Alteration of the isoform composition of plasma-membrane-associated rat sperm α-L-fucosidase during late epididymal maturation: comparative characterization of the acidic and neutral isoforms

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In a previous study, evidence was provided for the presence of a novel plasma-membrane-associated neutral-pH-optimum α-L-fucosidase in rat sperm. In the present study, rat sperm α-L-fucosidase was characterized during epididymal maturation. The pH 7 activity optimum of α-L-fucosidase and its subunit composition (one or two closely spaced immunoreactive protein bands of about 53 ± 2 kDa) did not appear to change during transit through the epididymis. Isoelectric focusing of α-L-fucosidase indicated the presence of a major isoform (B) with a pI near 7 in sperm from testis, caput, corpus and the proximal half of the cauda. α-L-Fucosidase from sperm from the distal half of the cauda, which contained a significant enrichment of sperm and α-L-fucosidase activity, contained isoform B and an additional minor isoform (A) with a pI near 5.2. Isoform B and small amounts of isoform A were present in sperm from the vas deferens. The two fucosidase isoforms present in sperm from the distal cauda were separated by isoelectric focusing and comparatively characterized. They had similar pH–activity curves (with optima near pH 7) and comparable apparent K_M values (0.4 ± 0.04 mM) for 4-methylumbelliferyl α-L-fucopyranoside. Preincubation of the isoforms at different temperatures indicated that isoform A is considerably more thermostable than isoform B. Immunoprecipitation studies using polyclonal antibodies against human liver α-L-fucosidase indicated that approx. 90 % of the enzymic activity for both isoforms was immunoprecipitable under conditions that immunoprecipitated essentially all the human liver enzyme. Neuraminidase treatment of sperm α-L-fucosidase from distal cauda (when compared with the appropriate heat-treated control) led to disappearance of isoform A and a concomitant increase in isoform B. The overall results suggest that isoform A is derived by sialylation of isoform B near the end of epididymal maturation.

INTRODUCTION

Epididymal maturation is a process during which sperm traverse the epididymis, become biochemically and morphologically mature, and develop the capacity to be motile and fertile [1–3]. This process appears to take place in all mammals and involves significant alterations in numerous components (e.g. lipids, proteins) of the sperm plasma membrane [1,4–6]. The total epididymal transit time (including storage) for mammalian sperm is 7–14 days [3], with rat sperm taking about 8 days [7]. During maturation in a number of mammals, approx. 50–80 % of the epididymal sperm are concentrated in the cauda epididymal section [8] at which time they attain their fullest epididymal fertilizing potential [1].

Mammalian α-L-fucosidases (EC 3.2.1.51) are ubiquitous and normally found as soluble components of the lysosome where they are involved in the hydrolytic degradation of fucose-containing molecules [9]. These enzymes exist as multimeric glycoproteins and are classified as acid hydrolases since they normally have relatively acidic enzyme activity optima between pH 4 and 6. Absence or gross deficiency of α-L-fucosidase activity leads, at least in some mammals (e.g. humans and dogs), to the genetic neurovisceral storage disease fucosidosis [10,11].

In a previous study evidence was provided for the presence of a novel α-L-fucosidase in rat sperm [12]. The α-L-fucosidase was immunocytochemically localized on the plasma membrane of the convex region of the principal segment of testicular and cauda epididymal sperm heads. Subcellular fractionation studies using sucrose-density-gradient centrifugation indicated that the fucosidase was present in the plasma-membrane-enriched fraction and provided further evidence for its plasma-membrane localization. Kinetic analysis of the plasma-membrane-associated α-L-fucosidase on intact sperm and from 0.5 M NaCl-solubilized extracts of sperm indicated an unusually high neutral-pH activity optimum between 6.9 and 7.1. Isoelectrical focusing of NaCl-solubilized extracts of rat epididymal sperm indicated the presence of one major isoform with a pI near 7.2 ± 0.1 and a minor more acidic isoform with a pI near 5.2 ± 0.1. SDS/PAGE and Western-blot analysis indicated that the NaCl-solubilized α-L-fucosidase from epididymal sperm contained one or two closely spaced subunits of about 54 and 50 kDa. The relationship of the novel plasma-membrane associated sperm α-L-fucosidase to the lysosomal α-L-fucosidase is not completely understood but both enzymes have two non-identical subunits and a multimeric structure with at least some antigenic epitopes in common.

