Characterization of sperm surface and seminal plasma glycoproteins of the chimpanzee

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Summary. Washed ejaculated chimpanzee spermatozoa and a 100 000 g supernatant of seminal plasma were subjected to radiolabelling by sequential treatment with galactose oxidase and sodium borol³H]hydride or with sodium metaperiodate and NaB³H₄. Sperm surface glycoproteins and seminal plasma glycoproteins radiolabelled by these procedures were compared by SDS–polyacrylamide gel electrophoresis. Spermatozoa labelled by galactose oxidase treatment showed a single labelled macromolecular component of 37 000 whereas spermatozoa labelled by sodium metaperiodate–NaB³H₄ treatment showed incorporation into macromolecular components of 37 000 and 25 000 mol. wt. Seminal plasma radiolabelled by galactose oxidase–NaB³H₄ treatment contained labelled components of 47 000, 37 000, 19 000 and 12 000 mol. wt, whereas seminal plasma radiolabelled with sodium metaperiodate–NaB³H₄ contained macromolecular components of 47 000, 37 000, and 19 000 mol. wt.

Introduction

The surface of mammalian spermatozoa undergoes progressive modification during post-testicular development. These modifications occur during sperm passage through the excurrent duct system of the male tract, after ejaculation due to interaction of spermatozoa with seminal plasma components and during sperm residence in the female tract (Bedford, 1975; Orgebin-Crist, Danzo & Davies, 1975; Bedford & Cooper, 1978, for reviews). The interaction of secretory products of the excurrent duct system and accessory glands with the sperm plasma membrane has been demonstrated by various immunological, biochemical and histochemical techniques (Hunter, 1969; Barker & Amann, 1971; Killian & Amann, 1973; Gordon, Dandekar & Bartoszewicz, 1975; Nicolson, Usui, Yanagimachi, Yanagimachi & Smith, 1977; Lea, Petrusz & French, 1978; Voglmayr, Fairbanks, Jackowitz & Colella, 1980). It is believed that these changes may be of significance for sperm maturation which occurs in the epididymis and for capacitation which occurs in the female tract. However, in many instances a characterization of the components binding to the surface of the spermatozoon has not been reported.

The present study was undertaken to provide data upon the potential modification of the sperm surface by seminal plasma constituents. We report here data obtained from radiolabelling experiments designed to identify macromolecular surface components of chimpanzee spermatozoa. The same techniques were employed to identify glycoprotein components of seminal plasma for subsequent comparison of the seminal plasma and sperm surface components.

Materials and Methods

Ejaculates were obtained from adult chimpanzees. Semen from 6 different animals was used in the present study; up to 4 samples were obtained from some animals to monitor consistency of the results. Semen was allowed to liquefy at 37°C and the liquid fraction was centrifuged at 500 g for 15 min. The sperm pellet and supernatant were then separated. The sperm pellet was immediately used in the radiolabelling procedures described below while the supernatant fraction was frozen and stored at -70°C until used.

The sperm pellet was resuspended in phosphate-buffered saline (PBS = 0.145 M-NaCl and 0.01 M-sodium phosphate, pH 7.1) and again centrifuged at 500 g for 10 min. The resulting sperm pellet was gently suspended with 2 ml PBS and divided into two equal aliquots. One aliquot was radiolabelled by galactose oxidase-sodium boro[³H]hydride (NaB³H₄) treatment, a procedure which labels terminal galactose or galactosamine residues of the oligosaccharide chains of plasma membrane glycoproteins and glycolipids (Gahmberg & Hakomori, 1973; Steck & Dawson, 1974). The second aliquot was radiolabelled by sodium metaperiodate-NaB³H₄ treatment to label sialic acid residues of glycoproteins or glycolipids preferentially (Morrell, Van Den Hamer & Scheinberg, 1966; Van Lenten & Ashwell, 1971). For these procedures 1 ml aliquots of the sperm suspension were incubated at 37°C for 15 min with 20 µl galactose oxidase (1 U/µl: Worthington Biochemicals, Freehold, New Jersey, U.S.A.) or with 20 µl 0.1 M-sodium metaperiodate (NaIO₄). The suspensions were then centrifuged at 500 g for 5 min. The pellets were re-suspended with 1 ml PBS and 20 µl sodium boro[³H]hydride (NaB³H₄; 25 mCi/ml: New England Nuclear, Boston, Massachusetts, U.S.A.) were added and the tubes incubated for 15 min at 37°C. After centrifugation at 500 g for 5 min the pellets were washed twice by re-suspension with 5 ml PBS followed by centrifugation at 500 g for 10 min. The final pellets were extracted at room temperature for 1 h with 250 µl of a demembrating solution composed of 1% sodium dodecyl sulphate (SDS), 1 mM-ethylenediaminetetraacetic acid (EDTA), 10% sucrose and 10 mM-Tris-HCl (pH 8.0). Non-solubilized components were removed by centrifugation in a microcentrifuge at 8000 g for 5 min. The supernatant solutions were used for SDS-polyacrylamide gel electrophoresis.

