Appearance of Autosomal Linked G6PD Isozymes in Rat Sperm during Passage through the Epididymis

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In mammals glucose-6-phosphate dehydrogenase (G6PD; E.C.1.1.1.49) is produced by both the X-chromosome and an autosomal linked gene [19–21]. The X-chromosome linked G6PD found in erythrocyte is highly specific for glucose-6-phosphate (G6P) as substrate, while the autosomal linked enzyme in liver microsomal fraction has affinity for both G6P and galactose-6-phosphate (Gal-6P) [11, 16]. Several investigations have indicated in mouse that the X-chromosome linked G6PD is synthesized in the premeiotic stage of spermatogenesis and that the G6PD of cauda epididymal sperm has the properties of X-chromosome linked form [2, 8, 9]. However, there is no evidence for the alteration of sperm G6PD zymogram during the epididymal transit of sperm. The present paper describes the alteration of rat sperm G6PD zymogram characterized by the appearance of autosomal linked G6PD during the transit of sperm through the epididymis.

Adult rats of Wistar strain (80–90 days old) were used throughout the experiment. The supernatants from both testicular and epididymal sperm homogenates were prepared as previously reported [10, 12]. An appropriate amount of the supernatant containing 90 or 100 μg of protein was charged for the analysis of G6PD zymogram. Erythrocytes, which were isolated from blood using Histopaque (Sigma) [23] were disrupted in a phosphate buffer (0.1 M, pH 7.4) by freeze-thawing and subjected to centrifugation at 8,200 × g for 5 minutes. The liver homogenized with an equal amount of buffer was also centrifuged at 8,200 × g for 5 minutes. Both the clear supernatants obtained from erythrocytes and liver were also used for the zymogram analysis. For analysis of the zymogram, vertical micro-slab gel electrophoresis with commercial equipment (Atto Co., Ltd., Tokyo) was carried out by the method of Davis [6]. After electrophoresis, the gel was stained in the dark to demonstrate the zymogram with a total volume of 20 ml of 0.4 M Tris-HCl buffer (pH 7.4) containing 7.3 μM of nitro blue tetrazolium, 13.1 μM of NADP, 0.14 μM of phenazine methosulphate, 5.0 μM of NaCN and 131.5 μM of substrate (G6P or Gal-6P) for 1 hr at 37°C. Relative activities of each G6PD isozymes were measured with a densitometer at 500 nm. Protein concentration was measured using Coomassie Brilliant Blue G-250 [4]. Bovine serum albumin was used as a standard.

Sperm obtained from both testes and epididymides were more than 95% pure (Table I). There were few epididymal spermatozoa with the cytoplasmic droplet. Two major G6PD isozymes (A and D) and two minor ones (B and C) were demonstrated in the rat sperm. The activity of isozyme-A with G6P and of isozyme-D with Gal-6P was higher than the others, though all of the isozymes showed affinity for both substrates to some

Table 1. Purities of testicular and epididymal spermatozoa obtained (%)

<table>
<thead>
<tr>
<th>Sperm Type</th>
<th>Purity (%)</th>
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<tbody>
<tr>
<td>Testicular sperm (6)</td>
<td>97.3±0.9</td>
</tr>
<tr>
<td>Caput epididymal sperm (12)</td>
<td>95.2±3.1</td>
</tr>
<tr>
<td>Cauda epididymal sperm (11)</td>
<td>96.3±3.2</td>
</tr>
</tbody>
</table>

Mean±SD: Numbers in parentheses indicate numbers of samples examined.

![Fig. 1. Rat sperm G6PD zymograms with glucose-6-phosphate as substrate.](image)
a. Testicular sperm; b. Epididymal caput sperm; c. Epididymal cauda sperm;
d. Erythrocyte; e. Liver. A, B, C and D indicate isozyme-A, -B, -C and -D, respectively.
extend (Figs. 1 and 2). The activity of isozyme-A was observed in all the sperm examined, whereas the activity of isozyme-D appeared only in epididymal sperm. The relative activity of isozyme-D to A increased in the sperm during their epididymal transit (Table 2). In the erythrocytes the activity of isozyme-A was prominent and no isozyme-D activity was demonstrated. On the other hand, both isozyme-A and -D were active in the liver.

