Changes in surface ATPase of rat spermatozoa in transit from the caput to the cauda epididymidis

M. Chulavatnatol and S. Yindepit

Department of Biochemistry, Faculty of Science, Mahidol University, Rama VI Road, Bangkok, Thailand

Summary. Rat spermatozoa from the cauda epididymidis were found to have a lower activity of the surface ATPase than the spermatozoa from the caput region. The enzyme from spermatozoa of both regions had the same Michaelis constant ($K_m$) for ATP of $5 \times 10^{-4}$ M. It was partly inhibited by ouabain and fluoride, but strongly inhibited by Cu$^{2+}$, Zn$^{2+}$, p-chloromercuribenzoate, 8-anilino-1-naphthalenesulphonate Triton X-100, Lubrol–PX, urea, guanidine hydrochloride, sodium dodecyl sulphate and glycerophosphorylcholine. The enzyme of the spermatozoa from the cauda epididymidis was more sensitive to inhibition by ouabain and fluoride but less sensitive to inhibition by Cu$^{2+}$ than that of the cells from the caput region. The Arrhenius plot of the temperature dependence of enzymatic activity varied for the cells from the caput and cauda epididymidis. The differences in the enzyme properties of spermatozoa from the two regions of the epididymis suggested that the decline in the activity during epididymal maturation may reflect changes in the lipids and sulphydryl groups of the sperm membrane.

Introduction

During their passage from the caput to the cauda epididymidis, mammalian spermatozoa acquire the ability to fertilize eggs. Some of the known changes in the spermatozoa associated with this process of maturation are oxidation of their sulphydryl groups into disulphide linkages (Calvin, Yu & Bedford, 1973), increase in the negative surface charges (Yanagimachi, Noda, Fujimoto & Nicolson, 1972), increase in glycolysis (Frenkel, Peterson & Freund, 1973; Hoskins, Munsterman & Hall, 1975), decrease in phospholipid content (Dawson & Scott, 1964; Grogan, Mayer & Sikes, 1966; Quinn & White, 1967), and lower incorporation of $[^{14}C]$glucose into glycerides and glycerophosphatides (Terner, MacLaughlin & Smith, 1975). However, little is known about enzymatic changes in the spermatozoa during epididymal maturation. Terner et al. (1975) observed no significant change in the activities of lactate dehydrogenase, hexokinase, glucose-6-phosphate dehydrogenase or α-glycerophosphate dehydrogenase, but a significant increase in monoglyceride lipase and decrease in alkaline phosphatase during the maturation process of rat spermatozoa.

Durr, Abla & Mrouch (1972) reported the presence of a (Na$^+$ + K$^+$)-dependent ATPase in intact human spermatozoa that can utilize exogenous ATP as its substrate, suggesting that it resided on the sperm surface with its active site facing outwards. Moreover, the properties of membrane-bound enzymes are known to be sensitive to changes in the composition of the membrane (Coleman, 1973; Farias,Bloj, Morero, Sineriz & Trucco, 1975; Skou, 1975; Schwartz, Lindenmayer & Allen, 1975), and it was anticipated that the properties of the surface ATPase of epididymal spermatozoa may change during maturation and that the change may reflect the alteration of the sperm surface during the process. In the present study, therefore, the properties of the surface ATPase of rat spermatozoa from the caput epididymidis were compared with those of the spermatozoa from the cauda epididymidis.
Materials and Methods

Preparation of epididymal spermatozoa. Male albino rats (200–300 g) were anaesthetized with ether. The epididymides were quickly removed, separated into caput and cauda portions and placed in ice-cold 0-01 m-tris–HCl buffer, pH 7.4. The tubules were punctured with a fine hypodermic needle and spermatozoa were extruded by squeezing. Each cell suspension was centrifuged at 600 g for 10 min at 4°C. The spermatozoa were then washed with the buffer and centrifuged. The washing was then repeated once more. This was sufficient to remove the cytoplasmic droplets, as judged by microscopic inspection. The washings also reduced the activity of the ATPase to a constant value. This was probably due to the removal of the droplets and membrane fragments from epididymal microvilli that possessed ATPase activity (Garbers, Wakabayashi & Reed, 1970; Reed & Takahashi, 1973). The cell concentration of the final suspension was determined by counting with a haemocytometer.

