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# Lectins as Membrane Components of Mitochondria from *Ricinus communis*

By DIANNA J. BOWLES,* CLAUS SCHNARRENBERGER and HEINRICH KAUSS
*Fachbereich Biologie der Universität, Postfach* 3049, 675 *Kaiserslautern, German Federal Republic*

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1. Mitochondria were isolated from developing endosperm of *Ricinus communis* and were fractionated into outer membrane and inner membrane. The relative purity of the two membrane fractions was determined by marker enzymes. The fractions were also examined by negative-stain electron microscopy. 2. Membrane fractions were sequentially extracted in the following way. (*a*) Suspension in 0.5M-potassium phosphate, pH7.1; (*b*) suspension in 0.1M-EDTA (disodium salt)/0.05M-potassium phosphate, pH7.1; (*c*) sonication in 0.05M-potassium phosphate, pH7.1; (*d*) sonication in aq. Triton X-100 (0.1%). The membranes were pelleted by centrifugation at 100000*g* for 15min, between each step. Agglutination activity in the extracts was investigated by using trypsin-treated rabbit erythrocytes. 3. The addition of lactose to inner mitochondrial membrane resulted in the solubilization of part of the lectin activity, indicating that the protein was attached to the membrane via its carbohydrate-binding site. Pretreatment of the membranes with lactose before the usual extraction procedure showed that lactose could extract lectins that normally required more harsh treatment of the membrane for solubilization. 4. Lectins extracted from inner membranes were purified by affinity chromatography on agarose gel. Polyacrylamide-gel electrophoresis of purified samples in sodium dodecyl sulphate indicated that at least part of the lectin present in inner mitochondrial membrane was identical with the *R. communis* agglutinin of mol.wt. 120000.

For many years it has been recognized that proteins and glycoproteins capable of binding to specific carbohydrate residues can be extracted from seeds of many different plants (Sharon & Lis, 1972; Lis & Sharon, 1973). The carbohydrate-binding ability of seed lectins has been extensively utilized in studies of the structure and topography of surface membranes of animal cells, both during normal growth conditions and in the transformed state (Hakomori, 1975; Nicolson, 1976).

In contrast with the increased knowledge of membrane structure brought about by the utilization of lectins as an investigative tool, the role of carbohydrate-binding proteins in plants has remained a largely unresolved mystery. Bowles & Kauss (1975) showed that carbohydrate-binding proteins could be extracted from cellular membranes (dictyosomes, endoplasmic reticulum, plasma membrane and mitochondria) of plant tissue. The plant intracellular-membrane system has been shown to be involved in the synthesis and transport of secretory material destined for incorporation into the cell wall (Bowles & Northcote, 1972, 1974; Kauss, 1974; Bowles & Kauss, 1976*a*). A preliminary study also indicated that a mixture of proteins extracted from cell walls of *Phaseolus aureus* Roxb. exhibited sugar-binding activity (Kauss & Glaser, 1974). The possibility therefore existed that lectins recoverable in isolated membrane fractions only represented secretory proteins contained within the membranes during transport to the cell surface. In addition, it also appeared possible that soluble lectins present in the cytosol could bind to the sugar moieties of the glycoprotein and glycolipid components of cellular membranes during homogenization of the tissue.

Thus, in order to confirm our suggestion that lectins are indeed membrane components, mitochondrial membranes have been chosen for investigation. Such organelles can be obtained intact in a high degree of purity, are not involved in secretory processes, and the possibility of artificial binding of cytoplasmic lectins to inner membranes of mitochondria during initial grinding of the tissue is most unlikely.

Evidence is presented in this report to show that lectins, among them *R. communis* agglutinin 120, a lectin previously only extracted from seeds of *R. communis*, can be recovered from inner mitochondrial membranes. Lectin can be extracted by lactose, indicating that it is held within the membrane by its carbohydrate-binding site. The implications of these results are discussed in relation to the physiological function of lectins in plants, and the use of seed lectins in membrane research.

