Location of the PH-20 Protein on Acrosome-Intact and Acrosome-Reacted Spermatozoa of Cynomolgus Macaques

JAMES W. OVERSTREET, YING LIN, ASHLEY I. YUDIN, STUART A. MEYERS, PAUL PRIMAKOFF, DIANA G. MYLES, DAVID F. KATZ, and CATHERINE A. VANDEVOORT

California Regional Primate Research Center and Division of Reproductive Biology and Medicine
Department of Obstetrics and Gynecology, School of Medicine, University of California-Davis
Davis, California 95616

ABSTRACT

Fluorescence microscopy and transmission electron microscopy (TEM) were used to determine the location of the membrane protein PH-20 on spermatozoa of cynomolgus macaques. Rabbit antiserum raised against recombinant cynomolgus macaque sperm PH-20 was used as the primary antibody, and the second antibody was goat anti-rabbit IgG conjugated with either fluorescein isothiocyanate or 15 nm gold particles. Spermatozoa were evaluated before capacitation and after capacitation and induction of acrosome reactions with calcium ionophore A23187. In sperm suspensions with a high percentage of intact acrosomes, fluorescence labeling was observed uniformly over most of the sperm head. The sperm midpiece and tail were not labeled. In sperm suspensions with a high percentage of acrosome reactions, most spermatozoa labeled intensely over the anterior sperm head, but labeling of the posterior sperm head was greatly reduced. TEM of acrosome-intact spermatozoa revealed gold particles distributed uniformly on the plasma membrane overlying the acrosome, the equatorial segment, and most of the post-acrosomal region. After the acrosome reaction, gold label was present on the inner acrosomal membrane and on the plasma membrane overlying the equatorial segment. Very little label was present on the plasma membrane in the post-acrosomal region of acrosome-reacted spermatozoa. The location of PH-20 on the surface of macaque spermatozoa suggests a function for this protein in primary and/or secondary binding to the zona pellucida. The apparent decrease in amount of PH-20 on the posterior head of macaque spermatozoa following the acrosome reaction is consistent with the migration of this protein to the inner acrosomal membrane, as demonstrated previously for the homologous PH-20 protein of guinea pig spermatozoa.

INTRODUCTION

The membranes of mammalian spermatozoa include a number of molecules that have strong affinity for the zona pellucida of the oocyte [1]. It is believed that one or more of these molecules may be specific sperm “receptors” for the zona and that such sperm receptors play an important role in the cellular mechanisms that regulate fertilization [1]. Recently, there has been significant progress in the identification of these sperm receptors and the complementary molecules to which they bind in the zona pellucida.

In the mouse, two zona components that bind to sperm have been identified. The ZP-3 glycoprotein is involved in primary binding to the plasma membrane of the acrosome-intact mouse spermatozoon [2], and ZP-3 also has the capability to induce the acrosome reaction of bound spermatozoa [3]. Acrosome-reacted mouse spermatozoa bind to the zona pellucida by the inner acrosomal membrane, and the ZP-2 glycoprotein is thought to be involved in this binding [4–6]. This phase of sperm-zona pellucida interaction is termed secondary binding and may involve the sequential binding and release of the spermatozoon as it traverses the thickness of the zona [1].

A variety of molecules in several species have been identified as potential sperm receptors for the zona pellucida, and one or more of them may be required for sperm-zona binding [1]. Many of these molecules are enzymes, although their zona binding function may not be enzymatic. They include galactosyl transferase [7, 8], a tyrosine kinase [9], and proacrosin/acrosin [10, 11], as well as the PH-20 protein [12], which recently has been shown to have hyaluronidase activity [13].

There is strong evidence that the PH-20 protein has a required role in sperm-zona pellucida binding in the guinea pig [12]. This 64-kDa protein is located on the plasma membrane of the sperm head and becomes localized to the post-acrosomal region during sperm maturation in the epididymis [14]. PH-20 also is present on the inner acrosomal membrane and is exposed at the time of the acrosome reaction [15]. This population of PH-20 molecules is joined by other PH-20 molecules that migrate from the posterior head plasma membrane to the inner acrosome membrane after the acrosome reaction [15].

