Aust. J. Biol. Sci., 1978, **31**, 149–60

Reaction of Some Invertebrate and Plant Agglutinins and a Mouse Myeloma Anti-galactan Protein with an Arabinogalactan from Wheat

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Abstract

Plant, invertebrate and vertebrate proteins which show anti-galactan combining specificities were used in precipitation and inhibition studies with arabinogalactan preparations from wheat and ryegrass (*Lolium multiflorum*). Of the agglutinins studied, only mouse anti-galactan myeloma protein J539 showed strong reactivity with wheat arabinoglactan–peptide. Weak reactions were observed with the agglutinins from the clam *Tridacna maxima*, the sponge *Axinella polypoides* and the anemone *Cerianthus membranaceus*. No reactions were detected with lectins from the plants *Abrus precatorius* and *Ricinus communis*. Reactions readily occurred between *Lolium* arabinogalactan–protein and the invertebrate and vertebrate agglutinins. Removal of terminal arabinosyl residues from the wheat and *Lolium* arabinogalactans either by mild acid hydrolysis or by treatment with an arabinofuranosidase increased the reactivity of both peptidoglycans with all of the agglutinins examined except the *Ricinus* RCA_I lectin. Results obtained with wheat arabinogalactan indicate that few D-galactose units are terminal and available for reaction. The difference in reactivities between the wheat and *Lolium* arabinogalactans may be due to the differences in the galactose:arabinose ratios or to differences in linkage of the galactosyl residues on the two peptidoglycans, or both. Results indicate that the mouse anti-galactan could be a useful reagent for the subcellular localization of wheat arabinogalactan and that tridacnin and *Axinella* agglutinins could be used to localize the arabinogalactan in *L. multiflorum* cells.

Introduction

The immunochemical specificities of the invertebrate anti-galactan agglutinins from the clam *Tridacna maxima*, the marine sponge *Axinella polypoides*, and the anemone *Cerianthus membranaceus* have been the subject of a number of recent papers (Baldo and Uhlenbruck 1975*a–d*; Baldo *et al.* 1977*a*, 1977*b*). This paper extends these observations and compares their immunological reactivity with two plant anti-galactan lectin preparations from *Ricinus communis* and *Abrus precatorius* and a mouse myeloma protein with specificity for 1,6-β-galactans.

Two representatives of a widely distributed group of water-soluble plant arabinogalactans (Aspinall 1973) and their derived galactans were examined in haemagglutination inhibition and precipitation studies. The first was an arabinogalactan–peptide isolated from aqueous extracts of wheat flour (Fincher and Stone 1974; Fincher *et al.* 1974; Neukom and Markwalder 1975) which has a branched β-galactan backbone onto which are attached arabinofuranosyl residues. The galactan chains are linked 1,3- and 1,6- and there are branch points through 1,3,6-linked galactosyl residues

(Neukom and Markwalder 1975; Neukom 1976; Anderson *et al.* 1977). Removal of the arabinosyl residues which are attached terminally to the molecule leads to a large increase in the proportion of 1,6-galactosyl residues, whilst the proportion of 1,3-galactosyl residues changes to a much smaller extent. This suggests that the arabinosyl residues are attached mainly to 1,6-linked galactosyl residues (Neukom 1976; Anderson *et al.* 1977). Experiments with both galactose oxidase and *Ricinus* lectin indicated that a few terminal unsubstituted galactosyl residues are present (Fincher *et al.* 1974).

The second polysaccharide studied was an arabinogalactan–protein isolated from the cells and culture media of *Lolium multiflorum* endosperm grown in tissue culture (Anderson *et al.* 1977). The polysaccharide portion of this molecule also has a 1,3:1,6 β-galactan core but its detailed structure, as shown by methylation analysis, differs from that of the wheat arabinogalactan–peptide (Anderson *et al.* 1977). It is also larger (molecular weight $2{\cdot}2 \times 10^5$–$2{\cdot}8 \times 10^5$) than the wheat arabinogalactan–peptide (molecular weight 2×10^4) (Fincher *et al.* 1974).