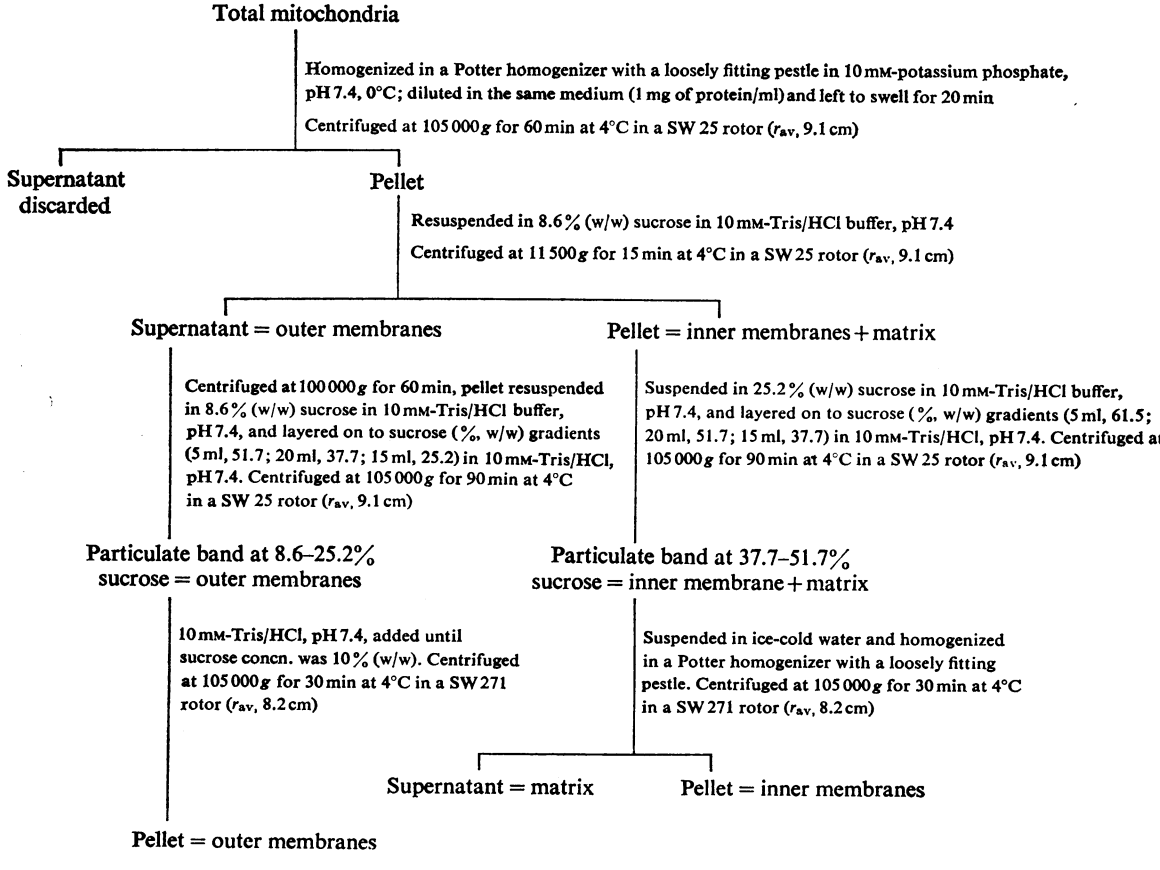
* Present address: Department of Biochemistry, University of Cambridge, Tennis Court Road, Cambridge CB2 1QW, U.K.

## Methods and Results

### *Isolation of structural compartments of mitochondria*

Mitochondria were isolated from endosperm (normally 66g) of 5-day-old germinating seeds of *R. communis* L. by isopycnic centrifugation in a sucrose gradient (Schnarrenberger *et al.*, 1971). Fractions corresponding to inner membrane, outer membrane and matrix were prepared by the method of Maisterrena *et al.* (1974). The fractionation procedure (Scheme 1) involved initial swelling and rupture of the mitochondria in 10mM-potassium phosphate buffer, pH 7.4, followed by separation of inner membrane, matrix and outer membranes by both differential and sucrose-gradient centrifugation. Rupture of the inner membrane by the swelling of isolated inner membrane and matrix in water resulted in the recovery of pure inner membranes and soluble matrix.

Marker enzymes were used for the determination of purity of the membrane fractions; methods for measurement of enzyme activities were identical with those used in the references cited. NADPH–cytochrome *c* reductase was used as a marker for outer membrane (Donaldson *et al.*, 1972), cytochrome *c* oxidase as a marker for inner membrane (Schnarrenberger *et al.*, 1971) and malate dehydrogenase as a marker for the matrix (Schnarrenberger *et al.*, 1971). The results of the enzyme activities are shown in Table 1. If protein contamination of the two membrane fractions by both matrix and intermembrane proteins is not taken into account, it can be calculated that the inner membranes are contaminated by 19% outer-membrane protein, and the outer membranes are contaminated by 8% inner-membrane protein. Thus cross-contamination of the two membranes is low. Malate dehydrogenase activity is still present




Scheme 1. *General procedure for the preparation of membrane fractions from mitochondria of Ricinus communis endosperm*

Table 1. *Specific activities of marker enzymes in fractions of mitochondria prepared from Ricinus communis endosperm*

Seeds of *R. communis* were germinated for 5 days. The endosperm was homogenized and used to prepare mitochondria by isopycnic sucrose-gradient centrifugation. Mitochondria were then fractionated into outer and inner membranes and matrix by differential and discontinuous sucrose-gradient centrifugation. Enzyme assays were made on 50 $\mu$l samples.

| Fraction | Vol. (ml) | Protein (mg/ml) | Specific activity (nmol/min per mg of protein) Malate dehydrogenase | Cytochrome *c* oxidase | NADPH–cytochrome *c* reductase |
|---|---|---|---|---|---|
| Total mitochondria | 30 | 4.2 | 18700 | 2050 | 1.88 |
| Outer membrane | 9 | 0.44 | 1720 | 400 | 9.45 |
| Inner membrane | 9 | 2.42 | 3160 | 3960 | 1.97 |
| Matrix | 55 | 0.2 | 8320 | 880 | 6.4 |

in the inner-membrane fraction, indicating that matrix proteins were not completely removed by the fractionation procedure. However, total activity of the enzyme was greatest in the soluble fraction. It was found that the specific activity of malate dehydrogenase increased linearly in outer and inner membranes and in the matrix fraction with increasing protein concentration used in the assay.

