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IMMUNODETECTABLE GALACTOSYLTRANSFERASE IS ASSOCIATED ONLY WITH HUMAN SPERMATOZOA OF HIGH BUOYANT DENSITY

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Human ejaculated spermatozoa are heterogeneous and can be separated into two distinct populations according to their respective buoyant densities.In order to investigate the functional differences between these two types of spermatozoa, we have searched for the presence of galactosyltransferase. A Western blot of sperm proteins following their electrophoresis was probed with an anti-galactosyltransferase serum revealing that this enzyme is present in human spermatozoa. Furthermore, galactosyltransferase is detectable only in those proteins isolated from the head of high density spermatozoa. These results suggest that ejaculated spermatozoa consist of two populations that are functionally different.

Compared to most mammals, human ejaculated spermatozoa are characterized by a high heterogeneity. The enrichment of high buoyant density spermatozoa results in greater motility, nuclear maturity and fertilizing ability when compared to unselected ejaculated spermatozoa (1,2). These parameters are known to characterize mature epididymal spermatozoa.

In the hamster, two populations of spermatozoa are also present along the epididymis that are characterized by different buoyant densities. Moreover, the proportion of dense spermatozoa increases during epididymal transit (3). Compared to the low density spermatozoa, dense spermatozoa are characterized by a glycoprotein that may be implicated in the species-specific recognition of the zona pellucida. These spermatozoa could then represent mature "functional" male gametes (3,4). Until now, no such protein has been identified in the human. In mice, strong evidence has been presented suggesting that sperm galactosyltransferase is involved in the recognition of the zona

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pellucida (5,6). Herein, we report that immunodetectable galactosyltransferase is present in human ejaculated spermatozoa and that it appears only on the head of those spermatozoa with a high buoyant density. These results suggest that human semen consists of two functionally distinct populations of spermatozoa.

MATERIALS AND METHODS

Sperm preparation. Semen from donors of a heterologous artificial insemination program were used in this study. Cryopreserved semen samples (7) were thawed at room temperature and pelleted by centrifugation. After two washes in Hepes-saline buffer (HSB:10 mM Hepes buffer, 150 mM NaCl, 50 mM benzamidine, pH 7.4), the spermatozoa were centrifuged on a continuous Percoll gradient (3). The spermatozoa were distributed into two bands in the gradient and were recovered, washed in HSB and the sperm number was evaluated using a hemacytometer. Equal numbers of spermatozoa of each buoyant density were subjected to sonication; three times at 50 watts/30s. Microscopic examination revealed that this treatment results in the dissociation of the spermatozoa at the level of the connecting piece. In order to separate the heads from the flagellae, these suspensions were centrifuged in an 80% (v/v) continuous Percoll gradient prepared in HSB using a fixed angle rotor (Beckman Ti-65) at 175000 rpm for 30 min. Pure preparations of heads and flagellae were then recovered from these gradients and washed by centrifugation in HSB.

Mouse (CD-1) spermatozoa were obtained by making incisions in the distal cauda epididymis. These spermatozoa were washed in HSB.

Biochemical procedures. The pellets containing the heads and flagellae of human spermatozoa or mouse spermatozoa were resuspended in ten volumes of 0.04% Triton X-100 in HSB. After 10 minutes at room temperature, these suspensions were pelleted by centrifugation in a microfuge and the supernatants were recovered. Detergent extracted proteins were precipitated with 90% (v/v) cold acetone, dried under a stream of nitrogen, solubilized in electrophoresis buffer (50 mM Tris-HCl buffer, 10% (v/v) glycerol, 5% (v/v) β-mercaptoethanol, 2% SDS, pH 6.8) and denatured at 100°C for 3 minutes. These samples were then subjected to SDS-PAGE and Western blotted on nitrocellulose membranes (8). The amount of protein applied to each lane corresponded to a constant number of spermatozoa.

