Bovine Sperm Forward Motility Protein

PARTIAL PURIFICATION AND CHARACTERIZATION*

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A protein, present in bovine seminal plasma, initiates forward motility in immature, immotile caput spermatozoa that have been incubated with a cyclic AMP phosphodiesterase inhibitor. An improved motility assay was developed to study this process and the protein involved. This forward motility protein exhibits multiple forms when fractionated on the basis of charge or molecular weight. Molecular sieving in urea or sodium dodecyl sulfate and dithiothreitol results in a single peak of activity which will re-form the larger aggregates in the absence of these agents. The molecular weight of this monomeric motility protein, as estimated from molecular sieving under these dissociating conditions, is 37,500.

The forward motility protein can be partially purified by heat treatment, gel chromatography in urea, and affinity chromatography on concanavalin A/agarose. Enzymatic treatments further suggest a glycoprotein nature, i.e., treatment with β-galactosidase, neuraminidase, α-mannosidase, or galactose oxidase reduces its activity by 50%; treatment with trypsin completely abolishes forward motility protein activity. On the basis of concurrent studies on the activity, properties, and distribution of forward motility protein in bovine body fluids, it is suggested that this protein is involved in the development of the capacity for motility as sperm traverse the epididymis.

The biochemical factors responsible for regulating motility in mammalian sperm are largely unknown. Recently, however, cyclic AMP (cAMP) has been shown to play an important role in both the initiation and maintenance of this process (1). For example, washed, mature bovine spermatozoa from either semen or the cauda epididymis become motile after CAMP levels are elevated by the addition of phosphodiesterase inhibitors. These cells become quiescent as the cAMP levels decline (1–5).

Although all of the components of the cyclic nucleotide second-messenger system, i.e., adenylate cyclase (3–6), cAMP phosphodiesterase (6), cAMP-dependent protein kinase (7–9), and phosphoprotein phosphatase (10) are present and functional in mammalian sperm, how an increase in cAMP is ultimately expressed as increased motility is not known. During studies on this process, specifically on identification of phosphorylated "motility proteins," i.e., protein kinase substrates, in mature sperm from the bovine cauda epididymis, we noted that cells inactivated by simple incubation at 37°C for 90 min took on several properties purportedly characteristic of immature sperm from the caput epididymis (5). That is, they became immotile, glycolyzed at reduced rates, and contained higher levels of ATP. Significantly, they also contained lower levels of cAMP than control (zero time) sperm cells. Since it is during passage through the epididymis, a process of approximately 11 days duration in the bull (11), that sperm acquire the capacity for motility, this suggested to us that caput sperm might be immotile as a consequence of a low cAMP level. Support for this idea was obtained when cAMP levels in the two cell types were compared and caput sperm were found to contain significantly lower cyclic nucleotide levels (9). Our subsequent attempts to initiate motility in these cells by adding phosphodiesterase inhibitors, however, resulted only in a twitching motion in some 30 to 50% of the cells (5). Forward motion was not induced. Recently, however, we have shown that forward progression can be induced in caput sperm by a factor present in seminal plasma, provided only that intracellular cAMP levels are increased simultaneously (5).

This report describes the development of an assay for this seminal plasma forward motility protein and its partial purification and characterization. It is likely that this protein is involved in the initiation of motility in the epididymis since parallel studies on epididymal fluids indicate that seminal plasma forward motility proteins are present in the epididymis and that these proteins may be bound to sperm during epididymal transit.

EXPERIMENTAL PROCEDURES

Materials—Theophylline, α-methyl-D-mannoside, phosphorylase α, cytochrome c, ribonuclease A, human γ-globulin, ovalbumin, lysozyme, aldolase, chymotrypsinogen A, Fraction V bovine albumin, soybean trypsin inhibitor, Bacillus subtilis α-amylase, almond β-glucosidase, yeast α-glucosidase, bovine epididymal α-L-fucosidase, jack bean α-mannosidase, Polyporus circinatus galactose oxidase, and jack bean concanavalin A (the latter insolubilized on hepdary agarose) were purchased from Sigma Chemical Co. Ampholine solutions and methyl-3-isobutylxanthine were purchased from LKB Instruments and Aldrich Chemical Co., respectively, and ICY 83, 197 was a gift from Imperial Chemical Industries, Ltd. Urea and sodium dodecyl sulfate (SDS) were recrystallized from absolute ethanol. Ficol, Sepharose, and Sephadex were obtained from Pharmacia, and DEAE-cellulose and Dowex AG 2-X100 (200 to 400 mesh) were from Bio-Rad Laboratories. Trypsin, Clostridium perfringens neuraminidase, and Escherichia coli β-galactosidase were purchased from Worthington Biochemical Corp. Freshly ejaculated bovine semen was supplied by All West Breeders, Inc. Burlington, Wash.