Isolated membranes were examined by negative staining with 2% (v/v) phosphotungstic acid, neutralized by NaOH to pH 6.8. Outer membranes were prefixed in 1% $OsO_4$ before negative staining. Samples were placed on Formvar-coated copper grids and viewed in a Zeiss electron microscope (Plate 1). The characteristic 'empty-sac' appearance of the vesicles (Plate 1*a* and 1*b*) are typical of isolated outer membranes from animal mitochondria (Maisterrena *et al.*, 1974), in contrast with the non-fixed preparations of inner membrane (Plates 1*c* and 1*d*). The convoluted nature of the cristae is clearly visible, and again agrees with negative-stained preparations of inner membrane from mitochondria of pig heart (Maisterrena *et al.*, 1974) and rat liver cells (Sottocasa *et al.*, 1967).

*Determination of agglutination activity in the isolated fractions*

All extraction procedures were carried out immediately on preparation of the isolated membranes, i.e. within 8h of the initial homogenization of the endosperm tissue. Pellets of inner and outer membrane were extracted sequentially in 4ml of extractant for 10min at 0°C, as follows. (1) Suspension in 0.5M-potassium phosphate buffer, pH 7.1 (extract 1). (2) Suspension in 0.1M-EDTA (disodium salt)/0.05M-potassium phosphate, pH 7.1 (extract 2). (3) Sonication for 5s (Braun Sonic 300) in 0.05M-potassium phosphate, pH 7.1 (extract 3). (4) Sonication for 5s in an aq. 0.1% Triton X-100 (extract 4). Each extraction step was performed twice, and between each step the membranes were re-pelleted by centrifugation at 100000*g* for 15min. All centrifugations were carried out at 4°C, in a Beckman Spinco L2 centrifuge, with a SW50 L rotor ($r_{av.} = 7.3$cm).

The two extracts from each step were combined, dialysed extensively for 20–40h against 4×5 litres of 0.05M-sodium/potassium, phosphate, pH 7.4, containing 0.9% NaCl phosphate-buffered saline). The non-diffusible material from each membrane extract was made up to equal volume with the phosphate-buffered saline and the matrix fraction was dialysed directly against this buffer. Protein in the non-diffusible material was determined by the method of Lowry *et al.* (1951), with bovine serum albumin (Sigma Chemical Co., München, Germany) as a standard. Agglutination activity was tested by using the Microtitre-plate method as described previously (Kauss & Glaser, 1974; Bowles & Kauss, 1975), against trypsin-treated rabbit erythrocytes prepared from fresh blood by the method of Sharon & Lis (1972).

The results of agglutination activity in extracts 1–4 of the membrane fractions are shown in Table 2. Only those membrane components of the outer membrane solubilized by high-molarity phosphate buffer show agglutination activity against rabbit erythrocytes. In contrast, the agglutination activity of all the extracts of the inner membrane is very high, indicating that all the extractants used are effective in the elution of proteins from inner membrane that exhibit carbohydrate-binding characteristics. The sequential extraction procedure ensures that there is a progressive solubilization of the membranes. The results indicate that agglutinins may be eluted both by extractants normally assumed to release proteins loosely bound to the membrane, e.g. extractants 1–3, and by those used for proteins tightly bound, e.g. detergent (Coleman, 1973; Helenius & Simons, 1975).

It is of interest that, when extractant 1 was replaced by acetic acid, a procedure that is generally used to

Table 2. *Agglutination activity exhibited by extracts of isolated inner and outer mitochondrial membranes*

Inner and outer membranes were isolated by differential and discontinuous sucrose-gradient centrifugation. Membrane pellets were extracted sequentially with the solutions indicated in the Table. Agglutination activity of the extracts (in total vol. 12.0ml) was determined by the titre-plate method with trypsin-treated rabbit erythrocytes.

| | Membrane type ... | Outer membranes | | Inner membranes | |
|---|---|---|---|---|---|
| Extract | Extractant | Total protein (mg recovered from 66g fresh wt. of endosperm) | Agglutination activity (titre/25 $\mu$l) | Total protein (mg recovered from 66g fresh wt. of endosperm) | Agglutination activity (titre/25 $\mu$l) |
| 1 | 0.5M-Potassium phosphate, pH7.1 | 0.181 | 128 | 1.380 | 131072 |
| 2 | 0.1M-EDTA (disodium salt)/0.05M-potassium phosphate, pH7.1 | 0.178 | 0 | 0.756 | 32768 |
| 3 | Sonication in 0.05M-potassium phosphate, pH7.1 | 0.065 | 0 | 2.880 | 4096 |
| 4 | Sonication in aq. 0.1% Triton X-100 | 0.175 | 0 | 6.840 | 524288 |