Mammalian G6PD linked with X-chromosome, which shows different behavior in electrophoresis, runs faster than the autosomal enzyme [16, 22]. It is also reported that the erythrocyte is dominant in terms of the amount of X-chromosomal G6PD but hardly contains the autosomal enzyme [15, 26]. In the present investigation, an isozyme designated as A, which had a higher affinity for G6P as substrate, ran faster in electrophoresis than the isozyme designated as D, that showed affinity for both substrates, G6P and Gal-6P. Isozyme-D was absent in rat erythrocyte. These findings suggest that isozyme-A and -D are the enzymes linked with X-chromosome and autosome, respectively.

Some histochemical investigations have indicated that sperm G6PD activity is localized in the midpiece [3, 27]. In the present study the cauda epididymal sperm obtained was more than 95% pure. The sperm with the cytoplasmic droplet was hardly observed. These data indicate that the appearance of isozyme-D in the rat cauda epididymal sperm is not due to the contamination of G6PD from both the epididymal epithelium and of the cytoplasmic droplet.

During the meiotic prophase the X-chromosome condenses, becomes genetically inactive and thus shows no uptake of (3H)-uridine [13]. However, some autosomes exhibit very active RNA synthesis during meiosis [18]. Erickson [9] has indicated that the X-chromosome linked G6PD of mouse sperm appears just before meiosis and retains its activity in sperm like lactate dehydrogenase isozyme-X (LDH-X), a long life enzyme [7]. Premkumar and Bhargava [17] have shown that RNA transcription and protein synthesis apparently occur in mature bovine sperm, and that they do not require concurrent transcription of nuclear information for cytoplasmic protein synthesis. Bragg and Handel [5] have also reported that mitochondrial proteins are synthesized in mouse epididymal sperm. In previous paper we have reported that two new minor isozymes of malate dehydrogenase (MDH) appeared in epididymal sperm associated with the change of relative activities of major MDH isozymes (mitochondrial and microsomal MDH) during epididymal transit of sperm [12].

Mammalian phosphoglycerate kinase isozyme-A (PGK-A), the production of which is controlled by the gene on X-chromosome, is found in both somatic cells and spermatogonia while isozyme-B (PGK-B) controlled by the autosomal gene appears in mouse spermatozoa [1, 20, 24, 25]. Recently, Nakaniishi and his co-workers [14] have indicated that the gene for PGK-A is in switch-off in contrast to the gene for PGK-B in switch-on during the pachytene stage of meiosis and suggested that timing of the m-RNA synthesis for PGK-B is not always coincident with the timing of synthesis of PGK-B.

Table 2. Relative activities of each G6PD isozymes (%)

<table>
<thead>
<tr>
<th></th>
<th>Isozyme-A</th>
<th>Isozyme-B</th>
<th>Isozyme-C</th>
<th>Isozyme-D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testicular sperm (3)</td>
<td>&gt;99.0</td>
<td>0.5±0.03</td>
<td>0.4±0.1</td>
<td>1.9±0.4</td>
</tr>
<tr>
<td>Caput sperm (3)</td>
<td>97.2±1.0</td>
<td>19.7±1.3</td>
<td>9.0±0.6</td>
<td>9.7±0.1</td>
</tr>
<tr>
<td>Cauda sperm (3)</td>
<td>46.7</td>
<td>46.7</td>
<td>6.6</td>
<td></td>
</tr>
<tr>
<td>Erythrocyte (1)</td>
<td>26.5</td>
<td>0.2</td>
<td>0.2</td>
<td>73.2</td>
</tr>
</tbody>
</table>

MD±S.D; Numbers in parentheses indicate the numbers of sample examined.
The following possibilities may be considered in connection with the appearance of autosomal linked G6PD isozymes in epididymal sperm: 1) the inactivated or masked G6PDs, which are synthesized before premeiotic stage, are re-activated or demasked during passage of sperm through the epididymis; 2) m-RNAs for autosomal G6PDs, which were synthesized during meiosis, survive to be activated in the epididymal sperm; 3) in epididymal sperm the autosomal gene with G6PD is activated to synthesize the enzyme; and 4) testicular sperm, which are dominant in the contents of the X-chromosome linked G6PD isozymes, receive preferential selection in epididymal environment and then the sperm which is rich in autosomal G6PD isozymes, increase in number. So epididymal sperm in total show higher activities of the autosomal than the X-chromosomal enzymes.

Further investigation is required, though the second possibility seems likely.

REFERENCES