PH-20 cDNA has been cloned, and the amino acid sequence of the protein has been determined [16]. The PH-20 gene has been shown to be conserved in many species including mouse, rat, hamster, bovine, human, and macaque [16]. Recombinant macaque PH-20 has been pro-
duced, and antibodies against this protein have been generated [13]. These anti-PH-20 antibodies have been used to demonstrate with immunofluorescence the presence of the protein that is homologous to PH-20 on spermatozoa of the mouse and human [13].

Although homologues of the PH-20 protein appear to be components of spermatozoa from several mammals, it has been difficult to propose a common role for this molecule in fertilization because of insufficient information on the comparative biology of gamete interaction in different model species. Sperm-zona pellucida interaction has been investigated most thoroughly with mouse gametes, and in this species only acrosome-intact spermatozoa initiate binding to the zona [17–21]. On the basis of this model, it is generally believed that the fertilizing spermatozoon is induced to undergo the acrosome reaction as a result of sperm-zona binding [1]. However, guinea pig spermatozoa can bind to the zona pellucida and fertilize oocytes after the acrosome reaction [22], and thus primary zona binding may be bypassed in this species. Acrosome-intact and acrosome-reacted guinea pig sperm can bind to the zona pellucida [23], as appears to be the case for human sperm [24] and macaque sperm [25]. Nevertheless, it has not been shown in the primate species that sperm are capable of penetrating the zona pellucida if sperm binding is initiated after the acrosome reaction as a result of sperm-zona binding [17–21]. On the basis of this model, it is generally believed that the fertilizing spermatozoon is induced to undergo the acrosome reaction as a result of sperm-zona binding [1]. However, guinea pig spermatozoa can bind to the zona pellucida and fertilize oocytes after the acrosome reaction [22], and thus primary zona binding may be bypassed in this species. Acrosome-intact and acrosome-reacted guinea pig sperm can bind to the zona pellucida [23], as appears to be the case for human sperm [24] and macaque sperm [25]. Nevertheless, it has not been shown in the primate species that sperm are capable of penetrating the zona pellucida if sperm binding is initiated after the acrosome reaction has occurred.

Recent studies have shown that acrosome-intact spermatozoa of mice and humans have a homologue of PH-20 protein on the plasma membrane of the sperm head [13]. Therefore, it is possible that this molecule could have the same role in sperm binding to the zona pellucida in other species as it appears to have in guinea pigs. In previous studies, immunofluorescence has been used to determine the location of PH-20 protein on spermatozoa. In this communication, we report observations at the ultrastructural level on the location of the PH-20 homologue in macaque spermatozoa with intact acrosomes and at various stages during the acrosome reaction. Our observations are consistent with the interpretation that the PH-20 homologue migrates from the plasma membrane of the posterior sperm head to the inner acrosomal membrane after the acrosome reaction, as has been shown previously for PH-20 protein in spermatozoa of the guinea pig [14].

**MATERIALS AND METHODS**

**Reagents**

Gold-labeled (15 nm) goat anti-rabbit IgG was obtained from EY Laboratories (San Mateo, CA). Fluorescein isothiocyanate-conjugated goat anti-rabbit IgG (FITC-GAR) was obtained from Sigma Chemical Company (St. Louis, MO). Fluorescein isothiocyanate-conjugated *Pisum sativum agglutinin* (FITC-PSA) was obtained from Vector Laboratories (Burlingame, CA). Dulbecco's phosphate-buffered saline (DPBS) was prepared with 1% BSA (Sigma). HPLC-grade pure water used in all media was obtained from Fisher Scientific (Santa Clara, CA). All other chemicals and salts were obtained from Sigma.

