Partial characterization of galactosyltransferase in human seminal plasma and its distribution in the human epididymis

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Galactosyltransferase activity has been partially characterized in human seminal plasma. K_m values of $130 \mu\text{mol l}^{-1}$ for UDP-galactose and 2.25 mmol l^{-1} for N-acetylglucosamine were calculated and the enzyme was found to be dependent on temperature and manganese and present as a highly active component of human seminal plasma. Galactosyltransferase was inhibited by nucleotides, glycosylated nucleotides, bovine and human α -lactalbumin but not by monosaccharides. Radiation inactivation studies revealed that the biologically active unit of seminal plasma galactosyltransferase has a molecular mass of 45 kDa. Although the majority of galactosyltransferase activity found in seminal plasma is probably of prostatic origin, we report for the first time that it is also present in human epididymal intraluminal fluid. Low activity was detected in the proximal caput region but activity increased to maximum values in the adjacent downstream segment, the intermediate caput region. Specific activity was relatively constant albeit at a lower value in the following epididymal segments and vas deferens. The significance of the epididymal and seminal plasma galactosyltransferase activities is unknown, but the enzyme could be implicated in glycosylation events that are known to be important in gamete interaction.

Introduction

Mouse sperm membrane-bound β -1,4-galactosyltransferase (E.C. 2.4.1.38, GalTase) has been the subject of numerous studies, which have shown that this enzyme is involved in gamete interactions (Macek and Shur, 1988). A soluble form of GalTase in the male reproductive tract has also been described in rats (Hamilton, 1981) and mice (McLaughlin and Shur, 1987). GalTase is a member of the glycosyltransferase family responsible for the synthesis of complex substrates such as glycosaminoglycans, glycolipids and glycoproteins (see Strous, 1986, for review). Although it is principally found in the Golgi apparatus, plasma membrane-bound and soluble forms have also been described. The presence of GalTase on the plasma membrane of various cell types has been associated with its role in cell adhesion and cellular interaction (Shur, 1984). Accordingly, GalTase has been implicated in morula compaction in the mouse (Bayna *et al.*, 1988), neural crest migration (Runyan *et al.*, 1986), and infiltration of adrenal carcinoma (Penno *et al.*, 1989) and melanoma cells (Passaniti and Hart, 1990). GalTase also plays a role in gamete interaction in mice (Macek and Shur, 1988). The enzyme is synthesized in the testis and gradually becomes localized on the plasma membrane overlying the acrosomal region of the spermatozoon (Scully *et al.*, 1987). It has been shown to have an affinity for the zona pellucida (Lopez *et al.*, 1985) and thus represents one of the components associated

with gamete recognition (Benau and Storey, 1988). GalTase has also been detected on equine and bovine spermatozoa (Fayrer-Hosken *et al.*, 1991). Moreover, an immunologically reactive sperm protein has been detected using anti-GalTase antibodies on a population of human spermatozoa separated on a Percoll gradient (Sullivan *et al.*, 1989). Humphreys-Beher *et al.* (1990) detected anti-GalTase antibodies in sera of patients with antisperm antibodies. Although some enzyme activity has been detected in human sperm cells (Humphreys-Beher and Blackwell, 1989; Humphreys-Beher *et al.*, 1990; Miller *et al.*, 1992), other investigators have been unable to detect significant GalTase activity associated with these cells (Tulsiani *et al.*, 1990). The role of this immunologically reactive protein on human spermatozoa is unknown.

A soluble form of GalTase that can galactosylate a 26 kDa sperm membrane protein has been described in the rat rete testis and epididymal fluids (Hamilton, 1981; Hamilton and Gould, 1982). It has also been detected in epididymal, prostatic and seminal vesicle cytosolic preparations in mice, hamsters and guinea pigs (Reddy *et al.*, 1976) and in the intraluminal fluid of the mouse caput epididymis (McLaughlin and Shur, 1987). Tadolini *et al.* (1976) were the first to detect GalTase activity in human seminal plasma and they measured the enzyme activity in a membrane-free fraction as well as in fluid obtained after prostatic massage. However, no further characterization of the enzyme has since been undertaken. In this paper, we report the partial characterization of GalTase activity in human seminal plasma. In addition, epididymides obtained from cadavers were used to measure the enzyme activity in the intraluminal fluid

*Reprint requests.