Preparation of Sperm and Seminal Plasma—Sperm were sedi-
ментed from seminal plasma by centrifugation at 800 x g for 20 min at 4°C and the resultant supernatant was recentrifuged at 178,000 x g at 3°C. Heat treatment consisted of incubation for 10 min at 90°C followed by centrifugation at 178,000 x g for 1 h at 0°C. Centrifugation was repeated, after the supernatant was decanted, to remove residual high molecular weight gelatious material.

Bovine testicles, with tunica intact, were obtained from a local abattoir and used within a few hours. The distal caput portion of the epididymis was removed, cleaned of connective tissue and blood vessels, and minced. Sperm and epididymal fluid were extracted from the minced tissue with 5 ml of room temperature assay buffer (40 mM KCl, 102 mM NaCl, 5 mM MgSO<sub>4</sub>, 10 mM KH<sub>2</sub>PO<sub>4</sub>, and 20 mM Hepes, pH 7.5) to which albumin (1 mg/ml) had been added. This extract was layered on 20 ml of 8% Ficoll in the same buffer and centrifuged first for 5 min at 900 x g and then for 5 min at 1150 x g (12). This two-step centrifugation reduced the amount of material trapped by the sperm as they moved into the Ficoll. The sperm pellet was resuspended in assay buffer containing albumin (5 mg/ml). The presence of albumin allowed sperm suspensions to be stored for several hours at room temperature prior to use.

**Forward Motility Assay**—An aliquot of this concentrated sperm suspension was added to a second tube, also at room temperature, containing albumin (5 mg/ml) and assay buffer. Aliquots, chosen to give 2.5 x 10<sup>6</sup> sperm/ml as a final density, were then added at timed intervals to a final incubation tube, now at 37°C, which contained a phosphodiesterase inhibitor. Usually 33 mM theophylline. 10 mM glucose, albumin (10 mg/ml), and some quantity of material to be tested for forward motility activity. After incubation for 20 min, activated sperm were placed on a thermostated (37°C) hemacytometer (0.1 mm thickness) and a photograph was taken with high field illumination. The length of each sperm track was measured and an average velocity (v), in micrometers per s, computed. The number of sperm tracks (n) and total number of sperm (N) were used to calculate a forward motility index (FMI) by means of the following equation:

\[
FMI = \frac{n}{N} \times 100
\]

Corrections were made for differences between the photographic field and the counting field and the units were micrometers per s at the standard assay conditions (see previous description).

**Separation Methods**—Sepharose 6B gel filtration and DEAE-cellulose chromatography were carried out as described under "Results." Molecular weight determinations were carried out on columns (1.5 x 60 cm) with flow adapters. All runs contained dextran blue and 0.1 M H<sub>2</sub>PO<sub>4</sub> as electrode solutions. A section of gel was removed, mixed with 50 to 500 mg of heat-treated, dialyzed seminal plasma, and focused for 16 h. The slab was divided into 30 fractions, and the counting field and the units were micrometers per s at the pH was determined at 4°C with a Brinkmann surface electrode. Slices containing 5 ml of Ampholine, mixed, poured into the bed, dried to either dinitrophenol-glycine or FMN as markers as well as the sample and molecular weight standards.

