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Changes in Rat Sperm Membrane Glycosidase Activities and Carbohydrate and Protein Contents Associated with Epididymal Transit

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ABSTRACT

Rat spermatozoa were recovered from the caput, corpus, and cauda epididymides and assayed for glycosidase activity, total nonamino (neutral) carbohydrate, and protein content. The activities of β-glucosidase, β-galactosidase, β-N-acetylglucosaminidase, and β-N-acetylgalactosaminidase were fluorometrically assayed in spermatozoa and membrane-enriched fractions. Except for β-glucosidase, the activities of the glycosidases based on protein content were greatest in whole sperm and membrane-enriched fractions obtained from the cauda epididymides. Based on sperm concentration, however, glycosidase activities increased proceeding from the caput to the corpus epididymides, then declined from the corpus to the cauda epididymides. Analyses of nonamino carbohydrate and protein content based on sperm number indicated regional trends similar to those of glycosidase activity. Total nonamino carbohydrate and protein content were highest in corpus sperm, and lowest in cauda sperm. These data indicate major quantitative changes in cell surface carbohydrate as spermatozoa traverse the epididymis. A positive correlation for the membrane-enriched fraction between increasing glycosidase activity and decreasing carbohydrate and protein content suggests that glycosidases may play a significant role in modifying the spermatozoon surface during epididymal transit and maturation.

INTRODUCTION

The physiological role of glycosidases in the mammalian epididymis and their significance in the acquisition of sperm fertility is uncertain. Based on their ability to hydrolyze alpha and beta N-glycosidic and O-glycosidic linkages, it is likely that epididymal glycosidases are involved in the degradation and/or modification of glycoproteins, glycopeptides, glycolipids, and mucoproteins associated with epididymal fluid and spermatozoa. Monosaccharides, liberated as a result of hydrolysis, may either serve as an energy source or be recycled by enzymes involved in the synthesis of complex carbohydrate chains associated with proteins at the sperm surface and in epididymal fluid.

Several studies have demonstrated qualitative changes in the carbohydrate complexity of the plasma membrane of spermatozoa during epididymal transit by using lectin-binding assays (Nicolson et al., 1977; Courtens and Fournier-Delpech, 1979; Hammerstedt et al., 1979; Lewin et al., 1979; Olson and Danzo, 1981). However, these studies provide little quantitative information on changes in sperm membrane carbohydrate. Considering that epididymal spermatozoa are limited in their biosynthetic ability (Abraham and Bhargava, 1963a,b; Witkin and Bendirch, 1977) and that carbohydrate chains associated with the sperm surface are not primary gene products (Beyer et al., 1979), modification of carbohydrate residues on the sperm surface may be regulated by glycosidases and glycosyltransferases (Hamilton and Gould, 1980). The unusually high activity levels of a variety of glycosidases found in epididymal tissue and fluid (Conchie et al., 1959; Jones and Glover, 1973; Khar and Anand, 1977; Kemp and Killian, 1978; Mayorga and Bertini, 1983; Chapman and Killian, 1984) lend support to the concept that these enzymes may alter complex carbohydrate chains associated with the sperm plasma membrane.

We previously demonstrated differences in glycosidase activities in epithelial cells and sperm isolated

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from different regions of the epididymis (Chapman and Killian, 1984). However, these studies utilized whole spermatozoa, and the contribution of membrane glycosidases to the total activities could not be distinguished from other sources, such as those present in the acrosome. Considering the potential importance of membrane carbohydrates and glycosidases in sperm maturation, the present study was undertaken to quantitate nonamino sugar, sialic acid, and protein contents and the activities of four glycosidases in membrane-enriched fractions from caput, corpus, and cauda spermatozoa.

MATERIALS AND METHODS

Animals and Isolation of Epididymal Spermatozoa

Adult male Sprague-Dawley rats ranging from 350–400 days of age and 400–600 g in body weight were used. The animals were bred and maintained in the Kent State University Department of Biological Sciences, and housed in 12L:12D cycle at 22°C with laboratory chow and water supplied ad libitum. For each experiment, 6–8 rats were killed by decapitation after a sharp blow to the head. Epididymides with vasa deferentia attached were excised and trimmed of extraneous adipose tissue. Each epididymis was sectioned into three parts: the caput, corpus, and cauda, with vas deferens attached (Hoffmann and Killian, 1981).

Cauda spermatozoa were recovered from an incision in the distal cauda and backflushing the vas deferens with Sorenson's phosphate buffer, pH 7.38 (SPB, made 0.0667 M with Na_2HPO_4/KH_2PO_4). The recovered fluid was reused for subsequent backflushings to minimize dilution of spermatozoa and associated fluid.