Received 8 June 1992.

obtained from the various epididymal regions. The possible role of this enzyme in seminal plasma and epididymal fluid is also discussed.

Materials and Methods

Seminal plasma samples

Frozen semen samples (1.1 l) collected in fertility clinics in the Montreal region were pooled and benzamidine hydrochloride was added to a final concentration of 50 mmol l⁻¹. The pooled semen was centrifuged for 10 min at 600 g to remove spermatozoa and the supernatant was subsequently ultracentrifuged for 30 min at 100 000 g to remove cellular debris. The seminal plasma was delipidated by precipitating the proteins by adding 8 volumes of 95% ethanol. The precipitate was left at -4°C for 24 h and centrifuged for 20 min at 8000 g to obtain a protein pellet. After washing twice with 95% ethanol, the pellet was resuspended in 800 ml water before being frozen and lyophilized. Half of this delipidated seminal plasma (DSP) was subsequently resuspended in a minimal volume and dialysed (cut-off of 12 kDa) against a 0.05 mol ammonium bicarbonate buffer l⁻¹ (pH 8.5). The dialysed and delipidated seminal plasma (DDSP) was subsequently frozen, lyophilized and aliquots were stored at -20°C.

GalTase assay

GalTase activity was measured according to Ram *et al.* (1989) with certain modifications. Samples were resuspended in the reaction buffer consisting of 120 mmol NaCl l⁻¹, 4.8 mmol KCl l⁻¹, 1.7 mmol CaCl₂ l⁻¹, 1.2 mmol MgCl₂ l⁻¹, 10 mmol MnCl₂ l⁻¹ and 25 mmol Hepes l⁻¹ at pH 7.2. Four microlitres of this sample preparation was placed in a 0.5 ml Eppendorf microfuge tube and evaporated in a Speedvac (Savant Instruments, Farmingdale, NY). The reaction was started by the addition of approximately 0.3 µCi of UDP-[4,5-³H]galactose (specific activity 0.8 Ci mmol l⁻¹), 23 mmol *N*-acetylglucosamine l⁻¹ (GlcNAc) and 8 mmol 5'-AMP l⁻¹ in a total volume of 4 µl. The tubes were tightly closed and incubated for 30–120 min in a shaking water bath set at 37°C. The reaction was stopped by adding 4 µl of cold 50 mmol EDTA l⁻¹ and by placing the tubes on ice. A control tube containing the substrate but no enzyme was included in each assay to evaluate non-enzymatic degradation.

The presence of an endogenous acceptor of [³H]galactose was verified by incubating 4 µl fractions of seminal plasma (7 µg), DSP (7 µg) or a commercial preparation of bovine milk galactosyl-transferase (1 ng) (Sigma) in the presence or absence of exogenous GlcNAc (23 mmol l⁻¹).

Separation of the radiolabelled product

Samples containing a final volume of 8 µl were mixed in a 100 µl Hamilton syringe with 50 µl of methanol and applied to a 10 × 20 cm polyethylenimine-cellulose thin layer chromatography plate using an automated sample applicator (Multi Spotter, AIS, Libertyville, IL). The plates were placed in a tank containing 1 mmol sodium phosphate buffer l⁻¹ (pH 8.0) and

following a 10 cm migration, the top 4.5 cm containing the radiolabelled product was scraped off and placed in a plastic vial to which 250 µl of sodium phosphate buffer at pH 3.0 was added to elute the product. The amount of [³H]galactose transferred was established by the addition of 10 ml of scintillation fluid (P989, NEN Dupont, Mississauga, Ontario) and assay of the radioactivity in an LKB Model 1217 beta counter.

