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ULTRASTRUCTURAL LOCALIZATION OF GLYCOCONJUGATES IN SPERMATOZOA OF THE NOCTUID MOTH *Anticarsia gemmatalis* (INSECTA, LEPIDOPTERA, NOCTUIDAE)

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ABSTRACT

The presence of glycoconjugates in apyrene and eupyrene spermatozoa of the noctuid moth *Anticarsia gemmatalis* was examined using ruthenium red and direct lectin-gold labeling techniques. Acid mucopolysaccharides of the plasma membrane of apyrene spermatozoa and the lacinate appendages of eupyrene spermatozoa were stained by ruthenium red. The lacinated appendages of eupyrene spermatozoa were the most intensely labeled structures when lectins were used as histochemical probes. Sugar residues were also present in the dense cap of apyrene spermatozoa and in the nucleus of eupyrene spermatozoa. There was no labeling in the acrosome of eupyrene spermatozoa. These results indicate that the two categories of spermatozoa have different types and distributions of glycoconjugates which probably reflect their distinct functions during differentiation and fertilization.

Key words: Insect, lectins, Lepidoptera, spermatozoa, ultrastructure

INTRODUCTION

Biological processes such as intercellular interactions, intercellular transport, enzymatic reactions and cellular differentiation involve the participation of carbohydrate-rich molecules. Carbohydrate interactions are important for spermatozoa to be able to distinguish eggs of other species from their own [27]. Lectins, which are proteins or glycoproteins of non-immune origin, are valuable tools for identifying, characterizing and localizing carbohydrate-containing sites [10,17,22]. Various studies have examined the distribution of carbohydrates in insect spermatozoa using lectin histochemistry [1-3,6,19,20].

The males of many insect species show sperm heteromorphism in which several sperm types are concomitantly produced [25]. Male lepidopterans produce apyrene spermatozoa that are anucleated, and eupryrene spermatozoa that are nucleated and fertilize eggs [8,9,15,16,18,24,26]. Another important feature of eupyrene spermatozoa is their elaborate lacinate appendage that extends from the plasma membrane [9].

In this study, we used staining with ruthenium red and gold-labeled lectins to identify specific glycoprotein conjugates in spermatozoa of the noctuid moth *Anticarsia gemmatalis*, an important soybean pest.

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MATERIAL AND METHODS

Adult males of *A. gemmatalis* were obtained from a colony maintained at the National Center of Genetic Resources (CENARGEN, Brasília, DF, Brazil). The lectins used (Table 1) were obtained from Sigma Chemical Co. (St. Louis, Mo, USA). The various glycoproteins were labeled with colloidal gold particles (8-10 nm in diameter), according to Roth [22].

The following techniques were used: **a)** The ruthenium red method according to Luft [14]. Tissues were fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.2, containing 0.5 mg of ruthenium red/mL, for 3 h. After fixation, the specimens were rinsed in buffer and then postfixed with 1% osmium tetroxide in the same buffer containing 0.5 mg of ruthenium red/mL. The material was dehydrated in acetone and embedded in Spurr's epoxy resin. Ultrathin sections were examined after additional staining with uranyl acetate and lead citrate. **b)** For labeling with lectins, testicles were fixed for 3 h at 4°C in a solution containing 4% paraformaldehyde, 1% glutaraldehyde, 0.2% picric acid, 3.5% sucrose and 1 mM $CaCl_2$ in 0.1 M sodium cacodylate buffer, pH 7.2. After washing the specimens with several changes of this buffer, free aldehyde groups were quenched with 50 mM ammonium chloride in the same buffer, for 1 h at 4°C, followed by block staining with 2% uranyl acetate in 15% acetone for 2 h at 4°C [4]. The specimens were dehydrated in 30-90% acetone and embedded in LR gold resin. Ultrathin sections were collected on nickel grids, pre-incubated for 1 h at room temperature (RT) in phosphate-buffered saline (PBS) containing 1.5% bovine serum albumin (PBS-BSA) and 0.01% Tween 20, and subsequently incubated for 1 h at RT with differ-