In the present study, rat sperm α-L-fucosidase has been characterized to determine whether any changes occur in the properties of this plasma-membrane-associated enzyme during epididymal maturation. Most of the studies were performed on fucosidase solubilized with 0.5 M NaCl from intact sperm from testis, from the three major sections of epididymis (caput, corpus and cauda), from the proximal and distal halves of the cauda, and from the vas deferens.

MATERIALS AND METHODS

Animals, tissues and sperm preparation

Sexually mature male Sprague–Dawley rats (Charles Rivers Laboratories, St. Constant, Quebec, Canada) ranging from 70 to

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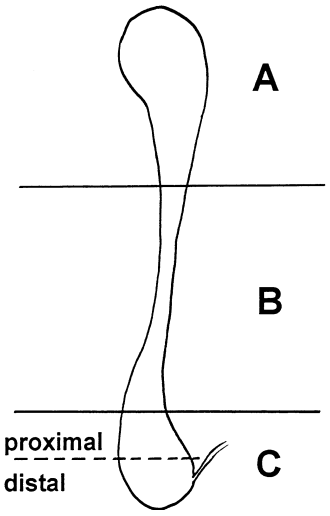

Figure 1 Schematic diagram of rat epididymis

A, Caput; B, corpus; C, cauda. In some experiments, the cauda was divided into proximal and distal halves as indicated by the dashed line.

300 days of age and weighing 340–610 g were given water and rodent chow *ad libitum* and used immediately after CO_2 asphyxiation. Testes, total epididymides (caput, corpus and cauda) and vas deferens were removed and trimmed of adipose tissue. For most experiments, epididymides were subdivided into three anatomical sections (caput, corpus and cauda) as illustrated in Figure 1. In some experiments, the cauda was divided into proximal and distal halves (see Figure 1). Sperm were prepared from specific sections of the male reproductive tract immediately after removal and dissection essentially as previously described [12] and were used as the source for the preparation of plasma-membrane-associated α-L-fucosidase. The number of sperm was estimated by using a Neubauer improved counting chamber.

Release of α-L-fucosidase from intact sperm

Intact sperm were shaken gently (75 rev./min on a Tak-Pro Variable Rotator) at 20 °C in phosphate buffer containing 0.5 M NaCl for 30 min as described [12], the suspensions were centrifuged (10000 ***g*** for 20 min), and the resultant supernatants were assayed for α-L-fucosidase activity using 1 mM 4-methylumbelliferyl α-L-fucopyranoside (Sigma Chemical Co., St. Louis, MO, U.S.A.) [12]. All studies were carried out on α-L-fucosidase solubilized with 0.5 M NaCl from intact sperm. One unit of α-L-fucosidase activity is defined as the amount of enzyme required to hydrolyse 1 nmol of substrate/min at 37 °C. Protein concentration was determined by the method of Lowry et al. [13] using human serum albumin (Sigma Chemical Co.) as the protein standard.

pH optimum, K_m and thermostability studies

The pH–activity curves were constructed as described [12] using duplicate assays for 5–10 min at 37 °C, and actual pH values of a third set of mock tubes were recorded. Fluorescences were corrected for tissue and substrate blanks.

Apparent K_m values were determined graphically by the Lineweaver–Burk double-reciprocal plot method [14] using 4-methylumbelliferyl α-L-fucopyranoside as substrate. Aliquots of enzyme were incubated in substrate of various concentrations (10 concentrations from 0.67 to 0.0067 mM) in 0.1 M NaH_2PO_4/Na_2HPO_4 buffer, pH 7.0, in duplicate for 20 min at 37 °C. Fluorescences were corrected for tissue and substrate blanks.

Thermostability studies were performed on the separated isoforms (A and B) of α-L-fucosidase from distal cauda sperm after preincubation at different temperatures (37, 43, 47 and 50 °C) for up to 90 min. These studies were carried out using a constant amount of α-L-fucosidase activity (0.2 unit) and using human serum albumin (Sigma Chemical Co.) to give a constant final protein concentration of 0.2 mg/ml. After thermal preincubation, the samples were assayed in duplicate for 20 min at 37 °C using 4-methylumbelliferyl α-L-fucopyranoside as substrate.