Characterization of seminal plasma GalTase activity

The Michaelis–Menten constants of seminal plasma GalTase were determined by solubilizing samples of DDSP (7 µg) in 4 µl of reaction buffer containing 5'-AMP and increasing amounts of GlcNAc (0.4–50 mmol l⁻¹) or UDP-galactose (1.7 µmol l⁻¹–50 mmol l⁻¹). After Speedvac evaporation of the sample, the reaction was started by adding the radiolabelled substrate. A one hour incubation time was used.

The effects of incubation for 90 min at different temperatures (24, 30, 32, 37, 40 and 45°C) or at 37°C with increasing amounts of manganese (0, 5, 10, 15, 20, 30 and 40 mmol MnCl₂ l⁻¹) were also evaluated.

Inhibition studies

The specificity of glycosylation was evaluated by the addition of various substances some of which are known GalTase inhibitors. Nucleotides (UMP, UDP and UTP), glycosylated nucleotides (UDP-mannose and UDP-glucose) as well as monosaccharides (β-D-glucose, D-galactose, D-mannose, myo-inositol, D-arabinose and α-fucose) were added in concentrations varying from 0.5 to 50 mmol l⁻¹ (in water) to dried samples of DDSP previously solubilized in the reaction buffer (final concentration was 7 µg ml⁻¹). Incubations were performed at 37°C for 90 min. The effect of increasing concentrations of bovine (Sigma) and human (Sigma) α-lactalbumin as well as BSA (Sigma) were also tested using the same experimental conditions.

Radiation inactivation studies

Radiation inactivation studies can be used to estimate the radiation inactivation size (RIS) of an enzyme which corresponds to the biologically active unit of the enzyme (Beauregard *et al.*, 1987). The advantage of this technique is that it can be used on crude preparations of the particular enzyme under study. Aliquots of DDSP (7 µg ml⁻¹ reaction buffer) were dried and kept at -80°C in 0.5 ml Eppendorf centrifuge tubes. The air in the tubes was evacuated by a gentle stream of nitrogen and the samples were subsequently placed in a ⁶⁰Co irradiator (Gammacell, model 220, Atomic Energy of Canada). Samples were submitted to irradiation times of 0, 1, 2, 3, 4.5, 6, 7.5, 9, 10.5 and 12 h corresponding to increasing radiation doses. Triplicate samples were irradiated for each dose and the experiment was repeated twice. The enzymatic activity was measured by adding the radiolabelled substrate as mentioned above using a 2 h incubation time at 37°C.

Epididymal samples

Epididymides were obtained from two 36-year-old male subjects in an organ donor programme within 4 h of death and

Fig. 1. Schematic drawing of the human epididymis representing the different regions from which intraluminal fluid was collected. P: proximal; I: intermediate; and D: distal regions of the caput and corpus epididymis. (Redrawn from Baumgarten *et al.*, 1971.)

were kept on ice. The various regions of the caput and corpus epididymides (see Fig. 1) were punctured with a fine needle and a capillary was placed in the opening to collect intraluminal fluid. The epididymis was not dissected into the different segments to maintain a certain tonicity which facilitated fluid expulsion. Samples from the cauda and vas deferens were obtained by flushing the tubule lightly with 200 μ l of reaction buffer through a small calibre syringe. The fluids of the epididymis and vas deferens were centrifuged (3000 *g*, 15 min) to remove spermatozoa and further ultracentrifuged (100 000 *g*, 30 min) to eliminate particulate matter. Protein determinations were measured using the method of Bradford (1976). GalTase assay was achieved by evaporating 4 μ l tubular fluid from the assay tubes and by the addition of reaction buffer containing the radiolabelled substrate and the acceptor followed by incubation as described above.

Spermatozoa

Fresh spermatozoa were obtained from two healthy donors and the volume was completed to 10 ml with the reaction buffer. After removing the seminal plasma by centrifugation (600 *g*, 10 min), the sperm cells were washed twice with 10 ml of reaction buffer and recentrifuged. The final pellet was resuspended in 5 ml of reaction buffer, the sperm cells were counted, separated into increasing amounts, and dried in the assay tubes. The enzymatic reaction was performed as described above. The sperm membranes have been shown to remain intact using this technique as we have described (Langlais *et al.*, 1981).