Caput and corpus spermatozoa were recovered by mincing tissue from the isolated corpus and caput segments of the epididymis into 1-mm pieces in glass petri dishes with a single-edged razor blade. Tissue suspensions were transferred into graduated Pyrex clinical centrifuge tubes with a Pasteur pipette and allowed to settle for 20 min in SPB (4 ml/epididymis) at room temperature. After the upper phase had cleared of tissue fragments, the supernatants containing caput or corpus spermatozoa were transferred to another graduated Pyrex clinical centrifuge tube and spun at 1000 × *g* for 10 min at room temperature to pellet spermatozoa.

Spermatozoon pellets obtained from the various regions of the epididymis were resuspended and pelleted twice from 4 ml of SPB by centrifugation at 1000 × *g* for 10 min at room temperature. The final washed pellets were resuspended in 3 ml of SPB and held temporarily on ice (10 min). Ten-μl aliquots were removed, diluted with 10% formalin, and counted with a hemocytometer to determine the number of spermatozoa recovered for each experiment. The preparation of sperm was contaminated by less than 0.1 percent somatic cells as revealed by phase-contrast microscopy.

Membrane Isolation

Membrane-enriched fractions isolated from rat spermatozoa recovered from different epididymal regions were purified according to the method of Olson and Hamilton (1978), with some minor modifications. Briefly, caput, corpus, and cauda spermatozoa were pelleted by centrifugation for 10 min at 1000 × *g* at room temperature. The pellets were resuspended in SPB or Na_2CO_3 buffer (100 mM, pH 11.3) and sonicated on ice for 3 min with a Model 300 Fisher Sonic Dismembrator equipped with a microtip and set at 35% relative output. Aliquots of samples sonicated in SPB or Na_2CO_3 buffer were used to determine protein content. However, only spermatozoon samples sonicated in SPB were used to assess glycosidase activities.

Sonicated samples were centrifuged at 500 × *g* for 5 min at room temperature. The supernatants were carefully removed and transferred into 15-ml Corex centrifuge tubes and spun at 48,000 × *g* for 1 h at 5°C in a Beckman J-21 centrifuge. The resulting membrane pellets (crude membranes) were homogenized in 3 ml of SPB and overlayered on a cushion of 1.7 M sucrose (2 ml), and spun again at 48,000 × *g* for 1 h at 5°C. The enriched membrane fractions, which banded at the interface, were carefully removed from the tubes with the aid of a curved-tipped Pasteur pipette, transferred to another 15-ml Corex centrifuge tube containing 3 ml of SPB, and spun at 48,000 × *g* for 2 h at 5°C.

Electron micrographs, prepared in our laboratory of the final membrane fraction, demonstrated that it was devoid of mitochondria, tails, and heads (Wang, 1984). No evidence for cytochrome oxidase, a mitochondrial membrane-marker enzyme, was detectable in the membrane preparation after electrophoresis (O'Farrell, 1975) in polyacrylamide gels (Fig. 1) or biochemical assay using the method of Wharton and