Materials and Methods

Polysaccharides and Glycopeptides

The wheat arabinogalactan–peptides were prepared as described previously (Fincher *et al.* 1974; Neukom and Markwalder 1975). Ryegrass (*L. multiflorum*) arabinogalactan–protein was obtained from cultures of endosperm cells as described by Anderson *et al.* (1977). These preparations are henceforth referred to as wheat arabinogalactan and ryegrass (or *Lolium*) arabinogalactan. Their compositions are shown in Table 1. Arabinose-'free' wheat galactan–peptide and ryegrass galactan–protein (henceforth termed wheat galactan and ryegrass galactan for convenience) were obtained by hydrolysis with dilute oxalic acid (Fincher *et al.* 1974) and by reaction of wheat arabinogalactan with an α-L-arabinofuranosidase. The enzyme treatment removed approximately 90% of the L-arabinose residues (Neukom and Markwalder 1975). The arabinoxylan from wheat endosperm cell walls was isolated from wheat flour by the method of Mares and Stone (1973*a*, 1973*b*). Other polysaccharides and glycopeptides examined were obtained as previously described (Baldo and Uhlenbruck 1975*c*; Uhlenbruck *et al.* 1975).

Invertebrate and Plant Agglutinins

Agglutinins from the elongate clam *Tridacna maxima*, the sponge *Axinella polypoides*, and the tube anemone *Cerianthus membranaceus* were prepared by affinity chromatography on Sepharose (Pharmacia) or acid-treated Sepharose. Tridacnin was isolated from previously dialysed and lyophilized *T. maxima* haemolymph using, as affinity adsorbent, the product formed from the co-polymerization of larch arabinogalactan with the *N*-carboxyanhydride of L-leucine (Baldo and Uhlenbruck 1975*b*) or from columns of acid-treated Sepharose (Ersson *et al.* 1973; Baldo and Uhlenbruck 1975*d*; Baldo *et al.* 1977*a*). Agglutinins from *A. polypoides* and from tentacles of *C. membranaceus* were isolated by adsorption to, and subsequent elution from, Sepharose columns (Eichmann *et al.* 1976; Baldo *et al.* 1977*a*, 1977*b*). In experiments with *A. polypoides*, agglutinins eluted from Sepharose were used in haemagglutination inhibition studies but, because of the small yields obtained (Baldo *et al.* 1977*a*, 1977*b*), a partially purified fraction was used for precipitation studies.

Castor beans (*Ricinus communis* cv. Zanzibarensis) were obtained commercially and saline extracts prepared by homogenization in a Waring blender and subsequent centrifugation. *Ricinus* lectins were purified by affinity chromatography on Sepharose 4B and gel filtration (Nicolson and Blaustein 1972; Tomita *et al.* 1972; Nicolson *et al.* 1974). The lectins RCA_I and RCA_{II}, both of which bind to Sepharose, were eluted with 0·2 M D-galactose and subsequently separated on a column of Sephadex G100 in phosphate buffered saline. Although β-lactose and D-galactose inhibit both lectins, the specificities of RCA_I and RCA_{II} can be distinguished by the fact that *N*-acetyl-D-galactosamine is a potent inhibitor of RCA_{II} but shows little activity against RCA_I (Nicolson and Blaustein 1972).

Abrus precatorius seeds were kindly donated by Dr B. P. Chatterjee, India. A crude extract was prepared by allowing the seeds to swell for 5 days at 4°C in saline containing 0·2% sodium azide and grinding in a mortar with the addition of sufficient saline-azide to give a potent haemagglutinating solution (titre against human group O erythrocytes 2^{14}–2^{16}). Extracts were allowed to stand for 2–3 weeks at 4°C before being centrifuged and used for the preparation of purified *Abrus* agglutinins. Purification was achieved by passing crude extract through a column of Sepharose–Con A (Pharmacia) and eluting the adsorbed agglutinins with 0·1 M α-methyl mannoside. After removal of the sugar, the eluate was concentrated before passing through a small column of acid-treated Sepharose 4B (Ersson *et al.* 1973). Bound lectin was eluted with 0·1 M D-galactose. Full details of this procedure will be published (Baldo and Uhlenbruck, unpublished data).

The isolated plant and invertebrate agglutinins were examined by disc gel electrophoresis using 5, 7 and 9% acrylamide gels (Davis and Ornstein 1968).

Table 1. Composition of purified wheat and ryegrass peptidoglycans examined

n.d., Not determined

Peptidoglycan	Composition (%)		% Polysaccharide as:			Ratio
	Polysaccharide	Protein	Arabinose	Galactose	Xylose	Gal:Ara
Wheat arabinogalactan–peptide[A]	93·9	6·1	40·8	58·4	0·8	1·4
Wheat arabinogalactan–peptide[B]	92	8	40·8	59·2	—	1·5
Lolium arabinogalactan–protein[C]	n.d.	n.d.	n.d.	n.d.	—	1·7

[A]Neukom and Markwalder (1975). [B]Fincher *et al.* (1974). [C]Anderson *et al.* (1977).