GalTase activity was also measured in murine spermatozoa. After killing the mice by cervical dislocation, the epididymides were dissected free and the caudal segments were punctured in a Petri dish containing reaction buffer. The spermatozoa were collected, washed and processed as described for human ejaculated spermatozoa.

Fig. 2. Galactosyltransferase activity in the presence (■) and absence (▨) of exogenous *N*-acetylglucosamine (GlcNAc). Seminal plasma (SP), delipidated seminal plasma (DSP), and bovine milk galactosyltransferase (bo/GalTase) were incubated for 1 h at 37°C in the presence and absence of 23 mmol GlcNAc l^{-1} . Activities obtained in the presence of exogenous GlcNAc are fixed at 100% activity, and the values obtained in the absence of GlcNAc were calculated as a percentage of activity relative to this.

Results

GalTase assay

The thin layer chromatography method used in these experiments separates sugar nucleotides which remain at the origin from glycosylated acceptors (Ram *et al.*, 1989). The reaction product is separated from the radiolabelled substrate and can be removed by scraping off the top 4.5 cm of the polyethylenimine-cellulose plate. GalTase activity is present in human seminal plasma (Fig. 2) and this activity is located in the protein pellet after precipitation. However, a galactose acceptor is lost in the latter step as demonstrated by the fact that, in seminal plasma, 83% of the enzyme activity is obtained in the absence of exogenous GlcNAc, whereas no product was formed when DPS or bovine milk GalTase were used as enzyme sources and GlcNAc was deleted (Fig. 2). GalTase activity was linear with time and non-enzymatic degradation of the radio-labelled substrate was low and represented only 4% of the total radioactivity migrating with the thin layer chromatography buffer after a 120 min incubation. This non-enzymatic degradation was evaluated in each assay and was subtracted from those values obtained in the presence of enzyme.

Kinetic analysis of GalTase activity

Seminal plasma GalTase activity was temperature dependent (Fig. 3). The activity increased from 24 to 40°C (from 37% to 100%, respectively), after which it decreased to approximately 80% of its maximum activity at 45°C. Bovine milk GalTase exhibited a slightly different pattern with maximal activity occurring between 24 and 40°C, to decrease subsequently by 30% at 45°C.

Activity was low in the absence of manganese but increased when 5 mmol manganese l^{-1} was added (Fig. 4). A plateau was obtained with a slight increase at concentrations varying from 20 to 40 mmol l^{-1} .

Fig. 3. Galactosyltransferase activity was calculated after a 90 min incubation at 24, 30, 32, 37, 40 and 45°C. Assays were done in triplicate and are expressed as percentages of the activity obtained at optimal temperature (32°C for bovine milk galactosyltransferase (■) and 40°C for dialysed and delipidated seminal plasma (○)).

Fig. 4. Dialysed and delipidated seminal plasma was incubated for 90 min with increasing amounts of manganese and the amount of lactosamine formed was then measured.

When GalTase activity was calculated in the presence of different UDP-galactose concentrations, a K_m value of $130 \mu\text{mol l}^{-1}$ and a $V_{\max}$ value of 148 pmol h^{-1} was obtained by plotting the Lineweaver–Burke double-reciprocal curve (Fig. 5a). Similarly, a K_m value of 2.25 mmol l^{-1} was calculated when GlcNAc concentrations were increased (Fig. 5b).

Specificity studies

The transfer of radiolabelled galactose was inhibited by the addition of several glycosylated products to the reaction mixture. The order of decreasing inhibition capability was UDP-mannose (IC_{50} of 0.02 mmol l^{-1}) and UDP-glucose (IC_{50} of 1 mmol l^{-1}) (Fig. 6a). Enzyme activity was also inhibited by the presence of uridine mono-, bi- and triphosphates (Fig. 6b). Uridine bi- and triphosphate produced a comparable effect (IC_{50} of 0.04 mmol l^{-1} and 0.06 mmol l^{-1} , respectively), whereas UMP was less potent (IC_{50} of 0.6 mmol l^{-1}). Galactose, glucose, mannose, inositol, arabinose and fucose did not inhibit

Fig. 5. Determination of Michaelis–Menten constants for (a) uridine diphosphate galactose (UDP-Gal) and (b) GlcNAc. Lineweaver–Burke double reciprocal curves of dialysed and delipidated seminal plasma galactosyltransferase activity measured in the presence of increasing amounts of (a) (UDP-Gal) or (b) *N*-acetyl glucosamine are presented. The K_m values obtained from the curves were $130 \mu\text{mol l}^{-1}$ for uridine diphosphate galactose and 2.25 mmol l^{-1} for *N*-acetyl glucosamine.