Table 3. *Inhibition of the agglutination activity recovered in extracts of inner mitochondrial membranes by sugars and glycosides*

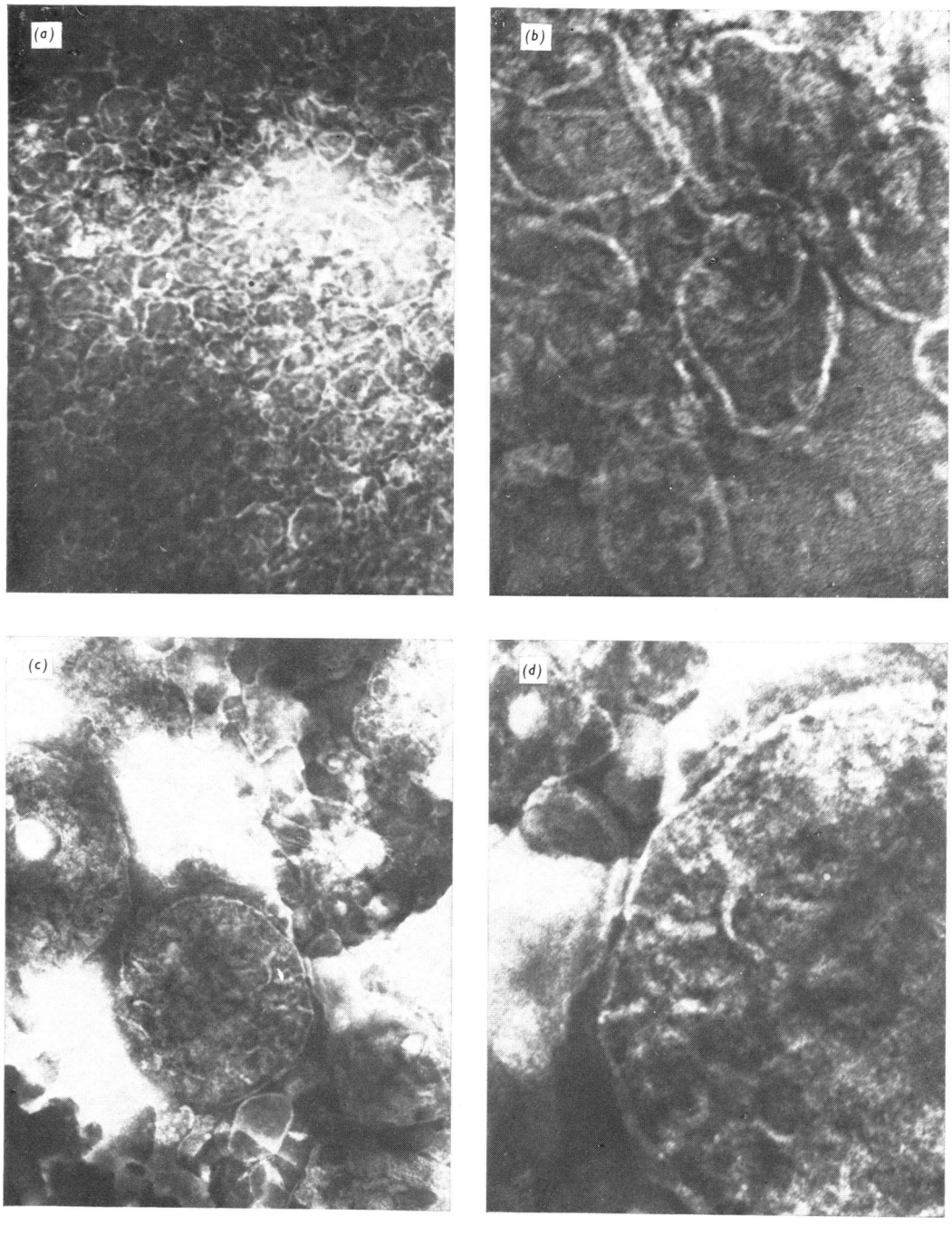
Inner mitochondrial membranes were isolated and sequentially extracted. The action of various sugars and glycosides on the agglutination activity of the extracts was investigated by the titre-plate method. Extracts were diluted differentially with phosphate-buffered saline before the inhibition tests. Results from one representative investigation out of ten experiments are given (see the text for degree of variation). For details of extraction see Scheme 1 and Table 2.