ent gold-labeled lectins in PBS-BSA. For *Arachis hypogaea* (PNA), *Griffonia simplicifolia IB4* (GS-IB4), *G. simplicifolia II* (GSII), *Triticum vulgaris* (WGA), *Ulex europaeus I* (UEA I) and *U. europaeus II* (UEA II) lectins, the incubations were done at a dilution of 1:5 at pH 8.0. For *Helix pomatia* (HPA) lectin, the incubation was done at a dilution of 1:50 at pH 8.0. After incubation, the grids were washed with PBS and distilled water, stained with uranyl acetate and lead citrate, and observed in a Jeol 100C transmission electron microscope. The controls consisted of the addition of 200-300 mM of the corresponding monosaccharides to the incubation medium (Table 1).

Table 1. Lectins used as histochemical probes.

Source of lectin	Abbreviation	Sugar specificity
Arachis hypogaea	PNA	β-galactose
Griffonia simplicifolia-IB4	GS-IB4	α-galactose
Griffonia simplicifolia II	GS-II	α- and β-N-acetylglucosamine
Helix pomatia	HPA	α-N-acetylgalactosamine
Triticum vulgaris	WGA	β-N-acetylglucosamine and N-acetylneuraminic acid
Ulex europaeus I	UEA-I	α-fucose
Ulex europaeus II	UEA-II	β-N-acetylglucosamine

RESULTS

Males of *A. gemmatalis* produced eupyrene (nucleated) and apyrene (anucleated) spermatozoa, as previously described in detail [8]. Staining with ruthenium red indicated the presence of acid mucopolysaccharides in the lacinate appendages of eupyrene spermatozoa. In cross-section, these molecules gave the stained structure a radial configuration (Fig. 1). The plasma membrane of apyrene spermatozoa also stained with the ruthenium red (Fig. 2).

Ultrathin sections of LR gold-embedded spermatozoa were used to locate the binding sites specific for β-galactose (PNA), α-galactose (GS-IB4), α- and β-N-acetyl-glucosamine (GS II, WGA and UEA II), α-N-acetyl-galactosamine (HPA), N-acetylneuraminic acid (WGA), and α-fucose (UEA I). All of the binding reactions were inhibited by including the appropriate sugar in the incubation medium. The pattern of labeling of the lacinated appendages in eupyrene spermatozoa varied among the lectins tested (Table 2).

Table 2. Summary of the labeling pattern in ultrathin sections of LR gold-embedded eupyrene spermatozoa.