$[^3\text{H}]$ galactose transfer to GlcNAc at concentrations varying from $0.5 \mu\text{mol l}^{-1}$ to 100 mmol l^{-1} (Fig. 6c).

The addition of α -lactalbumin to the reaction mixture inhibited GalTase activity in a dose-dependent manner (Fig. 7). This inhibition was very similar whether human or bovine α -lactalbumin was used. The IC_{50} value was 0.1% ($4 \mu\text{g}$ per assay) in both cases and total inhibition was achieved at a concentration of 1% . However, BSA had no effect on activity at similar concentrations.

Radiation inactivation studies

The radiation inactivation size (RIS) of seminal plasma GalTase was determined by calculating the residual GalTase activity in DDPS samples placed in a calibrated ^{60}Co Gammacell (Beauregard and Potier, 1982). RIS can be defined as the mass of one mole of protein which loses its biological activity when hit by a single ionizing radiation. This entity is based on Lea's (1955) target theory whereby the probability of a molecule being hit by an ionizing radiation increases with its size for any given dose. The RIS can thus be calculated by plotting the relative initial enzyme activity of a protein as a function of the irradiation dose on a semi-logarithmic scale (Beauregard *et al.*, 1987). The log of residual activity remaining following increasing radiation doses is shown (Fig. 8a). The enzymatic activity corresponding to a radiation dose of zero (A_0) was extrapolated

Fig. 6. Inhibition of seminal plasma galactosyltransferase activity with (a) sugar nucleotides, (b) nucleotides and (c) monosaccharides. Uridine diphosphate- ^3H galactose transfer to *N*-acetyl galactose was assayed in the presence of increasing amounts of the different components. The percentage of remaining activity refers to the amount of enzyme activity obtained in the presence of the inhibitor relative to that obtained in its absence. Concentrations required to inhibit 50% of the maximal activity (IC_{50}) were, in (a) 0.02 mmol l^{-1} (uridine diphosphate mannose, $\square$), 1 mmol l^{-1} (uridine diphosphate glucose, $\blacksquare$) and in (b): 0.6 mmol l^{-1} (uridine monophosphate, $\bullet$), 0.04 mmol l^{-1} (uridine diphosphate, $\circ$) and 0.06 mmol l^{-1} (uridine triphosphate, $\blacktriangle$). As shown in (c) galactose ($\triangle$), glucose ($\blacksquare$), mannose ($\square$), inositol ($\bullet$), arabinose ($\circ$) and fucose ($\blacktriangle$) did not inhibit the enzymatic reaction.

from the linear regression curve. The antilog of this value corresponds to 100% activity ($A_0 = 1.603$). On the basis of this value the residual activity obtained at each irradiation dose was calculated as a percentage of the initial activity and after log transformation (Fig. 8b). From this graph, it was calculated that, at 37% of the initial activity, this corresponds to an irradiation dose (D_{37}) of 14.2 Mrad. Kepner and Macey (1968) have determined, empirically, a factor that is inversely proportional to the molecular mass of a biologically active protein and its D_{37} :

$$M_r = \frac{6.4 \times 10^5}{D_{37}}$$

Fig. 7. Inhibition of seminal plasma galactosyltransferase activity by bovine and human α -lactalbumin. Increasing amounts of bovine ($\circ$), and human ($\blacksquare$) α -lactalbumin were added to assays containing dialysed and delipidated seminal plasma. Residual activity (as a percentage) was calculated after 90 min of incubation at 37°C . As controls, 0.0001, 0.1 and 1% albumin were also assayed in the presence of dialysed and delipidated seminal plasma ($\triangle$).

