Binding of *Ricinus communis* Agglutinin to the Mitochondrial Inner Membrane as an Artifact during Preparation

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Endosperm from *Ricinus communis* was homogenized in the presence of ^3H -labelled *Ricinus communis* agglutinin, with or without addition of lactose. In preparations without the binding-specific sugar the subfraction containing the mitochondrial inner membrane contained sufficient labelled agglutinin to account for the agglutinin reported to be associated with this membrane.

Although plant lectins (haemagglutinins) have brought increased knowledge in the fields of membrane biochemistry and animal cell biology, their function in plant cells remains unresolved. It was hoped that some relevant information might be gained from studies on the subcellular distribution of lectins. Bowles & Kaus (1975) showed that lectins could be extracted from various cellular membranes (dictyosomes, endoplasmic reticulum, plasma membrane and mitochondria) of several plant tissues. To evaluate these observations further, studies were performed on lectins contained in mitochondria isolated from endosperm of *Ricinus communis* (castor bean) (Bowles *et al.*, 1976). Mitochondria where chosen, as the function of their membranes is relatively well known and they do not take part in intracellular membrane flow. It was found that the content of extractable lectin is highest in the mitochondrial inner membrane. Part of the lectin could be eluted from this membrane with lactose and was identified as *Ricinus communis* agglutinin of mol.wt. 120000 (*R. communis* agglutinin 120). Further lectin was extracted by subsequent treatment with Triton X-100. The haemagglutinating activity of this fraction was only partially inhibited by lactose, indicating that in addition to *R. communis* agglutinin 120 some other lectin (or lectins) of unknown identity was present.

The fact that at least part of the *R. communis* agglutinin 120 was obviously bound to the membrane by its carbohydrate-binding site attracted particular interest since this might have indicated a role of this well-known lectin in membrane stabilization. At first glance it appeared reasonable to assume that this lectin was an integral membrane component, as the mitochondrial inner membrane was envisaged to have no contact with the soluble proteins present in the preparation. However, appreciable amounts

of soluble *R. communis* agglutinin 120 are also present (Bowles *et al.*, 1976) in the preparation, probably as a result of the rupture of protein bodies during homogenization of the endosperm in aqueous solution (Tully & Beevers, 1976; Youle & Huang, 1976). Before the performance of more detailed studies on the possible function of *R. communis* agglutinin 120 in mitochondria it was therefore necessary to establish whether this agglutinin is indeed an integral membrane component. We now report results suggesting that the *R. communis* agglutinin 120 is bound to the inner membrane as an artifact during preparation of the mitochondria.

Experimental

The growth of plant material and most of the other materials and methods were as described previously (Bowles *et al.*, 1976). Each 15g of endosperm was homogenized for 1 s in a Sorvall Omnimixer homogenizer at maximum speed with 8 ml of buffer as described by Bowles *et al.* (1976), but in the presence of either 1.0M-sucrose or 1.0M-lactose. After addition of 20 μCi of ^3H -labelled *R. communis* agglutinin 120 in 2 ml of the sucrose-containing buffer, the samples were homogenized for another 7 s, as above, then squeezed through four layers of muslin to result in suspensions of membranous organelles that were then layered on to 30 ml of a 20–57.5% (w/w) sucrose gradient. After centrifugation at 100000g for 2 h at 4°C in a Beckman L3-50 centrifuge equipped with an SW27.2 rotor (r_{av} 11.8 cm), the gradient tubes were punctured and fractions containing visible bands were collected in graduated tubes. The radioactivity in 100 μl of each fraction in 10 ml of Unisolve (Koch-Light Laboratories) was determined by using a Berthold Frieske BF 5000 scintillation counter. Sucrose

concentrations in gradient fractions were determined by means of a Zeiss refractometer.

The ^3H -labelled *R. communis* agglutinin 120 was prepared by the method of Bolton & Hunter (1973) with *N*-succinimidyl[2,3- ^3H]propionate (specific radioactivity 53 Ci/mmol; Amersham-Buchler) and *R. communis* agglutinin 120 from Boehringer Mannheim. The labelled product was purified by affinity chromatography by the method of Nicolson & Blaustein (1972). It had a specific radioactivity of 0.12 mCi/mg of protein and unaltered haemagglutination activity as indicated by a titre of 16384, measured against trypsin-treated rabbit erythrocytes (Bowles *et al.*, 1976). Protein was determined by the

method of Sedmak & Grossberg (1977), with bovine serum albumin as a standard.

