Binding of concanavalin A and ricin 120 to parenchymal and non-parenchymal cells of rat liver

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Postmitochondrial supernatants may be harvested by centrifugation to yield a microsomal fraction consisting of vesicular membrane fragments and unattached polyribosomes. In an attempt further to subfractionate these preparations, we have tested the ability of immobilized lectins to bind and hence remove plasma-membrane fragments from postmitochondrial supernatants (PMS) from rat liver.

PMS was prepared from the livers of male Sprague–Dawley rats as described previously (Palmer et al., 1978) except that the livers were homogenized in 2.5 vol of 20 mm-Hepes [(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid, pH 7.5, containing 25 mm-KCl and 5 mm-MgCl₂. The PMS was incubated with agarose-bound concavalin A (Con A) or agarose-bound castor-bean (Ricinus communis) agglutinin I (RCA₁₂₀) (purchased from BDH Chemicals Ltd., Poole, Dorset, U.K.) (6 mg of Con A or 4 mg of RCA/ml of settled gel) for 30 min at 7°C. The gel was separated from unbound material on a glass sinter.

Fig. 1 shows the recovery of 5'-nucleotidase, a marker for plasma membrane (Emmelot et al., 1964) in PMS after filtration through the agarose-bound lectin. The recovery of glucose 6-phosphatase, associated with endoplasmic membranes (Emmelot et al., 1964) was 99.9 ± 2% (n = 8). The recovery of 5'-nucleotidase depends on the amount and nature of the lectin used; Con A will completely remove 5'-nucleotidase from PMS, whereas RCA₁₂₀ will remove approx. 30% of the activity, over a wide range of PMS/lectin ratios. This suggests that Con A may be removing all membranes associated with 5'-nucleotides, whereas RCA₁₂₀ removes only a fraction of membrane fragments.

Rat liver is a complex tissue composed of several cell types. In order to compare the lectin-binding capacity of parenchymal cell membranes with those of non-parenchymal cells, liver was disaggregated by the method of Berry & Friend (1969) as modified by Seglen (1972). Parenchymal cells were recovered from filtered cell suspension by sedimentation at 50g, for 3 min; non-parenchymal cells were harvested from the supernatant after parenchymal-cell sedimentation by centrifugation at 600g, for 5 min. Both membrane pellets were washed in collagenase-free perfusing medium and re-pelleted twice. These fractions were then stained with fluorescent derivatives of Con A and RCA₁₂₀. Fractions were stained with fluorescein isothiocyanate-labelled Con A (Miles) at a concentration of 150 μg in 1 ml of phosphate-buffered saline for 20 min on ice, washed by centrifugation, resuspended and observed under fluorescence and phase-contrast optics on a Leitz Orthoplan microscope. Both fractions were found to be faintly positive, but some small cells in the non-parenchymatous fraction were found to be strongly positive. Other fractions were stained with fluorescein isothiocyanate conjugate of RCA₁₂₀ (Miles) at a concentration of 25 μg in 1 ml of saline (0.9% NaCl) under similar conditions. In this case parenchymatous cells were unstained, apart from occasional blebs on damaged cells, whereas non-parenchymatous cells were strongly positive and had commonly agglutinated in the presence of the lectin. This agglutination, together with the pattern of staining, indicated a surface labelling. A few larger cells were found in the preparation, and these were negative. Occasional sheets of free membrane were present, and these were positive.

An attempt was made to relate these results to the structure of the tissue in situ. Frozen sections (8 μm) of adult rat liver were similarly stained with fluorescent RCA₁₂₀. Hepatocytes were generally negative, but blood vessels, collecting ducts and interlobular elements stained brightly, thus suggesting the origin of at least some of the positive non-parenchymatous cells.


Fig. 1. Effect of preincubation with increasing concentrations of lectin and the recovery of 5'-nucleotidase activity from rat postmitochondrial supernatant

The supernatant was incubated at 7°C for 30 min with agarose-bound concavalin A (●) or castor-bean agglutinin (○). Unbound material was washed from the gel on a glass sinter and glucose 6-phosphatase and 5'-nucleotidase activities determined. 5'-nucleotidase recovery is expressed as a ratio of percentage recovery to that for glucose 6-phosphatase in the same preparation. The average recovery of glucose 6-phosphatase for the experiments summarized in this figure was 99.9 ± 2% (n = 8).


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