Isoelectric focusing

Isoelectric focusing was performed essentially as described [12] on aliquots of sample containing 50–125 units of α-L-fucosidase activity. Semipreparative focusing was performed on 600 units of α-L-fucosidase activity to separate the two isoforms (A,B) of α-L-fucosidase present in sperm from the distal half of the cauda. In some experiments, α-L-fucosidase activity (75 or 125 units) from sperm from the distal half of the cauda was subjected to isoelectric focusing after incubation for 6, 17.5 or 27 h at 37 °C in 0.1 M citric acid/sodium citrate buffer, pH 5 (negative control) or after incubation with 10 units of neuraminidase (*Clostridium perfringens*, type VI; Sigma Chemical Co.) for 6, 17.5 or 27 h at 37 °C in 0.1 M citric acid/sodium citrate buffer, pH 5.

SDS/PAGE and Western-blot analysis

Slab SDS/PAGE was performed as described [12] using 4% stacking and 12% separating gels run using 25 mM Tris/0.2 M Tris/0.2 M glycine buffer, pH 8.6, containing 0.1% SDS for approx. 1 h at 175 V at room temperature. Molecular-mass standards were from Sigma Chemical Co. (prestained SDS/PAGE proteins) and included *Escherichia coli* β-galactosidase (123 kDa), rabbit muscle fructose-6-phosphate kinase (89 kDa), chicken muscle pyruvate kinase (67 kDa), chicken egg ovalbumin (50 kDa) and rabbit muscle triose phosphate isomerase (34 kDa), and gels were stained with 0.1% Coomassie Blue R-250 (Bio-Rad Laboratories, Richmond, CA, U.S.A.). For Western blotting, the SDS/PAGE slab gels were equilibrated for 30 min in 25 mM Tris/192 mM glycine in 20% (v/v) methanol, and proteins were electrotransferred to 0.45 μm-pore-size Immobilon-P membrane (Sigma Chemical Co.) at 100 V for 1 h. After washing and blocking as described [12], the membrane was incubated with a 10^3-fold dilution of the IgG fraction of anti-(human liver α-L-fucosidase) polyclonal antibodies from a goat [15], washed three times, incubated with horseradish peroxidase-conjugated rabbit anti-(goat IgG) antibody (Sigma Chemical Co.) for 60 min, and washed three more times [12]. Development was accomplished by immersing the membrane in 0.05% 3,3′-diaminobenzidine and 0.015% H_2O_2 in PBS for 2–5 min. Molecular-mass standards and purified human liver α-L-fucosidase were used as negative and positive controls respectively for blotting analysis [12].

Immunoprecipitation studies

For the immunoprecipitation studies, approx. 1 unit of α-L-fucosidase activity (isoform A, or isoform B or human liver α-L-fucosidase) was brought to volume (150 μl) with 10 mM

NaH_2PO_4 buffer, pH 5.5. Variable amounts (0–100 μl) of a 1.5×10^3-fold dilution of the IgG fraction of anti-(human liver α-L-fucosidase) polyclonal antibodies from a goat [15] were added to each tube containing the α-L-fucosidase and 50 μl of 10 mM NaH_2PO_4 buffer, pH 5.5, containing 1.0 mg/ml human serum albumin. Each tube was brought to a total volume of 300 μl and preincubated for 6 h at 2–4 °C. After preincubation, 25 μl of 5-fold-diluted rabbit anti-(goat IgG) (Research Products International Corp.) was added, mixed and incubated for 12–16 h at 2–4 °C. The final mixture was centrifuged (48000 ***g*** for 30 min) and the supernatant was carefully removed (without disturbing the pellet) and assayed in duplicate for α-L-fucosidase activity (50 μl for 20 min at 37 °C).

RESULTS

The number of sperm and the amount of plasma-membrane-associated sperm α-L-fucosidase activity in the caput, corpus and cauda sections of rat epididymis are summarized in Table 1. The results are given for each of three trials (using three rats/trial), and the means ± S.D. are also included. The cauda section contained approx. 70 % of the recovered sperm compared with approx. 25 and 5 % for caput and corpus respectively. The finding that most sperm are found in the cauda section is consistent with previous findings in a number of mammals in which 50–80 % of sperm present in excurrent ducts were found in cauda epididymis [8]. The cauda contained approx. 75 % of the recovered α-L-fucosidase activity compared with approx. 18 and 7 % for caput and corpus respectively. The units of α-L-fucosidase activity normalized to 10^6 sperm were similar in caput (7.9 ± 5.0 units/10^6 sperm), cauda (10.5 ± 2.0 units/10^6 sperm) and corpus (13.9 ± 10.3 units/10^6 sperm) sperm.