Results and Discussion

The suspension of membranous subcellular organelles in the preparations with or without lactose contained 39 or 40% respectively of the added ^3H -labelled *R. communis* agglutinin 120, the remainder being trapped in the bulky homogenate. Radioactivity was carried into the gradient when membranes were present (Fig. 1). In contrast, on a control gradient without membrane material all the ^3H -labelled agglutinin remained in the supernatant. The gradient contained far more radioactivity when no lactose was present during homogenization, indicating that group-specific binding of the lectin to membrane carbohydrates was the most likely explanation for the occurrence of ^3H -labelled *R. communis* agglutinin 120 in the gradient fractions.

When the mitochondria present in the mitochondrial band were further fractionated (Table 1), to our great surprise most of the bound ^3H -labelled *R. communis* agglutinin 120 was recovered with the mitochondrial inner membrane. Almost all of the radioactivity could be eluted with lactose, a sugar specific for *R. communis* agglutinin 120, indicating that the labelled lectin was bound to membrane carbohydrate groups via its binding site. The fact that far less radioactivity was found associated with the mitochondrial membranes if lactose was present during homogenization also shows that binding of the ^3H -labelled agglutinin occurs during that step.

The haemagglutination titre of the supernatant fractions reached values of 65 536, and thus the added

Fig. 1. Labeled agglutinin in membrane fractions from *R. communis* endosperm

R. communis endosperm was homogenized in the presence of ^3H -labelled *R. communis* agglutinin 120 in a buffer containing sucrose (Δ) or lactose ($\blacktriangle$). The membranous fractions were isolated by centrifugation on a sucrose gradient as described in the text, and visible bands were collected. The radioactivity of samples of the respective fractions (a) and the sucrose content (b; $\circ$, preparation with sucrose; $\bullet$, preparation with lactose) were determined as described in the text.

Table 1. Distribution of radioactivity recovered from the gradients

The mitochondrial bands indicated in Fig. 1 were subfractionated after osmotic lysis of the mitochondria, and the membranes were isolated by differential centrifugation as described by Bowles *et al.* (1976). Values given represent total radioactivity in the subfractions; they do not sum up owing to losses during centrifugation and washing.

Fraction	$10^{-3} \times R. communis$ agglutinin 120 recovered after preparation of mitochondria (d.p.m.)	
	Lactose absent	Lactose present
Total supernatant	17300	17700
Total mitochondrial band	111	31
Outer membrane	3	1
Inner membrane	56	5
0.1 M-Lactose extract of inner membrane	50	4

^3H -labelled *R. communis* agglutinin 120 represented only a small fraction (about 1%) of the total soluble haemagglutination activity present in the supernatant. The radioactivity recovered in the mitochondrial inner membrane (Table 1, preparation without lactose) accounts for 0.3% of that in the supernatant. To evaluate these measurements we carried out experiments similar to those in Fig. 1 but without adding ^3H -labelled *R. communis* agglutinin 120 and measured the haemagglutinating activity of both fractions. Although such measurements are less accurate than those of radioactivity because of the semi-quantitative nature of titre determination, the lactose extract from the mitochondrial inner membrane contained about 0.6% of the haemagglutinating activity in the supernatant. The percentages are therefore in the same range. This indicates that the *R. communis* agglutinin 120 reported to reside in the mitochondrial inner membrane (Bowles *et al.*, 1976) might result partly, if not entirely, from binding as an artifact during preparation.

The methods employed do not enable us to say whether *R. communis* agglutinin 120 really binds to mitochondrial inner membrane, a process that would necessitate a transition of protein molecules through the outer membrane, possibly as a result of some membrane damage during preparation. Alternatively, the mitochondrial inner-membrane fraction could be contaminated by small amounts of other

material with the sugar groups necessary for binding of *R. communis* agglutinin 120. The results suggest, however, that further searches for a role of *R. communis* agglutinin 120 in the organization of the mitochondrial inner membrane are not promising. Nevertheless the unidentified lectins that were solubilized in addition to *R. communis* agglutinin 120 from the mitochondrial inner membrane of *Ricinus* by treatment with Triton X-100 (Bowles *et al.*, 1976) might still be of functional importance. However, work on this problem should be performed preferentially with plant tissues devoid of soluble lectins, e.g. hypocotyls of mung beans, for which high lectin titres were reported in extracts from total mitochondria (Bowles & Kauss, 1975).

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