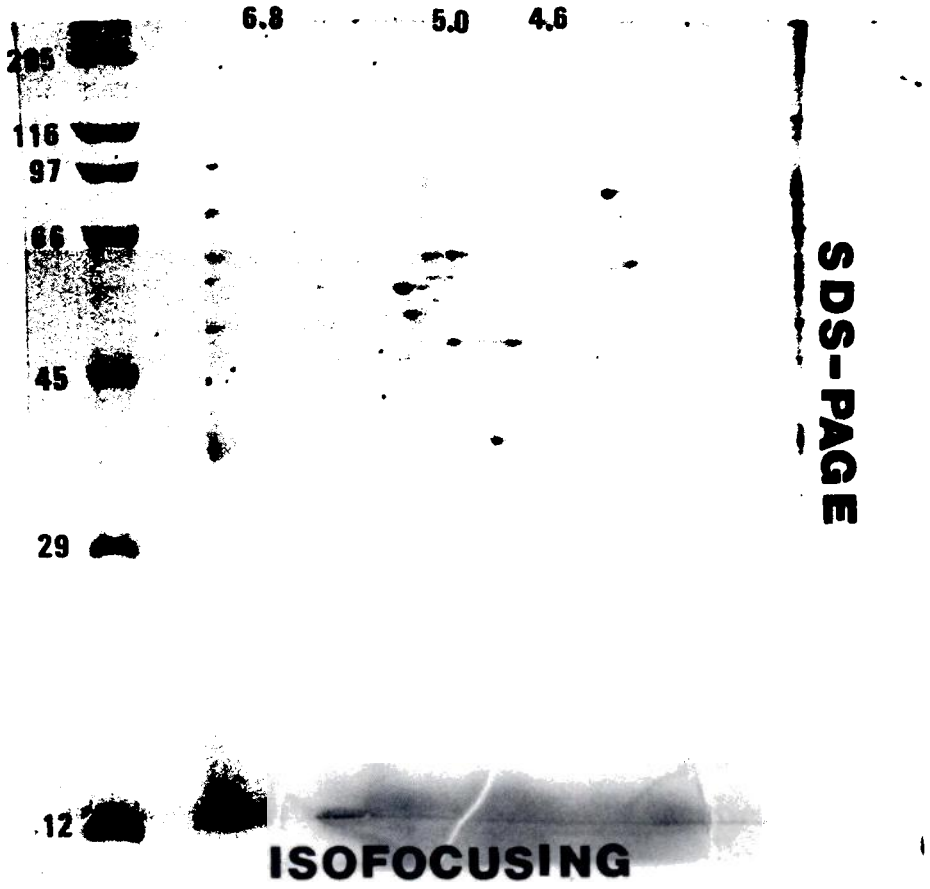

FIG. 1. Coomassie-stained two-dimensional gel profile of membrane polypeptides of cauda epididymal sperm. Approximately 150 μg total protein was applied to the isofocusing gel in the first dimension, and the second dimension was run in a 9–15% linear gradient acrylamide gel. The following proteins were used as molecular mass markers to calibrate the gel in the second dimension: myosin (rabbit muscle; 205 × 10^3 daltons), β-galactosidase (*E. coli*; 116 × 10^3 daltons), phosphorylase b (rabbit muscle; 97.4 × 10^3 daltons), albumin (bovine; 66 × 10^3 daltons), albumin (egg; 45 × 10^3 daltons), carbonic anhydrase (bovine erythrocytes; 29 × 10^3 daltons), and cytochrome C (horse heart; 12.4 × 10^3 daltons).

Tzagoloff (1967). Therefore, we concluded that plasma and acrosomal membranes were the major constituents of this preparation.

Enzyme Assays

Activity levels of the glycosidases were measured fluorometrically with their corresponding 4-methylumbelliferyl substrates (Sigma Chemical Co.). The glycosidases and their substrates were β-glucosidase, 4-methylumbelliferyl-β-D-glucose; β-galactosidase, 4-methylumbelliferyl-β-D-galactose; β-N-acetylglucosaminidase, 4-methylumbelliferyl-N-acetyl-β-D-glucosaminide; and β-N-acetyl-galactosaminidase, 4-methylumbelliferyl-N-acetyl-β-D-galactosaminide. Two buffer systems were used for assaying glycosidases. Buffer A consisted of 0.2 M sodium acetate/acetic acid at pH 5.0 and was used to assay β-N-glucosidase and β-galactosidase. Buffer B was 0.1 M sodium citrate/citric acid at pH 5.0 and was used to assay β-N-acetylglucosaminidase and β-N-acetylgalactosaminidase. The protein concentration of each sample was adjusted to 1 μg/μl. A 20-μl aliquot was then combined with the appropriate buffer and 4-methylumbelliferyl substrate at 2 mM to yield a final assay volume of 200 μl. After the assay mixture was incubated for 1 h at 37°C, the reaction was terminated by the addition of 3 ml of 2 M glycine/NaOH buffer at pH 10.8.

Fluorescence of the released 4-methylumbelliferone (4-MU) was measured in a Turner Fluorometer Model III in the 1X range. Filters used were a primary 7-60, which passes light of >365 nm wavelength, and a secondary 3, which passes light of >415 nm wave-

length. A fresh 0.001 N H_2SO_4 solution containing 4.0×10^{-8} g/ml of quinine sulfate was used to standardize the fluorometer scale at 5 units. The fluorescence intensity was converted to nanomoles 4-MU released by comparison to a standard curve derived from a serially diluted concentration (1 μg/ml) of 4-MU dissolved in 2 M glycine/NaOH at pH 10.8. At the concentrations used (0–30 ng/μl), fluorescence was linear for the standard plot. A unit was defined as the activity that produced 1 nmole of product (4-MU) per h at 37°C. Sp. act. was defined as units of activity per mg of protein.

To assess whether the enzyme activity of sperm isolated from the caput, corpus, and cauda regions was differentially affected by the time necessary to isolate the samples, we determined β-galactosidase activity of sperm immediately after their removal from the tissue and at timed intervals up to 60 min later.

An experiment also was performed to determine if β-galactosidase activity was linear over time for sperm continuously incubated for 60 min.

The method of Schwert and Takenaka (1955), with N-benzoyl-L-arginine ethyl ester HCl as the substrate, was used to assay acrosin activity. Before assessing each sample for acrosin activity, the samples were acidified to pH 3.5 with 0.1 N HCl. To 1.6 ml of substrate, which consisted of 0.5 mM N-benzoyl-L-arginine ethyl ester in 0.15 M Tris/HCl buffer, pH 8.0, containing 0.05 M $CaCl_2$ was added 100 μl of sample. The change in absorbance was recorded at 253 nm wavelength at 1-min intervals for a total of 8 min. Generally, the enzymatic rates were linear for 3–4 min, beyond which the enzyme activity progressively declined. A plot of absorbency versus time was used to determine the change in rate, which in turn was used to calculate the amount of product (N-benzoylarginine) released per min. One unit (U) of activity is defined as μmoles of substrate hydrolyzed per h at 37°C.