Figure 2 depicts pH–activity curves (representative of at least three experiments) for 0.5 M NaCl-solubilized α-L-fucosidase from intact testicular (Figure 2A), corpus (Figure 2B) and cauda (Figure 2C) sperm. All three curves are very similar and exhibit neutral optima between pH values of 6.9 and 7.1 with second optima (approx. 40–50 % of maximal activity) between pH 3 and 4. A possible shoulder of activity may be present near pH 6.0. No significant buffer effects were noted at the overlapping pH values between the oxalate and citrate buffers or between the citrate and phosphate buffers.

Table 1 Distribution of sperm and plasma-membrane-associated α-L-fucosidase activity in the caput, corpus and cauda sections of rat epididymis

Three rats were used per trial.

Epididymal section	Trial	Total no. of sperm	Total α-L-fucosidase activity (units)	Specific α-L-fucosidase activity (units/10^6 sperm)
Caput	I	35×10^6	475	13.6
	II	84×10^6	398	4.7
	III	84×10^6	443	5.3
	Mean ± S.D.	$(68 \pm 28) \times 10^6$	439 ± 39	7.9 ± 5.0
Corpus	I	11×10^6	279	25.4
	II	19×10^6	200	10.5
	III	12×10^6	68	5.7
	Mean ± S.D.	$(14 \pm 4.4) \times 10^6$	182 ± 107	13.9 ± 10.3
Cauda	I	173×10^6	2024	11.7
	II	222×10^6	1830	8.2
	III	138×10^6	1605	11.6
	Mean ± S.D.	$(178 \pm 42) \times 10^6$	1820 ± 210	10.5 ± 2.0

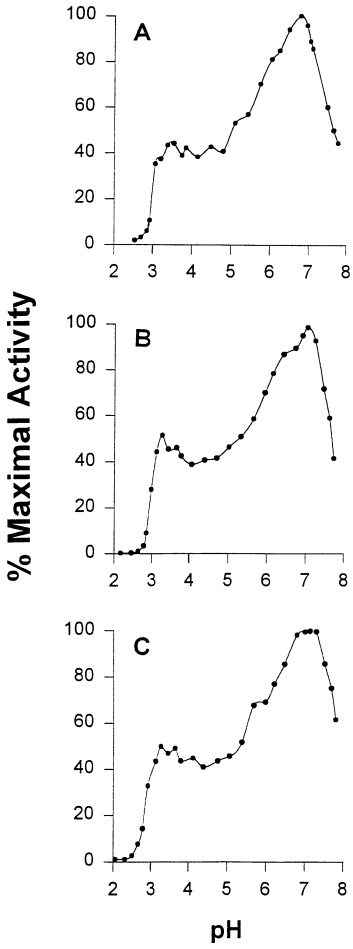

Figure 2 pH–activity curves of 0.5 M NaCl-solubilized α-L-fucosidase from testicular (A), corpus (B) and cauda (C) sperm

See the Materials and methods section for details.

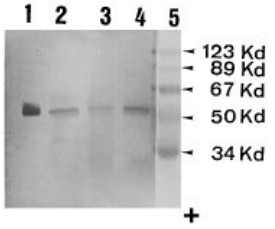

Figure 3 Western-blot analysis of 0.5 M NaCl-solubilized α-L-fucosidase from caput (lane 4), corpus (lane 3) and cauda (lane 2) sperm

Authentic purified human liver α-L-fucosidase is in lane 1, and molecular-mass standards in lane 5 (Kd, kDa). See the Materials and methods section for details.

Figure 3 depicts results (representative of at least three experiments) of Western-blot analysis of 0.5 M NaCl-solubilized α-L-fucosidase (0.5–1.0 μg) from cauda (lane 2), corpus (lane 3) and caput (lane 4) sperm. Lane 1 contains 1.5 μg of purified human liver α-L-fucosidase (as a positive control) and lane 5 contains the prestained molecular-mass standards from SDS/PAGE. All three fucosidases gave similar blots with one or two closely spaced immunoreactive bands (which co-migrate with