| | | Agglutination activity (titre exhibited by 25 $\mu$l of extract) | | | |
|---|---|---|---|---|---|
| Sugar or glycoside tested at a final concn. of 5mM in phosphate-buffered saline | Extract ... | 1 | 2 | 3 | 4 |
| No sugar added (control) | | 2048 | 64 | 64 | 1024 |
| Lactose | | 4 | 2 | 8 | 64 |
| *N*-Acetyl-D-galactosamine | | 512 | 32 | 32 | 512 |
| Methyl $\alpha$-D-galactopyranoside | | 16 | 16 | 16 | 512 |
| Methyl $\beta$-D-galactopyranoside | | 4 | 2 | 8 | 1074 |

remove extrinsic proteins of mitochondrial inner membranes (Fleischer *et al.*, 1968), lectin activity was also observed in the extract. However, when identical amounts (1.62mg) of inner membrane were used, the titre in comparable extracts (7ml) of acetic acid and high-molarity phosphate buffer was similar (respective titres: 128 and 256), whereas the protein in the same extracts was 324 and 117 $\mu$g respectively. Further extraction of the acetic acid-treated membranes indicated that substantial lectin activity was still present in the membrane and was not denatured by acid. The results show that acid extraction removed more non-lectin protein and was therefore less specific than extraction with high-molarity phosphate buffer.

The high agglutination activity recovered in extracts of inner membrane is in contrast with that obtained for the matrix fraction. A titre of 256 was given by 25 $\mu$l, from a 60ml matrix fraction containing 9.08mg of total protein, prepared from 66g of endosperm. In addition to the membrane-bound agglutinating activity appreciable activity was also found in the soluble fraction on the top of the initial sucrose gradient used to prepare mitochondria. It was not possible to directly compare these two types of binding activities, since there was always a gradual solubilization of agglutinins from the membranes during each of the numerous purification and washing steps (see also the results for the mannitol control in Table 4).

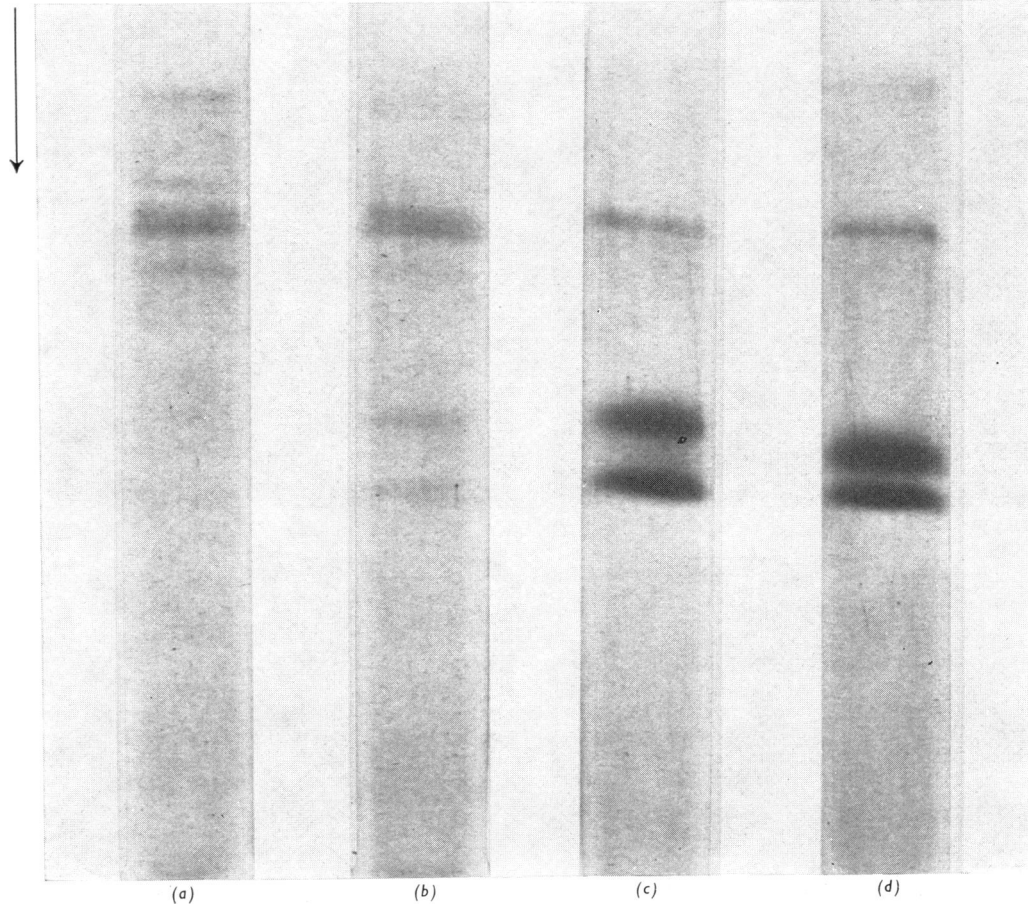
The ability of different sugars and glycosides to inhibit lectin activity in the four extracts of inner membrane was also investigated. Commercial samples of sugars were obtained from either Serva (Heidelberg, Germany), Sigma or Ferak (Berlin, Germany). Extracts were prepared as before from 2.7mg of inner membrane, dialysed against the phosphate-buffered saline, and the non-diffusible material for each extract was made up to 7.0ml. The results from a representative experiment are given in Table 3.