Western blots of SDS-PAGE electrophoretic patterns of sperm proteins or of purified galactosyltransferase (9) were probed with a rabbit polyclonal immune serum raised against galactosyltransferase and revealed with a second antibody coupled to peroxidase. For the preparation of the immune serum, twenty-five units (5.7 mg of protein) of crude bovine milk galactosyltransferase (Sigma #G-5507) were purified by affinity chromatography on N-acetylglucosamine Agarose (Sigma #A-2278) (9). Female rabbits were injected monthly with 400 µg of purified galactosyltransferase over a 4-month period. Immune serum was prepared from blood one week after the last injection.

RESULTS AND DISCUSSION

The antiserum used in this study has been produced against purified milk galactosyltransferase and specifically recognizes two protein bands of molecular weight (MW) of 43 and 51 kDa (Fig. 1). These results are in agreement with the previous report of Lopez et al (9). Detergent treatment of human ejaculated spermatozoa enables the extraction of proteins containing a band

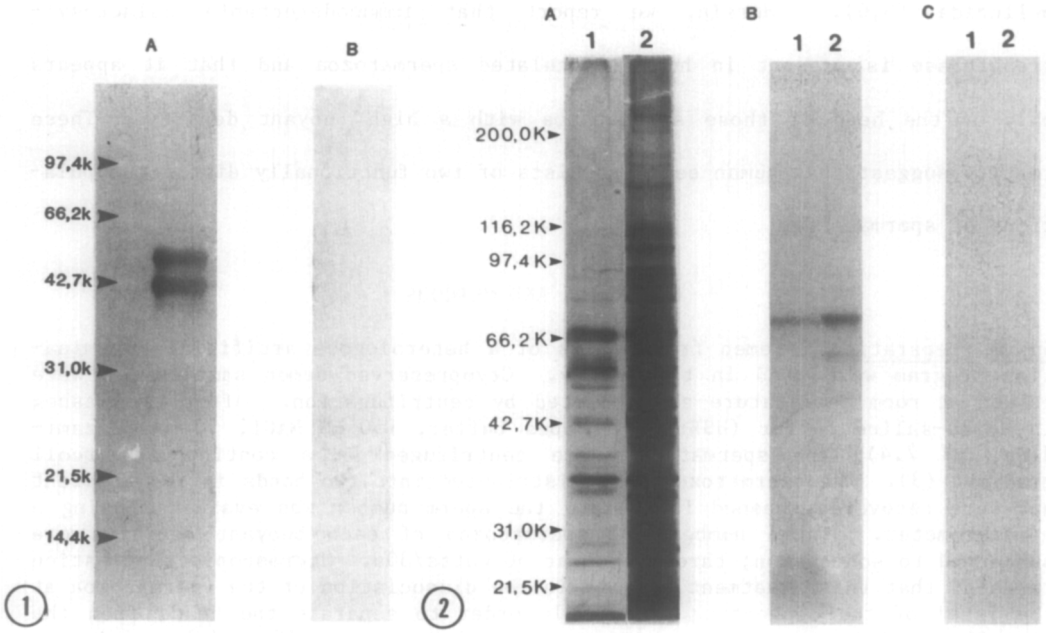

Figure 1: Immunodetection of galactosyltransferase. Purified milk galactosyltransferase was subjected to SDS-PAGE followed by Western blotting. It was incubated with (A) a rabbit anti-galactosyltransferase serum, or B) a control serum. MWs of standard proteins are indicated on the left of the figure.

Figure 2: SDS-PAGE of detergent-extracted proteins from mouse (1) and human (2) spermatozoa, and immunodectection of galactosyltransferase on a corresponding Western blot. A) Silver stain of the gel. The Western blots were incubated with B) a rabbit anti-galactosyltransferase serum, or C) a control serum. MWs of standard proteins are indicated on the left of the figure.

that is recognized by the antiserum directed against galactosyltransferase. This protein has the same MW as immunodetectable galactosyltransferase present in mouse sperm proteins (Fig. 2). The discrepancy between the MW of sperm and milk galactosyltransferase is probably due to the fact that this enzyme is an integral protein of the sperm plasma membrane (10). However, it has been shown that antiserum directed against milk galactosyltransferase recognizes sperm galactosyltransferase and interferes with its properties (review 6).