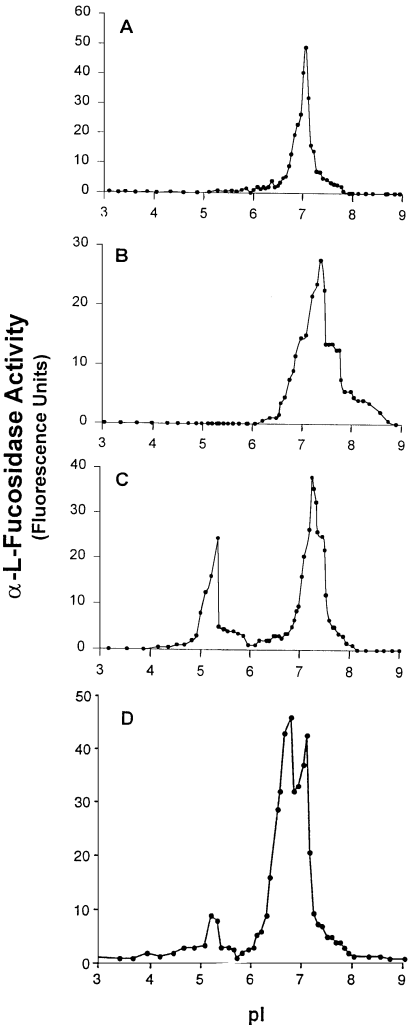

Figure 4 Isoelectric focusing profiles of 0.5 M NaCl-solubilized α-L-fucosidase from testicular (A), proximal cauda (B), distal cauda (C) and vas deferens (D) sperm

See the Materials and methods section for details.

authentic human liver α-L-fucosidase) with apparent molecular masses of about 53 ± 2 kDa (as determined by relative migrations compared with standard proteins). The upper band is more intense than the lower band, which can barely be seen for corpus α-L-fucosidase (lane 3), probably because of the smaller amount of protein available from the smaller number of sperm present in this epididymal section (see Table 1).

Isoelectric focusing experiments were run at least three times for 50–125 units of α-L-fucosidase solubilized with 0.5 M NaCl from intact sperm from testis, caput, corpus, cauda, the proximal and distal halves of the cauda and the vas deferens, and representative profiles are depicted in Figure 4. Sperm α-L-fucosidase from testis (Figure 4A), caput and corpus (profiles not shown) all have very similar isoform profiles with one major isoform with a pI of 7.0 ± 0.1. In some experiments, this isoform appeared with a shoulder or was even at times a split peak. Sperm α-L-fucosidase from cauda (profile not shown) also has the major isoform (with a possible shoulder) at a pI of 7.0 ± 0.1 (comparable with the isoform in testis, caput and corpus sperm) but also contains a minor isoform with a pI between 5 and 6.

Table 2 Distribution of α-L-fucosidase activity from sperm from the proximal and distal halves of cauda epididymis

Two rats were used per experiment. Values in parentheses are the percentage of recovered activity in the given portion of cauda.

Experiment	Age of rats (days)	Weight of rats (g)	α-L-Fucosidase activity recovered (units)		
			Proximal cauda	Distal cauda	Total cauda
1	70–84	337, 339	68 (16)	363 (84)	431
2	91–105	400, 441	216 (19)	924 (81)	1140
3	124–138	560, 595	230 (16)	1246 (84)	1476
			(17 ± 2)*	(83 ± 2)*	

* Mean ± S.D.

When the cauda was divided into proximal and distal halves, sperm α-L-fucosidase from the proximal half had an isoform profile (Figure 4B) comparable with that for the testis, caput and corpus sperm (with one isoform with a pI near 7). Sperm α-L-fucosidase from the distal half of the cauda had the major isoform as well as significant amounts of the more acidic isoform (designated A) (Figure 4C) seen in total cauda sperm. A representative α-L-fucosidase isoform profile from vas deferens sperm is depicted in Figure 4(D) for 6-month-old rats. The profile has a major split peak of fucosidase activity near pI 7 and a small peak of activity between pI 5 and 6. The major neutral peak was split in five of seven different vas deferens profiles examined, and the more acidic peak was identifiable in small amounts in six of these seven profiles. The relative amount of the more acidic peak did not appear to be related to the age (3–10 months) of the donor rats.