Fig. 8. Radiation inactivation studies. (a) The log of residual activity as a function of radiation dose is presented. Galactosyltransferase activity decreases with increasing radiation dose. By extrapolating the value obtained at a dose of 0 Mrad, the value corresponding to 100% activity ($A_0 = 1.603$) can be estimated. (b) From the value obtained for A_0 (100% activity), the percentage of residual activity was calculated as a function of the radiation dose. At 37% of the initial activity ($Y = 1.568$), a radiation dose of 14.2 mRad was obtained which corresponds to a radiation inactivation size (RIS) of 45 kDa.

Fig. 9. The relative activity of galactosyltransferase in the human epididymis and vas deferens. Samples of intraluminal fluid obtained from two subjects were assayed for 2 h at 37°C. Pooled duplicate measurements obtained with tissues from both subjects are represented. The activity is expressed as a percentage relative to the most active segment in each subject (intermediate region of the caput) (mean $\pm$ SD). P: proximal; I: intermediate; and D: distal regions of the caput and corpus epididymis.

This equation is commonly used to calculate the RIS of an enzyme (Beauregard *et al.*, 1987). The RIS value of GalTase can thus be calculated:

$$\text{RIS} = \frac{6.4 \times 10^5}{D_{37}} = \frac{6.4 \times 10^5}{14.2} = 45.1 \text{ kDa}$$

The mean value of RIS calculated from two radiation inactivation experiments was 44.8 kDa.

GalTase activity in the intraluminal fluids of the epididymis and vas deferens

Epididymal fluid was also studied for its ability to transfer [^3H]galactose from UDP-[^3H]Gal to GlcNAc (Fig. 9). The specific GalTase activity present in the proximal caput region was low (0.17 pmol h $^{-1}$ μg^{-1} of protein) but was found to increase in the following segment, the intermediate caput region, where it attained the highest specific activity (3.94 pmol h $^{-1}$ μg^{-1} protein) that was measured in the epididymis and vas deferens. The activity measured in downstream segments was lower and represented between 30% (cauda) and 67% (proximal corpus) of that obtained in the intermediate region of the caput. GalTase activity was maintained in the vas deferens where 1.30 pmol h $^{-1}$ μg^{-1} of protein were transferred to GlcNAc.

Human and mouse sperm GalTase activity

Only slight activity was detected when human washed ejaculated spermatozoa were added to the reaction buffer (Fig. 10a). Even at concentrations of up to 100 000 spermatozoa per assay, only 0.05 ± 0.02 pmol h $^{-1}$ of [^3H]galactose were transferred to GlcNAc. However, for purposes of comparison, mouse

Fig. 10. Human and murine sperm galactosyltransferase activity as a function of number of spermatozoa. Samples containing increasing amounts of (a) human ejaculated and (b) murine caudal epididymal spermatozoa were assayed for galactosyltransferase activity. Values represent means $\pm$ SD of duplicate experiments.

caudal sperm display GalTase activity that increased with sperm concentration (Fig. 10b).

Discussion

It is well known that GalTase participates in sperm-egg interaction in mice (Macek and Shur, 1988). As an immunologically reactive sperm membrane protein was detected on human spermatozoa using anti-GalTase antibodies (Sullivan *et al.*, 1989), we proceeded to verify further the presence of GalTase on human spermatozoa using a more direct assay, i.e. via its enzymatic activity. Very low activity was found to be associated with human ejaculated spermatozoa, whereas much higher activity was found in the seminal plasma. The low enzymatic activity associated with spermatozoa is not related to the technique used since mouse spermatozoa exhibited GalTase activity using the same procedure. Other groups have also reported very little GalTase activity associated with human spermatozoa (Tadolini *et al.*, 1976; Tulsiani *et al.*, 1990). This minimal activity, as well as that described in this study, is possibly due to a contamination by the seminal plasma enzyme. Miller *et al.* (1992) found that some activity was associated with human spermatozoa, although it was approximately 15-fold less than the activity previously measured with mouse spermatozoa using the same experimental conditions (Shur and Neely, 1988).