**Sperm Preparation**

Four individually caged adult male cynomolgus macaques were used as semen donors. Males were trained for chair restraint and were electroejaculated with a Grass 6 stimulator (Grass Medical Instruments, Quincy, MA) equipped with pad electrodes for direct penile stimulation [26]. All males were housed at the California Regional Primate Research Center in compliance with the Federal Animal Welfare Act and the National Institutes of Health Guidelines for Care and Use of Laboratory Animals. Animals were maintained on a 0600–1800-h light schedule at 25°–27°C and were fed a diet of Purina monkey chow and water ad libitum. Semen samples were allowed to stand for 30 min prior to removal of the coagulum. Semen was diluted with 6 ml of modified Biggers, Whitten, and Whittingham (BWW) medium [27] containing 3 mg/ml BSA, and centrifuged at 300 × g for 10 min. The sperm pellet was resuspended in 6 ml BWW and recentrifuged. The washed sperm pellet from each male was resuspended in 2 ml BWW to give a sperm concentration of 10 × 10⁶ to 20 × 10⁶ per milliliter. The sperm suspensions (two males per experimental day) were pooled and equilibrated by incubation for 30 min at 37°C in water-saturated air containing 5% CO₂. In order to produce large numbers of motile, acrosome-reacted spermatozoa, the sperm suspensions were incubated in a TEST-yolk skim milk (TSM) buffer [28] before treatment with calcium ionophore A23187. Sperm suspensions were centrifuged as previously described and resuspended in TSM diluted with BWW (1:2, v:v, hereafter TSM). After TSM treatment, one-half of each pooled sperm sample was chemically activated to induce capacitation by the addition of 1 mM each caffeine and dibutyryl cAMP [29]. The second sperm aliquot received no additional treatment and served as a control. The sperm suspensions were incubated for an additional 30 min, and A23187 (15 μM in dimethyl sulfoxide) was added to the capacitated samples to induce acrosome reactions. Immediately after the addition of A23187 (time 0) and at 10 min later, 50-μl aliquots were removed from each sample for assessment of sperm motility and acrosomal status, after which paraformaldehyde (4%) was added (1:1 v:v) to fix the sperm membranes and to stop acrosome reactions. The untreated control suspensions were processed similarly.

**Assessment of Sperm Motility and Acrosome Reactions**

The percentage of motile spermatozoa was determined by visual observation with duplicate counts of 100 spermatozoa in each suspension by means of an Olympus BH series phase contrast microscope (Scientific Instruments Co., Sunnyvale, CA).
The total percentage of spermatozoa with acrosome reactions was determined by a modification of the method of Cross et al. [30, 31]. Sperm aliquots were placed on multiwell fluorescence microscopy slides (Polysciences, Inc., Warrington, PA). An equal volume of cold ethanol (95%) was added to the sperm droplet, and the droplet was incubated for 10 min at ambient temperature and then air-dried onto the slide at 37°C. Slides were rinsed in DPBS, 50 µl FITC-PSA was placed over the dried sperm droplets, and the slides were incubated for 10 min at ambient temperature. Slides were rinsed again in DPBS, mounting medium in glycerol was added, and coverslips were placed over the slides. Acrosomal status was evaluated for 200 consecutive spermatozoa per slide [30, 31] with a Leitz Ultralux fluorescence microscope (50x dry bright field objective, 12.5x ocular).

Sperm Immunolabeling

Polyclonal antisera were obtained after immunization of New Zealand white rabbits with purified recombinant cynomolgus macaque sperm PH-20. Preimmune sera were obtained from the same animals for use in control preparations. The methods for production and purification of the recombinant protein and for production of polyclonal antibodies to the protein have been published previously [13]. The specificity of the anti-PH-20 antiserum was demonstrated by comparing the migration of the recombinant protein with PH-20 protein from a total extract of 5 × 10⁶ cynomolgus macaque sperm. Samples were run on nonreducing SDS-polyacrylamide gels followed by immunoblotting. The anti-PH-20 antiserum [13] was the primary antibody; anti-rabbit immunoglobulin conjugated to alkaline phosphatase was the secondary antibody.