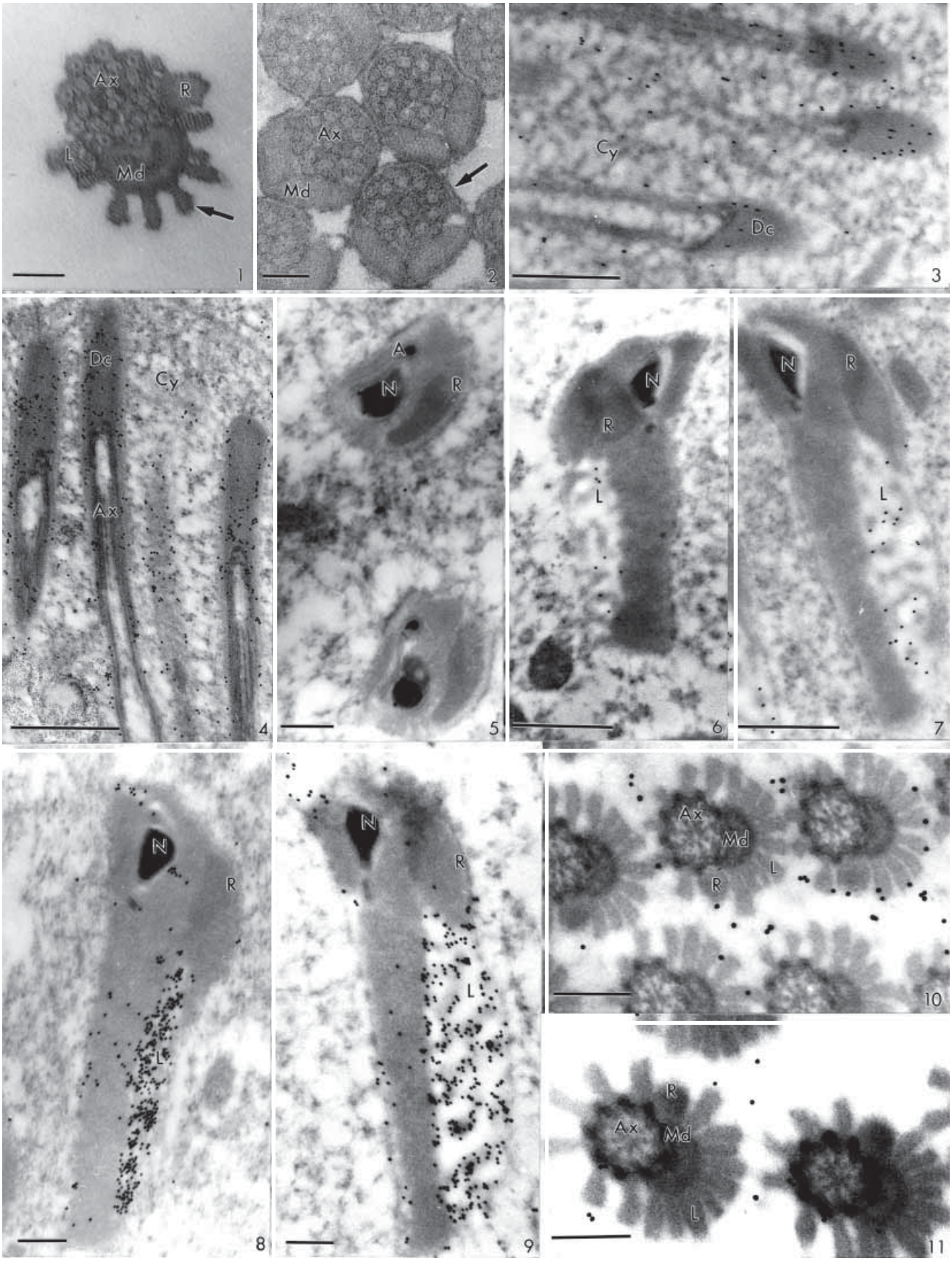
Lectin	Lacinated appendages	
	Head	Tail
PNA	+	+
GS-IB4	+	+
GS-II	±	++
HPA	++	++
WGA	++	±
UEA-I	+	+
UEA-II	++	++

++, Dense labeling (>1,000 gold particles/μm²); +, moderate labeling (10-1,000 gold particles/μm²); ±, sparse labeling (< 10 gold particles/μm²). The lectin abbreviations are given in Table 1.

In the anterior region embedded in the cystic cell, the apyrene spermatozoa had a dense cap which was labeled by GS-IB4 (Fig. 3) and HPA (Fig. 4), indicating the presence of α-galactose and α-N-acetyl-galactosamine residues, respectively.

Eupyrene spermatozoa had an elongated nucleus and an acrosome located anteriorly. Elaborate lacinate and reticular appendages extended from the plasma membrane. These structures were labeled by some lectins. The lectin WGA, which binds to β-N-acetyl-glucosamine and N-acetylneuraminic acid residues, labeled the whole nucleus of the spermatozoa, but no labeling was observed in the acrosome (Fig. 5). The lacinated appendages were loosely arranged in the head region and were labeled by GS II (Fig. 6), indicating the presence of α- and β-N-acetyl-glucosamine residues, by PNA, indicating the occurrence of β-galactose residues (Fig. 7), by HPA (Fig. 8) and UEA II (Fig. 9), indicative of α-N-acetyl-galactosamine, and α- and β-N-acetyl-glucosamine residues, respectively. The lectins UEA II (Fig. 10) and GS II (not shown), which bind to α- and β-N-acetyl-glucosamine residues, strongly labeled the lacinated appendages in the flagellar region, whereas the lectins UEA I (Fig.