The physiological significance of this activity thus remains to be clarified.

In agreement with the presence of an immunologically reactive protein similar to GalTase, Humphreys-Beher *et al.* (1990) reported the presence of anti-GalTase antibodies in the serum of infertile patients displaying anti-sperm antibodies. Moreover, they also provided evidence that certain patients whose spermatozoa were unable to fertilize zona-free hamster eggs have altered restriction patterns in their GalTase coding gene (Humphreys-Beher and Blackwell, 1989). Hence, an immunologically reactive form of GalTase that is devoid of activity may be present in human sperm membranes or this activity is inhibited in a manner that is not reversed by our experimental conditions. This phenomenon is similar to the trypsin inhibitor binding site on mouse spermatozoa which also does not possess catalytic activity (Aarons *et al.*, 1984).

Seminal plasma is a rich source of GalTase. As shown by the kinetic analysis, its activity is linear up to 2 h and it depends upon the presence of manganese, which is in agreement with all GalTases that have been characterized (Strous, 1986). The K_m value for UDP-Gal ($130 \mu\text{mol l}^{-1}$) is comparable to that found for GalTase in the epididymal fluid of the rat ($100 \mu\text{mol l}^{-1}$; Hamilton, 1980) and mouse spermatozoa ($30 \mu\text{mol l}^{-1}$; Shur and Neely, 1988). The K_m value of the enzyme for GlcNAc (2.25 mmol l^{-1}) is similar to that found on mouse spermatozoa (4 mmol l^{-1} ; Shur and Neely, 1988). The enzyme activity is temperature dependent. Activity increased between 24 and 40°C and subsequently decreased at 45°C . This dependency on temperature is similar to that observed for intact mouse sperm GalTase but different from the purified form of this enzyme (Shur and Neely, 1988). A different dependence on temperature is observed using human serum GalTase, in which maximal activity is observed at 30°C and complete inhibition is encountered at 40°C (Kim *et al.*, 1972).

Inhibition studies show that seminal plasma galactosyl-transferase is specific for glycosylated nucleotides and that the enzyme has more affinity for di- and triphosphorylated forms of the nucleotides. Monosaccharides do not compete for the active site as they do not inhibit [^3H]galactose transfer to GlcNAc.

The only known function described for α -lactalbumin is to bind GalTase to form a lactose synthetase complex by modifying substrate specificity (Strous, 1986). In a similar manner to the GalTases described in the reproductive tract of the mouse (McLaughlin and Shur, 1987) and rat (Hamilton, 1980) epididymis, human seminal plasma GalTase is also inhibited by α -lactalbumin. Both bovine and human α -lactalbumin inhibit enzyme activity in a similar manner. This is not surprising since these proteins possess an 85% homology in their amino acid sequence (Hurley and Schuler, 1987).

The biologically active unit obtained in the radiation inactivation experiments had a molecular mass of 45 kDa. This RIS represents the protein core that is necessary for the catalytic activity of the enzyme excluding glycosylation or lipid binding (Beauregard *et al.*, 1987). It is thus comprehensible to find higher molecular masses from GalTase purification experiments ranging from 43 to 69 kDa with higher molecular masses being characteristic of membrane-bound GalTase, whereas the lower molecular mass forms are found in soluble form (Strous, 1986). The molecular masses of GalTases purified from human serum, amniotic fluid, skin fibroblasts and pleural effusions range

between 43 and 55 kDa (Strous, 1986). The soluble GalTase of human milk and colostrum has a molecular mass of 64 kDa (McGuire *et al.*, 1989). The existence of a small biologically active unit of GalTase is also supported by molecular biology studies of a cloned fragment of the human GalTase gene (Aoki *et al.*, 1990). These authors cloned a recombinant form of the human GalTase gene in *E. coli* that produced an active 36 kDa polypeptide and they demonstrated, by site-specific mutagenesis, that residues Tyr284, Tyr309 and Trp310 are responsible for GlcNAc binding while Tyr309 is also implicated in UDP-galactose binding (Aoki *et al.*, 1990).

