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IDENTIFICATION OF MEMBRANE ANTIGENS OF GOAT EPIDIDYMAL SPERMATOZOA

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Summary : Purified goat sperm plasma membrane was used as antigen to raise the antibody in rabbit. Using this antisera four groups of antigenic membrane polypeptides are determined in caput and cauda epididymal sperm. The immunoresponsiveness of the polypeptides in caput and cauda sperm differs significantly. In case of cauda epididymal sperm, the polypeptides of region A (96KDa, 82KDa, 78KDa, 68KDa) and region D (24KDa, 20KDa, 18KDa) are highly immunoresponsive whereas in case of caput epididymal sperm the same antisera recognized the polypeptides of region B, C and D. By surface labelling with lactoperoxidase iodination and subsequent immunoprecipitation in the iodinated cell extract we demonstrate eight of these above polypeptides (96KDa, 82KDa, 68KDa, 50KDa, 29KDa, 24KDa, 20KDa and 18KDa) as surface antigen. The 96KDa, 82KDa and 68KDa surface polypeptides are highly immunoresponsive than the other lower molecular weight surface antigens in cauda epididymal goat spermatozoa.

To understand the involvement of the antigens in the event of fertility as well as the cause of infertility of male and female the isolation and characterization of the antigens of the sperm membrane are essential. The biochemical physiological and immunological changes of the spermatozoa during the passage through epididymis are mainly occur at the surface membrane level. The origin, nature and the role of some of the membrane proteins, which relate to the functional maturation process of the sperm cell are being studied by several investigators (1,2). Identification of the sperm surface antigens and their analysis have been reported in different species (3-5). It appears that the immunological approaches alongwith biochemical studies become a great help to evaluate the differences in the sperm cell of different stages of maturation process.

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Abbreviations : PM, Plasma membrane; TLCK, Na-p-tosyl-L-lysine chloromethyl ketone; PMSF, Phenyl Methyl Sulfonyl; PBS, Phosphate buffer saline; PAs, Periodic acid Schiff reagent.

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Most of these studies on determination of surface antigens were made using whole sperm or isolated antigens to raise the antibody. We report here the use of antibody against highly purified PM of goat spermatozoa for the determination of caput and cauda epididymal sperm by immunoblotting of PM proteins and by immunoprecipitation in the cell extract of goat spermatozoa. Since the antisera was raised against high purity PM, the polyclonal antisera used in the present study is devoid of the antibody against cytoplasmic, mitochondrial and microsomal proteins. In this communication we demonstrate the epididymal sperm immunoresponsive membrane protiens and identified some of them as surface antigens.

MATERIALS AND METHODS

Collection of spermatozoa

Mature motile spermatozoa were collected from goat cauda epididymis described earlier (6). The highly motile sperm cell were sedimented by centrifugation at 500 x g for 10 min at room temperature (31 ± 1°C) and the pellet was washed thrice gently with same medium to remove the epididymal fluid. The purity and motility of the washed cells were judged under light microscopy in absence and presence of epididymal fluid.

Isolation of membrane

The sperm cell membrane was isolated according to the method described in the reference (7).

Preparation of sperm membrane antibody

The rabbit was immunized with cauda epididymal sperm purified PM. The immunoglobulin of the rabbit serum was precipitated twice with 50% ammonium sulfate and then dialysed extensively against 0.02 M PBS, pH 7.4 containing 1 mM PMSF. This partially purified membrane antibody was used for the experiments.

SDS-PAGE electrophoresis and immunoblotting

SDS-PAGE was performed with 10% polyacrylamide gels by the method of Laemmli (8). After the completion of the electrophoresis, the gel was divided in two parts. One part was stained for coomassie blue and rest of the gel was transferred to transfer buffer for "Western blotting". From the gel the proteins were transferred electrophoretically to nitrocellulose. The immunoblot was carried out according to the procedure of Burnette (9). The blot was exposed to the dialysed polyclonal antibody at 100 fold dilution. Autoradiography was performed using Kodak X-omat AR film with an intensifying screen at -70°C.