Preparative isoelectric focusing was performed in a gerninar gel matrix on a horizontal slab apparatus (LKB Instruments) by the method of Radola (13, 14). Five grams of prewashed and dried Sephadex G-75 superfine was added to 100 ml of distilled water containing 5 ml of Ampholine, mixed, poured into the bed, dried to near the crack point, and prefocused for 2 h at 4°C with 0.1 M NaOH and 0.1 M H<sub>2</sub>PO<sub>4</sub> as electrode solutions. A section of gel was removed, mixed with 50 to 500 mg of heat-treated, dialyzed seminal plasma, and focused for 16 h. The slab was divided into 30 fractions, and the pH was determined at 4°C with a Brinkmann surface electrode. Slices were eluted with 2 volumes of assay buffer and dialyzed extensively against assay buffer at 4°C.

For affinity chromatography, concanavalin A, isobilrubinized on agarose, was washed with 1 bed volume of 0.5 M α-methyl-d-mannoside and then 100 bed volumes of 1 M NaCl, both in 0.1 M Tris-HCl, pH 7.4, before use. The column was equilibrated with 1 mM CaCl<sub>2</sub>, 1 mM MgCl<sub>2</sub>, 0.1 M NaCl, and 20 mM Hepes, pH 7.4. The dialyzed samples were applied in the same buffer and washed with several column bed volumes prior to elution with 0.25 M α-methyl-d-mannoside, also in this buffer. The pooled fractions were dialyzed extensively against assay buffer at 4°C.

**Enzymatic Degradation of Forward Motility Protein**—Several hydrolytic enzymes were tested for their ability to inactivate forward motility protein by prior incubation (0 h, 30°C) with forward motility protein at a level of 1 mg/ml. Trypsin (200 units/ml) was incubated at pH 7.2 and trypsin inhibitor (220 units/ml) was added before the incubation as a control or after the incubation in the tests. Neuraminidase (4.8 units/ml), α-fucosidase (1 unit/ml), α-mannosidase (27 units/ml), galactose oxidase (97 units/ml), and β-galactosidase (5 units/ml) were incubated at pH 6.0, α-glucosidase (6.3 units/ml) at pH 5.0, α-amylase (980 units/ml) at pH 7, and β-galactosidase (360 units/ml) at pH 7.5 with the forward motility protein. Equal aliquots of these mixtures were assayed in the motility assay at concentrations, which were below saturation for the untreated controls. Inclusion of the enzymes at those concentrations or heat inactivation of the enzymes prior to the forward motility assay, or both, did not change the observed forward motility index.

**Miscellaneous Methods**—Protein concentrations were determined by the method of Lowry et al. (15) with albumin as the standard. Hexose sugars were measured by the method of Ashwell (16) with D-glucose as the standard. In experiments where SDS was removed from proteins after chromatography, fractions were passed over Dowex AG 2-X10 (17) without using. Polyacrylamide gel electrophoresis in SDS was performed as described previously (18, 19).

**RESULTS**

**Characteristics of Forward Motility Initiation**—Induction of forward motion in bovine caput sperm, measured by the forward motility index, is dependent on the time of incubation, concentration, and type of phosphodiesterase inhibitor, and concentration of seminal plasma. Fig. 1 shows the time course for the development of forward motility at three concentrations of theophylline in the presence of a saturating level of seminal plasma. Within 10 or 15 min after exposure of sperm to theophylline and forward motility protein, the forward motility index of the suspension reaches a maximum and maintains this activity for 90 min. Increasing the concentration of theophylline markedly increases the final forward motility index attained. These data are in good temporal agreement with the theophylline-induced elevation in cAMP levels previously reported (5). Since a number of phosphodiesterase inhibitors and cAMP analogs are capable of increasing flagellar movement without producing forward progression in bovine caput sperm in the absence of forward motility protein (5), we tested the dependence of the forward motility protein on the concentration of two of these inhibitors, methyl-3-isobutylyxanthine and theophylline, in the presence of a saturating level of forward motility protein (Fig. 2). The figure shows that methyl-3-isobutylyxanthine is approximately 10 times as effective as theophylline, on the basis of the concentration required for half-maximal stimulation, although both inhibitors ultimately achieve the same maximum forward motility index. The ICI compound, 63, 197, reaches a half-maximal forward motility index at a level (1.5 mM) intermediate between the two xanthines, although it attains a similar maximum forward motility index (data not shown).

