Urol Res (1982) 10:253–257

Urological Research

Promotive Effect on Human Sperm Progressive Motility by Prostasomes

B. Stegmayr[1] and G. Ronquist[2]

1 Department of Internal Medicine, Central Hospital, Eskilstuna, Sweden
2 Department of Clinical Chemistry, University Hospital, Uppsala, Sweden

Accepted: July 14, 1982

Summary. Seminal plasma constituents were separated on Sephadex G200 gel columns. The column eluate was analysed with regard to protein content, ATPase activity and promotive activity on sperm progressive motility. Two different chromatographic fractions were also subjected to electron microscopy after sedimentation by preparative ultracentrifugation. A maximum promotive value on sperm progressive motility coincided with a maximum ATPase activity value in a single peak from seminal plasma eluted first on the column and containing less protein than the other peaks appearing later in the chromatogram. This first peak was the only one containing ATPase activity and membrane-surrounded organelles named prostasomes. Other peaks, rich in protein but lacking ATPase and prostasomes, displayed a moderate and rather irregular pattern in reference to promotive activity on sperm progressive motility. Evidence is given that the positive effect by prostasomes is specific on sperm progressive motility. Hence, procedures aiming at a change of membrane integrity of the prostasomes resulted in diminished effects on sperm progressive motility. This could be explained by a probable dissipation of the electrochemical gradient of calcium ions.

Key words: Prostasomes, Sperm progressive motility, Electronmicroscopy, ATPase activity, Calcium, Magnesium ions, Ionophore A23187, Deoxycholate.

Introduction

The role of seminal plasma in human reproduction with special regard to sperm motility is controversial [3]. One hypothesis is that the exposure of spermatozoa to seminal plasma is of limited functional importance, since it lasts for too short a time. There are also findings favouring the view that a contact between spermatozoa and prostatic fluid is not obligatory for the fertilising ability of oocytes [4, 16]. The observations of Lindholmer [5] are however contradictory to that, since he found that progressive motility of human epididymal spermatozoa was initiated by prostatic fluid. The complexity of the problem is also illustrated by the work of Mooney et al. [7] who found an increasing percentage of motile spermatozoa towards the cauda epididymidis in man. Nevertheless, it can be concluded that a man with completely immotile sperms is sterile in otherwise normal sexual union and his immotile sperms, live or dead, cannot penetrate cervical mucus [15].

We observed previously that a sedimentation of material from seminal plasma and prostatic fluid occurred on preparative ultracentrifugation after dilution 1:9 with a buffer and after spermatozoa and cell debris had been removed in a preceding centrifugation step at low speed [10]. This material, named pellet II, contained secretory granules and vesicles as well as an amorphous substance [10]. The organelles, later denoted prostasomes [2], contained calcium and to a lesser extent magnesium and zinc in concentrations that exceeded several fold those of seminal plasma [12]. Furthermore, the pellet II material containing prostasomes and amorphous substance was found to favour progressive motility of washed spermatozoa in a distinct way [13]. Hence, the progressive motility of washed spermatozoa resuspended in a buffer supplemented with pellet II material was threefold higher than when seminal plasma lacking pellet II material was the suspending medium [13]. However, it was not elucidated whether prostasomes or amorphous substance or both contributed to the positive effect on progressive motility.

The aim of the present study was to further investigate the possible role of prostasomes rather than amorphous substance in the sustenance of progressive motility and the mechanism or mechanisms behind such an activity.

