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GLYCOPROTEINS OF RAM SPERM PLASMA MEMBRANE. RELATIONSHIP OF PROTEIN HAVING AFFINITY FOR Con A TO EPIDIDYMAL MATURATION

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SUMMARY. Con A Receptors from the sperm plasma membrane were quantitated (using ^{3}H acetyl-Con A) along the epididymal duct; they diminished in the second part of the epididymis as compared to the epididymal head. Glycoproteins having affinity for Con A were partially characterized: washed spermatozoa from rete testis (= testicular spermatozoa), middle corpus and distal cauda epididymis were labelled (^{125}I Na). Proteins of their plasma membrane were extracted (Triton x100, 0.1% and chromatography affinity): differences appeared in ACA44 profiles from ^{125}I Con A Glycoprotein extractions between testicular spermatozoa (2 major peaks Kav= 0.41 and 0.52) and epididymal spermatozoa (3 major peaks Kav= 0.33-0.34, 0.41 and 0.52 and additionnal minor peaks between 0.66 and 1.00). The peak Kav= 0.41 diminished considerably on epididymal spermatozoa as compared to testicular spermatozoa.

INTRODUCTION

The distribution of glycoproteins which are characterized by their affinity for Concanavalin A (Con A) are quantitatively modified on the surface of spermatozoa during epididymal maturation in the rabbit (9, 18), rat (5, 16) and ram (3, 7, 24). However, little is known of molecular events which occur in these proteins of the surface of the maturing spermatozoa in spite of evidence for important changes in the different layers of the membrane (10).

This reports relates the modifications which have been observed in ram spermatozoa taken at different levels of maturation (6) into rete testis or epididymis, from the analysis of the ^{3}H acetyl-Con A binding and from ultrogel profiles of glycoproteins extracted on insolubilized Con A.

MATERIALS AND METHODS

A. Sperm preparation. Spermatozoa were obtained from Romanov and Prealpes rams by micropunture of the rete testis and various segments of the epididymis : distal head, middle and distal corpus, proximal and distal cauda. Spermatozoa were diluted in 10 ml PBS (KH_2PO_4/K_2HPO_4 0.05 M; NaCl, 0.1 M; pH 5.6) and centrifuged (500 g, 1 min) at room temperature to eliminate contaminant epithelial cells; 8 ml of the supernatant containing the spermatozoa were recovered and centrifuged (800 g, 5 min). Pelleted spermatozoa were then washed twice in 10 ml PBS after which the preparation was observed under microscope for absence of epithelial cells.

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B. Sperm labelling

1. Labelling of external saccharides with ^{3}H-acetyl-Concanavalin A. The experiments were done in duplicate or triplicate when there were sufficient number of spermatozoa. The suspension of cells was diluted (1-2x10^{6} cells/ml) in acid-acetate buffer 0.2 M, $MgCl_2$ 0.003 M, $CaCl_2$ 0.003 M, NaCl 0.1 M, pH 5.6. Incubations were carried out (100 ul of sperm solution in 500 ul final volume) at room temperature for 1h with ^{3}H acetyl-Con A (5-29 Ci/mMole, New England Nuclear 452 NEN). The specific activities of incubations were adjusted by dilution as the concentration of Con A increased (0.1 to 1 uCi/500 ul). As a control, 2α-DMethylmannose was used as an inhibitor to saturate Con A at a concentration of 0.05 M, twenty minutes before the addition of 100 ul of spermatozoa suspension. The number of sites was estimated from saturation curves or Scatchard plots assuming a molecular weight of 108,000 D for tetrameric Con A with 4 binding sites per molecule.

2. Iodination, extraction and fractionation of glycoproteins from sperm plasma membranes. Spermatozoa from five adult rams were labelled with ^{125}I by the lactoperoxidase technique (22); the proteins were dissolved from the surface of spermatozoa using Triton x100 (0.1% in acid-acid buffer 0.2 M NaCl 0.1 M pH 5.6) and glycoproteins were then extracted by affinity chromatography on Con A-Agarose (5); elution was performed using methyl-mannose 1 M in acid-acetic buffer. The Con A Agarose extracts were then fractionated on an acrylamide Agarose column (ultrogel ACA44 IBF, 80x1 cm) in acid-acetate buffer 0.2 M NaCl 1 M, pH 5.6, containing sodium azide, 2 g/l Bovine Serum Albumin, 5 g/l): Dextran Blue 2,000, myoglobin and cytochrom C were used for approximate calibration (Kav= 0.33, 0.58, 0.70 respectively). Kav 1.00 was determined with ^{125}I Na.