Protein Determination

The protein content of spermatozoa was determined by the Lowry method (Lowry et al., 1951), with bovine serum albumin (BSA) as the standard. Standards and samples were assayed in duplicate with the concentrations of BSA ranging from 10 to 60 μg/ml in 10-μg increments.

Carbohydrate Analyses

The phenol-sulfuric acid method (Dubois et al., 1956), with mannose as the standard, was used to determine total nonamino (neutral) carbohydrate content in sperm and membrane-enriched fractions. Since membrane-enriched fractions were purified on a sucrose cushion, membrane fractions were washed twice in 5 ml of SPB and centrifuged at 48,000 × *g* for 30 min at 5°C prior to carbohydrate analysis to ensure minimal contamination by sucrose.

Sialic acid content of membrane fractions was determined by the thiobarbituric acid method (Warren, 1959). Membrane-bound sialic acid associated with intact whole sperm was determined (Toowicharanont and Chulavatnatol, 1983) using 3-methyl-2-benzothialinone (MBTH; Eastman Kodak Chemical Co., Rochester, NY). Differential staining of live/dead spermatozoa was performed after initial and postwashing and centrifugation using nigrosin-eosin staining (Dott and Foster, 1972), and analyzed by light microscopy to assess cell death that might have resulted from excessive washing and centrifugation. Spermatozoa subjected to such treatment exhibited 74–83% viability.

For analysis of sperm membrane nonamino carbohydrate by gas liquid chromatography (GLC), membrane samples (2 mg total protein) were hydrolyzed with 3 N HCl at 100°C for 2 h (Churchill et al., 1976). The released nonamino sugars were then analyzed as their alditol acetate-derivatives, with arabinitol as the internal standard. Alditol acetate-derivates of mannose, fucose, galactose, glucose, and xylose were used as standards to identify major membrane nonamino sugar components, and the purity of each standard sugar was determined by GLC-mass spectroscopy. Quantitative analysis of membrane nonamino sugars was performed using a Gow-Mac Model 550 gas chromatograph (Gow-Mac Instruments Co., Bound Brook, NJ) equipped with a thermal conductivity detector, microprocessor temperature programmer, and a 1.83 m × 3.18 mm coiled glass column packed with ECNSS-M on 100/120 mesh Gas Chrom Q (Alltech Associates, Inc., Deerfield, IL).

Statistical Procedures

Significant differences ($p<0.05$) among whole cells and membrane fractions and the three epididymal sperm populations were determined by Student's *t*-test.

RESULTS

Regional differences were observed in glycosidase activities associated with whole sperm and their membrane-enriched fractions. Based on sperm concentrations, β-N-acetylglucosaminidase, β-N-acetylgalactosaminidase and β-galactosidase activities increased in whole sperm in the corpus relative to the caput and then declined in cauda sperm to a value which was still greater than that of caput sperm (Fig. 2A). However, except for β-glucosidase, the sp. act. of glycosidases in whole cauda sperm and cauda membrane-enriched fractions were significantly greater than that of whole caput and corpus sperm and their membrane-enriched fractions (Fig. 2B and C).

The assay of β-galactosidase activities in caput, corpus, and cauda sperm at the initial time of sperm isolation and up to 60 min later revealed no change in activities, with increased holding time for the three sperm types (Fig. 3). Furthermore, β-galactosidase activity was linear for caput, corpus, and cauda sperm during the 60-min incubation (Fig. 4).

Membrane fraction/whole sperm sonicate glycosidase ratios were not constant among sperm obtained from different epididymal regions (Table 1). Since it was possible that an enrichment of glycosidases in membranes may have occurred during membrane purification by artifactual binding of soluble glycosidases derived from epididymal tissue and fluid, β-N-acetylglucosaminidase was assayed during the steps of purification of cauda sperm membranes. Beta-N-acetylglucosaminidase is found in both the acrosome and epididymal fluid (Conchie et al., 1959; Jones and Glover, 1973; McRorie and Williams, 1974). In addition, acrosin, a serine proteinase associated with acrosomal membranes, and the matrix (Harrison et al., 1982; Straus and Polakoski, 1982; Johnson et al., 1983) was also assayed during the membrane purification. Although acrosin and β-N-acetylglucosaminidase were observed in whole sperm sonicates, only acrosin activity decreased during the course of cauda membrane purification (Table 2). Although an increase in β-N-acetylglucosaminidase activity was observed in the 48,000 × *g* supernatant, essentially no significant change was observed in either the crude or enriched membrane fractions. The enriched membrane fraction treated with SPB containing hypertonic NaCl (0.1 M) did not result in a significant loss in either acrosin or β-N-acetylglucosaminidase activity.

On the basis of sperm concentration, it was determined that total nonamino carbohydrate and protein contents were greatest in spermatozoa obtained from the corpus region and lowest in spermatozoa obtained from the cauda epididymides (Fig. 5A and B). With respect to protein, there was a slight increase in nonamino carbohydrate content of corpus sperm-enriched membranes compared to that of caput and cauda sperm membranes, but the differences were not significant (Fig. 5C). Total nonamino carbohydrate and protein contents of spermatozoa recovered from the caput, corpus, and cauda epididymides were positively correlated (r=0.99).

