Quantitative Determination of Plant Agglutinin Membrane Sites on Mammalian Spermatozoa (36847)

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(Introduced by L. Mastroianni, Jr.)

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The spermatozoon is a highly differentiated cell with extremely specialized cellular structures. These structures play important functional roles in sperm motility, ova recognition and other processes of fertilization (1–3). We have recently identified certain surface saccharide components on mammalian spermatozoa to initiate studies on their possible roles in cellular recognition and fertilization (4).

We report here on the quantitative determination of rabbit and hamster sperm surface saccharide receptors for three plant lectins or agglutinins: concanavalin A (Con A), specific for α-*D*-mannose-like receptors (5, 6); *Ricinus communis* agglutinin (RCA), specific for β-*D*-galactose-like receptors (7, 8), and wheat germ agglutinin (WGA), specific for N-acetyl-D-glucosamine receptors (9, 10).

Methods. Cells. Spermatozoa were isolated from rabbit and golden hamster cauda epididymis into 0.15 *M* NaCl at 5°. Care was taken not to contaminate the sperm suspension with blood. The spermatozoa were washed three times by centrifugation in 0.15 *M* NaCl, counted and stored at 5°.

Plant agglutinins. Each of the plant agglutinins was purified using affinity chromatography techniques. Concanavalin A, obtained as a twice crystalized product (Miles-Yeda), was further purified by absorption to a 2.5 × 30 cm column of Sephadex G-75. After washing, the agglutinin was eluted with 0.2 *M* sucrose by the method of Agrawal and Goldstein (5) and extensively dialyzed to remove bound sucrose. *Ricinus communis* agglutinins were purified according to the procedures of Nicolson and Blaustein (7). The ammonium sulfate-precipitated preparation (60% ammonium sulfate fraction) of *R. communis* agglutinins was applied to a 4 × 40 cm column of Biogel A–0.5 *m* agarose (Biorad). After washing the column with buffer, the agglutinins were eluted in a single peak with 0.2 *M* lactose. The 120,000 mol wt *R. communis* agglutinin (RCA_{120}) was separated from the 60,000 mol wt agglutinin on a 2 × 50 cm column of Sephadex G-100 (7). Wheat germ agglutinin was purified on an ovomucoid–Sepharose column according to the procedures of Burger (10). Crystallized ovomucoid (Sigma) was coupled to Sepharose using the cyanogen bromide activation methods of Cuatrecasas (11). After extensive washing to remove noncovalently bound proteins, the ovomucoid–Sepharose was used to make a 2 × 20 cm affinity column. A crude wheat germ lipase preparation (Worthington) was heat-inactivated at 56° for 10 min and then quickly cooled to 5°. After centrifuging to remove precipitated protein, the partially purified WGA preparation was applied to the ovomucoid–Sepharose column. After washing with buffer, 0.1 *M* acetic acid (pH 2) was used to elute the agglutinin (10).

^{125}I-Labeling. The affinity-purified agglutinins were iodinated using the iodine monochloride method of McFarlane (12). After extensive dialysis, the agglutinins had the following specific activities:

Con A, 2.02×10^7 cpm/mg
WGA, 11.43×10^7 cpm/mg
RCA_{120}, 2.78×10^7 cpm/mg.

Spermatozoa were labeled with the purified ^{125}I-agglutinins as follows: saline washed

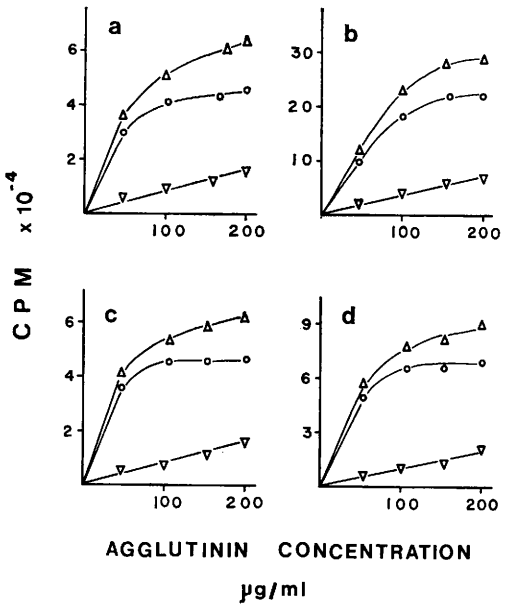

FIG. 1. The binding of purified ^{125}I-labeled plant agglutinins to rabbit and hamster sperm. (a) Rabbit sperm labeled with *Ricinus communis* agglutinin, (b) rabbit sperm labeled with wheat germ agglutinin, (c) hamster sperm labeled with *Ricinus communis* agglutinin and (d) hamster sperm labeled with concanavalin A. (△) counts per minute bound to sperm without inhibitor present; (▽) cpm bound to sperm in the presence of a saccharide inhibitor; (○) specific cpm bound to sperm (corrected for nonspecific binding of agglutinin).