Assay of surface ATPase. The activity of the surface ATPase was assayed by following the formation of ADP from added ATP. This was done by coupling the ADP formation with the pyruvate kinase reaction to yield pyruvate which could then be measured spectrophotometrically as pyruvic phenylhydrazone (Kornberg & Morris, 1965). An aliquot of the sperm suspension was incubated at 37°C with 0-1 m-tris–HCl buffer, pH 7-4, containing 0-01 m-MgCl₂, 0-01 m-NaCl, 0-1 m-KCl, 4 \times 10^{-3} m-ATP, 0-01 m-phosphoenolpyruvate and 4 units of pyruvate kinase. The total volume was 0-1 ml. After 20 min, the reaction was terminated by adding 0-33 ml 0-1% 2,4-dinitrophenylhydrazine in 2 n-HCl, followed by 0-9 ml double-distilled water. The mixture was then further incubated for 10 min at 37°C to allow the formation of pyruvic phenylhydrazone. At the end of the incubation, 1-66 ml 10% NaOH was added. The absorbance of the solution at 445 nm was then recorded against a blank without added ATP using a Zeiss PMQ II spectrophotometer. The reading was converted into μmoles using an ADP standard curve.

Although ATPase of intact spermatozoa has previously been studied by several groups (Uesugi & Yamazoe, 1966; Quinn & White, 1968; Durr et al., 1972; Abla, Mroueh & Durr, 1974), none of the assay methods used can measure exclusively the activity of the surface ATPase. The assay of the activity of ATPase employed in this study measures only the surface ATPase with an outwardly facing active site, for the following reasons. Firstly, the rate of uptake of exogenous ATP (5 \times 10^{-3} μ) by the rat epididymal spermatozoa is known to be 0-03 nmol/min/10⁶ cells (Sithiseree, 1975) which was far less than the specific activity of the surface ATPase (Table 1), suggesting that the exogenous ATP was not readily available as the substrate to intracellular ATPase. Secondly, the hydrolytic product, ADP, would be immediately used by the exogenously added pyruvate kinase to form pyruvate which was later converted into the pyruvic phenylhydrazone. Any endogenous pyruvate that leaked from the cells during this process was corrected for by the reagent blank.

Results

The specific activity of the surface ATPase of the rat epididymal spermatozoa decreased significantly during the passage from the caput to the cauda epididymis (Table 1). The cause of the variation of the specific activity from animal to animal was unknown. The decrease in the specific activity of the surface ATPase during maturation was not due to change in the Michaelis constant for ATP which remained at 5 \times 10^{-4} μ (Text-fig. 1).

Temperature effects. The Arrhenius plot (Text-fig. 2) for the activity of the surface ATPase of the spermatozoa from the cauda epididymis was linear over the temperature of 3°C to 38°C, with a break at 32°C. However, the linear plot for the activity of the enzyme of the spermatozoa from the caput region showed two breaks at 25°C and 7°C. In addition, the enzymatic activity of the spermatozoa from the cauda region was highest at 32°C while that of the spermatozoa from the caput region peaked at 25°C.

Inhibition by ouabain and fluoride. The surface ATPase of the rat epididymal spermatozoa was shown to be (Na⁺ + K⁺)-dependent because it was inhibited by ouabain, a specific inhibitor for this type of ATPase (Schwartz et al., 1975), and fluoride (Text-fig. 3). The enzyme of the spermatozoa
Table 1. The specific activities (nmol/min/10^6 cells) of surface ATPase of rat epididymal spermatozoa

<table>
<thead>
<tr>
<th>Rat</th>
<th>Spermatozoa from:</th>
<th>Ratio of specific activities</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Caput</td>
<td>Cauda</td>
</tr>
<tr>
<td>1</td>
<td>5.50</td>
<td>1.38</td>
</tr>
<tr>
<td>2</td>
<td>5.24</td>
<td>1.22</td>
</tr>
<tr>
<td>3</td>
<td>7.78</td>
<td>2.66</td>
</tr>
<tr>
<td>4</td>
<td>6.77</td>
<td>2.39</td>
</tr>
<tr>
<td>5</td>
<td>6.00</td>
<td>2.57</td>
</tr>
<tr>
<td>6</td>
<td>5.33</td>
<td>3.03</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>6.10 ± 1.00*</td>
<td>2.21 ± 0.74*</td>
</tr>
</tbody>
</table>

* P < 0.01 (paired Student's t test).

Text-fig. 1. ATP saturation curves of the surface ATPase of the spermatozoa from the caput epididymidis (Δ) and from the cauda epididymidis (○) of the rat. The insert is the Lineweaver–Burk plot of the same data for determination of the Michaelis constant (K_m).