EXPLANATION OF PLATE 1

*Electron microscopy of negative-stained preparations of mitochondrial membranes*

(*a*) and (*b*), Outer membranes fixed in $OsO_4$ before negative staining with sodium phosphotungstate (*a*, ×28000; *b*, ×150000); (*c*) and (*d*), inner membranes, negative-stained with sodium phosphotungstate (*c*, ×31000; *d*, ×111000).




## EXPLANATION OF PLATE 2

*Disc electrophoresis of extracts from mitochondrial inner membrane*

The direction of migration is from the top. Electrophoresis of the samples was performed in 7.5% (w/v) acrylamide gel containing 0.1% sodium dodecyl sulphate, as described in the text. Gels were stained for protein by using Coomassie Brilliant Blue B250. (*a*) Sucrose extract of the membranes; (*b*) lactose extract; (*c*) *Ricinus* agglutinin, mol.wt. 120000; (*d*) *Ricinus* agglutinin, mol.wt. 60000.

Table 4. *Effect of* 0.1 M-*lactose on solubilization of agglutinins and proteins from mitochondrial inner membrane*

Portions (6.47mg) of isolated inner membrane were first extracted in 0.05M-Hepes/NaOH, pH7.0, containing either 0.1M-lactose or 0.1M-mannitol. After centrifugation at 100000*g* for 15min at 4°C, the membrane pellets were sequentially extracted in the solutions indicated. Agglutination activity of the various extracts (total vol. 7ml) was determined by the titre-plate method; data from two parallel determinations on each extract are given.

| Composition of the first extractant ... | Lactose | | Mannitol | |
|---|---|---|---|---|
| Extractant | Titre | Protein (μg/ml) | Titre | Protein (μg/ml) |
| Material solubilized by first extractant | 2048; 4096 | 51 | 8; 8 | 33 |
| 0.5M-Potassium phosphate, pH7.1 | 2048; 4096 | 52 | 4096; 4096 | 50 |
| 0.1M-EDTA (disodium salt)/0.05M-potassium phosphate, pH7.1 | 64; 128 | 21 | 512; 512 | 24 |
| Sonication in 0.05M-potassium phosphate, pH7.1 | 16; 16 | 24 | 64; 128 | 40 |
| Sonication in aq. 0.1% Triton X-100 | 4096; 4096 | 255 | 8192; 8192 | 301 |

Galactosides containing both anomers of the sugar were the best inhibitors of lectin action. The β-anomers generally exhibited a more pronounced effect than the α-anomers. In other experiments, a similar influence was found by D-galactose, *p*-nitrophenyl α-D-galactoside and β-D-galactoside, raffinose, melibiose and stachyose, whereas smaller inhibitory effects were found with L-rhamnose and L-arabinose. The degree of inhibition by lactose, the most potent general inhibitor, varied considerably among the ten experiments. The inhibition was always high with extracts 1 and 2, and less pronounced with extract 3. The variation in the degree of inhibition by lactose and other sugars was greatest with extract 4 (ranging, for example, from almost complete inhibition by lactose with ten dilution steps difference to little inhibition with only one dilution step difference). However, despite these variations, the inhibition studies strongly suggest that an agglutinin in the crude extracts from the inner membrane has binding properties very similar to those of lectins isolated from seeds of *R. communis* (Kruppe, 1955; Drysdale *et al.*, 1968; Pardoe *et al.*, 1969; Nicolson & Blaustein, 1972; Nicolson *et al.*, 1974).

*Solubilization of lectins from the inner mitochondrial membrane by lactose*

The observations that lectin activity was high in extracts of inner membranes and that the activity was strongly inhibited by lactose led to an investigation into the extractability of lectins from inner membranes by lactose.

Portions (e.g. 6.47mg of protein/sample) of membrane were suspended in 4.5ml of either 0.1M-lactose or 0.1M-mannitol, dissolved in 0.05M-Hepes [2-(*N*-2-hydroxyethylpiperazin-*N'*-yl)ethanesulphonic acid] adjusted to pH7.0 by the addition of NaOH. Membranes were re-pelleted by centrifugation at 100000*g* for 15min at 4°C by using a SW50 L rotor head. Each subsequent pellet was then sequentially extracted with different solutions as described above. All extracts were dialysed against phosphate-buffered saline, and lectin activity in the non-diffusible material from each extract was determined.

Table 4 shows that suspension of inner membrane in 0.1M-lactose causes an extraction of lectin activity. This effect cannot be explained by a non-specific solubilization of proteins by the relatively high concentration of the extractant, since lectin activity in the 0.1M-mannitol extract is low. Similar results were observed when sucrose was used instead of mannitol in the control experiment.

To determine the effectiveness of only one extraction by 0.1M-lactose, a portion (1.62mg of protein) of inner membrane was sequentially extracted three times by the sugar, by using methods identical with those already described. The titres exhibited by 25μl samples (from total extract vol. 7ml) were 128 for the first extraction, 4 for the second extraction and 2 for the third. After the three lactose treatments an appreciable amount of lectin activity was still left in the membranes, as shown by further sequential extraction with high concentrations of phosphate buffer and Triton X-100 (titres of 16 and 512 respectively). However, the data do indicate that one extraction by 0.1M-lactose is sufficient to solubilize a great proportion of that part of the lectin that is accessible to the sugar.

