Galactosyltransferase, pyrophosphatase and phosphatase activities in luminal plasma of the cauda epididymidis and in the rete testis fluid of some mammals

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Galactosyltransferase activity was measured in the luminal plasma of the cauda epididymidis of mice, rats, rabbits, rats and boars, and in the rete testis fluid of rats and boars. The activities of nucleotide pyrophosphatase and alkaline phosphatase, which compete with galactosyltransferase for substrate, were also determined. In these species, galactosyltransferase activity in the luminal plasma of the cauda epididymidis was similar when the inhibitory effect of pyrophosphatase and phosphatase was minimized by assay conditions. However, under assay conditions that did not minimize the effect of these enzymes, the galactosyltransferase activities of these species were very different and were inversely correlated with the activities of pyrophosphatase and phosphatase. The ratio of galactosyltransferase activity to pyrophosphatase and phosphatase activity was much higher in the rete testis fluid than in the luminal plasma of the cauda epididymidis in both rats and boars. In rats, galactosyltransferase in the luminal plasma of the cauda epididymidis was more heat resistant than that in serum. These results suggest that there is a species difference in the availability of galactosyltransferase activity in the luminal plasma of the cauda epididymidis and that in some species, galactosyltransferase in the luminal fluid is unlikely to have any function. The results are also discussed with respect to the possible function of galactosyltransferase, pyrophosphatase and phosphatase in epididymal luminal plasma and rete testis fluid.

Introduction

The presence of β-1,4-galactosyltransferase (GalTase) in the reproductive tract of mammals has been widely reported. Soluble GalTase has been located in rete testis luminal plasma (commonly referred as rete testis fluid, RTF) (Hamilton, 1980), epididymal luminal plasma (Hamilton, 1980; McLaughlin and Shur, 1987; Ross et al., 1993) and seminal plasma (Tadolini et al., 1977; Ross et al., 1993). Membrane-bound GalTase has been located on spermatogenic cells (Scully et al., 1987), spermatozoa (Shur and Bennett, 1979; Scully et al., 1987; Humphreys-Beher and Blackwell, 1989; Fayrer-Hoskin et al., 1991; Miller et al., 1991; Suganuma et al., 1991), sperm cytoplasmic droplets (Oko et al., 1993) and a few types of testicular and epididymal somatic cells (Suganuma et al., 1991). Mouse sperm surface GalTase has been intensively studied and is known to be a receptor (Shur and Hall, 1982a; Lopez et al., 1985; Youakim et al., 1994) for zona pellucida during sperm–zona binding. In addition, it is redistributed during sperm maturation (Scully et al., 1987) and is responsible for sperm capacitation (Shur and Hall, 1982b). The role of GalTase in the sperm acrosome reaction has also been investigated (Macek et al., 1991; Cardullo and Wolf, 1995; Gong et al., 1995). However, there have been a few reports that showed species differences in sperm surface GalTase. Tulsiani et al. (1990) reported minimal GalTase activity on human intact spermatozoa and purified sperm plasma membrane. Tulsiani et al. (1993) showed that rat sperm surface sialyltransferase and fucosyltransferase, but not GalTase, exhibit maturation dependent changes.

The function of soluble luminal GalTase has received much less attention compared with sperm surface GalTase, although an investigation in rats found that the majority of the epididymal luminal GalTase activity was due to the soluble form of the enzyme (Tulsiani et al., 1993). Shur and Hall (1982b) reported that in mice, soluble GalTase in suspensions of cauda epididymidis specifically catalyses the galactosylation of a small molecular weight endogenous glycopeptide. In rats, a 24 kDa membrane glycoprotein on the surface of spermatozoa is galactosylated during epididymal maturation. Galactosylation of this protein was detected in the cauda epididymidis but not in the caput epididymidis (Olson and Hamilton, 1978; Jones et al., 1981; Brown et al., 1998).
1983; Zeheb and Orr, 1983; Hamilton et al., 1986) or the corpus epididymidis (Jones et al., 1981). This glycoprotein can be galactosylated in vitro by soluble luminal GalTase (Hamilton and Gould, 1982). It is not known whether similar events also occur in other species, or whether there are also some species differences in the presence and function of GalTase in cauda epididymal luminal plasma (CEP) as in the case of sperm surface GalTase. In addition to mice and rats, only humans (Ross et al., 1993; Tulsiani et al., 1993) have been examined for soluble GalTase activity in epididymal fluid.