Materials and Methods

Patients

Semen was obtained from 22 men, mean age 33 years, range 26–45. Twelve (A) of them were admitted due to infertility in their mar-

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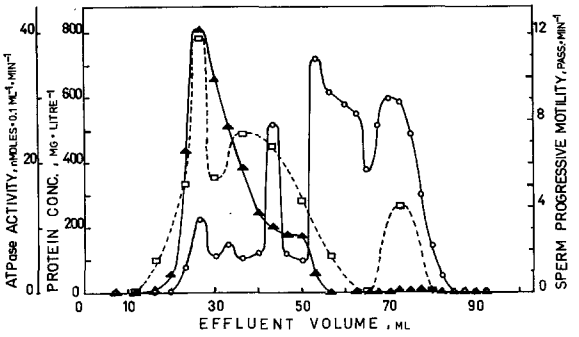

Fig. 1. Sephadex G200 column chromatography of seminal plasma from one man. ATPase activity (▲——▲), total protein content (○——○) and promotive effect on sperm progressive motility (□– – –□) were determined in each tube containing 4–6 ml of eluate

Table 1. Failure of resuspended pellet II material on dilution to promote sperm progressive motility. Pellet II was obtained from 1 ml of seminal plasma and resuspended (and diluted) to initial volume in an isotonic Tris-HCl buffer (0.05 M, pH 7.32) containing 138 mM NaCl and 1 mM $MgCl_2$. The amount of pellet II material was decreasing in accordance with the scale given while the number of spermatozoa was kept constant in all experiments

Dilution of resuspended pellet II material	Sperm progressive motility, passages x min^{-1}
1/1	56
1/2	24
1/4	15
1/8	10

riages. Ten (B) of the men were examined before and after voluntary sterilisation by vasectomy. Spermiograms of these men showed variations in sperm contents from 123 to 540 x 10^6. Morphologically abnormal spermatozoa varied between 29%–78%. Sperm progressive motility was subjectively graded in all samples and they displayed at least good sperm motility in a 4-graded scale [13]. Semen from vasectomised subjects (B) was devoid of spermatozoa. In addition, spermatozoa were obtained from 21 other men (26–39 years) with good sperm motility, i.e. 71%–82% and 7.2–27.8 passages/1 x 10^6/min [13]. These freshly voided spermatozoa were used for the progressive motility experiments.

General Procedure

Ejaculates from all men were initially centrifuged for 10 min at 2,500 x g and 22 °C. The supernatant fluid (seminal plasma) was separated from the sedimented spermatozoa and possible cell debris.

The seminal plasma was chromatographed directly in some experiments (Fig. 1). In others it was subjected to a preparative ultracentrifugation in order to obtain pellet II material (containing prostasomes) and supernatant II. This was in accordance with a previously described method [10] with the exception that the seminal plasma was not diluted. This was necessary, since dilution of seminal plasma resulted in volumes too large for subsequent column chromatography.

The pellet II material was resuspended in the isotonic Tris buffer mentioned above to the initial volume of seminal plasma. This pellet II suspension was used in some experiments as such (Table 1). It was also subjected to different pretreatment procedures before incubation with washed spermatozoa such as freezing and thawing four times under hypotonic conditions to destroy membrane integrity of prostasomes. Alternative pretreatment procedures were preincubation for 20 min at 22 °C with either 10 nmoles/l of ionophore A23187 or 0.01%–0.1% of deoxycholate to induce leakiness of the prostasomes for calcium ions (Table 2). In other experiments it was subjected to column chromatography on Sephadex G200 (Fig. 2).

Table 2. Effect of different pretreatment procedures of pellet II material on ATPase and promotive activity on sperm progressive motility. Pellet II was obtained from 1 ml seminal plasma and resuspended to initial volume in an isotonic Tris-HCl buffer (0.05 M, pH 7.32) containing 138 mM NaCl and 1 mM $MgCl_2$. Freezing and thawing four times was performed under hypotonic conditions and isotonicity was restored before ATPase and progressive motility measurements. The concentration of ionophore, A23187 was 10 nmoles/l in resuspending buffer while that of deoxycholate was 0.1%

Pretreatment procedure of pellet II material	ATPase activity, percent of control value	Pellet II-promotive activity on sperm progressive motility, percent of control value
None (control)	100	100
Freezing and thawing	88(85[a])	71(29[a])
Ionophore, A23187	98–101(n = 2)	0–52(n = 4)
Deoxycholate	14(93[b])	4(41[b])