GLC analyses of nonamino carbohydrates in membrane-enriched fractions of the three sperm

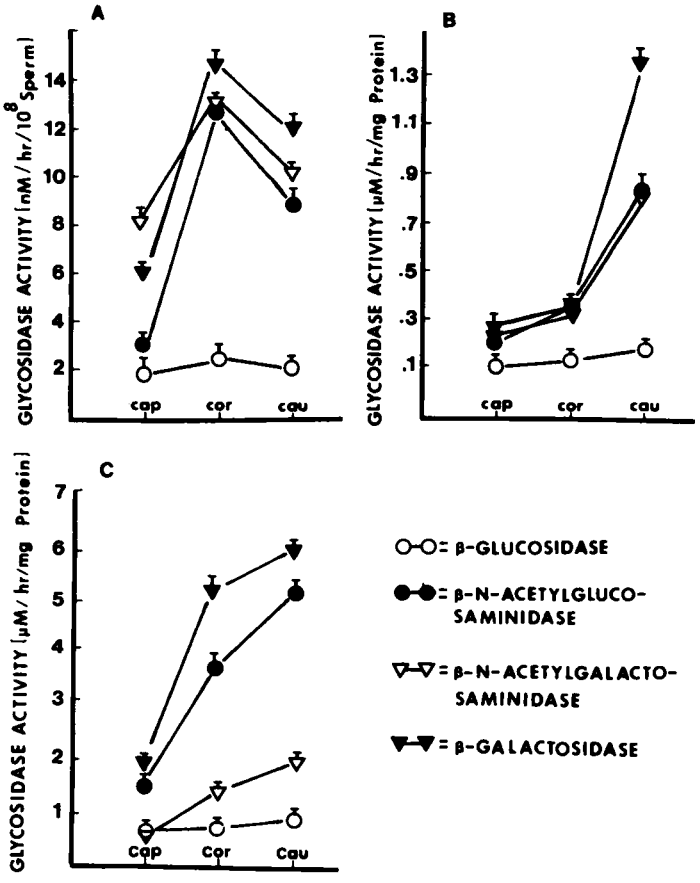

FIG. 2. Levels of β-glucosidase, β-N-acetylglucosaminidase, β-N-acetylgalactosaminidase, and β-galactosidase in caput (*cap*), corpus (*cor*), and cauda (*cau*) epididymal sperm and their membrane-enriched fractions. (*A*) Glycosidase activities based on sperm concentration (nM of 4-methylumbelliferone liberated per h per 10^8 sperm). (*B*) Glycosidase activities based on cellular protein content (μM of 4-methylumbelliferone liberated per h per mg sperm protein). (*C*) Levels of glycosidase activities associated with membrane-enriched fraction (μM of 4-methylumbelliferone liberated per h per mg membrane protein). Means and standard errors are indicated for four experiments.

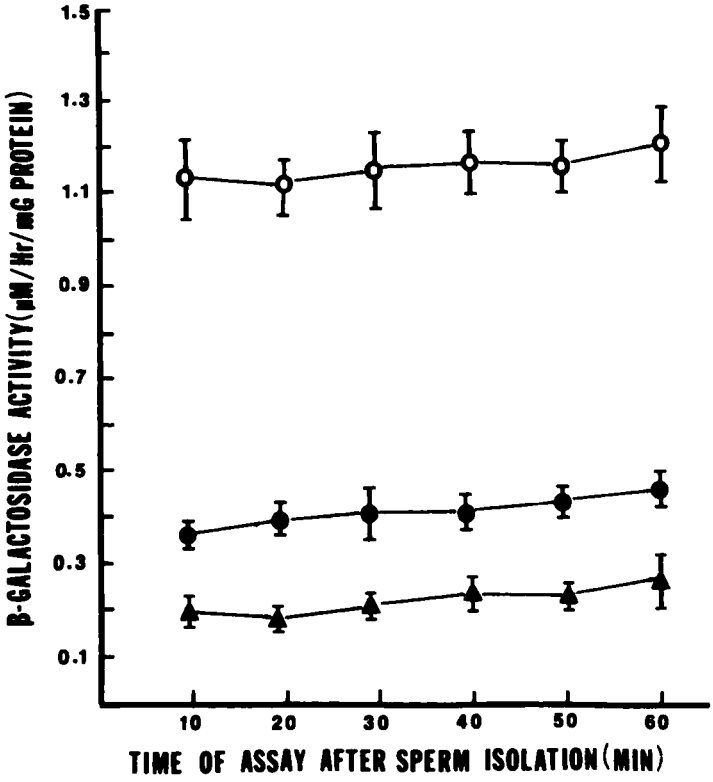

FIG. 3. β-Galactosidase activity (μmoles 4-methylumbelliferone release/h/mg protein^{-1}) as a function of time after the initial isolation of caput (▲), corpus (●), and cauda (○) sperm. Means and standard errors for five experiments.