The different α-L-fucosidase isoform profiles found for the proximal and distal halves of the cauda led to a study of the distribution of sperm α-L-fucosidase activity between these two sections (summarized in Table 2). Three experiments were carried out with rats (two per experiment) of different ages (and weights). The total amount of fucosidase activity recovered from cauda sperm went up significantly with the age of the rats from 431 units (70–84 days) to 1140 units (91–105 days) to 1476 units (124–138 days). Despite this increase in total cauda fucosidase activity with age, the percentage distribution between the two halves remained remarkably constant, with 17 ± 2 and 83 ± 2 % associated with the proximal and distal halves respectively. It thus appears that the great majority of fucosidase activity is found concentrated in sperm from the distal half of the cauda, the portion of the epididymis in which the A isoform is first observed.

Semipreparative isoelectric focusing was performed on 600 units of sperm α-L-fucosidase from the distal half of the cauda so that the two isoforms could be separated and comparatively characterized. The pH–activity curves for isoform A (Figure 5A) and isoform B (Figure 5B) are similar with optima near 7, a possible shoulder near pH 6, and significant activity between pH 3 and 4. The individual isoform pH–activity curves are also quite similar to that of total cauda sperm α-L-fucosidase (Figure 2C) except that the latter curve contains a more well-defined secondary optimum between pH 3 and 4. Apparent K_m values were determined for isoforms A and B using 4-methylumbelliferyl α-L-fucopyranoside as substrate. The Lineweaver–Burk double-reciprocal plots yielded straight lines with apparent K_m values of 0.4 ± 0.04 mM (mean ± S.D. for three experiments) for both isoforms. Thermostability studies were carried out on the iso-

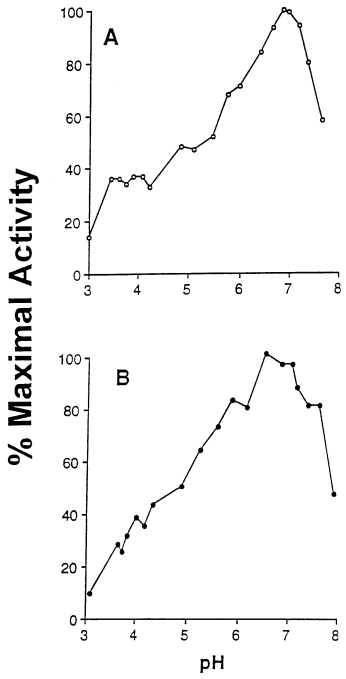

Figure 5 pH–activity curves of isoform A (A) and isoform B (B) of distal cauda sperm α-L-fucosidase

See the Materials and methods section for details.

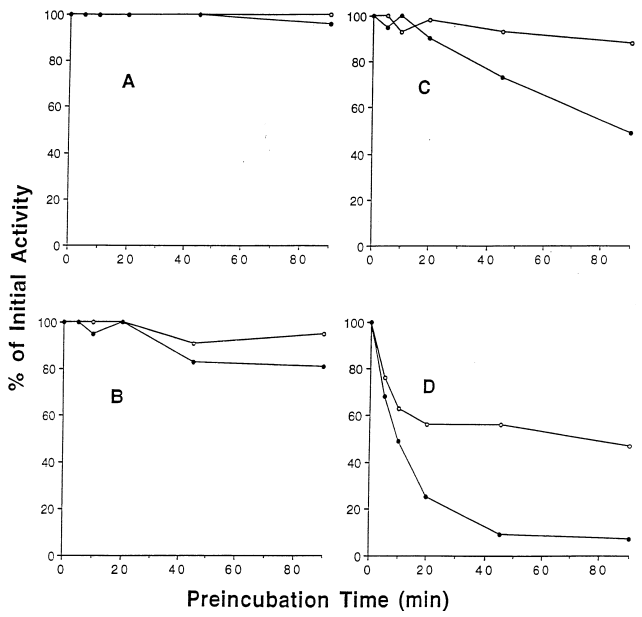

Figure 6 Thermostability curves of isoform A (○) and isoform B (●) of distal caudal sperm α-L-fucosidase after preincubation for various times at 37 °C (A), 43 °C (B), 47 °C (C) and 50 °C (D)

See the Materials and methods section for details.

forms under conditions of constant amounts of enzyme activity and constant protein concentration. Preincubation for up to 90 min at 37 °C led to comparable and essentially complete thermostability for both isoforms (Figure 6A). However, as the