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**FIG. 1.** Time course for development of motility. Incubation conditions are described under "Experimental Procedures." Activation was initiated with saturating (10 μl) levels of bovine seminal plasma and either 33 mM (○), 11 mM (△) or 3.3 mM (□) theophylline. Each time course is the average of duplicate experiments.
The effect of forward motility protein concentration on the forward motility index under standard assay conditions is shown in Fig. 3. Classical saturation kinetics are evident, i.e., the forward motility index increases linearly to a maximum and then levels off. This pattern is reproducible among the batches of seminal plasma and at different stages of factor purification and, although the specific activity varies, the maximum forward motility index achievable is constant. It is noteworthy that the seminal plasma forward motility index has no effect on the percentage of sperm showing flagellar activity. Its sole effect is on the number of sperm showing forward progression.

Assay of forward motility index results in a 10 to 15% standard error in the mean as judged from 10 replicate determinations of the same assay. The precision between assays also fell within this range. Infrequently, the sperm from one caput epididymidis show diminished activity or head-to-head aggregation. A secondary standard is therefore included in each assay to detect the former; the latter is easily observable and the samples were discarded.

**FIG. 2. Effects of varying the concentration of two phosphodiesterase inhibitors on the forward motility index.** Assay conditions are described under "Experimental Procedures" and crude bovine seminal plasma is at saturation (10 μl). The forward motility index is plotted against theophylline (□) and methyl-3-isobutylxanthine (○) concentration. Both curves are extrapolated (---) to an optimum concentration. The forward motility index for these conditions. Each curve is the average of duplicate experiments.

**FIG. 3. Activation of forward motility by various concentrations of forward motility protein.** Heat-treated bovine seminal plasma containing 15 mg of protein/ml was added to the standard assay. The forward motility index is plotted versus the micrograms of protein added per ml of final assay volume. The point at 750 μg of protein is an average of six points between 240 and 750 μg of protein.

**Fractionation of Seminal Plasma Forward Motility Protein—**Bovine seminal plasma is fractionated into four separate peaks of activity on DEAE-cellulose (Fig. 4). The first peak of activity is not retarded and the bound activity is eluted at approximately 0.05, 0.25, and 0.35 M KCl with the application of a 0 and 0.5 M KCl gradient. This activity profile is reproducible and the shoulder at 0.15 M can be partially resolved on a larger column (2.6 x 20 cm). An alternative separation, also based on charge, was achieved with preparative slab isoelectric focusing. With this technique, bovine seminal plasma is separated into six major activity peaks between pH 4 and 7 (data not shown). All six fractions contain both protein and hexose. The rather complex picture resulting from these charge separations led us to try molecular sieving of seminal plasma. Fig. 5 shows that seminal plasma can be separated on a Sepharose 6B column into four peaks displaying forward motility protein activity. The apparent molecular weights, based on globular protein standards, were between 10⁴ and 1.6 x 10⁵. The elution profile of the forward motility proteins was quite reproducible, but variations in column conditions such as temperature (4°C and room temperature), buffer, and ionic strength (0 or 0.5 M KCl added), produced significant changes in the relative activities of one peak to another. This observation and the heat stability of proteins of this size, especially from extracellular fluids, suggested to us that forward motility proteins might be glycoproteins or exist in aggregate equilibrium, or both.

**Aggregate Equilibrium of Forward Motility Protein—**An interrelationship between the various fractions from Sepharose 6B was first indicated by the fact that changing the ionic strength of the column eluant changed the relative amounts of activity found in each of the four peaks. Elution at low ionic strength (20 mM Tris, pH 7.4) produced more of the larger molecular weight form. When elution was performed with 0.5 M KCl added to this buffer, activity was shifted toward the lower molecular weight peaks. Further evidence of an aggregate equilibrium among the proteins was demonstrated by the observation that rechromatography of each of the individual forward motility protein fractions shown in Fig. 5, resulted in the formation of four new peaks of activity. Perhaps most
FIG. 5. Chromatography of bovine seminal plasma on Sepharose 6B. Heat-treated bovine seminal plasma (40 mg of protein in 2 ml) was applied to a column (1.5 cm X 30 cm) of Sepharose 6B. The chromatography was carried out at 5°C in assay buffer. Forward motility assays were performed (standard conditions and 100-μl aliquots of the collected fractions/ml of assay). Forward motility index (O) and absorbance at 280 nm (-----) are plotted against V_e/V_o (elution volume/void volume).