[a] determination after further incubation for 5 h at 22 °C
[b] 0.01% deoxycholate

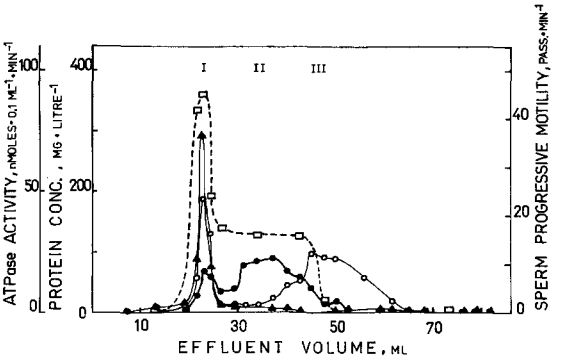

Fig. 2. Sephadex G200 column chromatography of resuspended pellet II material obtained after preparative ultracentifugation of seminal plasma of one man. ATPase activity (▲——▲), TCA-precipitable (●——●) and non-precipitable (○——○) protein as well as promotive effect on sperm progressive motility (□– – –□) were determined in each tube containing 4–6 ml of eluate

Column Chromatography on Sephadex G200

Sephadex G200 was obtained from Pharmacia Fine Chemicals, Uppsala, Sweden. It was allowed to equilibrate with the isotonic Tris buffer. This and subsequent procedures were performed at 4 °C.

Material (Seminal plasma, pellet II, supernatant II) in volumes never exceeding 7% of Sephadex volume, was applied to columns containing 35 ml Sephadex G200. Elution was performed with the isotonic Tris buffer mentioned before, that was brought in with the aid of a peristaltic pump.

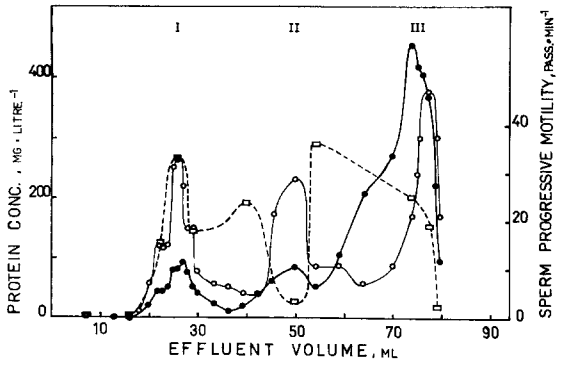

Fig. 3. Sephadex G200 column chromatography of residing supernatant (supernatant II) after recovery of pellet II material from one man. Measureable ATPase activity was absent. TCA-precipitable (●——●) and non-precipitable (○——○) protein as well as promotive effect on sperm progressive motility (□– – –□) were determined in each tube containing 4–6 ml of eluate

For the purpose of progressive motility studies, spermatozoa were carefully washed three times at 22 °C in an isotonic, 0.05 M Tris-HCl buffer, pH 7.32 containing 138 mM NaCl and 1 mM $MgCl_2$. During washing the centrifugations never exceeded 1,800 x g. Attempts were made to remove the supernatant buffer-solution as completely as possible after each centrifugation. The washed spermatozoa were resuspended in the above-mentioned buffer. The volume was adjusted to give convenient sperm densities for the progressive motility studies and final sperm concentration in the assays was the same within series (although not between series).

Analytical Procedures. Sperm progressive motility was determined by recording the number of sperm passages over a 0.25 mm line per min in a Bürker hematocytometer as described [13]. ATPase was measured spectrophotometrically in the presence of magnesium ions in accordance with a previous method [10].

Protein was determined according to Lowry et al. [6] with an equine serum (Seronorm, Nygaard Company, Oslo, Norway) as standard. In some experiments the protein content was measured directly in the eluted samples (total protein content). In others the determination was preceded by precipitation with trichloroacetic acid (TCA) in a final concentration of 7%. Hence, protein was determined in the acid-precipitated material as well as in the residing non-precipitable fluid (Figs. 2 and 3).

