SPECIES DIFFERENCES IN THE LECTIN-BINDING SITES ON THE ZONA PELLUCIDA OF RODENT EGGS

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Mammalian eggs are surrounded by a moderately thick transparent coat, the zona pellucida, which is believed to be an important site of sperm recognition and of the block to polyspermy (Braden, Austin & David, 1954; Austin, 1961; Dickmann, 1962; Yanagimachi, 1964; Barros, 1968; Barros & Yanagimachi, 1972; Hanada & Chang, 1972; Hartmann, Gwatkin & Hutchison, 1972; Yanagimachi, 1972; Gwatkin, Williams, Hartmann & Kniazuk, 1973; Hartmann & Hutchison, 1974). Little is known of the molecular structure of the zona pellucida, but available evidence indicates that it is composed of glycoproteins or glycopeptides (Braden, 1952; Konecny, 1959; Stegner & Wartenberg, 1961; Lowenstein & Cohen, 1964; Jacoby, 1962; Soupart & Noyes, 1964; Fléchon, 1970; Inoue & Wolf, 1974; Kang, 1974). Previously we found that the hamster zona pellucida contains receptors for a variety of plant lectins, glycoproteins that bind to specific saccharide determinants (Oikawa, Yanagimachi & Nicolson, 1973; Oikawa, Nicolson & Yanagimachi, 1974; Nicolson, Yanagimachi & Yanagimachi, 1975). Some of these lectins can bind to the zona pellucida and induce egg agglutination and/or cause changes in the ability of the exterior region of the zona to scatter visible light (Oikawa *et al.*, 1973, 1974; Oikawa, Nicolson & Yanagimachi, 1975). We report here that rodent eggs show species-specific differences in lectin-induced agglutination and light scattering properties of their zonae pellucidae when treated with *Ricinus communis* agglutinin I (RCAI), which recognizes β -linked D-galactose residues (Nicolson & Blaustein, 1972; Nicolson & Lacorbier, 1973), or wheat germ agglutinin (WGA), which recognizes N-acetyl-D-glucosamine and sialic acid residues (Burger & Goldberg, 1967; Greenaway & LeVine, 1973).

Unfertilized eggs of the golden hamster, rat (Fisher) and mouse (Swiss albino) were obtained as follows. Adult hamster females were injected intraperitoneally with 20 to 30 i.u. PMSG (Ayerst, New York) on the morning of ovulation. This was followed by an intraperitoneal injection of 20 to 30 i.u. HCG (Ayerst) in the evening 2 days later. Adult mice were injected with 5 to 10 i.u. PMSG regardless of the stage of their oestrous cycle followed by an injection of 5 to 10 i.u. HCG 48 hr later. Prepubertal rats were injected with 20 to 30 i.u. PMSG

followed by an injection with 20 to 30 i.u. HCG 48 to 56 hr later. Between 15 and 18 hr after injection of HCG, the animals were killed and their oviducts were flushed with Tyrode's solution to collect eggs. The eggs were freed from the surrounding cumulus cells by treating them for 10 to 15 min at 25°C with Tyrode's solution containing 0.1% crystalline bovine serum albumin (Reheis Chem. Co., Chicago, Illinois) and 0.1% bovine testicular hyaluronidase (300 USP units/mg: Nutritional Biochem., Cleveland, Ohio). After they had been rinsed with Tyrode's solution containing 0.1% serum albumin, the eggs were placed in albumin-saline (0.01 M-tris-HCl:0.9% NaCl:0.1% serum albumin, pH 7.4) with various concentrations of RCA₁ or WGA. The RCA₁ (mol. wt 120,000) was prepared from Hale hybrid strain castor beans by affinity chromatography according to procedures of Nicolson & Blaustein (1972). The WGA was prepared from an impure wheat germ lipase preparation (Sigma Chem. Co., Saint Louis, Missouri) by affinity chromatography as described by Nicolson & Lacorbier (1973). After incubation in these solutions for 30 min at 25°C,

Table 1. Effects of *Ricinus communis* I agglutinin on the agglutination and light-scattering property of the zonae pellucidae of the eggs of three rodent species