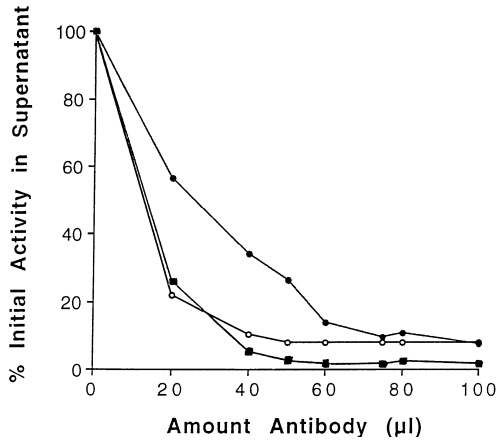

Figure 7 Immunoprecipitation curves of isoform A (○) and isoform B (●) of distal cauda sperm α-L-fucosidase compared with human liver α-L-fucosidase (■)

The IgG fraction of polyclonal antibodies against human liver α-L-fucosidase [15] was used for these studies. See the Materials and methods section for details.

preincubation temperature increased from 37 °C to 43, 47 and 50 °C (Figures 6B, 6C and 6D respectively), there were increasing differences between the two isoform curves, with isoform A consistently exhibiting greater thermostability than isoform B. Immunoprecipitation studies using the IgG fraction of polyclonal antibodies against human liver α-L-fucosidase [15] indicated that approx. 90 % of the activity for both fucosidase isoforms was immunoprecipitable (Figure 7) under conditions of near-quantitative immunoprecipitation of human liver fucosidase activity (run as a positive control). However, the antibodies appear to have less affinity for the B than the A isoform (as seen at lower antibody concentrations).

The results of isoelectric focusing of sperm α-L-fucosidase from the distal half of the cauda after incubation at 37 °C (negative control) or after incubation with 10 units of *Cl. perfringens* neuraminidase at 37 °C for different amounts of time (6, 17.5, 27 h) and different amounts of α-L-fucosidase (75 or 125 units) are summarized in Table 3. There was quantitative recovery of fucosidase activity even after heat treatment for 27 h, and both the major B and minor A fucosidase isoform peaks were present in approximate relative amounts of 80 and 20 % respectively (as determined by weighing the peaks on a Mettler analytical balance). There was also quantitative recovery of fucosidase activity after neuraminidase treatment for 27 h (suggesting that little or no protease activity is present in the neuraminidase), but isoform A was no longer present. Four different neuraminidase experiments were carried out and each neuraminidase treatment was compared with its appropriate heat-treated control incubated for the same amount of time at 37 °C in buffer without neuraminidase. In general, there was a greater decrease in the peak for isoform A and a greater increase in the peak(s) for isoform B as incubation time and the ratio of neuraminidase to α-L-fucosidase increased (Table 3). The collective results suggest that isoform A is chemically related to isoform B by increased sialylation.

DISCUSSION

Evidence is accumulating that sperm–egg interactions, which are for the most part species-specific and necessary for fertilization to occur [1], involve carbohydrate-containing molecules [16,17].

Table 3 Summary of results of neuraminidase treatment of α-L-fucosidase from distal cauda sperm

Each experiment involved a different preparation of distal cauda sperm α-L-fucosidase. Heat-treated controls were in buffer without neuraminidase.

Experiment number	Treatment	Incubation time at 37 °C (h)	Amount of α-L-fucosidase treated (units)	Recovery of α-L-fucosidase activity (%)	Post-treatment activity (%) associated with:	
					Isoform A	Isoform B
1	Heat (control)	6	125	100	21	79
	Neuraminidase	6	125	95	18	82
2	Heat (control)	17.5	125	100	39	61
	Neuraminidase	17.5	125	100	20	80
3	Heat (control)	17.5	75	100	9	91
	Neuraminidase	17.5	75	100	0	100
4	Heat (control)	27	75	100	20	80
	Neuraminidase	27	75	100	0	100

A number of studies have provided evidence that L-fucose and/or the enzyme α-L-fucosidase may be involved in gamete interactions in a number of species. Early studies indicated the presence of fucosyl sites on the vitelline coat of the egg of the ascidian *Ciona intestinalis* [18], and that α-L-fucosidase on the sperm surface might bind to these sites by an enzyme–substrate-type complex [19]. Very recently, a plasma-membrane-associated α-L-fucosidase has been found to be present on sperm from the mollusc bivalve *Unio elongatulus* [20] and sperm from rats [12] (see the Introduction). The unusual location and/or properties of these α-L-fucosidases suggests that they may be involved in sperm–egg interactions. Further evidence for an involvement of α-L-fucosidase in sperm–egg interactions comes from studies in which L-fucose and/or L-fucose-containing molecules have been shown to inhibit sperm–egg interactions in hamsters [21], mice [22], rats [23] and humans [24–26].

