasomes are prostate-derived submicron organelles occurring in human semen. They have several biological activities but their physiological function is still not settled.

The membrane surrounding the prostasomes exhibits a very high cholesterol/phospholipid ratio yielding high molecular ordering. The organelles have an immunosuppressive capacity by inhibiting the lymphoproliferation and the phagocytosis of macrophages. In addition, they regulate the complement activation and possess an antioxidant capacity. It is assumed that the prostasomes, by coating the sperm cells, convey their various abilities to the sperm cells, and as a consequence the sperm cells would be garded against attack from the female immune system. Prostasomes also promote forward motility of sperm cells. Hence, prostasomes seem to be pluripotent in their ability to sustain and promote the sperm cells in their ultimate goal to reach and fertilize the ovum.

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