Fig. 5 Chromatography of bovine seminal plasma on Sepharose 6B. Heat-treated bovine seminal plasma (40 mg of protein in 2 ml) was applied to a column (1.5 X 30 cm) of Sepharose 6B. The chromatography was carried out at 5°C in assay buffer. Forward motility assays were performed (standard conditions and 100-μl aliquots of the collected fractions/ml of assay). Forward motility index (O) and absorbance at 280 nm (-----) are plotted against V_e/V_o (elution volume/void volume).

significantly, preincubation (2 h, 37°C) and subsequent chromatography of heat-treated seminal plasma on Sepharose 6B in a solution containing 1% SDS, 2 mM dithiothreitol, 2 mM EDTA, and 50 mM Tris (pH 7.4), produced a single major peak of forward motility protein (Fig. 6A). In this experiment, SDS was removed prior to assay by passage of each fraction over Dowex (see "Experimental Procedures"). When this SDS-free forward motility protein peak (Fig. 6A) was rechromatographed under the same conditions used to obtain the multiple peaks shown in Fig. 5, the same four peaks of activity in essentially the same relative amounts seen in Fig. 5 were re-formed.

One more related observation is of interest. When heat-treated seminal plasma was chromatographed under dissociating conditions identical with those in Fig. 6A, except for the omission of the dithiothreitol, a single, predominant peak of forward motility protein was again observed, but it eluted at a volume commensurate with a protein of approximately twice the molecular weight of the major peak in Fig. 6A. This suggests that a sulfhydryl bridge is involved in formation of a forward motility protein dimer.

Partial Purification and Properties of Forward Motility Protein—Although the chromatographic separations previously detailed provide useful information regarding the nature of forward motility protein, they are of limited use as purification techniques. Heat treatment (90°C, 10 min) on the other hand, is an effective purification step. The activity per volume of seminal plasma is unchanged or even slightly increased by heating, while between 50 and 75% of the total protein is removed by the subsequent high speed centrifugation (178,000 x g, 60 min). More dilute protein solutions, e.g. the four individual peaks from the Sepharose 6B column in Fig. 5, are also heat-stable under the same conditions.

Although nearly all the protein in the heat-treated bovine seminal plasma cochromatographed on Sepharose 6B with the forward motility protein in SDS (Fig. 6A), this is not observed when the chromatography is carried out in urea (Fig. 6B). In the latter case, a large portion of the total protein is retarded by the column and a moderate separation of the forward motility protein from the bulk of the protein is achieved. The forward motility protein is associated with a small preceding shoulder of protein and with the aid of analytical SDS-polyacrylamide gel electrophoresis, it is apparent that a group of proteins with molecular weights between 11,000 and 17,000 are responsible for the major protein peak in Fig. 6B. This group of proteins contribute between 50 and 75% of the total seminal plasma proteins, which can be stained with Coomassie blue, and are not present in the peak activity fractions in Fig. 6B. The specific activity is increased by a factor of 3.3 with this step, therefore producing an approximate 15-fold increase over crude bovine seminal plasma.