Soluble constituents of seminal plasma were subjected to the same radiolabelling procedures as spermatozoa. The 500 g supernatant obtained by centrifugation of liquefied semen was diluted to 1-2 ml with PBS containing 1 mM-phenyl methyl sulphonylfluoride as a protease inhibitor and clarified by centrifugation at 100 000 g for 30 min. Protein concentration in the supernatant was determined by the method of Bradford (1976). Aliquots (1 ml) of the seminal plasma supernatants were incubated with 20 µl galactose oxidase (1 U/µl) or 20 µl 0.1 M-NaIO₄ for 15 min at 37°C. Labelled seminal plasma was then dialysed at 4°C against several changes of deionized water and freeze-dried or the protein components were precipitated by the addition of 9 volumes of ice-cold acetone followed by incubation on ice for 1 h and centrifugation at 8000 g for 5 min. This acetone pellet was washed twice with acetone and then dried under nitrogen gas. Both the freeze-dried seminal plasma and the acetone-precipitated seminal plasma were solubilized at 60°C in SDS sample buffer composed of 1% SDS, 1 mM-EDTA, 10% sucrose, 40 mM-dithiothreitol (DTT) and 10 mM Tris-HCl (pH 8.0).

For SDS-polyacrylamide gel electrophoresis the protocol of Fairbanks, Steck & Wallach (1971) was employed. Before electrophoresis all samples were made 40 mM in DTT and heated to 100°C for 3-5 min. Samples were electrophoresed at 100 V on 5 mm × 11 cm tube gels. Gels for scintillation counting contained *N,N'*-diallyltartardiamide (BioRad Laboratories, Richmond, California, U.S.A.) as the cross-linker. Gels were stained with Coomassie Brilliant Blue R or sliced into 2 mm segments for scintillation counting. Gel slices were solubilized with 0.5 ml 2% periodic acid. Molecular weights of macromolecular components were estimated by comparing their relative mobilities to the mobility of standard proteins of known molecular weight.

Results

Spermatozoa showed incorporation of radioactivity into macromolecular components with both the sodium metaperiodate- NaB^3H_4 and galactose oxidase- NaB^3H_4 radiolabelling protocols. SDS-polyacrylamide gels revealed two labelled macromolecular components in sodium metaperiodate- NaB^3H_4 -labelled spermatozoa. The apparent mean molecular weights were 37 000 for one component and 25 000 for the other (Text-fig. 1). In all samples more counts were incorporated into the component of lower molecular weight. There was also a bipartite peak of radioactivity running close to the tracking dye (Text-fig. 1). The gels of galactose oxidase- NaB^3H_4 -labelled spermatozoa revealed a single peak of radioactivity for a macromolecule of mean molecular weight 37 000 and a peak of radioactivity migrating near the tracking dye front (Text-fig. 1).

Text-fig. 1. Profiles of radioactivity resolved by SDS-polyacrylamide gel electrophoresis of chimpanzee spermatozoa labelled with (a) NaIO_4 - NaB^3H_4 and (b) galactose oxidase- NaB^3H_4 . The mean molecular weight values are indicated.