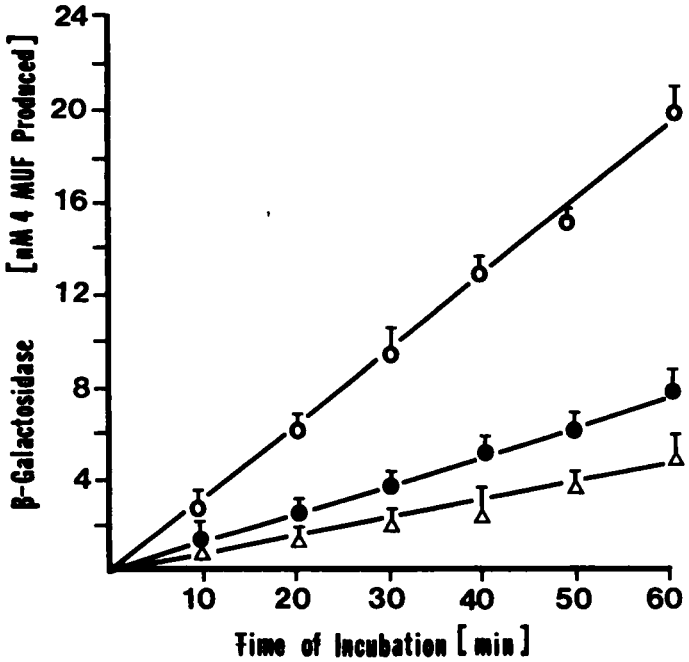

FIG. 4. Amount (nmoles) of 4-methylumbelliferone released from β-galactosidase substrate during incubation of caput (△), corpus (●), and cauda (○) sperm for up to 60 min. Means and standard errors for five experiments.

TABLE 1. Glycosidase activity ratios of purified sperm membrane to whole sperm.[a]

Enzyme	Caput	Corpus	Cauda
β-Glucosidase	9.42	10.40	6.29
β-N-acetylglucosaminidase	5.95	10.24	11.23
β-Galactosidase	1.74	3.98	2.0
β-N-acetylgalactosaminidase	7.12	15.98	7.35

[a]Values calculated from sp. act. data (Fig. 1, panel B and C).

populations (Fig. 6) revealed that the levels of galactose were unaltered during epididymal transit, whereas mannose levels increased 4-fold and fucose levels decreased 3-fold. Neither glucose nor xylose were detected. Sialic acid content was significantly greater in caput sperm membranes than in corpus and cauda sperm membranes.

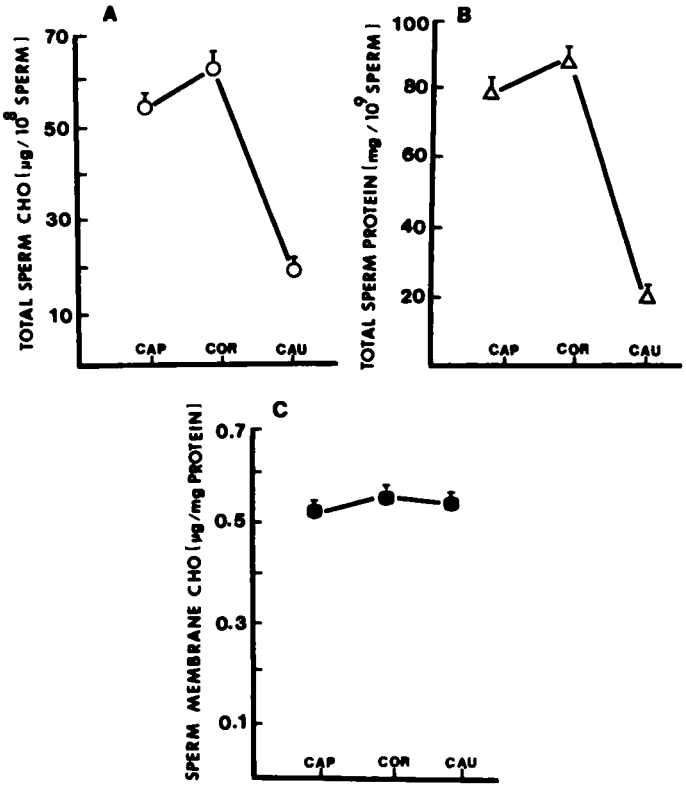

FIG. 5. Total nonamino carbohydrate and protein content in caput, corpus, and cauda epididymal sperm and their membrane-enriched fractions. (*A*) Total nonamino carbohydrate content based on sperm concentration (μg carbohydrate per 10^8 sperm). (*B*) Total sperm protein based on sperm concentration (mg cellular protein per 10^9 sperm). (*C*) Nonamino carbohydrate content associated with membrane-enriched fractions (μg carbohydrate per mg membrane protein). Means and standard errors for four experiments.