Nucleotide pyrophosphatase and alkaline phosphatase act together to decompose UDP-galactose to galactose 1-phosphate and finally to galactose, and therefore limit GalTase activity by reducing its substrate (Spik et al., 1979; Faltynek et al., 1981). The concentration of alkaline phosphatase activity in CEP varies considerably among mammalian species (Jones, 1978). If this is also the case for nucleotide pyrophosphatase, then even if GalTase is present in mammalian CEP, it may not be able to function in some species.

The aim of the present study was to examine CEP GalTase and pyrophosphatase and phosphatase activities in mice, rats, rabbits, rams and boars. CEP GalTase activity was measured under two sets of conditions: (i) the inhibitory effect of pyrophosphatase and phosphatase on GalTase was minimized by assay conditions of low pH and high UDP-galactose concentration, enabling the absolute activity of the enzyme to be determined; and (ii) the inhibitory effect of these two enzymes was not controlled, enabling investigation of GalTase activity under conditions closer to the normal physiological environment. ATP, which inhibits pyrophosphatase and phosphatase activity, was used to investigate the degree of its inhibitory effect on GalTase. Measurements were also made of GalTase and pyrophosphatase and phosphatase activities in RTF from rams and boars. To date, RTF has only been investigated in rats (Hamilton, 1980). The heat resistant property of GalTase in CEP was compared with that in serum as well as that reported for RTF (Hamilton, 1980) to investigate the source of this enzyme in CEP.

Materials and Methods

Chemicals

N-acetylglucosamine (GlcNAc) and N-acetyllactosamine (LacNAc) were obtained from Sigma (St Louis, MO), UDP-galactose and galactose 1-phosphate from Boehringer (Mannheim) and UDP-[3H]galactose from New England Nuclear (Bedford, MA). AG 1-X8 (200–400 mesh, acetate form) anion exchange resin was obtained from Bio-Rad (Richmond, CA). The paper used for high voltage electrophoresis was 3MM paper from Whatman (Maidstone). All the chemicals and organic solvents used were of analytical reagent grade.

Animals

All the animals used were sexually mature. Mice (LACA), rats (Porton) and rabbits (New Zealand White) were obtained from the Central Animal House of the University of Adelaide. Testes and epididymides of rams (Border-Leicester × Merino) and boars (Large White) were collected from the abattoirs of the South Australia Meat Corporation.

Sample collection

Mice and rats were killed by CO2 inhalation. Rabbits were killed by an intravenous injection of an overdose of Avertin (5 g methyibutanol and 3 ml tribromoethanol dissolved in 20 ml absolute ethanol with 0.9% (w/v) NaCl to make up the volume to 250 ml). CEP was collected by retrograde pumping of saline via the vas deferens (Brooks and Tiver, 1983) soon after the animals were killed. The testes and epididymides of rams and boars were obtained and placed on ice soon after the animals were killed, and samples were collected after 1.0–1.5 h (the time required for transport to the laboratory). For CEP collection, a small area of the tunica albuginea of the cauda epididymis was removed, the exposed epididymal tubules were punctured at points in which there were no visible blood vessels, and the fluid was squeezed out by forceps and collected by Pasteur pipette. RTF was collected by puncturing the rete testis with a syringe needle and collecting the fluid with a Pasteur pipette. The epididymal and testicular fluids were centrifuged at 4000 g for 2 min at 4°C and the supernatants separated for assay of enzyme activities.

Rat serum was prepared as follows. Blood was collected from the jugular vein into a glass tube. The blood was allowed to stand at room temperature for 3 h and then at 4°C for 1 h to clot. The sample was then centrifuged at 1300 g for 20 min at 4°C. The supernatant was collected and stored at −20°C.