For immunolabeling, sperm suspensions were fixed for 10 min in paraformaldehyde at ambient temperature and then were centrifuged at 300 × g for 10 min. Pelleted spermatozoa were washed twice by centrifugation at 1000 × g and resuspension in 1 ml DPBS containing 1% BSA (DPBS-BSA). Sperm suspensions were incubated with rabbit anti-PH-20 antiserum or with preimmune rabbit serum (1:100) for 60 min at 37°C in 5% CO₂. Sperm suspensions were divided into two 500-µl aliquots, and each was washed twice by centrifugation. The sperm aliquots were resuspended either in 500-µl DPBS-BSA and gold-conjugated goat anti-rabbit IgG (1:20; v:v) or in 100-µl DPBS-BSA and FITC-GAR (1:20; v:v). After incubation for 60 min at 37°C in 5% CO₂ in darkness, 1 ml DPBS-BSA was added to each sample, and samples were washed twice by centrifugation at 1000 × g and resuspension. Gold-labeled sperm samples were resuspended in 1 ml 0.2 M cacodylate buffer containing 2.5% glutaraldehyde prior to preparation for transmission electron microscopy.

**FIG. 1.** Specificity of antiserum raised against recombinant cynomolgus PH-20 protein. Samples were run on nonreducing SDS-PAGE followed by immunoblotting. The anti-PH-20 antiserum [13] was the primary antibody; anti-rabbit immunoglobulin conjugated to alkaline phosphatase was the secondary antibody. Lane 1, total extract of 5 × 10⁶ cynomolgus macaque sperm; lane 2, purified recombinant PH-20.

**Fluorescence Microscopy**

Fluorescence-labeled sperm aliquots (7 µl) were placed on multiwell fluorescence microscopy slides, and coverslips were added. Spermatozoa were evaluated routinely by fluorescence microscopy as described previously. For production of micrographs, sample slides were viewed on an Olympus BH-2 inverted microscope with a 60x oil immersion objective. The microscope was attached to the scanning head of a Bio-Rad MRC-600 laser scanning confocal system equipped with a 15-mW krypton-argon mixed gas multiline laser. Images were photographed after digital
conversion with customized software using a Polaroid CI-3000 Digital Parlette film recorder and Kodak TMAX 100 film.

**Electron Microscopy**

After gold labeling, sperm suspensions were fixed overnight (4°C) in a fresh solution of 2.5% glutaraldehyde. The buffer throughout the fixation process was 0.2 M cacodylate (pH 7.6). After overnight fixation, the samples were washed twice in buffer for 30 min. Washed samples were incubated in 1% tannic acid-buffer solution (pH 7.2) for 1 h. The samples were washed twice in buffer prior to postfixing in 1% osmium tetroxide for at least 2 h. After being washed, the samples were dehydrated through a graded ethanol series (50–100%) and embedded in Spurr’s epoxy (Ted Pella, Inc., Redding, CA) for 60 min at 37°C in 5% CO2 in darkness. Sections were cut on a diamond knife and were stained with uranyl acetate and lead citrate before being viewed on a Phillips 410 transmission electron microscope.

**RESULTS**

**Immunofluorescence Studies**

The rabbit antiserum used in this study was raised by immunizing with purified recombinant cynomolgus PH-20, synthesized in S9 cells [13]. The specificity of the antiserum was tested in a Western blot of a total cynomolgus sperm extract (Fig. 1). The antiserum blots a single band that migrates very close to the purified recombinant PH-20. The small difference in migration probably reflects a difference in glycosylation between the sperm protein and the recombinant protein.

