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Characterization of plasma membrane associated type II α-D-mannosidase and β-N-acetylglucosaminidase of *Aquarius remigis* sperm

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ABSTRACT

For successful fertilization to occur, molecules on the surface of male and female gametes must recognize each other in a complementary manner. In some organisms, sperm possess a glycosidase on the plasma membrane overlying the head while eggs have glycoproteins that are recognized by those glycosidases resulting in sperm-egg recognition. In this study, two glycosidases, mannosidase and β-N-acetylglucosaminidase, were identified and biochemically characterized in *Aquarius remigis* sperm. The mannosidase had a K_m of 2.36 ± 0.19 mM, a V_{max} of 27.49 ± 0.88 pmol/min and a Hill coefficient of 0.94 ± 0.18 at its optimal pH of 7.0. The mannosidase was extracted most efficiently with CHAPSO but was also efficiently extracted with sodium chloride. Mannosidase activity was effectively inhibited by swainsonine, but not by kifunesine, and was significantly reduced in the presence of Mn^{2+} and Mg^{2+}, but not Zn^{2+}. N-acetylglucosaminidase had a K_m of 0.093 ± 0.01 mM, a V_{max} of 153.80 ± 2.97 pmol/min and a Hill coefficient of 0.96 ± 0.63 at its optimal pH of 7.0. N-acetylglucosaminidase was extracted most efficiently with potassium iodide but was also efficiently extracted with Triton X-100 and Zn^{2+}, but not Ca^{2+}, Co^{2+}, Mn^{2+} or Mg^{2+}, significantly inhibited its activity. Taken together, these results indicate that the *A. remigis* sperm surface contains at least two glycosidases that may recognize complementary glycoconjugates on the surface of water strider eggs.

1. Introduction

Complementary ligand–receptor pairs between gametes play important roles in intercellular signaling, adhesion, and physiological transformations that are necessary for successful fertilization in animals. Carbohydrates appear to be particularly important for these events in both vertebrate and invertebrate systems that have been studied to date and carbohydrates have been suggested to be important in binding and recognition during fertilization. Specifically, glycans may play roles in maintaining sperm in storage or binding to the egg surface (DeMott et al., 1995; Dobrinski et al., 1996; Green et al., 2001; Wagner et al., 2002; Tulsiani et al., 1997; Martinez et al., 2000; Miranda et al., 2000; Mengerink and Vacquier, 2001; Rodeheffer and Shur, 2002; Koyanagi and Honegger, 2003; Claw and Swanson, 2012; Chiu et al., 2008; Pang et al., 2011). Thus, glycosidases could be involved in maintaining sperm in storage reservoirs. In mammals, sperm are stored in the oviduct by binding the epithelial surface (Harper, 1973; Hunter, 1981; Hunter and Nichol, 1983; Overstreet and Cooper, 1978; Suarez, 1987; Wilmut and Hunter, 1984; Yanagimachi and Chang, 1963) and oligosaccharides competitively inhibit sperm-epithelium binding in hamster (DeMott et al., 1995), horse (Dobrinski et al., 1996), and pig (Green et al., 2001; Wagner et al., 2002) suggesting that carbohydrates mediate sperm-epithelium binding.

Despite evidence that carbohydrates mediate sperm-egg recognition, the involvement of glycosidases as recognition molecules remains controversial. Additionally, both sperm surface lectin-like molecules and conjugate sugars on the egg

Abbreviations: ZP, Zona Pellucida; GlcNAc'ase, β-N-acetylglucosaminidase; β-GlcNAc, β-N-acetylglucosamine; 4NP-Man, 4-Nitrophenyl α-D-mannopyranoside; 4NP-NAG, 4-Nitrophenyl N-acetyl-β-D-glucosaminide; BCA, bicinchoninic acid; BSA, Bovine serum albumin.

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surface have been proposed to mediate the gamete recognition step (Tulsiani et al., 1997; Martinez et al., 2000; Miranda et al., 2000; Mengerink and Vacquier, 2001; Rodeheffer and Shur, 2002; Koyanagi and Honegger, 2003; Claw and Swanson, 2012; Chiu et al., 2008; Pang et al., 2011). Some of these molecules interact through a non-catalytic complex between sperm surface glycosidases or glycosyltransferases and complementary carbohydrates on the extracellular matrices surrounding the egg plasma membrane (Rodeheffer and Shur, 2002). As intracellular enzymes, glycosidases catalyze the hydrolysis of glycosidic linkages within lysosomes, but on cell surfaces their enzymatic cycle is inactivated leading to the formation of a receptor–ligand pair. Enzymes represent an important potential class of receptors since they exhibit a high degree of absolute, group, linkage or stereochemical specificity with their substrate (Seager and Slabaugh, 2010).

Several sperm surface glycosidases have been identified as putative egg surface (e.g., chorion, zona pellucida, etc.) recognition molecules. Examples of glycosidases present on the sperm plasma membrane include mannosidases in mammalian sperm (Tulsiani et al., 1989, 1990; Tulsiani et al., 1995), fucosidases and N-acetylglucosaminidases in tunicates (Godknecht and Honegger, 1991; Matsumoto et al., 2002; Downey and Lambert, 1994), and mannosidases, fucosidases and N-acetylglucosaminidases in flies (Perotti, 2001; Pasini et al., 1999; Intra et al., 2006, 2011; Cattaneo et al., 1997). In mammals, sperm from rats and mice possess a sperm surface mannosidase and the zona pellucida (ZP) surrounding the egg plasma membrane is heavily mannosylated (Tulsiani et al., 1997). In the ascidians *Phallusia mammillata* (Hoshi et al., 1985; Hoshi, 1986; Godknecht and Honegger, 1991, 1995) and *Ascidia* sp. (Lambert, 1989), the sperm plasma membrane contains a β-N-acetylglucosaminidase (GlcNAc'ase) and the egg vitelline coat possesses complementary β-N-acetylglucosamine (β-GlcNAc) residues.