Sperm cell iodination and immunoprecipitation

Washed cauda sperm cell (10^8/ml) have been iodinated with lactoperoxidase and glucose-glucose oxidase according to the procedure of Hubbard and Cohen (10). The iodinated sperm cell was extracted at 37°C for 30 min with buffer (A) containing 10 mM Tris-HCl, pH 7.5; 10 mM EDTA; 0.2 M NaCl; 0.2 mM TLCK, 1 mM PMSF and 3% triton X-100 or 1% NP-40 and 0.1% SDS. After the centrifugation the supernatant was dialysed against 0.02 M PBS containing 0.2 mM TLCK and 1 mM PMSF. The antibody (1 : 25 dilution) was added to the dialysed aliquot (100 ug protein) for immunoprecipitation. After 24 hrs. of the addition of first antibody, second antibody anti guineapig IgG was

added to the mixture to enhance the immunoprecipitation. For non-specific interaction a control experiment of anti guineapig IgG (Sigma chemicals) with dialysed iodinated cauda sperm cell extract was carried out. The immunoprecipitate was washed twice with PBS buffer and was analyzed finally on 10% SDS-PAGE and radioautography.

RESULTS

In order to investigate the goat sperm membrane antigens the caput and cauda epididymal sperm PM proteins were resolved on SDS-PAGE and then transferred to nitrocellulose. The antigenic peptides of sperm PM were then identified by immunoblotting. BY this method mainly eight major antigenic and several other minor bands of polypeptide were found (Fig.1A, Lane 3 and 4). In case of caput sperm PM, this same antibody recognized mainly lower molecular weight polypeptides under the same experimental conditions (Lane 1 and 2). The components of region A (96KDa, 82KDa, 78KDa, 68KDa) which are found to be the major antigenic polypeptides of cauda epididymal sperm PM, are almost absent in caput epididymal sperm PM as illustrated in radioautogram (Lane 1 and 2). This finding agrees with the coomassie stain of the polypeptide profile of caput and cauda epididymal sperm cell (Panel B). The pattern of the PM protein of caput and cauda differ quantitatively but qualitatively they are almost similar with one or two exception. It is significant to note that region A polypeptide is found to be present in high concentration in cauda sperm PM as judged by the coomassie stain of the gel, present only in trace amount in caput sperm PM, although an equivalent amount of protein applied on the gel. On the other hand, an appreciable amount of protein comprising of region B and C (62KDa, 50KDa and 32KDa, 30KDa) is present in both the caput and cauda epididymal sperm PM (Lane 2 and 4) but the polypeptides of these region are more immunoresponsive in caput than the cauda sperm PM as shown in the radioautogram (Panel A). The results suggest that due to some modification in the antigenic polypeptides the immunoresponsiveness of caput and cauda sperm may differ during the passage through the epididymal tubule.

To determine whether these major and minor immunoreactive polypeptides of cauda sperm PM are selectively located on the outer surface of the membrane, the highly motile sperm cell were iodinated with lactoperoxidase and subsequently immunoprecipitated with the antisera. The best selection of the detergent for the extraction of the membrane protein has been made in earlier experiment (results not shown). In the present experiment since the profile of the labelled mambrane protein extracted with the medium containing nonidet-40 or triton X-100 is similar, the immunoprecipitation of the PM protein was carried out in triton X-100 cell extract. Figure 2