Previous electron microscopic studies have shown that the extraction of human spermatozoa with 0.04% Triton X-100 (v/v) is optimal for the solubilization of the plasma membrane and the outer acrosomal membrane and has a minimal effect on other intracellular structures (11). Furthermore, we have detected galactosyltransferase activity in suspensions of washed ejaculated human spermatozoa (data not shown; see also 5). It is then reasonable to hypothe-

size a surface origin for this human sperm galactosyltransferase. In the mouse, this enzyme is believed to be involved in zona pellucida recognition and has been shown to be an externally-oriented plasma membrane constituent of the spermatozoa (10). Due to the fact that it has also been detected in spermatozoa of other mammalian species, this mechanism of gamete recognition may be of a general nature (5).

When submitted to a Percoll gradient centrifugation, human ejaculated spermatozoa are distributed into two distinct bands. The corresponding buoyant densities of these bands are 1.03 and 1.11 and these values are reproducible from one donor to another. Hamster epididymal spermatozoa also distribute into two bands when submitted to a gradient density centrifugation (3,12) and it has been proposed that dense spermatozoa could represent "functional" mature male gametes (3,4). In the human, the situation may be identical. This is reinforced by the fact that ejaculated spermatozoa, as selected according to their high buoyant density, exhibit greater fertility and maturational characteristics (1,2).

Western blots of proteins extracted from isolated heads and flagellae of both dense and less dense spermatozoa from the same donor show that galacto-

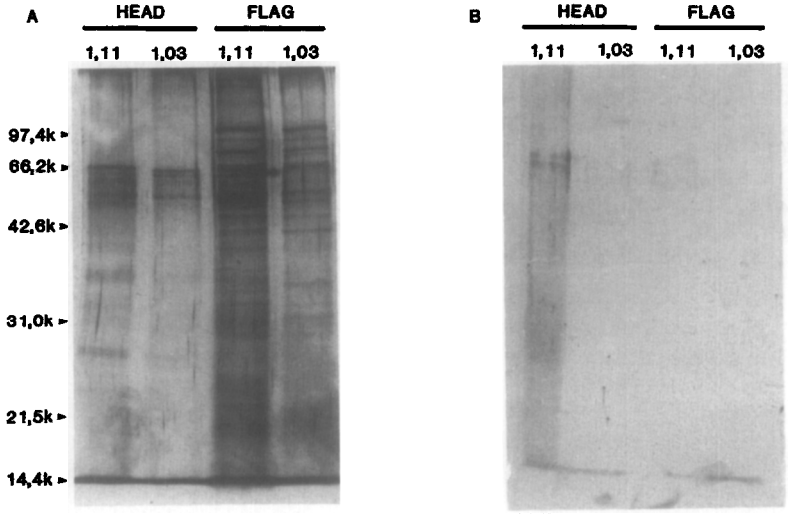

Figure 3: SDS-PAGE of proteins extracted from heads and flagellae (Flag) of both dense (1,11) and less dense (1.03) spermatozoa from one donor. A) Silver stain of SDS-PAGE of detergent-extracted proteins. B) Immunodectection of galactosyltransferase on a corresponding Western blot. The amount of proteins applied to each lane correspond to a constant number of spermatozoa. MWs of standard proteins are indicated on the left of the figure.

syltransferase is immunodetectable only on the heads of dense spermatozoa (Fig. 3). In view of the proposed implication of this sperm enzyme in the binding to the zona pellucida, we would expect to find this protein associated with the head of the spermatozoa.

These results demonstrate that the two populations characterized by different buoyant densities that are present in the human ejaculate show a major difference in terms of the presence of detergent extractable galactosyltransferase. Since this enzyme is presumed to be implicated in the process of fertilization, this difference could represent an important physiological distinction between these two types of spermatozoa.

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