TABLE 2. Activity levels of acrosin and β-N-acetylglucosaminidase in cauda sperm fractions.

Sperm fraction	Acrosin activity	β-N-acetyl-glucosaminidase (μM/h/mg protein)[a]
Sperm + cauda fluid	1191 ± 62	261 ± 28
Cauda fluid	2.3 ± 0.31	464 ± 21
Whole sperm sonicate	1014 ± 55	0.81 ± 0.04
Crude-membrane supernatant	955 ± 9.7	6.31 ± 0.13
48,000 X *g* supernatant	594 ± 26	20 ± 1.41
48,000 X *g* membrane pellet	282 ± 13	6.63 ± 0.32
Enriched plasma membrane	162 ± 7.8	5.66 ± 0.54
Hypertonic (0.1 M) NaCl treated membrane	119 ± 3.49	4.93 ± 0.28

[a]Mean ± SEM based on three separate isolations.

DISCUSSION

Numerous studies have presented evidence to support the concept that a structural reorganization of the sperm surface occurs during epididymal transit (Cameo and Balquier, 1976; Nicolson et al., 1977; Lea et al., 1978; Hammerstedt et al., 1979; Brooks, 1981; Jones et al., 1981; Olson and Danzo, 1981; Jones and Brown, 1982; Zeheb and Orr, 1984). The results of the present study confirm our earlier findings of regional differences in epididymal glycosidase activity in sperm (Chapman and Killian, 1984) and demonstrate for the first time that glycosidase activity is considerably higher in the sperm membrane fraction than in whole sperm. It is possible that glycosidases secreted by the epididymal epithelium become associated with, tightly bound to, or incorporated into the plasma membrane. Immunocytochemical studies have provided evidence that glycoproteins are secreted by the epididymal epithelium into the lumen where they are bound to spermatozoa (Kohane et al., 1980; Moore, 1981; Byers et al., 1984). Therefore, the higher glycosidase activities associated with sperm membranes may represent soluble glycosidases bound from epididymal fluid. These enzymes may subsequently function in membrane-mediated events leading up to fertilization, such as binding to and penetrating the zona pellucida, contact and fusion with the vitelline membrane, and penetration of the egg vitelline (Allison and Hartree, 1970; Farooqui and Srivastava, 1980; Rodger and Young, 1981).

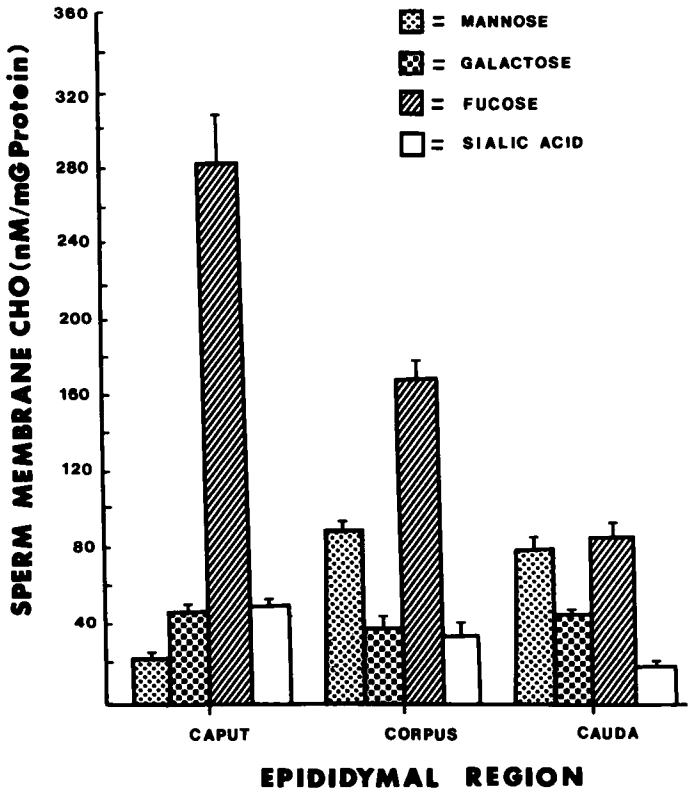

FIG. 6. Nonamino carbohydrate and sialic acid contents associated with caput, corpus, and cauda epididymal sperm membrane-enriched fractions. Nonamino carbohydrates were determined by GLC as their alditol acetate derivatives using arabinitol as the internal standard. For each preparation, three gas chromatographic runs were performed. N-acetylneuraminic acid (sialic acid) was determined by the thiobarbituric method. Means and standard errors are indicated for four experiments.