Fluorescence microscopy demonstrated that the PH-20 label was present only on the sperm head and not on the sperm midpiece or flagellum (Fig. 2, a-c). No fluorescence labeling was observed on spermatozoa incubated in preimmune serum. Before treatment with A23187 most of the sperm head was labeled (Fig. 2a). Fluorescence labeling was uniform over the anterior head, equatorial region, and post-acrosomal region, and the intensity of fluorescence in these regions was similar. This pattern of labeling was designated pattern I. After the addition of A23187, a new pattern of intense fluorescence, designated pattern II, was ap-

![Fig. 2. Fluorescence labeling patterns of spermatozoa from cynomolgus macaques incubated with antiserum raised against recombinant cynomolgus PH-20 protein and an FITC-conjugated anti-rabbit secondary antibody. a) labeling pattern I, predominant pattern observed in sperm suspensions with low percentages of acrosome-reacted spermatozoa. Fluorescence labeling was uniform in distribution and intensity over the sperm head. b and c) labeling pattern II, predominant pattern observed in sperm suspensions with high percentages of acrosome-reacted spermatozoa. Fluorescence labeling was intense over anterior head, but greatly reduced over posterior head. Bar = 5 μm.](https://academic.oup.com/biolreprod/article-abstract/52/1/105/2761393)

**TABLE 1. Fluorescence labeling patterns, percentage motility and acrosomal status of cynomolgus macaque spermatozoa following incubation with rabbit anti-PH20 antiserum and FITC-goat anti-rabbit antibody.**

<table>
<thead>
<tr>
<th>Percentage of spermatozoa*</th>
<th>Acrosome reacted</th>
<th>Pattern I</th>
<th>Pattern II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>90 ± 2*</td>
<td>8 ± 3*</td>
<td>80 ± 4*</td>
</tr>
<tr>
<td>A23187 - Treated 0 min</td>
<td>75 ± 3*</td>
<td>9 ± 1*</td>
<td>81 ± 3*</td>
</tr>
<tr>
<td>A23187 - Treated 10 min</td>
<td>75 ± 3*</td>
<td>85 ± 5*</td>
<td>11 ± 3*</td>
</tr>
</tbody>
</table>

*Data are presented as means ± SEM, n = 4. Means with different superscripts within columns are significantly different (p < 0.01). Data were analyzed using the Kruskal-Wallis Test and one-way ANOVA with Fisher's LSD for post-hoc comparisons.

![Fig. 3 and 4. Immunolocalization of PH-20 on acrosome-intact spermatozoa from cynomolgus macaques using antiserum raised against recombinant cynomolgus PH-20 protein. Figure 3 demonstrates that gold particles uniformly labeled plasma membrane (PM) of sperm head, including anterior acrosome, equatorial segment (ES), and post-acrosomal region of head (PH). On areas of sperm surface in which plasma membrane was disrupted and outer acrosomal membrane (OAM) was exposed, no gold particles were observed. Bar = 0.3 μm. Figure 4 demonstrates that gold label was found only on sperm head and not on midpiece or principal piece of flagellum. Gold labeling on plasma membrane of posterior head (PH) was similar in density to that on equatorial segment but ended abruptly at a point anterior to midpiece (arrow), which may correspond to striated band or posterior ring. Bar = 0.15 μm.](https://academic.oup.com/biolreprod/article-abstract/52/1/105/2761393)
FIG. 5. Immunolocalization of PH-20 on acrosome-reacted spermatozoon recovered 10 min after treatment with A23187. At this initial stage of acrosome reaction, gold particles were found in association with vesicles (V) formed by fusion of sperm plasma membrane and outer acrosomal membrane. Acrosomal matrix (AM) had not yet dispersed and inner acrosomal membrane was not exposed. Gold label was present on plasma membrane of equatorial segment (ES) and posterior head (PH). Bar = 0.25 μm.

parent over the anterior head (Fig. 2, b and c). Some spermatozoa with labeling pattern II also had fluorescence labeling over the posterior head, but the intensity of the labeling was not uniform (Fig. 2b). In other spermatozoa with this labeling pattern fluorescence was greatly reduced in the region of the posterior head (Fig. 2c).