In insects, the mechanisms of sperm-egg interactions are poorly understood and sperm-egg interactions have been studied in only two Dipteran species. *Drosophila* sperm possess fucosidase, mannosidase and β-N-hexosaminidase that have been proposed to recognize complementary fucose, mannose and β-N-acetylglucosamine residues on the surface surrounding the micropyle of the egg chorion (Intra et al., 2006, 2011; Perotti, 2001). The presence of multiple glycosidases suggests that multiple molecules may be involved in a complex sperm-egg recognition event (Perotti et al., 2001; Intra et al., 2006; Cattaneo et al., 2002). Similar to *Drosophila*, *Ceratitis capitata* sperm possess surface associated fucosidase, mannosidase and β-N-acetylglucosaminidase (Intra et al., 2011), although the presence of cognate sugars on the egg chorion was not determined.

The role of β-N-acetylhexosaminidase in sperm-egg recognition in *Drosophila melanogaster* is further supported by genetic evidence. In the *Casanova* mutant, the sperm head lacks β-N-acetylhexosaminidase and the flies are sterile due to the inability of the sperm to penetrate the egg (Perotti, 2001). The presence of β-N-acetylhexosaminidase on the sperm head is conserved across the *Drosophila* genus, and together with the finding that *Ceratitis capitata* sperm also have glycosidases in their plasma membrane and complementary glycoconjugates on the egg surface (Intra et al., 2011), suggests that these glycosidases may be necessary for successful fertilization in flies.

Despite these advances, the precise details of insect sperm-egg recognition are unknown in most insect taxa. In order to understand the molecular basis of fertilization in insects, it is critical to study multiple insect orders and to understand the key parameters that ultimately determine the molecular interactions that lead to successful sperm-egg recognition. In this study, we investigated the role of glycosidases in a Hemipteran, the North American semi-aquatic water strider *Aquarius remigis*. *A. remigis* provides an optimal system to examine the biochemical basis of sperm-egg recognition in Hemipterans since the sperm-egg interaction is likely to occur between receptors on the plasma membrane overlying the acrosomal region of the sperm and complementary ligands on an extracellular matrix surrounding the egg (Swanson and Vacquier, 2002; Vacquier and Moy, 1977; Vacquier et al., 1990; Burkin and Miller, 2000; Mengerink and Vacquier, 2001; Wassarman et al., 2001; Evans, 2000; Bleil and Wassarman, 1980a, 1980b, 1986; Vazquez et al., 1989; Yanagimachi, 1994) and *A. remigis* sperm contain an unusually long acrosomal process that is approximately 2.5 mm in length (Miyata et al., 2011). In most organisms, the acrosome makes up a minor fraction of the sperm and, therefore, acrosomal proteins, and the plasma membrane proteins surrounding the head, constitute a minor fraction of the total sperm protein. In contrast, the *A. remigis* sperm represents a markedly different situation that allows for copious quantities of protein to be extracted and examined for biochemical characterization.

In this study, two glycosidases, a mannosidase and an N-acetylglucosaminidase, from the *A. remigis* sperm plasma membrane were identified and characterized. As with Dipterans, their presence on the sperm plasma membrane suggests that these molecules may play a role in sperm-egg interactions and the results from this study will help to elucidate the role that these molecules play in insect reproduction. This report represents the first study of molecules potentially involved in fertilization in Hemipterans.

2. Materials and methods

2.1. Materials

Sodium citrate, KI, Na_2HPO_4, NaH_2PO_4, NaCl, $CaCl_2$, $MnCl_2$, $MgCl_2$, and $ZnCl_2$ were purchased from Fisher Scientific (Waltham, MA) and $CoCl_2$ was purchased from Mallinckrodt (Paris, KY). Tris–HCl, Triton X-100, CHAPSO, CHAPS, 4-Nitrophenyl α-D-mannopyranoside (4NP-Man), 4-Nitrophenyl N-acetyl-β-D-glucosaminide (4NP-NAG), mannosidase and β-N-acetyl-glucosaminidase were purchased from Sigma–Aldrich (St. Louis, MO). Kifunensine and swainsonine were purchased from ToCris (Ellisville, MO). The Pierce BCA Protein assay kit was purchased from Thermoscientific (Waltham, MA).

2.2. Animals

A. remigis males and females were kept at room temperature in tanks partially filled with water. Frozen crickets were provided ad libitum daily and Styrofoam™ cups were placed in the water to provide shelter and oviposition sites. Tanks were cleaned daily and the water changed weekly. Males were isolated from females for two weeks or longer to ensure that sufficient amounts of mature sperm were present in the seminal vesicles.

2.3. Sperm collection

Adult males were euthanized using chloroform and the seminal vesicles were then dissected from the animals. The seminal vesicles were placed in phosphate buffered saline (PBS; 10 mM Na_2HPO_4, 2 mM NaH_2PO_4, 135 mM NaCl, pH 7.2) and the intact sperm were removed from the seminal vesicles according to previously published procedures (Miyata et al., 2011). The methods used to remove sperm for this study have been previously used for motility assays (Miyata et al., 2012).