Text-fig. 2. Arrhenius plot of the dependence on temperature of the activity of the surface ATPase of the spermatozoa from the caput (Δ) and cauda (○) epididymidis of the rat. The activity at 38°C of each line is taken as 100%. The breaks are indicated by the arrows.
from the cauda region was more sensitive to inhibition than that of the cells from the caput epi-
didymidis. The enzyme was not a Ca\textsuperscript{2+}-activated ATPase since its activity was not inhibited by EGTA or 2,4-dinitrophenol, known specific inhibitors for Ca\textsuperscript{2+}-activated ATPase (Wins & Schoffeniels, 1966).

\[\text{Text-fig. 3.} \quad \text{The inhibition by (a) ouabain and (b) NaF on the surface ATPase of the spermatozoa from the caput (\(\triangle\)) and cauda (\(\odot\)) epididymidis of the rat.}\]

\textit{Inhibition by Zn\textsuperscript{2+} and Cu\textsuperscript{2+}.} The surface ATPase was strongly inhibited by Zn\textsuperscript{2+} and Cu\textsuperscript{2+} (Table 2) while Ca\textsuperscript{2+} and Ba\textsuperscript{2+} showed no effect. The enzyme of the spermatozoa from the cauda epididymidis seemed to be less sensitive to the inhibition by Cu\textsuperscript{2+} than that of the cells from the caput region, but there was little difference in the degree of inhibition by Zn\textsuperscript{2+}.

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|c|c|c|c|}
\hline
 & & & & & & & & \\
 & Conc. of inhibitor (mM) & & & & & & & \\
\hline
 & 0 & 0.2 & 0.4 & 0.6 & 0.8 & 1 & 2 & 5 \ 
\hline
\text{CuSO\textsubscript{4}} & & & & & & & & \\
\text{Caput} & (100) & 78 & 66 & 62 & 58 & 59 & 47 & 22 \ 
\text{Cauda} & (100) & 81 & 77 & 70 & 69 & 66 & 59 & 37 \ 
\text{ZnSO\textsubscript{4}} & & & & & & & & \\
\text{Caput} & (100) & 64 & 55 & 46 & 40 & 38 & 29 & 21 \ 
\text{Cauda} & (100) & 71 & 56 & 44 & 38 & 32 & 22 & 21 \ 
\hline
\end{tabular}
\caption{Effect of metal ions on the activity (expressed as \% of control) of surface ATPase of rat epididymal spermatozoa}
\end{table}

\textit{Inhibition by sulphydryl reagents.} Among the sulphydryl reagents tested, p-chloromercuribenzoate was the only one that showed pronounced inhibitory effect on the surface ATPase which was similar for spermatozoa from both epididymal regions. N-ethylmaleimide displayed only a slight effect (Text-fig. 4), but no inhibitory effects were induced by 1 mM-5,5'-dithiobis-(2-nitrobenzoate), 1 mM-dithiothreitol, 10 mM-\(\beta\)-mercaptoethanol, 2 mM-iodooacetate or 2 mM-iodoacetamide.

\textit{Effects of other reagents.} Several reagents were screened for their effects on the activity of the surface ATPase of rat epididymal spermatozoa. Particular attention was given to those that can interfere with the structure of proteins and lipids on the membrane (Razin, 1972) and to those present in high concentrations in the epididymal fluid (Brooks, Hamilton & Mallek, 1974). The reagents listed in Table 3 caused strong inhibition, but 40 mM-carnitine, 1 mM-phosphorylcholine, \(2 \times 10^{-4}\) M-cyclic 3',5'-AMP, 0.02 M-caffeine, 0.02 M-phosphate and 0.02 M-arsenate had no effect.
Inhibitor cone. (mM)

Text-fig. 4. The inhibition of the activity of the surface ATPase of the spermatozoa from the caput (△, Δ) and cauda (○, O) epididymidis of the rat by the sulphydryl reagents p-chloromercuribenzoate (solid symbol) and N-ethylmaleimide (open symbol).

Table 3. Effect of several inhibitors on the activity of surface ATPase of rat epididymal spermatozoa

<table>
<thead>
<tr>
<th>Addition (conc. for maximum effect)</th>
<th>% ATPase activity of spermatozoa from:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Caput</td>
</tr>
<tr>
<td>None</td>
<td>(100)</td>
</tr>
<tr>
<td>8-Anilino-1-naphthalenesulphonate (1 mM)</td>
<td>7</td>
</tr>
<tr>
<td>Triton X-100 (4%)</td>
<td>12</td>
</tr>
<tr>
<td>Lubrol-PX (4%)</td>
<td>17</td>
</tr>
<tr>
<td>Urea (0·2 m)</td>
<td>4</td>
</tr>
<tr>
<td>Guanidine hydrochloride (0·8 m)</td>
<td>12</td>
</tr>
<tr>
<td>Sodium dodecyl sulphate (0·4 mm)</td>
<td>3</td>
</tr>
<tr>
<td>Glycerylphosphorylcholine (1 mm)</td>
<td>35</td>
</tr>
</tbody>
</table>

Discussion

The decrease in the activity of the surface ATPase of rat epididymal spermatozoa during the maturation process is quite clear (Table 1), but its cause is still obscure. It is not due to any change in the $K_m$ value for ATP (Text-fig. 1). It may be due to certain changes in the sperm surface or membrane associated with the epididymal maturation.