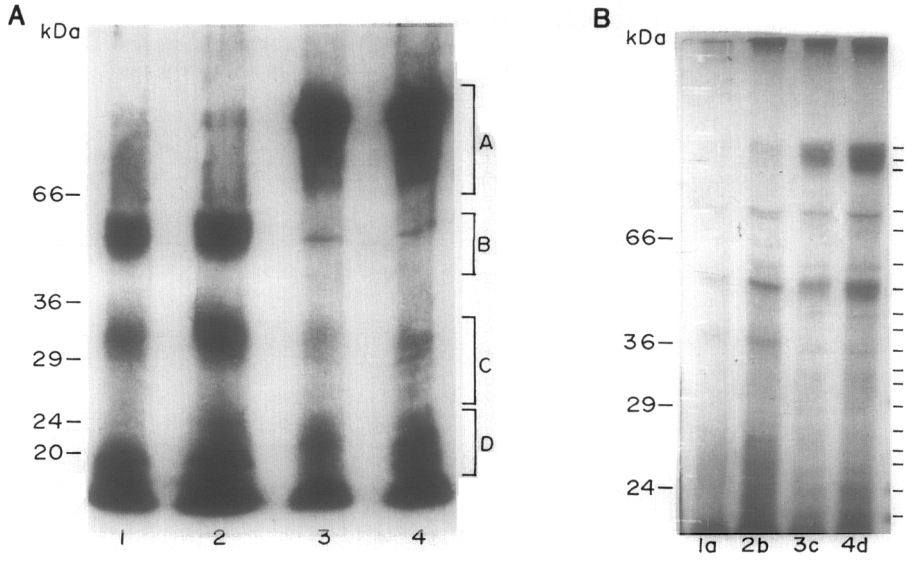

Fig. 1(A) Immunoblot with polyclonal antibody (1 :100) against cauda epididymal goat sperm PM. The antisera was partially purified by 50% $(NH_4)_2SO_4$ precipitation and was extensively dialysed against 0.02 M PBS, pH 7.4. PM protein of caput (lane 1, 40 ug; lane 2, 80 ug) and cauda (lane 3, 40 ug; lane 4, 80 ug) epididymal sperm were electrophoresed in 10% SDS-PAGE and were immunoblotted. We have designated following region according to the approximate molecular weights of group of polypeptides : A (96KDa, 82KDa, 78KDa, 68KDa); B (57KDa, 50KDa, 48KDa, 43KDa, 36KDa); C (34KDa, 32KDa, 31KDa, 28KDa, 26KDa, 25KDa); D (24KDa, 22KDa, 20KDa, 18KDa).

(B) Coomassie blue stained of 10% SDS-PAGE of duplicate of gel (A). Lane 1a and 2b, caput epididymal sperm PM,(40 ug and 80 ug). Lane 3c and 4d, cauda epididymal sperm PM (40 ug; 80 ug).

shows the radioautogram of the iodinated cell extract and their immunoprecipitation analyzed on 10% SDS-PAGE. It is clear from the result that at least ten polypeptides of cauda sperm PM are accessible for iodination with glucose-glucose oxidase, lactoperoxidase and Na[^{125}I] indicating that these polypeptides are located on the outer surface of the membrane. Among these [^{125}I]-labelled surface polypeptides five are highly immunoresponsive which have approximate molecular weights of 96KDa, 82KDa, 68KDa, 50KDa, 29KDa. Besides these several other lower molecular weights polypeptides have also been detected by the antisera. From this result it is clear that the polypeptides (96KDa, 82KDa and 68KDa) of region A, 50KDa of region B, 29KDa of region C and polypeptides (24KDa, 20KDa, 18KDa) of region D (Fig.1A) are identified as surface antigens of cauda epididymal sperm. The 96KDa, 82KDa surface antigens are strongly PAS positive (results not shown here).

Tissues and species specificity of this polyclonal antibody was assessed by the 'Western transfer' of proteins extracted from rat liver, rat testis, rat epididymis, goat epididymis, ham uterus, ham kidney and ham heart to

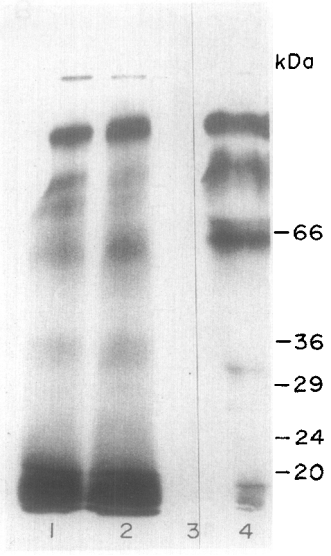

Fig. 2 Radioautography of immunoprecipitation of [^{125}I]-labelled cauda epididymal sperm cell extract.

Lane 1; [^{125}I]-labelled sperm extract in buffer A containing 3% triton

Lane 2; [^{125}I]-labelled sperm extract in buffer A containing 1% NP-40 and 0.1% SDS.

Lane 3; Lane 1 + Anti guineapig IgG (5 ug).
Lane 4; Lane 1 + membrane antibody.

nitrocellulose and was probed with the antibody at 25 fold dilution. Results from the one week exposure of the radioautograme (Fig.3A) show that the antibody has cross reactivity with four polypeptides of molecular weight (104KDa, 90KDa, 85KDa and 71KDa) of goat epididymal tissue extract. A single polypeptide of rat testis of 71 KDa has also cross reactivity with the antibody of goat sperm PM. Panel B is the coomassie stain of SDS-PAGE of different tissue extract from the different species.