The results from a representative study of the effect of treatment of inner membranes with different extractants is shown in Table 5. Portions (1.62mg of protein) of inner membrane were initially suspended in 4ml of the following extractants: 0.1M-lactose in 0.05M-Hepes/NaOH, pH7.0; 0.5M-potassium phosphate; aq. 1% Triton X-100; 0.05M-Hepes/NaOH, pH7.0. Each suspension was centrifuged at 100000*g* for 15min (4°C, SW50 L rotor head), and the resulting membrane pellets were sequentially extracted as described above. Equivalent samples from each

Table 5. *Effect on solubilization of agglutinins and proteins from inner membranes by different extractants*

Equivalent samples (1.62mg of protein) of isolated inner membrane were suspended in different extractants. After centrifugation at 100000*g* for 15min at 4°C, the membrane pellets were then sequentially extracted in the solutions indicated. Agglutination activity was determined by the titre-plate method.

| Composition of the first extractant ... | 0.1M-Lactose in 0.05M-Hepes/NaOH, pH7.0 | | 0.5M-Potassium phosphate, pH7.1 | | 0.1% aq. Triton X-100 | | 0.05M-Hepes/NaOH, pH7.0 | |
|---|---|---|---|---|---|---|---|---|
| Extractant | Titre | Protein (μg/ml) | Titre | Protein (μg/ml) | Titre | Protein (μg/ml) | Titre | Protein (μg/ml) |
| Material solubilized by first extractant | 256 | 26 | 512 | 26 | 512 | 64.8 | 8 | 23 |
| 0.5M-Potassium phosphate, pH7.1 | 32 | 5.6 | 1 | 1.7 | 512 | 8.5 | 512 | 11 |
| 0.1M-EDTA (disodium salt)/0.05M-potassium phosphate, pH7.1 | 8 | Trace | 8 | Trace | 8 | Trace | 8 | Trace |
| Sonication in 0.05M-potassium phosphate, pH7.1 | 8 | 5.2 | 16 | 6.9 | 4 | 0.8 | 16 | 4.7 |
| Sonication in aq. 1% Triton X-100 | 64 | 54.0 | 512 | 56.6 | 16 | 14.0 | 512 | 53.3 |

extract were tested for agglutination activity and the amount of protein present was determined. The action of either 0.5M-phosphate buffer or detergent on the membrane is clearly different from that of lactose. The proportion of lectin recovered in extract 1, as determined by titre, was unaffected by detergent pretreatment. Similarly, pretreatment with high-molarity of phosphate buffer did not result in a decrease in the proportion of lectin recoverable in extract 4.

*Purification and characterization of the agglutinins from mitochondrial inner membrane*

Lectins in inner-membrane extracts 1 and 4, and those solubilized by lactose, were purified by affinity chromatography on Bio-Gel-A/0.5M-agarose by the method of Nicolson *et al.* (1974). The column (bed vol. 12.0ml, height 25.0cm) was washed with 50ml of phosphate-buffered saline to remove unbound protein, and the agglutinins were eluted with 50ml of 0.2M-lactose in the buffer. The column eluates and the total extracts were dialysed against water, and the non-diffusible material was freeze-dried. Samples were taken up in 100μl of the sample buffer containing 0.1% sodium dodecyl sulphate and 2μl of β-mercaptoethanol, heated for 10min at 70°C, and subjected to electrophoresis in 7.5% (w/v) polyacrylamide gels containing sodium dodecyl sulphate (Davis, 1964). Under these conditions, commercial *Ricinus communis* agglutinin RCA 120 (Miles–Yeda, Lausanne, Switzerland) is split into subunits of 37000 and 29500 mol.wt. (Nicolson *et al.*, 1974). These two bands could be observed in all of the crude extracts 1–4 from inner membranes. Further, the agglutinins purified from extracts 1 and 4 by affinity chromatography exhibited the two bands characteristic of the agglutinin RCA 120 subunits. In some experiments a faint band representative of the 34000-mol.wt. subunit of agglutinin RCA 60 (Nicolson *et al.*, 1974) was also observed. The distribution of protein bands on gels, after electrophoresis of lactose and sucrose extracts of inner membrane, is shown in Plate 2. It is clearly evident that the lactose extract contains the protein bands characteristic of those derived from agglutinin RCA 120. In addition, other proteins common to those of the sucrose extract are also present, and are therefore not released specifically by lactose.

## Discussion

Agglutinins isolated from plant seeds have been widely used as investigative tools in membrane research because of their ability to bind specific carbohydrate groups exposed at the surface of animal cells (Sharon & Lis, 1972; Lis & Sharon, 1973; Nicolson, 1974). The physiological role of lectins in plants has been an intriguing but unresolved problem. One report, on the formation of root nodules in *Glycine max*, indicated that lectins may be involved in a recognition of symbiotic bacteria by the root surface, since an extract isolated from root tissue of soya-bean could agglutinate only those strains of bacteria known to form nodules (Bohlool & Schmidt, 1974).