The Arrhenius plots for the enzyme of the spermatozoa from the caput epididymidis and that from the cauda portion show breaks at different temperatures (Text-fig. 2). The breaks reflect the phase changes of the lipids in the sperm membrane and such changes are usually associated with the lipid composition of the membrane (Esfahani, Limbrick, Knutton, Oka & Wakil, 1971; Cobon & Haslam, 1973; Chapman, 1975). Therefore the lipids of the sperm membrane may change during epididymal maturation and may be associated with the decline in the total phospholipid content of the spermatozoa observed previously (Dawson & Scott, 1964; Grogan et al., 1966; Quinn & White, 1967).

The importance of lipids for the activity of membrane-bound ATPase is well known (Skou, 1975; Schwartz et al., 1975; Farias et al., 1975). Equally well known is the fact that sodium dodecyl sulphate, Triton X-100 and Lubrol-PX can solubilize membrane lipids (Razin, 1972). Thus, the inhibitory effect of these reagents on the surface ATPase (Table 3) implies a dependence of enzymatic activity on lipids. The hydrophobic reagent 8-anilino-1-naphthalenesulphonate is known to bind to the lipids of sperm membranes (Mercado & Rosado, 1973; Azzi, 1975) and immobilize spermatozoa (Edelman &
Millette, 1971); its inhibition of the surface ATPase further supports the dependence of this enzyme on membrane lipids.

Although the intracellular sulphhydryl groups in rat epididymal spermatozoa have been reported to decrease during maturation (Calvin et al., 1973), the change of the sulphhydryl groups on the sperm membrane has not been specifically demonstrated. Cu2+ which can oxidize sulphhydryl groups into disulphide linkages (Cecil, 1963) inhibits the surface ATPase of the spermatozoa from the caput epididymidis more than that of the cells from the cauda portion (Table 2), suggesting that there are more oxidizable sulphhydryl groups on the membrane of spermatozoa from the caput epididymidis, although sulphhydryl oxidase is also known to occur in the epididymis (Chang & Morton, 1975).

Since no differential effect was observed between the surface ATPases of the spermatozoa from the caput and cauda epididymidis with regard to the inhibition by Zn2+ or by p-chloromercuribenzoate, they must be interacting with some specific sulphhydryl group known to be essential for the activity of (Na+ + K+)-dependent ATPase (Patzelt-Wenczler, Erdman & Schoner, 1975). Zn2+ is known to interact strongly with sulphhydryl groups (Calvin, Hwang & Wohlrab, 1975), to inhibit the respiration of human spermatozoa (Eliasson, Johnson & Lindholmer, 1971), to inhibit alkaline phosphatase from bovine brain (Brunel & Cathala, 1973) and to bind tightly to biological membranes (Chvapil, 1973). It is also inhibitory to membrane-bound (Na+ + K+)-dependent ATPase in pulmonary alveolar macrophages (Mustafa, Cross, Munn & Hardie, 1971). p-Chloromercuribenzoate is reported to specifically inhibit (Na+ + K+)-dependent ATPase of other sources (Schwartz et al., 1975).

The inhibition by other protein-denaturating agents (Table 3) may be a general interference of the hydrogen bonds (by urea) and of the negative charges (by guanidine hydrochloride) of the surface ATPase itself or of other membrane proteins that can influence enzymatic activity.

The function of the surface ATPase of rat epididymal spermatozoa is presently unknown. It is probably not involved in the ATP-dependent surface reaction of spermatozoa which causes head-to-head association and which is activated by Mg2+, Ca2+ and Mn2+ but not inhibited by Zn2+ (Lindahl, 1973). It may participate in cation transport which is a well-known function of (Na+ + K2+)-dependent ATPase of other sources. The inhibition of the surface ATPase by Zn2+ and Cu2+ may be of some physiological importance when the spermatozoa encounter a high content of Zn2+ in the seminal plasma or of Cu2+ in the contents of a uterus containing a copper IUD. Although glycerylphosphorylcholine and carnitine are present at high concentrations (40 and 60 mM respectively) in the epididymal fluid (Brooks et al., 1974), only the former has a strong inhibitory effect on the surface ATPase of the epididymal spermatozoa. To our knowledge, this seems to be the only known effect of glycerylphosphorylcholine on spermatozoa and its significance requires further investigation.

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References


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