DISCUSSION

The sperm cell acquires its functional maturity by gaining the motility during the transit through epididymis from caput to cauda segment. At this stage the spermatozoa undergo a series of changes at the cell surface which direct the cell to react with zona pellucida. The present results illustrate that our partially purified antibody recognized different groups (based on mol. wt.) of polypeptides in immotile and motile sperm cell. The polypeptides of region B, C and D of immotile cell PM and A and D of motile sperm cell PM are found to be immunogenic. The partial loss of antigenicity of the polypeptides of region B and C in case of motile cauda epididymal sperm cell may be due to the modification of the surface components by limited proteo-

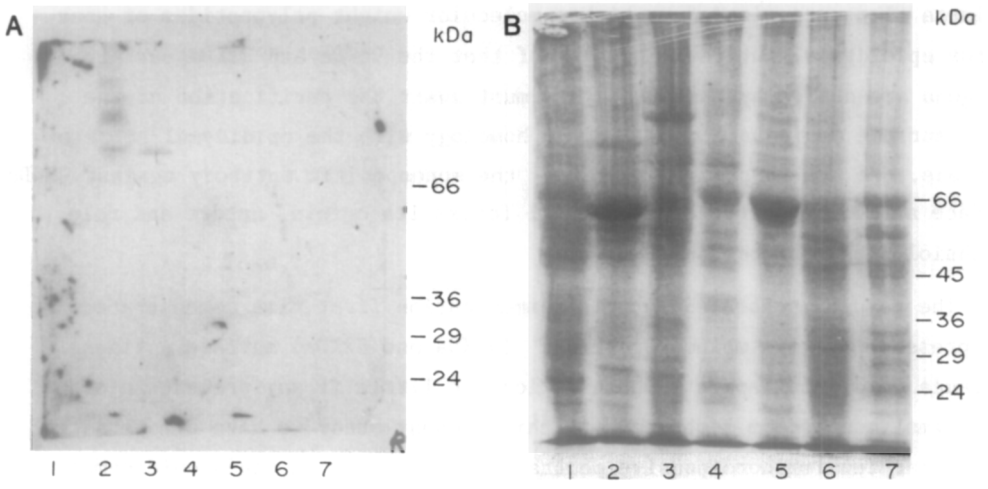

Fig. 3(A) Western transfer of proteins extracted from various tissues and probed with partially purified polyclonal antibody. Each lane contained 60 ug of protein.
Lane 1, Rat liver; 2, Goat corpus epididymis; 3, Rat testis; 4, Rat epididymis; 5, Hamster uterus; 6, Hamster kidney; 7, Hamster heart.
(B) Coomassie stain of 10% SDS-PAGE of duplicate of gel (A).

lysis or masking of the preexisting molecules at the cell surface during the epididymal maturation process. The possibility of the addition of a new moiety to the existing molecule so that the components are partially accessible to the antibody is also to be considered.

There is a report that a 110KDa surface protein in rat testicular spermatozoa was identified by glucose oxidase-tritium borohydride technique but the same protein could not be identified in cauda epididymal sperm surface by the same technique (11). From lactoperoxidase catalysed iodination studies it has also been demonstrated that the rat caput epididymal sperm have surface proteins of 94KDa, 72KDa and 59KDa which are not iodinated on cauda epididymal sperm (1). Previous report on the appearance of the components on sperm surface are mainly of low molecular weight protiens of 37KDa, 26KDa, 24KDa, 23KDa and 20KDa (2,12,13). The mouse sperm maturation antigen of 85KDa which was found to be present in the epithelium cells of corpus epididymis becomes associated on sperm surface after being trimmed to 54KDa component (14). However, most of these proteins secreted by the epididymal epithelium cells are glycoproteins.

All these studies on the sperm maturation by different investigators reveal that the epididymal secretory components have some influence on the modulation of the preexisting molecule, appearance of the new moiety and the expression of the antigenic determinants at the surface of the sperm

during the passage through the tubule. Although in the present study our antisera also recognized four higher molecular weight polypeptides of goat corpus epididymis, but the actual proof that the 96KDa and 82KDa surface antigens are shared by the epididymis must await the purification of the sperm surface antigens and to see the homology with the epididymal secretory protiens. We are in process to raise the monospecific antibody against 96KDa surface antigen and further study will focuss its origin, nature and role in phsiological phenomenon.

The results presented in this communication first time demonstrated the presence of high molecular weight (96KDa and 82KDa) surface antigens in cauda epididymal sperm membrane which are little if any present in caput epididymal goat sperm membrane. In this present study we have demonstrated altogether ten immunoresponsive membrane polypeptides in cauda epididymal goat sperm and identified eight of them as surface antigens of different intensity of immunoresponsiveness.

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