Electron Microscopy. Sephadex G200-chromatographed seminal plasma was used for this study. Four different semen samples were investigated in this way. Preparative ultracentrifugation was performed for 14 h at 38,000 x g and at 2 °C on initial eluate corresponding main ATPase activity and volumes of 25–35 ml. The same procedure was employed for the eluted protein-enriched samples. They appeared later in the chromatogram, separated from the ATPase peak, and comprised 22–32 ml of eluate. The sedimented material thus obtained was pre-fixed in an isotonic, phosphate-buffered, glutaraldehyde solution (3%). The subsequent procedures were in accordance with a previous paper [14].

Results

The separation pattern of seminal plasma on Sephadex G200 chromatography from a healthy man with a normal spermiogram is given in Fig. 1. The first peak contained the ATPase activity parallel to a moderate amount of total protein. Furthermore, a coinciding peak for stimulation of sperm progressive motility was distinct. Three major peaks of total protein were additionally obtained (Fig. 1). Less distinct peaks representing stimulatory effects on sperm progressive motility were also found. All ATPase activity was confined to the first single peak. This chromatogram was representative for all seminal plasmas investigated in this way.

When instead resuspended pellet II material was chromatographed, fewer peaks were obtained. Again, one single peak representing ATPase activity was seen. This peak also contained elevated recordings for sperm progressive motility, the maximum value of which coincided with that of the ATPase activity. A TCA-precipitable protein peak was also corresponding, although the major part of TCA-precipitable protein appeared later in the chromatogram. The distribution pattern for TCA non-precipitable protein was similar (Fig. 2). The same type of chromatogram could be repeated using pellet II material from azoospermic semen of vasectomised men with normal, preoperative spermiograms.

The resulting supernatant after recovery of pellet II material upon preparative ultracentrifugation of seminal plasma was also subjected to chromatography on Sephadex G200. Very low or no activity of ATPase could be detected anywhere in the eluted material and accordingly, no distinct peak for ATPase was achieved. However, three major peaks for TCA precipitable and non-precipitable proteins were found. Additionally, a rather irregular pattern for the promotive activity on sperm progressive motility was seen (Fig. 3).

The promotive activity of pellet II material on sperm progressive motility was dose-dependent with decreasing activity on dilution (Table 1). Table 2 illustrates the effects of different pretreatment procedures on ATPase activity and on the motility-promotive effect of pellet II material vis à vis washed spermatozoa. Repeated freezing and thawing as well as preincubation with either ionophore A23187 or deoxycholate resulted in small effects on the ATPase activity (except for 0.1% deoxycholate) while the decreased effect on sperm progressive motility was conspicuous.

The eluted material corresponding to peak I in Fig. 1 containing ATPase activity (effluent volumes 20–50 ml) was after preparative ultracentrifugation further investigated in transmission electron microscopy. Prostasomes were clearly identified while amorphous substance was absent (Fig. 4a). When instead the same procedure was repeated for the major protein component eluted concomitantly with effluent volumes 53–80 ml in the chromatogram (Fig. 1), exhibiting no ATPase activity, only amorphous substance was identified in transmission electron microscopy (Fig. 4b). These ultrastructural findings were the same regardless of whether the starting material originated from a fertile man with a normal spermiogram or a voluntarily infertile man after vasectomy.