spermatozoa were incubated with ^{125}I-Con A, ^{125}I-RCA, or ^{125}I-WGA for 15 min at room temperature. Controls contained additionally 100 m*M* α-methyl-mannoside (Con A control), lactose (RCA_{120} control) or *N*-acetyl-D-glucosamine (WGA control). After the 15 min incubation, the spermatozoa were washed twice in saline (or in the case of the controls, saline plus the appropriate saccharide inhibitor) by centrifugation and were counted in a Packard gamma scintillation counter. Several of the samples were finally diluted into a known volume of saline and the number of sperm per sample determined using a hemocytometer.

Results. Rabbit and hamster cauda epididymal spermatozoa were specifically labeled with the ^{125}I-agglutinins (Fig. 1). With increasing ^{125}I-agglutinin concentration, the amount of bound ^{125}I increased linearly until saturation, which was around 100 μg/ml for each of the agglutinins (Fig. 1). With inhibitory concentrations of competitive saccharides present, the amount of ^{125}I-agglutinin bound to spermatozoa was usually around 15–20% of the samples without saccharides present. From the amount of ^{125}I-agglutinins specifically bound, the number of binding sites per spermatozoon for Con A, RCA_{120} and WGA was estimated (Table I).

Discussion. We have recently determined from specific agglutination studies that mammalian spermatozoa have several classes of plant lectin or agglutinin sites on their surfaces (4). By observing which lectins caused agglutination and also which sperm structures were involved in the agglutination process, we were able to identify Con A, RCA, WGA and influenza virus receptors on rabbit and hamster sperm surfaces. Con A, RCA and WGA agglutinated hamster spermatozoa

TABLE I. Quantitation of Concanavalin A, *R. communis* and Wheat Germ Agglutinin Sites on Rabbit and Hamster Spermatozoa.

		Molecules of agglutinin specifically bound at saturation			
		Rabbit		Hamster	
Agglutinins	Saccharides recognized	Per sperm	(Per μm²)[a]	Per sperm	(Per μm²)[b]
Concanavalin A	α-D-mannose-like	1.0×10^7	8.3×10^4	1.9×10^7	5.4×10^4
R. communis[c]	β-D-galactose-like	0.22×10^7	1.8×10^4	0.55×10^7	1.5×10^4
Wheat germ	*N*-acetyl-D-glucosamine-like	1.2×10^7	10×10^4	3.3×10^7	9.4×10^4

[a] The surface area of a rabbit spermatozoon was taken to be 120 μm².

[b] The surface area of a hamster spermatozoon was taken to be 350 μm².

[c] The 120,000 mol wt *R. communis* agglutinin [Ref. (7)] was used for these studies.

more strongly tail-to-tail when compared with rabbit spermatozoa, indicating more α-D-mannose-like, β-D-galactose-like and *N*-acetyl-D-glucosamine-like sites on hamster tail surfaces (4). From the data presented here it appears that hamster spermatozoa have quantitatively more of these saccharide sites per cell than rabbit spermatozoa. This data may explain the differences in spermatozoa agglutinability, but these differences are probably also related to the topographic distribution of the lectin surface sites (13). Also the average surface area of a rabbit spermatozoon is about one-third of that of a hamster spermatozoon, so quantitative differences in lectin sites are not significant when corrected to average surface areas.

Edelman and Millette (14) have recently cleaved mouse spermatozoa into the head and tail portions and have shown that the mouse heads contain about 80% of the Con A sites. They also observed with intact spermatozoa that Con A strongly agglutinates mouse spermatozoa involving both heads and tails in the agglutination reaction. The discontinuous distribution of Con A sites on mouse spermatozoa indicates that quantitative labeling of whole spermatozoa and agglutination data will have to be augmented with Con A localization studies utilizing electron microscopy. We are currently examining the surface distributions of these and other agglutinin sites using ferritin-conjugated plant agglutinins (15) and techniques for topographical visualization of ferritin-agglutinin labeled spermatozoan structures (16).

Summary. Rabbit and hamster surface terminal saccharides were quantitatively determined using ^{125}I-labeled plan agglutinins. Hamster spermatozoa specifically bound more concanavalin A, wheat germ agglutinin and *Ricinus communis* agglutinin molecules (1.9×10^7, 0.55×10^7, 3.3×10^7, respectively) per spermatozoon than rabbit spermatozoa (1.0×10^7, 0.22×10^7, 1.2×10^7, respectively). However, when corrected to sperm surface area, these differences were not significant.

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