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Direct visualization of endogenous lectins in fish oocytes by glycosylated fluorescent cytochemical markers

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The serological and biochemical studies which have appeared during the two last decades have demonstrated that the ovary of many species of the Teleost fish families *Salmonidae, Percidae, Cyprinidae, Siluridae, Cobitidae* and *Clupeidae* contain powerful agglutinins in various cells. These lectins possess expressive serological and sugar-binding properties. Several were recently purified by affinity chromatography and partially characterized (Krajhanzl *et al.*, 1978a,b; Voss *et al.*, 1978; Sakakibara *et al.*, 1981; Nosek *et al.*, 1983). Our previous studies (Nosek *et al.*, 1982) suggest that these lectins are localized in previtellogenic oocytes in cortical granules. In order to confirm this observation from indirect immunofluorescence staining with antilectin antibody, we made use of the ability of lectins to bind specifically to the carbohydrate components of glycoproteins. This phenomenon offers the possibility of detecting lectins directly e.g. by using Fluorescein-labelled glycosylated markers as prepared by Kieda *et al.* (1977, 1979). These fluorescent neo-glycoproteins have been used to advantage for the localization of some membrane-bound endogenous lectins, e.g. the lymphocyte membrane lectin (Kieda *et al.*, 1979) or the nuclear membrane lectins of hepatocytes (Schulte & Monsigny, 1981). In our case, bovine serum albumin (BSA) served as a non-glycosylated macromolecular carrier of both the specific sugars (i.e. D-mannose and D-galactose) and the fluorescent label. Fluoresceinyl thiocarbamyl derivatives of glycosylated BSA were prepared by a substitution reaction in the presence of ethylene glycol, purified by gel filtration on Sephadex G-50, and finally dialysed and lyophilized (Kieda *et al.*, 1979; Kieda, 1980). The number of sugar residues and fluorescent molecules were calculated from absorbance data as described by Monsigny *et al.* (1979). Fluoresceinyl thiocarbamyl BSA (FTC–BSA) was prepared for control experiments in the same way.

Glutaraldehyde-fixed cryostat sections (10 μm) of vitellogenic oocytes (1–2.5% glutaraldehyde in phosphate buffered saline, pH 7.4) of rudd (*Scardinius erythrophthalmus L.*) and early previtellogenic oocytes of tench (*Tinca tinca L.*) were

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incubated with α-D-galactosyl FTC–BSA (250 μg/ml, 4–12 h, 4° C) (Fig. 1a,b). In agreement with the results obtained by the indirect immunofluorescence method (Nosek *et al.*, 1983), the fluorescent marker was observed to be bound to the lectin in cortical granules. The labelling of the cortical granules and follicular epithelium was totally abolished when the sections were treated in the presence of 0.2 M D-galactose or 0.1 M L-rhamnose. As well as the specific fluorescence staining of cortical granules, the plasma membrane and egg yolk also emitted a weak fluorescence. By application of the non-glycosylated FTC–BSA, it was found that this fluorescence was probably due to a non-specific interaction. Thus, the tench lectin specific for L-rhamnose and D-galactose, first detected *in situ* by this method, may be isolatable on a suitable affinity carrier (Nosek *et al.*, 1983).

In unfixed cryostat sections of mature activated eggs of the perch (*Perca fluviatilis L.*), the presence of lectin specific for D-glucose, D-mannose and L-fucose was studied with the use of α-D-mannosyl FTC–BSA (300–500 μg/ml). Fluorescence staining appeared only on the egg jelly coat and in the follicular epithelium (Fig. 1c). The interaction of the lectin bound to the jelly coat with fluorescent marker was specifically inhibited by 0.2 M D-mannose and 0.2 M D-glucose but not by 0.2 M L-rhamnose. Incubation of control sections with FTC–BSA in the same concentration did not yield fluorescence-emitting sections. Although this is not direct evidence for any role of fish ova lectins during the fertilization, this observation at least supports our hypothesis that the binding of lectins to the egg jelly may be involved in the formation of a permanent barrier to polyspermy (Nosek *et al.*, 1982, 1983). The binding of the glycosylated marker to the jelly-bound

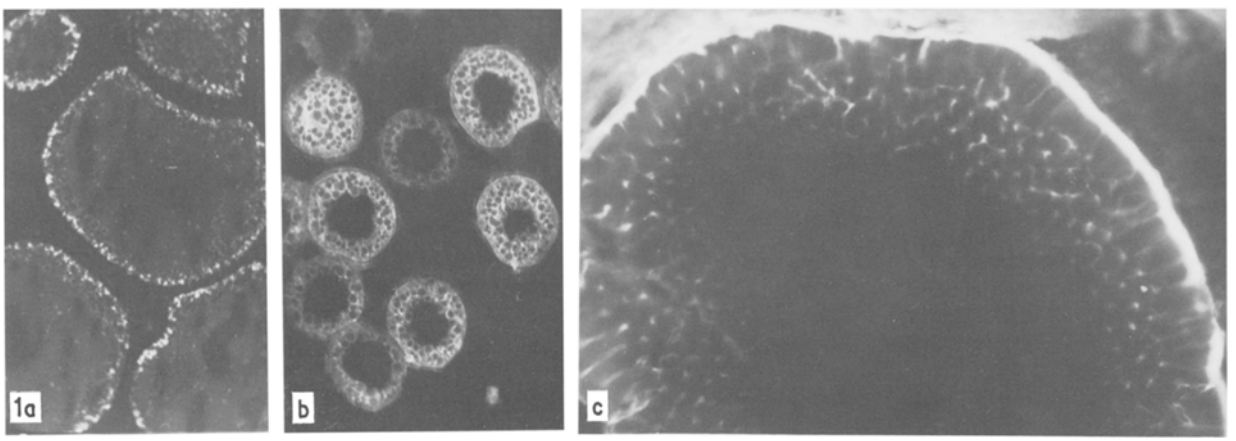

Fig. 1. Direct localization of endogenous lectins in fish oocytes by fluorescent glycosylated markers. Fluorescent bovine serum albumin with covalently bound α-D-galactosyl residues was employed for the detection of lectins in cortical vesicles of glutaraldehyde-fixed oocytes (fixation time, 30 min) of (a) rudd and (b) tench. × 50. In (c), unfixed cryostat sections of mature activated perch eggs were treated with α-D-mannosyl fluorescent marker. Lectin is found exclusively at the outer surface of jelly envelope. × 100.

lectin suggests that not all the binding sites of the 'immobilized' lectin are occupied and that the lectin is probably able to bind with, for instance, some bacteria as well (Köhler, 1970; Voss *et al.*, 1978).

In comparison with the indirect immunofluorescence technique, direct detection by Fluorescein-labelled glycosylated markers is advantageous in that it is a simpler procedure; it is also possible to apply it in those cases where the lectin is to be detected but has not yet been isolated. On the other hand, the fluorescence staining is not as intense and sensitive as in the indirect immunofluorescence technique. It is also necessary to take some precautions in handling the sections since the denaturation of lectins results in a loss of their binding activity.

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