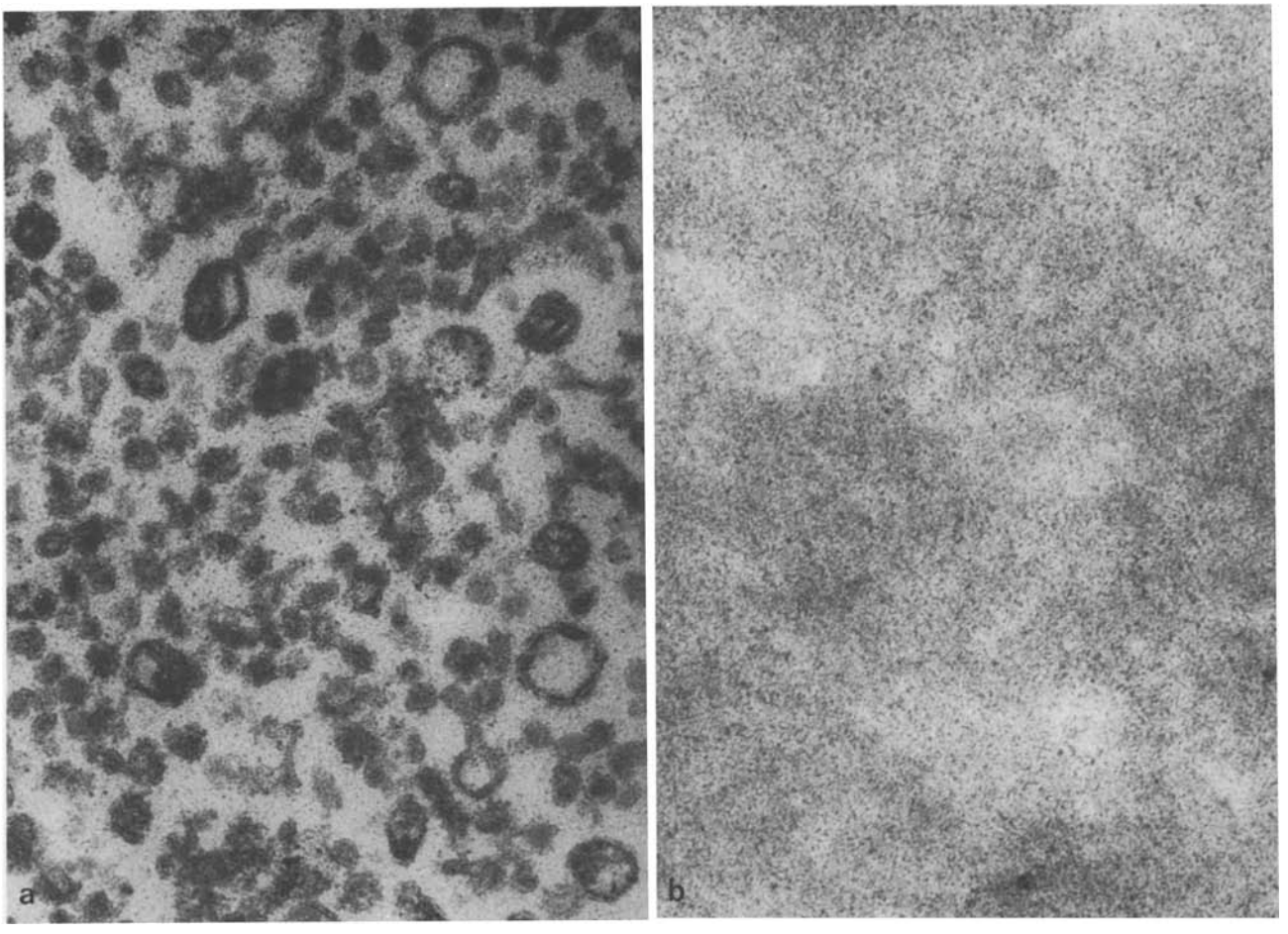

Fig. 4. Ultrastructure of two different fractions of seminal plasma eluted on Sephadex G200 column chromatography (Fig. 1). These fractions were subjected to preparative ultracentrifugation and the sedimented material was pre-fixed and embedded for electron microscopy as has been given in Methods (**a**) First fraction, containing ATPase activity and a minor protein peak, consists exclusively of prostasomes with no amorphous substance present (x 122,000). (**b**) Second fraction with major protein content but with no ATPase activity consists only of amorphous substance (x 118,000)