Assay of GalTase, pyrophosphatase and phosphatase activities

GalTase, pyrophosphatase and phosphatase activities of CEP were determined in the same assay. Two assay methods were used. The first, referred as the ordinary conditions method, was used to investigate the effect of pyrophosphatase and phosphatase on GalTase activity in CEP under conditions close to the normal physiological environment. The second method, referred as the low pH and high UDP-galactose method, was used to compare GalTase activities quantitatively in various species under conditions in which the inhibitory effects of pyrophosphatase and phosphatase were minimized.

Ordinary conditions method. The assay medium contained 50 mmol Hepes 1, pH 6.9, 20 mmol MnCl2 1, 0.4 mmol UDP-galactose 1 containing 8 nCi UDP-[3H]galactose, and CEP containing 100 μg protein in a total volume of 50 μl. 25 mmol GlcNAc 1 was present in half of the samples. The assay was conducted at pH 6.9, since the pH of the CEP of most of the species used is known to be between 6.8 and 7.0 (Jones, 1978); the pH value of mouse CEP is unknown. The concentration of UDP-galactose was similar to that used elsewhere for assaying GalTase activity in epididymal fluid (Hamilton, 1981;
Limpaseni and Chulavatanrat, 1986; Mclaughlin and Shur, 1987; Hölpert and Cooper, 1990). ATP at a concentration between 0 and 6 mmol l⁻¹ was added to the samples to inhibit the activity of pyrophosphatase and phosphatase. It is known that ATP can inhibit the activity of pyrophosphatase and phosphatase in the breakdown of UDP-galactose by replacing this sugar as the substrate of pyrophosphatase (Mookerjea and Yung, 1975; Hölpert and Cooper, 1990). However, when the activity of pyrophosphatase and phosphatase is low, ATP can also inhibit GalTase activity, although the inhibition is less effective than of the other two enzymes (Hölpert and Cooper, 1990). The assay mixture was incubated at 37°C for 30 min, and the reaction was stopped by placing the samples on ice and adding 20 μl of 0.3 mol EDTA 1⁻¹. The samples were then applied to AG 1-X8 columns (0.5 cm × 1.5 cm) and washed twice with 0.5 ml H₂O to separate the sugars. The remaining UDP-galactose and the galactose 1-phosphate were bound to the column, and the LacNAc and galactose were eluted. High voltage paper electrophoresis showed that LacNAc and galactose were the only two radioactive products in the eluate. The washings were mixed with scintillation fluid and counted for radioactivity. The difference between the radioactivities washed from the parallel samples in the presence and absence of GlcNAc was considered to be the GalTase activity. Radioactivity washed from the sample in the absence of GlcNAc was considered to be the activity of pyrophosphatase and phosphatase. Radioactive background, which was the radioactivity washed from the assay medium containing no biological fluid, was subtracted from all the samples. Approximately 85% of the total radioactivity in the assay mixture could be recovered from the column when UDP-galactose was maximally decomposed.

Low pH and high UDP-galactose method. The inhibitory effects of pyrophosphatase and phosphatase were minimized by using a low pH and a high UDP-galactose concentration in the assay medium. The assay mixture contained 50 mmol 4-morpholine-ethanesulphonic acid 1⁻¹, pH 6.5, 20 mmol MnCl₂ 1⁻¹, 25 mmol GlcNAc 1⁻¹, 2 mmol UDP-galactose 1⁻¹ containing 0.8 μCi UDP-[β-³²P]galactose, and CEP containing 100 μg protein in a total volume of 50 μl. The assay mixture of each sample was incubated at 37°C for 30 min in an Eppendorf tube with a parallel sample incubated at 0°C as a control for GalTase activity and pyrophosphatase and phosphatase activity. After incubation, 200 μl methanol and 85 μl chloroform were added and the mixture was vortexed and centrifuged at 4000 g for 15 min (Jones et al., 1986). The proteins were precipitated at the bottom of the Eppendorf tube after centrifugation, and the supernatant was transferred to a new Eppendorf tube and dried in a Speed vac vacuum centrifuge (Savant). The dried samples were then dissolved in 15 μl H₂O and applied to high voltage paper electrophoresis in 1% sodium tetraborate at 2000 V for 75 min. LacNAc, galactose, UDP-galactose and galactose 1-phosphate were used as standards. The UDP-galactose was located by 245 nm UV light. The other sugars were stained using silver nitrate (Trevyelyan et al., 1950). LacNAc migrated a short distance towards the anion electrode (Rg = 0.13), galactose migrated much further (Rg = 0.60), and UDP-galactose and galactose 1-phosphate migrated the farthest (Rg = 0.85 and 0.89, respectively). Each lane was cut at the positions corresponding to LacNAc and galactose, respectively, for counting radioactivity in scintillation fluid. The total radioactivity of each lane was also counted. The following equation was used to calculate the amount of the products in each sample:

\[
\text{pmole sugar} = \frac{\text{pmole UDP-galactose}}{\text{in assay medium}} \times \frac{\text{radioactivity at the position of sugar product}}{\text{total radioactivity of the lane}}
\]

The values of the control samples were subtracted from their parallel samples.

**Assay of protein concentration**

The Coomassie blue method was used to assay protein concentration (Sedmak and Grossberg, 1977).

**Statistical analysis**

Analysis of variance and Student’s t test were used for statistical analyses.

**Results**

**Effect of pyrophosphatase and phosphatase activity on GalTase activity in the CEP of various species**

In rats, pyrophosphatase and phosphatase did not have any significant inhibitory effect on the activity of GalTase (Fig. 1a), which was detectable in the absence of ATP. ATP at a concentration of 1 mmol l⁻¹ inhibited most of the pyrophosphatase and phosphatase activity and increased GalTase activity to a maximum. GalTase activity was slightly inhibited at an ATP concentration of 3 mmol l⁻¹ and was markedly inhibited at 6 mmol ATP l⁻¹. The results from the rabbits (Fig. 1b) were intermediate to those from the rats and rams. In rams (Fig. 1c), in the absence of ATP, all the UDP-galactose was degraded by pyrophosphatase and phosphatase, and GalTase activity was not detectable. In the presence of 1 mmol ATP l⁻¹, the activity of pyrophosphatase and phosphatase started to decrease and GalTase activity was detectable. However, only at ATP concentrations of 3 mmol l⁻¹ and above did ATP inhibit most of the pyrophosphatase and phosphatase activity and GalTase activity increased to a maximum. In boars (Fig. 1d), UDP-galactose was completely degraded by pyrophosphatase and phosphatase in the presence of 2 mmol ATP l⁻¹ and GalTase activity was not detectable. Pyrophosphatase and phosphatase activity started to decrease and GalTase activity was first detectable at 3 mmol ATP l⁻¹. Higher concentrations of ATP further inhibited the activity of pyrophosphatase and phosphatase and increased the activity of GalTase.

Although the concentration of ATP in CEP is unknown, it is known that ATP is present in blood and other extracellular sites in mammals at low micromolar or submicromolar
concentrations (Born and Kratzer, 1984; Gordon, 1986). Therefore, it is likely that the ATP concentration used to inhibit CEP pyrophosphatase and phosphatase in the present study greatly exceeds physiological concentrations.

GalTase activity in CEP in various species under conditions that minimize the effects of pyrophosphatase and phosphatase

Among the species investigated, there were no significant differences ($P > 0.05$) in the activity of CEP GalTase under conditions in which the effects of pyrophosphatase and phosphatase were minimized (Table 1). The activity of pyrophosphatase and phosphatase was significantly higher in boars compared with the other species ($P < 0.05$). In addition, the activity of these enzymes was significantly higher in rams compared with rabbits, mice and rats and was significantly higher in rabbits compared with rats (all $P < 0.05$) (Table 1).