It is unlikely that the regional differences observed in sperm glycosidase activity were due to differential post-mortem effects. The β-galactosidase activity assayed immediately after sperm recovery was not significantly different from that of sperm assayed up to 60 min later, suggesting that the activity of the enzyme for each sperm type remained stable during this period. The activity of β-galactosidase also remained constant during the enzyme assay since plots of 4-MU released over time were linear. These studies

lead us to suggest that biological differences exist in glycosidase activities among sperm from different epididymal regions. Further studies are necessary, however, to determine whether these differences in activity are the result of differences in the amount of enzyme associated with caput, corpus, and cauda sperm or whether structurally different forms of the enzyme are associated with the different sperm types.

The presence of acrosin activity in the cauda membrane fractions (Table 2) indicates contamination by acrosomal membranes. The observation that hypertonic salt treatment did not significantly reduce acrosin or β-N-acetylglucosaminidase activity in the purified membrane fraction suggests that at least one form of each of these enzymes is membrane-bound. However, since the sp. act. of cauda acrosin declined during membrane purification, it would appear that the majority of acrosin activity in the crude membrane preparations was only loosely associated with membrane and subsequently eluted during the purification procedure. This observation is supported by the work of others, which suggests that acrosin is not truly membrane-bound, but merely membrane-associated (Harrison et al., 1982; Straus and Polakoski, 1982). Glycosidases may be more tightly bound to sperm membranes than acrosin to account for the maintenance of high levels of β-N-acetylglucosaminidase during membrane purification.

The dramatic decline in total sperm protein is in agreement with an earlier finding (Hoffmann and Killian, 1981) and may reflect a major loss of membrane, since there is not a significant change in the membrane protein/phospholipid ratio as spermatozoa traverse the epididymis (Wang, 1984). The cytoplasmic droplet is normally found in association with caput and corpus sperm, but not cauda sperm (Bedford, 1975). The loss of the cytoplasmic droplet from cauda sperm may account for the decline in total sperm protein and the parallel decline in total sperm nonamino carbohydrate.

Values from GLC analyses of sperm membrane nonamino carbohydrates cannot be considered maximum values associated with the spermatozoon surface, since losses are likely to occur resulting from incomplete hydrolysis and reactions during derivation or destruction of carbohydrate residues. However, the results of GLC analyses do establish quantitative differences in surface carbohydrate of spermatozoa as they traverse the epididymis. There is a general reduction in certain membrane nonamino carbohydrates of sperm during epididymal transit, and these data are consistent with the observed reduction in membrane nonamino carbohydrate determined by the phenol-sulfuric acid method.

Electron microscopic studies of spermatozoon surface charge, as deduced from the selective binding of colloidal iron hydroxide, suggest an increase in negative surface charge during epididymal transit (Yanagimachi et al., 1972). An increase in negative surface charge of rabbit spermatozoa during epididymal maturation has been attributed to an increase in bound sialic acid (Nicolson et al., 1977). However, our findings demonstrate a decrease in membrane-bound sialic acid, with the highest levels found in caput sperm and the lowest levels in cauda sperm. Our observed decreases in membrane-bound sialic acid during epididymal transit are in agreement with those of other investigators using washed and unwashed rat spermatozoa (Gupta et al., 1974; Hoffmann and Killian, 1981; Toowicharanont and Chulavatnatol, 1983). It is possible that the decline in membrane-bound sialic acid may be relevant to sperm maturation in the rat, but not in other species.

An alternative explanation may rest with the fact that the electron microscopic studies measured negative charges associated with the sperm surface whereas our assay measured total sialic acid associated with the membrane. It is possible that while total membrane-bound sialic acid decreases, unmasking of surface sialic residues could account for observed increases in negative surface charge associated with epididymal transit.

The present study represents the first report of quantitative differences in nonamino carbohydrate associated with membranes of spermatozoa obtained from different regions of the epididymis. There is a drastic reduction in the amount of total nonamino carbohydrate and protein as spermatozoa traverse the epididymis. Coincident with these findings is the gradual increase in sp. act. of glycosidases associated with whole sperm and membrane-enriched fractions proceeding from the caput to the cauda epididymal region. It is possible that post-translational modification, such as deglycosylation of sperm membrane glycoproteins by glycosidases, may result in significant alterations in size and/or structure of these glycoproteins. Alterations in the size and structure of glycoproteins may also modify their physicochemical properties such as tertiary conformation, viscosity, and charge, and trigger membrane-related events leading to the acquisition of motility and fertility.

When the data were expressed as a function of sperm number, peak levels of glycosidase activity, nonamino carbohydrate and protein were observed in

the corpus epididymides. These observations suggest that the corpus region is a major site where sperm membrane modifications occur within the epididymis. Although the mechanisms are not understood, it is possible that membrane-associated glycosidases play a significant role throughout the life history of spermatozoa to modify the sperm surface and enable carbohydrate chains to serve as potential "signals" for the transfer of biological information (Olden et al., 1985). This modified surface carbohydrate may be important in sperm capacitation, the acrosome retention (Schwartz and Koehler, 1979), and sperm-egg interactions (Rosati and De Santis, 1980; Lambert and Van Le, 1984). A more complete understanding of the physiological role of glycosidases in the epididymis and in utero awaits further investigation.

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