RESULTS

a) External saccharide Con A receptors.

The number of sites per spermatozoa with affinity for ^{3}H acetyl-Con A decreased significantly in the corpus epididymis (m$\pm$sd): distal head, 12.3$\pm$2.5 x10^{6}; middle corpus, 12.8$\pm$8.7x10^{6}; distal corpus: 3.1$\pm$2.3x10^{6}; proximal cauda, 3.8$\pm$2.3x10^{6}, distal cauda, 3.6$\pm$2.0x10^{6}.

b) Glycoproteins from sperm plasma membranes.

^{125}I labelled cell surface proteins increased significantly on spermatozoa as they moved from the rete testis to the middle corpus epididymis (1410$\pm$160 vs 1990$\pm$245 cpm per 1x10^{4} washed spermatozoa; $P < .05$) and decreased significantly in the cauda epididymis (910$\pm$300 cpm/10^{4} spermatozoa; $P < .05$). The Triton extract contained 81$\pm$5% of the total radioactivity. The radioactivity of glycoprotein extracts did not vary significantly throughout the epididymis as shown by the number of cpm per 1x10^{4} spermatozoa: 231$\pm$36 in the rete testis, 218$\pm$20 in the middle corpus epididymis and 214$\pm$64 in the distal cauda epididymis.

Several glycoproteins were extracted by Triton from testicular or epididymal spermatozoa which differed in their Kav after ultrogel fractionation. One additional major peak (component 1: Kav= 0.33-0.34, in addition to component 2: Kav= 0.41 and component 3: Kav= 0.54) was observed in proteins

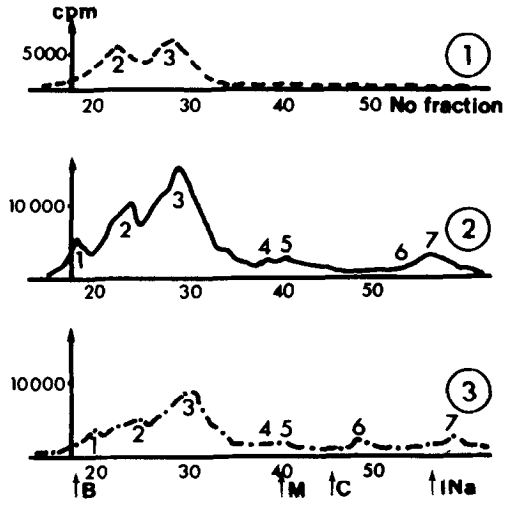

Fig. 1 : Elution profiles of glycoproteins extracted from ram sperm plasma membrane (ACA44 ultrogel; acid-acetate buffer 0.2 M containing BSA 5g/l; pH 5.6; B= Dextran Blue 2,000; M= Myoglobin; C= Cytochrom C; I= ^{125}I Na).

1= Testicular spermatozoa
2= Spermatozoa from middle corpus epididymis
3= Spermatozoa from distal cauda epididymis.

of spermatozoa from corpus and cauda epididymis as compared to testicular spermatozoa (components 2 and 3) (Fig. 1). The additional component of epididymal spermatozoa appeared in the exclusion peak labelled with dextran blue (B, Fig. 1). Moreover, minor peaks which were absent in proteins of testicular spermatozoa were observed between the elution of myoglobin and the excluded total volume (Kav: 0.66-1.00) in proteins of spermatozoa from corpus and cauda epididymis.

The distribution of the radioactivity between the different peaks (Table 1) was : 8±3% and 10±2% in peak 1 of the spermatozoa from the corpus and cauda epididymis respectively; 40±9%, 16±6%, 14±8% in peak 2 of the spermatozoa from the rete testis, corpus and cauda epididymis respectively; 50±10%, 47±9%, 54±12% in peak 3 of spermatozoa from the rete testis, corpus and cauda epididymis, respectively.