Preliminary experiments showed that when measured over the pH range 6.0-7.5, CEP pyrophosphatase and phosphatase activities of all species investigated increased with pH (Fig. 2). pH 6.5 was chosen for the assay medium since it is optimal for GalTase activity in most of the species studied, although it appeared to be slightly inhibitory in rats. A high UDP-galactose concentration (2 mmol l$^{-1}$) was used to reduce competition for this sugar between GalTase and pyrophosphatase and phosphatase. The concentration of UDP-galactose in CEP is unknown. However, it is known that UDP-galactose is present at a concentration of about 0.35 mmol l$^{-1}$ in bovine and ovine milk and at micromolar or submicromolar concentrations in human milk (Hernández and Sánchez-Medina, 1981; Arthur et al., 1991). The mammary gland is the principal location for GalTase, in which it catalyses the synthesis of lactose under the control of $\alpha$-lactalbionin by transferring galactose from UDP-galactose to glucose (Brodbeck and Ebner, 1966). Therefore, it is likely that 2 mmol UDP-galactose l$^{-1}$ is supraphysiological. UDP-galactose at a concentration of 2 mmol l$^{-1}$ had a slightly inhibitory effect ($P > 0.05$) on CEP GalTase activity in rats, but not in the other species (data not shown), compared with 0.4 mmol UDP-galactose l$^{-1}$. The lower GalTase activity in rats (Table 1), though not statistically significant, may have been caused by the low pH and high UDP-galactose.
Table 1. Comparison of galactosyltransferase and pyrophosphatase and phosphatase activities in the luminal plasma of the cauda epididymis of various mammals

<table>
<thead>
<tr>
<th>Species</th>
<th>Number of animals</th>
<th>Enzyme activity (pmole product per µg protein per 30 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Galactosyltransferase</td>
</tr>
<tr>
<td>Mouse</td>
<td>5 (groups)*</td>
<td>9.2 ± 3.2*</td>
</tr>
<tr>
<td>Rat</td>
<td>6</td>
<td>4.8 ± 0.8*</td>
</tr>
<tr>
<td>Rabbit</td>
<td>8</td>
<td>10.5 ± 2.3*</td>
</tr>
<tr>
<td>Ram</td>
<td>8</td>
<td>6.8 ± 0.6*</td>
</tr>
<tr>
<td>Boar</td>
<td>10</td>
<td>6.6 ± 1.2*</td>
</tr>
</tbody>
</table>

Values are mean ± se.
The low pH and high UDP-galactose method was used for the assay.
Values with different letters are significantly different (P < 0.05).
*Five groups each comprising > five mice were used to obtain sufficient cauda epididymal plasma for the assay. Each group was considered equivalent to one animal of the other species.

Table 2. Galactosyltransferase and pyrophosphatase and phosphatase activities in the luminal plasma of the cauda epididymis and the rete testis fluid of rams and boars

<table>
<thead>
<tr>
<th>Species</th>
<th>Sample</th>
<th>Total protein (µg)</th>
<th>ATP (mmol l⁻¹)</th>
<th>Galactosyltransferase</th>
<th>Pyrophosphatase and phosphatase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>per 10 µl fluid</td>
<td>per µg protein</td>
<td>per 10 µl fluid</td>
</tr>
<tr>
<td>Ram</td>
<td>RTF</td>
<td>5.7*</td>
<td>1126 ± 304</td>
<td>198</td>
<td>837 ± 208</td>
</tr>
<tr>
<td></td>
<td>CEP</td>
<td>117 ± 25</td>
<td>Not detectable</td>
<td>–</td>
<td>All UDP-galactose (20000 pmole) was decomposed</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Not applicable</td>
</tr>
<tr>
<td></td>
<td>–</td>
<td></td>
<td>919 ± 240</td>
<td>7.8</td>
<td>–</td>
</tr>
<tr>
<td>Boar</td>
<td>RTF</td>
<td>7.9*</td>
<td>2154 ± 396</td>
<td>272</td>
<td>1345 ± 257</td>
</tr>
<tr>
<td></td>
<td>CEP</td>
<td>191 ± 37</td>
<td>Not detectable</td>
<td>–</td>
<td>All UDP-galactose (20000 pmole) was decomposed</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Not applicable</td>
</tr>
<tr>
<td></td>
<td>–</td>
<td></td>
<td>1516 ± 364</td>
<td>8.1</td>
<td>–</td>
</tr>
</tbody>
</table>

Values are mean ± se.
CEP, luminal plasma of cauda epididymis; RTF, rete testis fluid.
The ordinary conditions method was used for the assay. In each species, rete testis fluid samples were from three different groups of animals and CEP samples were from three different animals.
*Owing to the limited amount of rete testis fluid, the protein concentration was only measured from a pooled sample in each species.