The data in Table 1 demonstrate the prevalence of spermatozoa with the two fluorescence labeling patterns after treatment with A23187, as well as the percentage of motility and acrosomal status of spermatozoa recovered from the same suspensions. Approximately 90% of control spermatozoa were motile, and less than 10% were acrosome-reacted; approximately 80% of spermatozoa in these suspensions had fluorescence labeling pattern I. Sperm motility was decreased immediately after treatment with A23187, but at this initial time point the percentage of acrosome reactions remained less than 10%, and more than 80% of spermatozoa had fluorescence labeling pattern I. By 10 min after treatment with A23187, more than 80% of spermatozoa were acrosome-reacted, although the percentage of motility was unchanged. In these suspensions, almost all spermatozoa had fluorescence labeling pattern II.

Electron Microscopy

Electron microscopy revealed that gold particles were located on the plasma membrane of the acrosome-intact spermatozoa and were distributed uniformly over most of the anterior and posterior head (Figs. 3 and 4). There was no evidence of gold particles on the midpiece or principal piece of the sperm flagellum (Fig. 4). There was a clear limitation of gold particles on the posterior head, with a boundary anterior to the midpiece (Fig. 4). This boundary
FIGS. 6 and 7. Immunolocalization of PH-20 on inner acrosomal membrane of acrosome-reacted spermatozoa recovered 10 min after treatment with A23187. Figure 6 demonstrates gold particles on inner acrosomal membrane in association with dispersal of acrosomal matrix. Gold particles remained associated with acrosomal vesicles (arrows). Bar = 0.27 μm. Figure 7 demonstrates intense gold labeling of inner acrosomal membrane. In this spermatozoon, membrane vesiculation had not yet reached equatorial segment (ES). Non-vesiculated plasma membrane was gold-labeled (arrow), but underlying inner acrosomal membrane was not labeled because of its inaccessibility to gold-labeled second antibody. Equatorial segment continued to be labeled but there was marked reduction of gold label on plasma membrane of posterior head (PH) in comparison with Figure 4. Bar = 0.28 μm.
FIG. 8. Immunolocalization of PH-20 on acrosome-reacted spermatozoon recovered 10 min after treatment with A23187. Although recovered at same time as spermatozoa in Figures 6 and 7, this spermatozoon was at final stage of acrosome reaction. Acrosomal vesicles and acrosomal matrix had completely dispersed. Gold particles were present on inner acrosomal membrane (IAM) and on plasma membrane of equatorial segment (ES). Only a few gold particles were present on plasma membrane of posterior head (PH); none were found on sperm flagellum. Bar = 0.25 μm.

appeared to correspond to the striated band or posterior ring that encircles the sperm head in this region [32]. The same labeling patterns were observed for acrosome-intact spermatozoa in control suspensions and for acrosome-intact spermatozoa in sperm suspensions treated with A23187. No gold label was observed on spermatozoa incubated with preimmune serum.

Immediately after the acrosome reaction, gold particles remained associated with the acrosomal vesicles, presumably the plasma membrane components of the vesicles (Figs. 5 and 6). The gold particles did not appear to be associated with the acrosomal matrix (Figs. 5–7). At the earliest stages of the acrosome reaction (Fig. 5), gold particles were not present on the inner acrosomal membrane, presumably because the acrosomal contents limited access of the second antibody. At this initial stage of acrosomal vesiculation, gold particles were still present on the plasma membrane underlying the equatorial segment and on the plasma membrane of the post-acrosomal region of the sperm head (Fig. 5). As the acrosomal matrix dispersed, exposing the inner acrosomal membrane, gold particles were observed in this region (Figs. 6 and 7). After the acrosome reaction, gold particles continued to be present on the plasma membrane underlying the equatorial segment, but were reduced in number on the plasma membrane of the post-acrosomal region (Figs. 7 and 8).
DISCUSSION