DISCUSSION

Con A is a lectin for glycopyranose residues depending on the structure and form of the oligosaccharidic chains in glycolipids and glycoproteins (14). In this work, glycoconjugates characterized by their affinity for Con A were shown to exist on the spermatozoal membrane. This is in agreement with results obtained on several species of rodents (2, 9, 15, 16, 17, 18). From

Table 1 : RELATIVE MOBILITY (A) AND % OF THE TOTAL RADIOACTIVITY (B) OF THE VARIOUS COMPONENTS OF GLYCOPROTEINS EXTRACTED FROM THE RAM SPERM PLASMA MEMBRANE AND FRACTIONATED ON ACA44 ULTROGEL.
Effect of maturation in the male genital tract

Origin of spermatozoa		Major peak			Minor peak			
		1	2	3	4	5	6	7
Rete testis	A		0.41±0.05	0.54±0.02				
	B		(40±9)	(50±10)				
Epididymal middle corpus	A	0.33±0.1	0.41±0.03	0.52±0.01	0.67±0.01	0.71±0.01	0.82±0.05	1
	B	(8±3)	(16±6)	(47±9)	10	10	10	10
Epididymal distal cauda	A	0.34±0.02	0.41±0.03	0.52±0.02	0.66±0.03	0.73±0.03	0.86±0.04	1
	B	(10±2)	(14±8)	(54±12)	10	10	10	10

A= Relative motility. Mean ± sd epididymis of 5 different rams. Peak 7 represents radioactivity in excluded volume.

B= % of the total radioactivity recovered from the elution profile.

previous observations, the Con A affinity of the intact spermatozoa was shown to be located on the plasma membrane (3). Consequently, ^{3}H acetyl-Con A is probably bound to surface residues; glycoproteins extracted from Con A-Agarose chromatography are also membrane material since the ^{125}I Na lactoperoxidase technique labelled membrane proteins exclusively (5, 7, 19, 21). Fractionation using ultrogel yields native proteins; for this reason it was used in preference to the SDS polyacrylamide gel elctrophoresis technique in which the proteins are denaturated.

In this study, the membranes of the epididymal spermatozoa differed greatly from testicular spermatozoa as previously shown by Hammerstedt et al. (10). The principal facts of these differences are the appearance of the component Kav= 0.33-0.34, the diminution of component Kav= 0.41 and the presence of new components, in small quantities, whose Kav values are between 0.66 and 1.

The protein(s) of the major peak (Kav= 0.33-0.34) may be more than 110,000 D in MW and could be heterogeneous since they appear in the excluded volume of ACA44. This MW is higher than the additional component of the epididymal spermatozoa of the rat which is 37,000 D (20). The new component of the membrane could arise from the binding of secretory epididymal proteins (4, 8) to the sperm surface. This suggestion is supported by the observation that epididymal specific proteins have affinity for immature sperm in the rat (4, 13, 15), bull (12), ram (7, 24) and pig (23) and may be involved in the functional activities of sperm (1, 16). In the rat, existence of a sperm coating antigen which is synthesized in the initial segment of the epidiymis has been demonstrated (11).

The minor peaks which appear on the epididymal spermatozoa provide further evidence of these differences in surface structure of the sperm according to time of transit in the epididymis. They are quite variable and thus quite difficult to analyse. A possible explanation of their existence is that they are degradation products from the component Kav= 0.41 which diminishes consistently in the epididymis: 14$\pm$8% of the radioactivity in the cauda epididymis versus 40$\pm$9% in the rete testis. This diminution (2/3) cannot explain the decrease of the total labelled proteins (1/2) since the quantities of ^{125}I glycoproteins extracted from the spermatozoa surface did not vary along the epididymis. From this, we can conclude that the diminution of total proteins involves proteins which are not the glycoproteins extracted on Con A-Agarose. Similar losses of proteins on the membrane of the maturing spermatozoa were reported in different species (4, 24). In spite of a lack of variation in glycoprotein quantities on the sperm surface in the rete testis or epididymis,

the number of sites quantitated using ^{3}H acetyl-Con A decreased in the epididymis. A possible explanation could involve the covering of saccharidic residues by components secreted by epithelial cells as discussed above.

In conclusion, this study showed that a molecular differentiation occurs in glycoproteins of the membrane on the maturing male gamete, raising the question of their significance in maturational changes during the epididymal transit.

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