Concentration assay conditions. The effect of pyrophosphatase and phosphatase on GalTase under these assay conditions was examined using boar CEP which contained the highest pyrophosphatase and phosphatase activity among the species studied. Addition of 2-6 mmol ATP l⁻¹ inhibited pyrophosphatase and phosphatase activity by 45-90%, but did not increase the activity of GalTase (data not shown). Therefore, under the assay conditions of the low pH and high UDP-galactose method, the CEP pyrophosphatase and phosphatase activities do not have an inhibitory effect on GalTase activity in any of the species used.

GalTase, pyrophosphatase and phosphatase activity in RTF

A small amount of RTF was collected from rams and boars using the method described earlier. GalTase, pyrophosphatase and phosphatase activities were detected in the RTF of both species (Table 2). The activities of these enzymes in the same volume of CEP of each species were assayed for comparison with those in RTF (Table 2). In both species, GalTase activity was higher in RTF than in CEP. This is particularly apparent when expressed by unit protein. Pyrophosphatase and phosphatase activities in RTF were much lower compared with CEP.
Comparison of the effects of heat treatment on GalTase in CEP and serum

Ram CEP and serum were incubated at 50°C for 0.5 and 1 h, respectively, before GalTase activity was assayed. A parallel subset of each sample was placed on ice for the same period as a control. After 0.5 and 1.0 h of pre-heating, the activity of serum GalTase was reduced by 78.1 and 97.6% of the control value, respectively, whereas the activity of CEP GalTase was reduced by only 56.9 and 78.0%, respectively (Fig. 3). The difference between the activities of these two samples after either 0.5 or 1.0 h of pre-heating was significant at \( P < 0.05 \) (Fig. 3).

Discussion

The results of the present study and previous investigations (Hamilton, 1980; Tulsiaini et al., 1993; Ross et al., 1993) show that soluble GalTase is typically present in both the RTF and the epididymal luminal plasma of mammalian species. The results of this study suggest that absolute CEP GalTase activity in the species investigated is similar, whereas the activity under physiological conditions is very different and is inversely correlated with CEP pyrophosphatase and phosphatase activity.

Many factors in biological fluids affect GalTase activity, such as nucleotides and nucleotide sugars and their related enzymes. In the present study, crude fresh samples, which constituted 10–30% of the assay volume, were used. Therefore, the results should reflect the balance of the positive and negative factors acting on the enzyme in CEP and RTF. Pyrophosphatase and phosphatase are the major factors that affect the activity of GalTase in luminal fluid. This study showed that in both rams and boars, the ratio of GalTase activity to pyrophosphatase and phosphatase activity, as well as GalTase activity per unit protein, was much higher in RTF than in CEP. Hamilton (1980) also showed that in rats, GalTase activity per unit protein was much higher in RTF than in epididymal luminal plasma, and little or no pyrophosphatase was detected in RTF. Einarsson et al. (1976) reported that ovine RTF contained much lower
alkaline phosphatase activity than the same volume of CEP. Therefore, these may be features that are common in mammals.

Hamilton (1980) reported that in rats, GalTase in RTF was more resistant to pre-heating at 50°C than GalTase in serum. The present study showed that in rams, GalTase in CEP was also more resistant to pre-heating than GalTase in serum. This observation suggests two possibilities: first, some or all of the GalTase in the epididymal plasma is derived from the RTF; or, second, the genes that encode the soluble GalTases are the same in the testicular and epididymal cells, at least in terms of the heat resistant properties of the enzyme.

