MOUSE SPERMATOZOAL GLUCOSE-6-PHOSPHATE DEHYDROGENASE IS THE X-LINKED FORM

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Received February 25, 1975

SUMMARY. Mouse spermatozoal "glucose-6-phosphate dehydrogenase" was characterized for substrate utilization, electrophoretic mobility, and by immunoinactivation with an antibody to human, erythrocytic glucose-6-phosphate dehydrogenase. The enzymatic activity was found to have the properties of the X-linked form (Gpd-2).

Several histochemical studies have reported the presence of glucose-6-phosphate dehydrogenase (G6PD) in spermatozoa (1-3) but no attempts were made to distinguish the autosomal, hexose-6-phosphate dehydrogenase (E.C.1.1.1.47, gene symbol Gpd-1 in mice) from the X-linked glucose-6-phosphate dehydrogenase (E.C.1.1.1.49, gene symbol Gpd-2 in mice). Nonetheless, Blackshaw (2) estimated a K_m for "G6PD" (on the basis of quantitating the acetone-extracted diformazans) which was similar to that reported for the erythrocytic (X-linked) G6PD. The nature of the G6PD in spermatozoa is important in view of a large amount of histological (5-7), and autoradiographic (8) evidence for X-inactivation during spermatogenesis. Would a long-lived spermatozoon be unable to synthesize the X-linked G6PD and have the autosomal form or might it, like the erythrocyte, retain X-linked G6PD?

EXPERIMENTAL. Assays. Spermatozoa were prepared from the epididymides and/or ducti deferentes of Swiss random-bred mice by a slicing and screening technique which results in suspensions containing less than 2% epithelial cells although red blood cell contamination, which was reduced by using exsanguinated animals, may be somewhat higher (9). The samples were assayed in microcuvettes at 37° C using a micromethod previously described (10). Human ejaculated spermatozoa, washed in 0.9% NaCl, were used for one set of determinations. Protein was determined by the method of Lowry.

Electrophoresis. An aqueous extract of spermatozoa was prepared by adding dH_2O

TABLE I

Substrate Utilization by Spermatozoal Extracts

substrate	mouse spermatozoa (μmole/min/mg protein)	human spermatozoa (μmole/min/mg protein)
6-phosphogluconate	0	--
glucose-6-phosphate	0.076	0.054
galactose-6-phosphate	0.005	0.008

and performing a cycle of freeze-thawing. A deoxycholate extract (DOC) was prepared using 0.5% DOC. Horizontal starch gel electrophoresis was performed for 18 hrs at 150 v and 4° C with 0.045 M Tris, 0.025 M boric acid, 0.001 M EDTA and 0.0005 M NADP in the gel and 0.18 M Tris, 0.125 M boric acid, and 0.004 M EDTA in the bridge: both at pH 8.6. The gels were stained for G6PD activity (11).

Immunoinactivation. Immunoinactivation of murine G6PD was performed with anti-human erythrocytic G6PD (antiserum courtesy of Dr. Akiro Yoshida). Aliquots of enzyme extracts (erythrocyte lysate was diluted to an activity comparable to that of the spermatozoal extract) were incubated 30 min at 25° C with aliquots of antibody and returned to 0° C until assayed for residual activity.

RESULTS.

Assays of glucose-6-phosphate dehydrogenase in spermatozoa disclosed that all the activity was extractable by combined osmotic and freeze-thaw lysis. There was no detectable activity when 6-phosphogluconate was used as the substrate (Table I). When galactose-6-phosphate was used as the substrate much lower activity levels resulted. The ratio of G6PD to galactose-6-phosphate dehydrogenase activity was 15, a ratio highly compatible with that of the X-linked, erythrocytic glucose-6-phosphate dehydrogenase (12) but incompatible with the ratio of the autosomal, microsomal hexose-6-phosphate dehydrogenase (13). Since the spermatozoal preparations contained a few percent of erythrocytes, a reconstruction experiment was performed to see if this contamination was responsbile for any of the G6PD activity. Red