We have shown that carbohydrate-binding proteins could be extracted from isolated plant membrane fractions of endoplasmic reticulum, dictyosomes and plasma membranes (Bowles & Kauss, 1975, 1976*a*). However, since such membranes constitute the intracellular transport system, it was not possible to differentiate unequivocally between the alternatives that the lectins recovered in membrane extracts were secretory proteins contained within isolated vesicles, or were structural components of the membranes.

Our demonstration that lectins can be extracted from mitochondrial inner membranes of *R. communis* endosperm provides strong evidence to suggest that lectins are located generally in membranes of plant tissue. It is probable that the presence of lectins in soluble fractions of seeds may be due only to progressive solubilization of the proteins during the extraction of the seeds. The fact that the lectins can be extracted from the mitochondrial membrane by lactose indicates that the specific carbohydrate-binding ability of the lectin is involved in holding the protein within the membrane. The implications of these observations with respect to the physiological function of lectins and the structure of membranes are considerable.

Much evidence has accumulated to suggest that an essential feature of the general process of membrane fusion is that it is highly specific and shows a high degree of spatial and temporal organization (Poste & Allison, 1973). If, as our results suggest, lectins are indeed plant-membrane components, the stage of initial recognition and contact between membranes in the fusion process may be mediated by a reversible binding between a lectin of one membrane and a complementary receptor on another. This mechanism could provide a basis for recognition and contact between different intracellular membranes during the fusion processes that form the basis of endomembrane flow within the cell.

It is considered most probable that membranes from both plant and animal sources have many structural and functional features in common. The presence of lectins in plant cell membranes allows the suggestion that analogous sugar-binding proteins or glycoproteins may also be present in membranes of animal cells. Recent experimental evidence provides support for the suggestion, since developmentally regulated lectins can be isolated from muscle cells (Nowak *et al.*, 1976), and a lectin extracted from plasma membrane of bovine liver can bind to receptors on cellular membranes *in vitro* (Bowles & Kauss, 1976*b*). Thus the possibility exists that the action of seed lectins *in vitro* mimics the action of endogenous membrane lectins.

The sequential extraction procedure used in the present investigation was an attempt to disrupt the mitochondrial inner membrane progressively, and to some extent to discriminate between characteristics of peripheral and integral membrane proteins (Singer & Nicolson, 1972). It is generally assumed that only integral proteins are critical for the maintenance of structural integrity of membranes and that the peripheral proteins, defined as those easily dissociated from the membrane, possess a relatively unimportant structural role (Singer & Nicolson, 1972; Coleman, 1973; Gulik-Krzywicki, 1975). However, lectin activity was recovered in all extracts of the inner membrane. This indicated that lectins were solubilized both by procedures normally regarded to release mainly peripheral-bound membrane components (high-molarity buffer or chelating agents) and by those that solubilize integral components (detergent). The release of lectins by this variety of procedures may be due either to the direct extraction from the membrane of the lectin molecule itself, or by an indirect release of the lectin by solubilization of the endogenous lectin receptor. The lectin receptors may be glycoproteins, intercalated hydrophobically into the lipid bilayer of the membrane, or glycolipid molecules, either of which would be only solubilized by the disturbance of the hydrophobic regions of the membrane by detergent action. The presence of both lectin and lectin receptor in the detergent extract may therefore be a possibility, and the titre subsequently exhibited by the extract against trypsin-treated rabbit cells may reflect the relative binding affinities of the lectin to the endogenous and assay receptors.

Results of pretreatment of inner membrane with lactose, before the usual sequential extraction, indicated that the lactose was able to extract those lectins usually solubilized by the full spectrum of extractants, i.e. pretreatment of the membrane with lactose led to a loss in recovery of lectin activity in subsequent extracts obtained by high-molarity buffer, chelating agent and detergent. Pretreatment of the membrane with extractants other than lactose gave very different results. High-molarity phosphate buffer did not elute lectins usually extractable with detergent, and, similarly, detergent action did not result in the elution of lectins extractable with high-molarity buffer.

Thus it is clear that at least some of the lectin is held within the membrane by its sugar-binding site and is released by the addition of the hapten inhibitor, lactose. However, there is also considerable lectin activity that cannot be released by the hapten, but is released by more harsh treatment of the membrane. Whether this lectin is also held within the membrane via sugar-binding sites inaccessible to the lactose molecule, or whether the lectin itself is intercalated into the lipid bilayer and requires detergent action for its removal, remains an unresolved question.

The observations strongly suggest, however, that lectin–receptor binding occurs within membranes and may therefore be involved in the maintenance of membrane structure. Interaction between carbohydrate-binding membrane components and sugar moieties of adjacent glycoprotein or glycolipid macromolecules could provide a sensitive reversible mechanism for rapid structural rearrangements and lateral movements of components within a fluid lipid matrix.

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