In the current study, 20 mM MnCl₂, mmol L⁻¹ was used in the enzyme assays. Preliminary experiments showed that GalTase activities in the CEP from different species reached a maximum at 1–3 mmol MnCl₂, L⁻¹ and remained constant at 25–40 mmol MnCl₂, L⁻¹ (data not shown). The Mn²⁺ concentrations for assays of mammalian GalTases from different sources ranged from 2 to 43 mmol L⁻¹ (see Ram and Munjal, 1985). However, the Mn²⁺ concentrations used in the present study and in previous investigations greatly exceed those in tissue and body fluids, which are submicromolar (Versieck et al., 1974; also see Powell and Brew, 1976; Kuhn et al., 1992). Various divalent ions, such as Co²⁺, Ba²⁺; Zn²⁺, Ca²⁺ and Mg²⁺, can substitute for Mn²⁺ with lower efficiency (Hudgin and Schachter, 1971; Powell and Brew, 1976; Gmeiner, 1988). In the present study, experiments with CEP from rams showed that only Co²⁺ and Mg²⁺ could stimulate GalTase activity by 13 and 3.2% of that promoted by Mn²⁺, respectively (data not shown). The suggestion that in vivo GalTase activity is stimulated by other ions, either independently or in association with Mn²⁺, is not well supported by evidence. In addition, a possible mechanism explaining how organic cations participate in the stimulation of the enzyme has been proposed (see Kuhn et al., 1992).

GalTase is the most widely studied glycosyltransferase in the male reproductive tract. However, more studies have been carried out on sperm surface GalTase, particularly in mice, although various observations suggest that soluble GalTase is the major source of luminal GalTase. In the epididymal lumen of rats, 80–90% of the activity of GalTase and three other glycosyltransferases is from soluble enzymes, and only 10–20% of the activity is associated with spermatozoa. In humans, although the epididymal luminal plasma and the seminal plasma are both rich in GalTase activity (Ross et al., 1993), very low GalTase activity is present on the sperm surface (see Ross et al., 1993). Preliminary results of the current study showed that in boars, more than 90% of CEP GalTase activity was present in the luminal plasma (data not shown). Understanding of the functions of soluble GalTase in the male reproductive tract remains very limited.

The results of this study suggest that soluble GalTase is able to function in the epididymal lumen of species that do not have high pyrophosphatase and phosphatase activity in CEP, such as mice and rats. Furthermore, soluble GalTase in the CEP is unable to function in other species, such as pigs and sheep, because of the strong inhibitory effect of very high pyrophosphatase and phosphatase activities. This may also apply to other glycosyltransferases in CEP. It is known that in mice, the soluble GalTase of the cauda epididymis specifically galactosylates a low molecular weight endogenous glycopeptide (Shur and Hall, 1982b). In rats, galactosylation of an integral 24 kDa glycoprotein (Olson and Hamilton, 1978; Jones et al., 1981; Brown et al., 1983; Zeheb and Orz, 1983; Hamilton et al., 1986) and some other glycoproteins (Brown et al., 1983) on the sperm surface have been detected in the cauda epididymis as part of the sperm maturation process. The 24 kDa glycoprotein and other glycoproteins can be galactosylated in vitro by soluble luminal GalTase (Hamilton and Gould, 1982). These results are consistent with the current finding that GalTase readily functions in the CEP of mice and rats. However, the present results also suggest that in some other species, such as pigs and sheep, the galactosylation of CEP glycopeptides is unlikely to occur; and the galactosylation of sperm surface glycoproteins is unlikely to be part of the sperm maturation process in the cauda epididymis. Many questions await further investigation, for example whether the galactosylation of CEP glycopeptides or of spermatozoa in the cauda epididymis occurs in other species with low or medium pyrophosphatase and phosphatase activity in addition to mice and rats, and what the physiological relevance is of the galactosylation of the glycopeptides in mouse CEP.

The functions of soluble GalTases in the caput and corpus lumen are unknown. The luminal environment in these sections of the epididymis is probably more favourable for GalTase activity than that of the cauda epididymis, since it is known that in boars, caput luminal plasma contains much smaller concentrations of alkaline phosphatase than cauda luminal plasma (Einarsson et al., 1976). GalTase in caput and corpus luminal plasma may have a role in the galactosylation of sperm surface glycoproteins during sperm maturation in some species, although the galactose residues may not be at
the terminals of the oligosaccharide chains and would therefore not be labelled with the same technique used to detect the 24 kDa glycoprotein mentioned earlier. This is because glycosylation of the sperm membrane is part of the maturation process in the epididymis (see Jones, 1989).