The location of the PH-20 homologue on the plasma membrane of the acrosome-intact macaque spermatozoon differs from the location of the PH-20 protein on spermatozoon of the guinea pig, where it is absent from plasma membrane of the anterior acrosomal region [33]. Guinea pig spermatozoon can initiate binding to the zona pellucida and fertilize oocytes after the acrosome reaction [22], so the initial events of sperm-zona binding that lead to zona penetration may involve molecules associated with the inner acrosomal membrane. It is interesting that macaque spermatozoon, which appear to initiate zona binding before the acrosome reaction [25], have a component that is homologous to PH-20 protein on the plasma membrane of the acrosome, as well as the inner acrosomal membrane. Sperm-zona binding also is thought to precede induction of the acrosome reaction in spermatozoa of mice [18, 19] and humans [34]. In spermatozoa of these species as well, the PH-20 homologue is located on the plasma membrane of the acrosome [13]. Taken together, these observations suggest that the PH-20 protein, which may be a component of spermatozoon in a wide range of mammalian species [16], may also play a common role as one of the sperm receptors for binding to the zona pellucida during mammalian fertilization.

The presence of the PH-20 homologue on the inner acrosomal membrane of macaque spermatozoa suggests that this protein also may have a function in secondary binding or during sperm penetration through the zona pellucida. Recombinant macaque PH-20 has hyaluronidase activity, and it is possible that PH-20 on the plasma membrane of the acrosome-intact spermatozoon may be an important source of hyaluronidase, which facilitates sperm passage through the cumulus investment of the oocyte [13]. While there is little doubt of the presence of hyaluronic acid in the extracellular matrix of the granulosa cell investment [35], its location in the zona pellucida is less certain. Although some investigators have reported hyaluronic acid in the outer third of the hamster zona pellucida [36], others have attributed the presence of this material to extensions of the cumulus matrix into the porous structure of the zona [35].

Even if there is little hyaluronic acid in the macaque zona pellucida, PH-20 could still function non-enzymatically as a binding molecule during zona penetration. The apparent decrease in amount of the PH-20 homologue on the posterior head of macaque spermatozoon after the acrosome reaction is consistent with migration of this protein to the inner acrosomal membrane. It has been suggested that migration of PH-20 protein to the inner acrosomal membrane may have a dual function in increasing the avidity of sperm-zona binding and in removing zona binding proteins from the posterior head so that binding is restricted to the inner acrosomal membrane [37]. In guinea pig spermatozoon, approximately two-thirds of the PH-20 protein on the inner acrosomal membrane preexists in that region, and one-third of the protein migrates from the plasma membrane of the posterior head [15]. The phenomenon of PH-20 migration in guinea pig spermatozoon has been investigated extensively, but the underlying cellular mechanisms are not fully understood [37]. The PH-20 protein located in the posterior head region has restricted mobility, but the protein has a glycosyl phosphatidylinositol anchor and is not a transmembrane protein [38]. Interactions of PH-20 with extracellular elements have been proposed to restrict its mobility [39]. PH-20 migration is regulated by intracellular calcium concentrations and may involve as yet unidentified cytoskeletal elements [37]. PH-20 protein on the inner acrosomal membrane is freely diffusible [39] and appears to be maintained in that location by a diffusion barrier in the equatorial region [37, 39].

The rate of migration of the PH-20 protein in vivo is not known. For guinea pig spermatozoon observed in vitro, the period of PH-20 migration may last for 1 h or longer [37]. However, this migration is considerably faster when sperm are treated with ionophore: PH-20 migration was completed in 100% of the spermatozoa within 10 min after ionophore treatment [33]. The speed of sperm passage through the zona pellucida in vivo also is not certain, but in most species a period of less than 10 min appears to be required for sperm binding and penetration of the zona [1]. If PH-20 has an important role in secondary sperm-zona binding, migration of the protein may augment or replace sperm binding molecules that remain attached to zona material as the sperm progresses through the zona thickness. Additional ultrastructural studies of sperm-zona pellucida interaction that incorporate immunolabeling techniques may clarify the relationship of PH-20 migration to zona penetration and provide additional insight into the biological importance of PH-20 migration preceding fertilization.

ACKNOWLEDGMENT

The authors thank Dr. Stanley Meizel for his critical review of the manuscript.

REFERENCES