The site of synthesis and secretion of GalTase in the male reproductive tract is unknown. We present the first evidence that GalTase activity is present in the intraluminal fluid of the human epididymis and that it is low in the proximal caput region and increases to its maximal specific activity in the adjacent downstream segment. This suggests that GalTase may be secreted by the intermediate region of the caput epididymis. However, the activation of a testicular or rete testis inhibited form of the enzyme by the addition of a cofactor or by removal of an inhibitor cannot be ruled out in these experiments. The cytoplasmic droplets are another possible source of GalTase activity. In many species, the cytoplasmic droplet that contains numerous lysosomal enzymes (Garbers *et al.*, 1970; Roberts *et al.*, 1976) slides off the flagellum during epididymal transit (Hermo *et al.*, 1988), although no GalTase activity has been associated with these particles up to the present. However, the samples used in this study are unlikely to contain cytoplasmic droplets since the intraluminal fluid was first centrifuged and subsequently ultracentrifuged to remove particulate matter. In humans, the loss of the cytoplasmic droplet by spermatozoa in the epididymis has not been the subject of extensive research. Bedford *et al.* (1973) reported that the detachment of the droplet from the flagellum is variable within the human epididymis, and that cytoplasmic droplets are frequently found on caudal spermatozoa. This possible source of GalTase activity thus remains a possibility that warrants further study.

This is the first report concerning the distribution of GalTase activity along the human male reproductive tract. This activity is present in the epididymal lumen and persists in the vas deferens. The presence of such soluble forms of GalTases has also been reported in the male reproductive tracts of rodents. McLaughlin and Shur (1987) identified GalTase in mouse caput epididymal fluid and it is also present in rat rete testis and epididymal fluids (Hamilton, 1981). GalTase activity is also present in cytosolic preparations of male reproductive tract where the activity is associated with epididymal tissues and important sources of activity were described to be of prostatic (rat and mouse) and seminal vesicle (hamster) origin (Reddy *et al.*, 1976). However, these authors reported that prostatic GalTase is membrane bound and is not found in a soluble form. The existence of GalTase activity in human seminal plasma was investigated by Tadolini *et al.* (1976) who showed that it is not membrane bound and is also present in fluid obtained after prostatic massage; the presence of GalTase activity in epididymal fluids was not studied.

The role of GalTase in the male reproductive tract remains to be elucidated. The importance of sugar moieties in gamete interaction is well documented in mammals (Miller and Ax, 1990) and the acquisition of fertilizing ability in the epididymis

coincides, in part, with the modification of components on the plasma membrane of spermatozoa (Cooper, 1986; Fournier-Delpech and Courot, 1987; Eddy, 1988). Thus, it is possible that glycosyltransferases or glycosidases present in the intraluminal fluid are involved in this modification. Accordingly, rat epididymal GalTase can galactosylate a 26 kDa sperm surface protein (Hamilton and Gould, 1982; Hamilton *et al.*, 1986). In humans, galactose residues and a potential acceptor, GlcNAc, are present on the sperm surface (Kallajoki *et al.*, 1985). GlcNAc is bound to human sperm plasma membrane lipids (Kurpisz *et al.*, 1989) which are also suitable GalTase acceptors of galactose (Strous, 1986).

Another interesting fact is that a potential endogenous acceptor of [³H]galactose is present in seminal plasma and is lost after ethanol precipitation. Although its nature is unknown, it could be a sugar component such as inositol, present in high quantities in the human reproductive tract, which can act as a substrate in the rat (Hamilton, 1981); glycolipids and glycopeptides are also potential candidates as galactose acceptors (Strous, 1986). Thus, in addition to its potential implication in murine gamete interaction, GalTase may also play a role in rat and mouse sperm maturation in the epididymis. In humans, however, its role remains unknown but further studies appear to be warranted to reveal its implication in some of these processes.

This research was supported by the Medical Research Council of Canada (PG-14) and the Fonds de Recherche en Santé du Québec. The authors would like to thank G. Beauregard for his helpful discussions and Sainte-Justine Hospital for the use of their Gammacell reactor for the irradiation studies and C. Boudreault for her assistance in typing this manuscript.

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