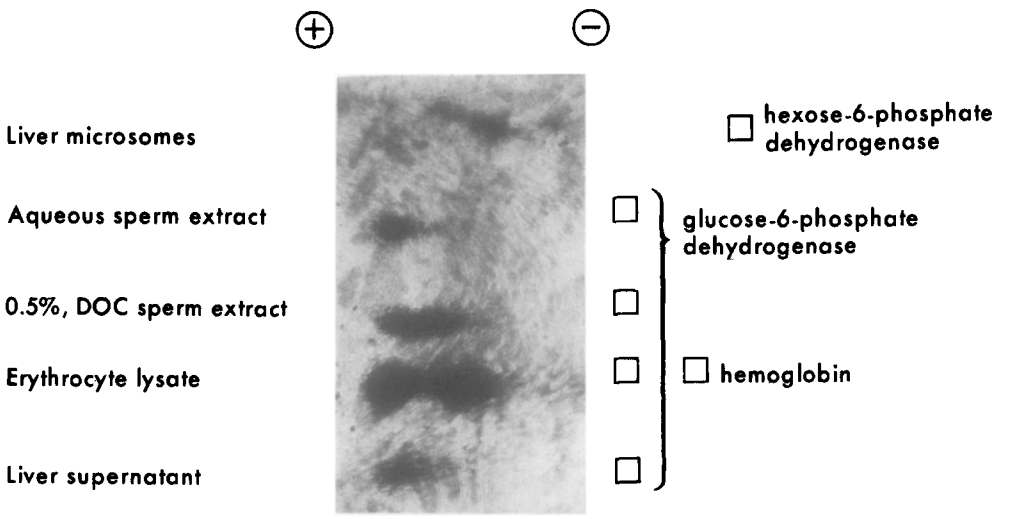

Fig. 1 Starch gel electrophoresis followed by glucose-6-phosphate dehydrogenase activity stain of mouse extracts.

cell hemolysates diluted to the same OD_{540} as the sperm extracts had no detectable G6PD activity. Ejaculated human spermatozoa which are free of erythrocytes, also had a glucose-6-phosphate/galactose-6-phosphate utilization ratio most compatible with the X-linked form of G6PD.

Electrophoretic studies confirmed that spermatozoa contained the erythrocytic, X-linked enzyme rather than the autosomal form (Fig. 1). The aqueous extract of spermatozoa gave a single band of activity co-migrating with erythrocytic extracts. (The double band appearance with the erythrocyte extract is due to the trailing hemoglobin band, the red color of which does not show up in the black and white photo). DOC extracts of spermatozoa showed a wider band, no part of which migrated as slowly as the DOC extract of liver microsomes.

We have previously shown that antibody to human erythrocytic G6PD cross-reacts with mouse erythrocytic G6PD in immunoinactivation studies (14). The incomplete cross reaction results in a failure to inactivate more than 75% of the activity as shown in the dashed line in Fig. 2 taken from that study. The inactivation of erythro-

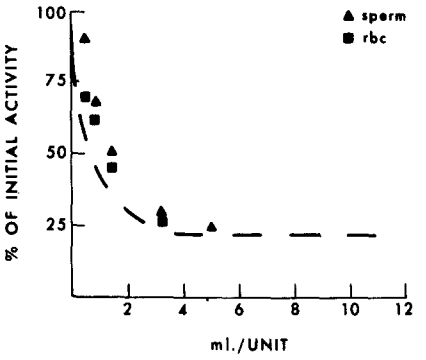

Fig. 2 Immunoinactivation of mouse extracts assayed for G6PD activity by antibody to human erythrocytic G6PD.

cytic G6PD compared to spermatozoal G6PD determined in one experiment is shown by the data points. Points for both extracts fall on the same curve and spermatozoal G6PD activity is inactivated to the maximal extent possible by this antibody.

DISCUSSION.

The data presented demonstrate that mouse spermatozoa contain the X-linked form of G6PD (*Gpd-2*) despite X-inactivation during spermatogenesis. Many proteins are synthesized post-meiotically during spermatogenesis, as shown by autoradiography (15) and studies on the time of synthesis of sperm-specific proteins such as the protamine-like histone (16). Thus, the presence of the X-linked G6PD in spermatozoa could either represent 1) synthesis after meiosis, suggesting that X-inactivation is incomplete or that the X-chromosome is reactivated after meiosis, or 2) persistence of enzyme from pre-meiotic stages. Since the inactivation of the paternally derived X chromosome seen in female marsupials (17) is most easily interpreted as persistence of the inactivation during spermatogenesis of the paternal X-chromosome, the second possibility seems the more likely. Glucose-6-phosphate dehydrogenase retains its activity for long time periods in erythrocytes despite no new synthesis and could very well do so in spermatozoa.

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