Figure 1. Cross-section through the tail of a eupyrene spermatozoon showing staining with ruthenium red (**arrow**) in the lacinate appendages (**L**). **Ax** - axoneme, **Md** - mitochondrial derivatives, **R** - reticular appendage. Bar = 0.1 μm.
Figure 2. Section through the flagella of apyrene spermatozoa showing staining with ruthenium red in the plasma membrane (**arrow**). **Ax** - axoneme, **Md** - mitochondrial derivatives. Bar = 0.1 μm.
Figures 3-4. Longitudinal section through the anterior region of apyrene spermatozoa. The dense caps (**Dc**) were labeled with GS-IB4 (Fig. **3**) and HPA (Fig. **4**), respectively. **Ax** - axoneme, **Cy** - cystic cell cytoplasm. Bar = 0.5 μm.
Figure 5. Section through the head region of eupyrene spermatozoa showing the nucleus (**N**) labeled with WGA. **A** - acrosome, **R** - reticular appendage. Bar = 0.2 μm.
Figures 6-9. Sections through the head of eupyrene spermatozoa showing the regions of loose lacinated appendages (**L**) labeled by GS-II (Fig. **6**), PNA (Fig. **7**), HPA (Fig. **8**) and UEA-II (Fig. **9**). **N** - nucleus, **R** - reticular appendage. Bars = 0.5 μm (**6** and **7**) and 0.2 μm (**8** and **9**).
Figures 10-11. Cross-sections through the tail of eupyrene spermatozoa. Note the labeling of the lacinated appendages (**L**) by UEA-I and UEA-II, respectively. **Ax** - axoneme, **Md** - mitochondrial derivatives, **R** - reticular appendage. Bar = 0.2 μm.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11), GS I, PNA and WGA (not shown) produced moderate labeling of the lacinated appendages in this region.

DISCUSSION

The plasma membrane of apyrene spermatozoa and the lacinate appendages of eupyrene spermatozoa are covered by acid mucopolysaccharides, as shown by staining with ruthenium red. These acid mucopolysaccharides are part of the cell coat (glycocalyx) and are most likely involved in cellular recognition, adhesion and the regulation of proliferation [21].

Several functions have been suggested for apyrene spermatozoa, including the digestion of material necessary for sperm maturation [18], facilitation of the acquisition of motility by eupyrene spermatozoa, the transport of eupyrene sperm from the spermatophore to the spermatheca, and the dissociation of eupyrene bundles [9,26]. The detection of α-galactose and α-N-acetylglucosamine in *A. gemmatalis* apyrene spermatozoa is insufficient to explain the function of these sugars, but may indicate that these carbohydrates are important in the recognition and activation of apyrene and/or eupyrene spermatozoa.

Since eupyrene spermatozoa are responsible for fertilization, it seems natural that they should have sugar residues in their outer coat, which is important for the cell-to-cell interaction with the egg during fertilization. This conclusion is supported by our results demonstrating numerous lectin binding sites in these spermatozoa. The precise roles of nuclear glycoconjugates are unknown. However, there is evidence for the involvement of glycoproteins in transcription [11-13]. Furthermore, qualitative and quantitative variations in lectin binding sites in the cell nucleus have been correlated with changes in nuclear activities [5,23]. The presence of certain glycoproteins as intrinsic chromatin components, suggests the involvement of sugar-containing macromolecules in chromatin condensation. These glycoproteins may modulate the physicochemical environment of the nucleoplasm and participate directly in molecular interactions at specific sites of the genome, as suggested by Kan and Pinto da Silva [12].

The lectins used here did not label the acrosomal region. This may be because eupyrene spermatozoa in the testis are still immature and their sugar residues are either absent or unexposed. A similar situation was observed in the spermatozoa of a phytophagous bug, in which sugar residues were detected only during the initial phase of the acrosomal complex formation [7].

The lacinate and the reticular appendages are the main particularities of eupyrene spermatozoa, and are associated with the sperm plasma membrane. The presence of several sugar residues, such as α- and β-galactose, α- and β-N-acetylglucosamine and α-fucose, may indicate the participation of these appendages in cell-to-cell recognition, in protection, or in a system that triggers the activation and subsequent maturation of apyrene sperm.

In summary, our data revealed different distribution patterns for glycoconjugates in the apyrene and eupyrene spermatozoa of *A. gemmatalis*. These differences probably reflect the divergent developmental functions of these sugars during fertilization.

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