The results of studies using affinity chromatography with

FIG. 6. Sepharose 6B chromatography of seminal plasma in dissociating buffers. Samples were dialyzed against the respective column buffers and chromatographed at room temperature. After removal of the dissociating buffers, the fractions were assayed in the forward motility assay (O-----O) and Lowry proteins were done (O-----O). A, the sample was 30 mg of heat-treated bovine seminal plasma in 2 ml and the buffer was 1% SDS, 2 mM dithiothreitol, 2 mM EDTA, and 50 mM Tris-HCl (pH 7.5). The dissociating buffer was removed after chromatography by the passage of the fractions over 1 ml Dowex AG 2-X10 columns and then dialysis against assay buffer. B, the sample was 15 mg of heat-treated bovine seminal plasma in 1 ml and the buffer was 4 M urea, 100 mM NaCl, 10 mM dithiothreitol, and 10 mM Tris-HCl (pH 7.5). This buffer was removed after chromatography by dialysis against assay buffer.
concanavalin A, immobilized on an agarose column, suggest that forward motility protein is a glycoprotein. Fig. 7 shows an experiment in which heat-treated seminal plasma was fractionated on a concanavalin A/agarose column. Two peaks of protein containing a small amount of forward motility protein were not bound, but were sieved on the agarose matrix. Elution of the bound material with 0.25 M α-methyl-d-mannoside (marked by the arrow) produced a single peak of protein and forward motility activity which contained 30% of the total protein was recovered. The extent of purification is confused by the apparent removal of an inhibitor, which results in over 100% recovery of the activity. This eluted material, when electrophoresed in SDS in polyacrylamide gels, is not pure and several bands are evident whether the gels are stained for protein with Coomassie blue, or for carbohydrate with periodic acid-Schiff stain.

Further support for a glycoprotein nature of forward motility protein comes from data (Table I) which demonstrate the extent to which several degradative enzymes inactivate forward motility protein. Trypsin is quite effective and several glycoprotein-degrading enzymes, e.g. β-galactosidase, neuraminidase, galactose oxidase, and α-mannosidase, reduce the activity by approximately 50%. Other enzymes produce little, if any, inactivation. Sequential neuraminidase-β-galactosidase treatments, longer β-galactosidase treatments, and higher concentrations of β-galactosidase did not change the percentage inactivation. Increasing the concentration of β-galactosidase-inactivated forward motility protein in the forward motility assay did not produce over 50% of the maximum control activity.

Finally, the molecular weight of the monomeric form of the forward motility protein can be estimated from the chromatographic profiles presented in Fig. 6. Several molecular weight standards were used to calibrate each column under exactly the same conditions used to chromatograph the samples. The calibration curves are presented in Fig. 8; Panel A (SDS) gives a molecular weight estimate of 37,000 while the data in Panel B (urea) gives an estimate of 37,500. Similar estimates are obtained, when the alternative plot, i.e. molecular weight versus \( K_d^{2} \) is used.

**DISCUSSION**

The data in this report establish that a protein found in bovine seminal plasma is able to convert the ineffectual, flagellar activity exhibited by sperm from the caput epididymis in the presence of phosphodiesterase inhibitors into the forward progression pattern exhibited by caudal or ejaculated sperm under similar conditions.

The forward motility protein exists in association-dissocia-

![Fig. 7. Affinity chromatography of seminal plasma on concanavalin A. An extensively prewashed 10 ml column (0.8 × 26 cm) of concanavalin A, immobilized on agarose, was equilibrated with a buffer containing 1 mM CaCl₂, 1 mM MgCl₂, 100 mM NaCl, and 20 mM Hepes (pH 7.4). Heat-treated dialyzed bovine seminal plasma (10 mg) was incubated in the same buffer, applied to the column, and eluted with this buffer. After approximately 4 bed volumes of washing, marked by the arrow, the elution buffer was changed to include 0.25 M α-methyl-d-mannoside. Fractions were read at 280 nm (○), pooled, and dialyzed against assay buffer prior to their use in the forward motility assay (hatched histogram).](http://www.jbc.org/)

![Fig. 8. Calibration curves for Sepharose 6B chromatography in dissociating buffers. The columns used for the data in Fig. 6, A and B, respectively, were calibrated with several protein standards. These proteins are: 1, phosphorylase a; 2, bovine serum albumin; 3, ovalbumin; 4, aldolase; 5, chymotrypsinogen A; 6, lysozyme; 7, ribonuclease A; and 8, cytochrome c. The arrows indicate the position of the peak of forward motility activity from Fig. 6. The buffers and conditions are identical with the respective column in Fig. 6.](http://www.jbc.org/)