Macromolecular components of seminal plasma were radiolabelled by both protocols. With the conditions used the incorporations were up to 2700 c.p.m./ μg protein with the NaIO_4 probe and up to 5500 c.p.m./ μg protein with the galactose oxidase probe. SDS-polyacrylamide gels of NaIO_4 - NaB^3H_4 -labelled seminal plasma revealed 3 prominent macromolecular peaks of radioactivity corresponding to molecular weights of 47 000, 37 000 and 19 000 (Text-fig. 2). A peak of radioactivity was present near the tracking dye front and a peak was present at the top

Text-fig. 2. Profiles of radioactivity seen in SDS gels of chimpanzee seminal plasma labelled with (a) $\text{NaIO}_4\text{-NaB}^3\text{H}_4$ or (b) galactose oxidase- NaB^3H_4 .

of the gel; it is not yet clear whether the latter peak represents a very high molecular weight component or aggregated material.

SDS-polyacrylamide gels of galactose oxidase- NaB^3H_4 -labelled seminal plasma resolved 4 macromolecular peaks of radioactivity, including components of molecular weights of 47 000, 37 000, 19 000 and 12 000 (Text-fig. 2). The mean molecular weight values for the first 3 peaks were identical to those obtained in seminal plasma labelled with $\text{NaIO}_4\text{-NaB}^3\text{H}_4$ but the distribution of radioactivity amongst the peaks was different. There was variation between animals in the relative labelling of the 19 000 component by the galactose oxidase probe; usually it was less labelled than the 47 000 and 37 000 components, but in some samples it was the most heavily labelled component. The basis for this variation is unclear. The fourth peak noted in galactose oxidase-labelled seminal plasma has a mean molecular weight of 12 000 and it had the least incorporated label. There was also a radioactive peak at the top of the gel but not one at the front.

Coomassie blue-stained gels of seminal plasma revealed several bands of molecular weight between 92 000 and 10 000 (Text-fig. 3). In addition to the presence of well-defined bands all the gels displayed a homogeneous background staining.

Text-fig. 3. Coomassie blue-stained SDS gel of seminal plasma proteins. The positions of the molecular weight markers are indicated.

Discussion

The results presented above demonstrate similarities and differences in the glycoprotein components of the sperm surface and seminal plasma. The data indicate that a component of 37 000 mol. wt is present on the sperm surface and in seminal plasma. The 37 000 components of spermatozoa and seminal plasma were reactive with both radiolabelling protocols, suggesting that they possess multiple or branched oligosaccharide chains some of which possess terminal galactose-like residues while others possess terminal sialic acid residues. The 37 000 component of the sperm surface appears to be tightly bound since it is not readily removed by multiple post-labelling washes, but further work is required to establish its identity. A component of molecular weight 37 000 has been identified on the rat sperm surface (Olson & Hamilton, 1978) and a 37 000 sialoglycoprotein which is a secretory product of the epididymal epithelium has also been identified in the rat (Lea *et al.*, 1978). Moreover the forward motility protein of bovine seminal plasma also has a subunit molecular weight of 37 000 and it, too, is thought to bind to

the sperm surface (Hoskins, Brandt & Acott, 1978). The peaks at the tracking dye fronts were probably labelled glycolipids (Steck & Dawson, 1974; Gahmberg, Häyry & Andersson, 1976).

Several differences in the labelling pattern of the spermatozoa and seminal plasma were noted. Spermatozoa but not seminal plasma possessed a 25 000 mol. wt sialoglycoprotein which reacted with the sodium metaperiodate probe only, while the 47 000, 19 000 and 12 000 components of seminal plasma were not present in spermatozoa. However, this result does not preclude a weak interaction between spermatozoa and these seminal plasma components which could have been disrupted by the washing of the cells before and after the labelling protocols.

The 47 000, 37 000 and 19 000 components of seminal plasma are probably sialoglycoproteins as they are labelled by the sodium metaperiodate- NaB^3H_4 treatment. An interaction of any of the sialoglycoproteins of seminal plasma with the spermatozoon could affect the relative charge at the cell surface and could contribute to the distribution of the anionic sites that have been demonstrated over the surface of primate spermatozoa by electron microscopic studies using positively charged colloidal iron as a marker (Cooper & Bedford, 1971; Gould, 1977). This would be compatible with data obtained from other species for which a change at the sperm surface in both the distribution and abundance of specific saccharide residues has been demonstrated after ejaculation (Yanagimachi, Noda, Fujimoto & Nicolson, 1972; Gordon *et al.*, 1975; Nicholson *et al.*, 1977). Further work will be required to see if the individual seminal plasma components interact with specific segments of the sperm surface and to determine how they contribute to the fertilization process.

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