Species	No. of females from which eggs were recovered	Experiment	No. of eggs used	Concentration of RCA ₁ (µg/ml)				
				0	3	10	30	100
Hamster	4	Agglutination	43	0	4+	4+	4+	4+
		Light scattering	25	—	—	±	++	+++
Mouse	4	Agglutination	40	0	1+	4+	4+	4+
		Light scattering	25	—	—	—	—	—
Rat	4	Agglutination	42	0	2+	3+	4+	4+
		Light scattering	25	—	—	—	+	++

the eggs were tested for their ability to agglutinate and for changes in the light scattering properties of their zonae pellucidae. For agglutination experiments, the eggs in a lectin solution were aggregated by pushing about ten of them together with a needle. The dishes were then agitated to disperse the aggregates. Agglutination was qualitatively scored from 0 to 4+ as follows: 0, agitation caused the aggregates to disperse completely; 1+, aggregates containing two eggs each remained; 2+, aggregates contained about three to four eggs each; 3+, aggregates contained four to six eggs each; 4+, the egg aggregates remained completely agglutinated. Light scattering of the zona pellucida was qualitatively scored under dark field illumination from — to +++ as follows: —, light scattering equivalent to untreated control eggs; +, light scattering slightly greater than controls; ++, moderate light scattering; +++, strong or intense light scattering of the exterior region of the zona. Lectin-treated controls contained 0.1 M concentrations of appropriate saccharide inhibitors (lactose for RCA₁ and N-acetyl-D-glucosamine for WGA).

Treatment of rodent eggs with RCA₁ demonstrated species differences in the lectin-induced agglutination and light scattering properties of the zona (Table 1). Only 3 µg RCA₁/ml was required to produce 4+ agglutination of hamster

eggs, but 10 $\mu\text{g/ml}$ were required for mouse eggs and 30 $\mu\text{g/ml}$ for rat eggs to produce an equivalent agglutination. The hamster zona pellucida also showed the strongest RCAI-induced scattering of visible light (+++ at 100 $\mu\text{g/ml}$), while the rat zona pellucida showed intermediate light scattering (++ at 100 $\mu\text{g/ml}$). Mouse eggs failed to show light scattering of their zonae pellucidae after treatment with 100 μg RCAI/ml in spite of being maximally agglutinated at this lectin concentration. The egg agglutination and light scattering of the zonae pellucidae mediated by RCAI were specific because inclusion of an appropriate saccharide inhibitor (0.1 M-lactose or D-galactose) in RCAI solution completely abolished the effects.

In contrast to RCAI, WGA proved to be a poor agglutinin for rodent eggs (Table 2). Only rat eggs were appreciably agglutinated with WGA (1+ at 100 $\mu\text{g/ml}$). Both hamster and rat eggs showed strong WGA-induced light scattering of their zonae pellucidae (+++ at 100 $\mu\text{g/ml}$), and mouse zonae showed moderate WGA-induced light scattering (++ at 100 $\mu\text{g/ml}$) (Table 2). The agglutination and light scattering of the zonae pellucidae mediated by WGA were specific because inclusion of an appropriate saccharide inhibitor (0.1 M-N-acetyl-D-glucosamine) in WGA solution abolished the effects.

Table 2. Effects of wheat germ agglutinin on the agglutination and light-scattering property of the zonae pellucidae of the eggs of three rodent species

Species	No. of females from which eggs were recovered	Experiment	No. of eggs used	Concentration of WGA ($\mu\text{g/ml}$)				
				0	3	10	30	100
Hamster	4	Agglutination	40	0	0	0	0	0
		Light scattering	24	—	—	±	++	+++
Mouse	4	Agglutination	42	0	0	0	0	0
		Light scattering	25	—	—	—	+	++
Rat	4	Agglutination	39	0	0	0	0	1+
		Light scattering	20	—	—	+	++	+++

These results indicate that there are species differences in the oligosaccharide lectin receptors on the zonae pellucidae of rat, mouse and hamster eggs. Although the significance of the receptor differences is unknown, it is tempting to speculate that the oligosaccharides on the mammalian zona pellucida are important in species recognition by gametes. It is possible that oligosaccharide residues of the zona glycopeptides have species-specific differences in sequence as well as in the relative amounts of each component, and that these differences are recognized by sperm surface receptors as a means of species recognition at fertilization.

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