**TABLE I**

<table>
<thead>
<tr>
<th>Enzymatic treatment of forward motility protein</th>
<th>Relative forward motility index ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>100 ± 8</td>
</tr>
<tr>
<td>Trypsin</td>
<td>0 ± 12</td>
</tr>
<tr>
<td>Trypsin + trypsin inhibitor</td>
<td>99 ± 11</td>
</tr>
<tr>
<td>β-Galactosidase</td>
<td>51 ± 7</td>
</tr>
<tr>
<td>Neuraminidase</td>
<td>92 ± 13</td>
</tr>
<tr>
<td>α-Glucosidase</td>
<td>110 ± 6</td>
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<tr>
<td>α-Amylase</td>
<td>99 ± 6</td>
</tr>
<tr>
<td>β-Glucosidase</td>
<td>102 ± 5</td>
</tr>
<tr>
<td>Galactose oxidase</td>
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<tr>
<td>α-Mannosidase</td>
<td>67 ± 4</td>
</tr>
<tr>
<td>α-L-Fucosidase</td>
<td>94 ± 11</td>
</tr>
</tbody>
</table>

*Conditions for treatments are detailed under "Experimental Procedures."
tion equilibria in vitro and forms four aggregate species. Our results indicate that the aggregates are composed of combinations of a single monomer of approximately 37,500 daltons. This molecular weight estimate is based on studies with molecular sieving in urea and in SDS. The problems inherent in these molecular weight determinations have been discussed in detail (20–23). Molecular weight and Stokes radius measurements are prone to discrepancies, especially in studies of large, asymmetric particles. The excellent agreement between the results of the SDS and urea chromatography provides reassurance that the forward motility protein does not behave anomalously under these conditions. Interestingly, the smaller proteins, which chromatograph with the forward motility protein in SDS, are retarded in urea and therefore separated from the forward motility protein. Polyacrylamide gel electrophoresis in SDS clearly demonstrates that it is several proteins (11,000 to 17,000 daltons) which are retarded in urea chromatography.

The presence and importance of carbohydrate in the forward motility protein is suggested strongly both by the binding of the protein to concanavalin A and by its susceptibility to partial destruction by β-galactosidase, neuraminidase, α-mannosidase, and galactose oxidase. The binding data indicate that either a mannosyl or glucosyl group is present in the carbohydrate portion of the protein. The enzyme treatments apparently show that carbohydrate residues are involved in the activity. Although it is frequently argued that these types of enzyme treatments may reflect the activity of proteases which contaminate the major enzyme, it seems unlikely that these proteases would produce 50% inactivation in each case. The variation in conditions for the β-galactosidase treatments make it even less likely that proteases produced the effects. The observation that these enzymes can reduce by 50%, but not destroy the activity, suggests either microheterogeneity or a complicated biochemical mechanism. The latter possibility is currently under study. The ion exchange and isofocusing data are also suggestive of the microheterogeneity often observed with glycoproteins.

The forward motility assay itself is an extension of previous techniques (24–26) and is a satisfactory compromise between subjective evaluations, which are still frequently used, and several recent methods, which require expensive instrumentation and elaborate computer analysis. Our data demonstrate the quantitative usefulness of this assay for expressing the motility state of sperm.

Certainly one of the most intriguing questions is how forward motility protein works. We have previously shown (5), and corroborated herein, that forward motility protein does not produce motility in the absence of phosphodiesterase inhibitors. It appears that cAMP is a primary regulator of mature sperm motility (27, 28), although this does not preclude the probable importance of other regulatory substances. The two components, cAMP and forward motility protein, thus appear to work in concert, probably effecting separate aspects of sperm forward motility.

The important questions of the biological role of forward motility protein in the development of the potential for forward motility have not been defined. It does, however, seem probable that this glycoprotein is involved in the final stage of sperm maturation since, in parallel studies, we have found higher levels of forward motility protein in the epididymis than in any other body fluid or tissue (29). We have suggested that forward motility protein is of testicular or epididymal origin and that it is bound to or incorporated into the sperm membrane during epididymal transit (29). This would be consistent with the fact that the sperm’s surface charge changes considerably (30, 31) and that there is an increase in the number of membrane lectin-binding sites (32–34) on sperm during epididymal transit. Although it is well known that the testis (35–37) and epididymis (36, 38–40) produce a number of specific proteins not found in any other body fluids and tissues, none of these has a known function. The forward motility protein described in this paper may well be the first to which a function is ascribed.

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REFERENCES

6750 Sperm Forward Motility Protein