In the present investigation, the recently described plasma-membrane-associated α-L-fucosidase from rat sperm [12] was characterized during epididymal maturation. The unusual neutral pH optimum of α-L-fucosidase and its subunit composition of one or two closely spaced bands with apparent molecular mass near 53 ± 2 kDa did not appear to change during transit through the epididymis. Isoelectric focusing indicated the presence of a major neutral-pI isoform (B) of α-L-fucosidase in sperm from testis, caput, corpus and the proximal half of the cauda. The peak of this isoform was split in some experiments suggesting that it may represent two unresolved isoforms. α-L-Fucosidase from sperm from the distal half of the cauda, which contained a significant enrichment of sperm and α-L-fucosidase activity, contained isoform B and an additional minor isoform A with a pI near 5.2. The higher-molecular-mass form of α-L-fucosidase seen on Western blotting is not related to the presence of the A isoform since it is also found in caput and corpus sperm α-L-fucosidase which does not contain the A isoform. Sperm from the vas deferens also contained isoforms A and B but the more acidic isoform was present in small amounts. It thus appears that a second minor isoform of rat sperm α-L-fucosidase is first expressed during epididymal maturation in the distal half of the cauda. The expression of isoform A on sperm at the end of epididymal maturation when sperm first become motile and fertile [1–3] suggests that this isoform of α-L-fucosidase may play a role in sperm–egg interactions and/or fertilization.

To understand the relationship between isoforms A and B better, α-L-fucosidase from sperm from the distal half of the cauda was subjected to semipreparative isoelectric focusing to separate the isoforms in sufficient quantities to be characterized comparatively. The pH–activity curves were similar for the two isoforms (and similar to those for α-L-fucosidase from testis, caput, corpus and cauda sperm) with optima near pH 7. The two isoforms had the same apparent K_m (0.4 ± 0.04 mM) for the synthetic 4-methylumbelliferyl substrate, similar to values previously found for a number of mammalian α-L-fucosidases [9]. Of course, the similar K_m values for the synthetic substrate may not reflect any possible differences that might be detected if a more appropriate natural substrate (e.g. a fucoglycoconjugate from rat zona pellucida) was available for kinetic analysis. A decreased K_m for such a fucoglycoconjugate by isoform A would provide evidence for increased affinity of this isoform for zona pellucida fucoglycoconjugates. A similar and large amount of the enzymic activity of both isoforms was immunoprecipitable (using antibodies to human liver α-L-fucosidase) under conditions that immunoprecipitated essentially all of the human liver enzyme. This was not an unexpected finding since previous studies have shown that rodent liver α-L-fucosidases can be immunoprecipitated by the antibody to human liver fucosidase [27] and that rat liver α-L-fucosidase exhibits 82% sequence identity with the human enzyme [28]. The close antigenic similarity of isoforms A and B suggests that these proteins are products of the same gene which might be related to one another by post-translational modifications. The disappearance of isoform A after neuraminidase treatment (compared with a heat-treated control) and the concomitant increase in isoform B provided evidence that isoform A is derived from isoform B by sialylation of the neutral isoform(s). This result is consistent with the results of previous studies from several laboratories which have provided evidence that the isoforms of human and mouse α-L-fucosidases are related to each other, at least in part, by sialic acid residues (reviewed in ref [9]). Previous studies on the differentially sialylated isoforms of human liver α-L-fucosidase have indicated a significant trend of increasing thermostability with increasing sialylation [29]. The present thermostability results on rat sperm α-L-fucosidase are consistent with the previous results since the more acidic isoform A was significantly more thermostable than isoform B. It is possible that the increased sialylation of isoform A contributes to an increased stability which would be advantageous in the oviduct where sperm–egg interactions take place.

The place from which isoform A originates in the distal cauda epididymis is unclear at the present time. It is possible that this more acidic isoform is derived by the addition of sialic acid residues by sialyltransferase to isoform B while it is present on the rat sperm plasma membrane. Alternatively, isoform A may

be independently derived from epithelial cells of the distal cauda epididymis. What specific role, if any, isoform A has in rat sperm–egg interactions remains to be determined.

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