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Preliminary communication

Isolation and identification of spermatozoon-surface glycoproteins from *Macaca radiata* *

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The motility of spermatozoa critically affects the process of reproduction. Despite their involvement in immunological infertility[1], the membrane-bound glycoproteins have not been structurally investigated, and their nature and composition remain unclear. Bonnet-monkey semen was obtained by electroejaculation[2] (0.25–0.4 mL), and kept for 1 h at 22° to allow liquefaction. The semen was then washed three times with 4% Tris hydrochloride (pH 7.0, 10 mL), and centrifuged at 500*g* to remove the seminal plasma and sperm-coating antigens. The supernatant liquor was removed, and spermatozoa (1.2×10^8), having a viability of 90% as judged by dye exclusion[3], were recovered from the pellet.

Sialic acid (260 μg/10^9 sperm) was detected[4] after treatment of the sperm with *V. cholerae* neuraminidase (Boehring, 150 U/mL) in 0.1M phosphate-buffered saline (PBS), pH 7.0, for 80 min at 37°. A suspension of the washed sperm in PBS (10 mL) containing TPCK-treated trypsin (Worthington, 20 μg/mL) was shaken for 30 min at 4°, and the sperm were then centrifuged at 500*g*. The procedure was repeated twice, the supernatant liquors were combined and dialyzed, and the retentate was lyophilized. Although human sperm are known to swell in trypsin solution[5], no alteration was detected under the mild conditions used in our experiments.

The residue was dissolved in 20mM phosphate buffer, pH 7.0 (0.5 mL), and treated with D-galactose oxidase (40 U) and peroxidase (150 U) for 12 h at 22°. The solution was then treated with 2M sodium boro[^{3}H]hydride (150 μL, 2 mCi) in mM

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sodium hydroxide for 6 h at 4°, followed by addition of another batch of sodium boro-[^{3}H]hydride, and the mixture was incubated for 2 h at 22°. The pH of the solution was adjusted to 5.4 with 4M acetic acid, and the solution was dialyzed, and lyophilized, to obtain the sperm-surface glycoproteins. These were chromatographed on a column (2.1 X 48 cm) of Bio-Gel P-200 in 0.1M pyridine–acetic acid, pH 5.4 (300 mL). The tritium- and carbohydrate-containing (phenol–sulfuric acid[6]) fractions were combined, and lyophilized, to give the glycoproteins. The polymers contained carbohydrates and amino acids (see Tables I and II).

In a separate experiment, the glycoproteins from the sperm surfaces were isolated by treatment first with D-galactose oxidase–sodium boro[^{3}H]hydride, according to the method of Gahmberg and Hakomori[7], and then processed as described earlier.

TABLE I

CARBOHYDRATE COMPOSITION OF SPERM-SURFACE GLYCOPROTEINS OBTAINED BY CHROMATOGRAPHY ON A COLUMN OF BIO-GEL P-200

Sugar components[a]	*Molar ratio*[b]
L-Fucose	2.35
D-Galactose	1.00
D-Glucose	1.57
D-Mannose	7.14
2-Acetamido-2-deoxy-D-glucose	3.81
2-Acetamido-2-deoxy-D-galactose	0.52
Sialic acid[c]	1.70

[a] Determined by gas–liquid chromatography[12]. [b] Molar ratio relative to D-galactose. [c] Determined by the Warren procedure[4].

TABLE II

AMINO ACID COMPOSITION OF SPERM-SURFACE GLYCOPROTEINS OBTAINED BY CHROMATOGRAPHY ON A COLUMN OF BIO-GEL P-200

Amino Acids	*Residues/1000 residues*
Ala	75
Val	43
Gly	79
Ile	46
Leu	97
Pro	63
Thr	88
Ser	108
Phe	69
Asp	184
Glu	109
Lys	39

The elution profiles of the glycoproteins obtained by use of the alternative procedures (*i.e.*, first trypsinizing and then labeling, or, first labeling and then trypsinizing) were identical on a Bio-Gel P-200 column.

The carbohydrate composition of this glycoprotein is unlike that of either the *N*-glycosyl or the *O*-glycosyl type, as it contains 2-acetamido-2-deoxygalactose, as well as fucose and glucose, in addition to mannose, galactose, 2-acetamido-2-deoxyglucose, and sialic acid residues. However, in the protein moiety, a significant content of asparagine suggests a preponderant proportion of an *N*-glycosylated type of glycoprotein. The structure and function of sperm-surface glycoproteins are still unclear, and their contribution to HL-A antigens is controversial[8], although their significance in immunological infertility in males, as well as females, has been considerably emphasized[1,9–11].

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