The physiological function of GaTase in the RTF is not well understood. The results of the present study and of earlier investigations (Einarsson et al., 1976; Hamilton, 1980) show that the RTF provides a favourable environment for GaTase activity due to the low pyrophosphatase and phosphatase activity. It is known that the majority of the RTF originates in the seminiferous tubules and there is little secretion of fluid from the rete testis itself (see Setchell, 1979). Hence, the GaTase, pyrophosphatase and phosphatase activities in the RTF may reflect the activities of these enzymes in the luminal plasma of the seminiferous tubules. This implies that the tubules also provide a favourable environment for the function of GaTase. It is possible that GaTase in the luminal plasma of the tubules has some function in spermatogenesis, since a few galactosylated glycoproteins have been detected on testicular spermatozoa in rats (Brown et al., 1983) and glycosylation of mammalian spermatogenic cells has also been reported (Millette et al., 1987). It is possible that specific galactosylation of sperm surface glycoproteins occurs at multiple sites in the male reproductive tract.

The physiological roles of pyrophosphatase and phosphatase in CEP are unknown. Little research has been carried out on nucleotide pyrophosphatase in the mammalian male reproductive tract. The order of pyrophosphatase and phosphatase activity in the CEP of the species in the present study (boar > ram > rabbit > rat) is similar to that of alkaline phosphatase activity in the CEP of the same species reported by Jones (1978) (boar > rabbit > ram > rat). However, the order in the current study was not only determined by the activity of alkaline phosphatase, but also by that of pyrophosphatase. The amount of ATP, which competes with UDP-galactose as the substrate of pyrophosphatase (Mookerjea and Yung, 1975), that was required to inhibit pyrophosphatase and phosphatase activity and allow GaTase to function in the CEP was also in the order boar > ram > rabbit > rat. Investigations with boars (Einarsson et al., 1976) and dogs (Frenette et al., 1986) have suggested that most of the alkaline phosphatase in the seminal plasma is derived from the epididymis and in particular the cauda epididymis (boar). Bell and Lake (1962) reported that alkaline phosphatase activity in the seminal plasma was highest in boars, lower in rabbits and even lower in bulls. In the current study, some preliminary experiments were carried out using bovine CEP. The results showed that pyrophosphatase and phosphatase activities in bovine CEP were intermediate to those in CEP from rabbits and rats, with values closer to those of rats (data not shown). Therefore, the concentration of seminal plasma alkaline phosphatase is likely to be correlated with the concentration of this enzyme in CEP in mammals, and this is consistent with the proposal that the cauda epididymis is the origin of seminal plasma alkaline phosphatase. Whether alkaline phosphatase in CEP functions locally or is prepared for a later stage in reproduction is not known. To date, there has only been general speculation on this matter. The hydrolytic enzymes in the cauda epididymidis may have a role in sperm maturation, such as modification of surface glycoproteins (Jones, 1978). The acid and alkaline phosphatases in seminal plasma may influence the normal activity of spermatozoa (see Bell and Lake, 1962).

The results of this study suggest that nucleotide pyrophosphatase and alkaline phosphatase in CEP act together to fulfil some of their physiological roles. These functions may be species-specific because of the differences in their activities among different species. In some species such as pigs and sheep, these enzymes may hydrolyse nucleotide sugars and thus inhibit glycosylation of sperm surface glycoproteins in the cauda epididymidis by GaTase and other glycosyltransferases. Furthermore, the enzymes may hydrolyse nucleotide sugars and thus inhibit glycosylation of soluble glycoproteins in the cauda epididymis in some species, although galactosylation is known to occur in mice (Shur and Hall, 1982b). The possible function of pyrophosphatase in modifying glycoconjugate synthesis by hydrolysing the substrate of glycosyltransferase has been discussed elsewhere (Nelson et al., 1977; Van Dijk et al., 1983). Further research is required to fully elucidate the functions of soluble GaTase, pyrophosphatase and phosphatase in the mammalian male reproductive tract.

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