Discussion

The present study demonstrates a link between stimulatory activity on sperm progressive motility and ATPase activity of eluates from seminal plasma using Sephadex G200 chromatography. Accordingly, the maximum value for ATPase activity coincided with maximum stimulation of sperm progressive motility. Since the ATPase activity is firmly associated with prostasome structures [10, 12] a dose-response-relationship existed between the prostasomes and spermatozoa under these conditions (cf. Table 1). What is more, the presence of prostasomes in the first ATPase-bearing peak was established by electron microscopy. However, the promotive activity on sperm progressive motility was not solely confined to this single peak. Minor peaks containing this property vis à vis spermatozoa were additionally found (Figs. 1 and 3). These latter peaks may represent an "unspecific" protein activity, where metal-chelating properties of some proteins may favour progressive motility (cf. Fig. 1. in [13]).

It is less probable that such a protein activity is the working principle also for the prostasomes eluted first in the chromatogram, since this fraction represented less protein than the other fractions. Hence, it is quite clear from the present investigation that the first portion eluted on the column (comprising prostasomes and ATPase activity) contained the minor part and the last portion the major part of protein. This is interesting to note, since we found the same phenomenon in a previous study [1] when employing quite a different technique for separation of prostasomes and amorphous substance. The ultrastructural examination of the first fraction from column chromatography revealed membrane-surrounded organelles, i.e. mainly intact prostasomes. These prostasomes were also reported of in previous reports [1, 10, 14]. Such ordered structures of glycoproteins, lipoproteins, lipids and phospholipids probably do not react as easily with metals in such an unspecific way.

Evidence for a specific behaviour of the prostasomes vis à vis spermatozoa was also given by the data of Table 2. Here, different pretreatments, that had in common a decreased or possibly a dissipated chemical gradient of calcium ions across the prostasome membrane, all led to diminished effects by the prostasomes on sperm progressive motility. Pretreatment with 0.1% deoxycholate also resulted in decreased ATPase activity confirmatory of a previous finding [10]. A reduction in sperm progressive motility occured after this treatment. Freezing and thawing on the other hand may well preserve ATPase activity while membrane integrity and thereby ion fluxes are changed (cf. [11]). An even more specific effect on calcium fluxes is rendered by the ionophore A23187, that has been reported to bind calcium ions and transport them along the gradient across lipid membranes [9]. An effect of this antibiotic on ATPase activity in vitro should therefore not be anticipated. Hence, it is reasonable to believe that changed fluxes of calcium ions through the prostasome membranes resulted in a decreased promotive effect on sperm progressive motility.

In conclusion, there are strong reasons to believe that the motility-promoting effect of the prostasomes is specific. Sperm cells exhibit a pronounced responsiveness to variations in the calcium ion content of the environment [8]. Therefore, we suggest a role for prostasomes, mainly by their modulation, of calcium ion composition in the microenvironment of spermatozoa, resulting ultimately in stimulation of sperm progressive motility. The possible role of the ATPase system of prostasomes in vectorial transport of calcium ions across the membrane has been discussed elsewhere [10] and the calcium concentration within the prostasome exceeds that of the surrounding medium many times [12].

The method presented here to separate constituents of seminal plasma seems to be a promising tool for further studies in this area. The method is simple and rapid. Furthermore, it seems to be a satisfactory way to isolate constituents of physiological importance in reproduction research.

Acknowledgements. This study was supported by grants from the Swedish Medical Research Council, project No B81-03X-05916-01A and the Södermanland County Council. We wish to thank Mrs. B. Stegmayr, Mrs. A.-B. Henriksson, Mrs. K. Smidt and Mrs. A. Sörensen for skilful technical assistance.

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Dr. G. Ronquist
Department of Clinical Chemistry
University Hospital
Uppsala
Sweden