Mouse Myeloma Antiserum

Ascites fluid from mice bearing myeloma J539 was generously provided by Dr M. Potter, National Institutes of Health, Bethesda, Maryland, U.S.A. This tumour cell line was originally induced in the laboratory of Dr M. Cohn, Salk Institute, San Diego, U.S.A. The galactan-specific antibodies in ascites fluid J539 belong to the IgA class. Due to the small amount of ascites fluid available, unfractionated fluid was used in most experiments but a few experiments with the purified IgA antibodies were carried out as a check. Purification was achieved using affinity chromatography on Sepharose as already described (Eichmann *et al.* 1976).

Immunological Methods

The haemagglutination and haemagglutination inhibition methods used have been described (Baldo and Boettcher 1970; Baldo 1972). Human erythrocytes were obtained from the Red Cross Blood Transfusion Service, Perth, W.A. Solutions used in inhibition experiments were prepared by techniques applicable to quantitative analytical procedures. Quantitative precipitin experiments were carried out on a microscale as described (Kabat 1961; Baldo and Uhlenbruck 1975*a*) using the ninhydrin procedure for colour development (Schiffman *et al.* 1964). Gel immunodiffusion examinations were carried out in Petri dishes using 1·5% Special Noble Agar (Difco Labs, Detroit, Michigan) containing 0·85% sodium chloride, 0·1% sodium azide and 0·001 M Ca^{2+}. Immunoelectrophoresis was performed according to the procedures of Scheidegger (1955) as described by Baldo (1973). Gels consisted of 1% Oxoid purified agar in barbiturate buffer pH 8·6. Both immunodiffusion and immunoelectrophoresis gels were washed with saline–Ca^{2+} before staining with amido black.

Results

Haemagglutination Inhibition Studies

The wheat arabinogalactan, the *Lolium* arabinogalactan, and derived galactans were examined for their capacities to inhibit the agglutination of human group O erythrocytes by the tridacnin, *Axinella*, *Cerianthus*, *Abrus* and *Ricinus* agglutinins.

Detailed inhibition studies using these agglutinins with a wide range of saccharide-containing preparations have been published (Pardoe *et al.* 1969, 1970; Nicolson and Blaustein 1972; Nicolson *et al.* 1974; Baldo and Uhlenbruck 1975*c*; Uhlenbruck *et al.* 1975; Baldo *et al.* 1977*a*). Both preparations of the native wheat arabinogalactan tested proved weak inhibitors of tridacnin, *Axinella* and *Cerianthus* agglutinins and they did not inhibit the *Abrus* and *Ricinus* agglutinins at the concentrations tested. Both wheat galactan preparations were potent inhibitors of tridacnin, *Axinella* and *Ricinus* RCA_{II} agglutinins and weaker inhibitors of the *Cerianthus* and *Abrus* agglutinins. Agglutinations induced by the mixed *Ricinus* agglutinins (RCA_I+RCA_{II}) and RCA_I alone were not inhibited by wheat galactan (Table 2). Although the RCA_I and RCA_{II} lectins were each inhibited by D-galactose and lactose, the latter agglutinin could be distinguished by its reaction with *N*-acetyl-D-galactosamine. The inhibition

Table 2. Inhibition by wheat and ryegrass peptidoglycans of invertebrate and plant anti-galactan agglutinins

—, No inhibition up to a concentration of 2 mg/ml. n.t., Not tested

Test substance	Minimum amount of substance (μg/ml) completely inhibiting the agglutination of human group O erythrocytes by agglutinins[A] from:						
	Tridacna maxima	*Axinella polypoides*	*Cerianthus membranaceus*	*Abrus precatorius*	*Ricinus communis* RCA_I+RCA_{II}[B]	*Ricinus communis* RCA_I[C]	*Ricinus communis* RCA_{II}[C]
Wheat arabinogalactan–peptide[D]	1000	289	336	—	—	—	—
Wheat galactan–peptide[D]	0·5	1·1	134	33·6–67·2	—	—	13–26
Wheat arabinogalactan–peptide[E]	1200	254	250	—	—	—	—
Wheat galactan–peptide[E]	3·6	3·6–7·2	114	450	—	—	11–22
Wheat arabinoxylan[F]	1950	—	—	—	—	—	—
Lolium arabino-galactan–peptide[G]	9–18	18–36	36–72	—	—	n.t.	n.t.
Lolium galactan–protein	0·98	7·8	15–30	63	—	—	3·9

[A] Eight haemagglutination doses of each agglutinin preparation used. Results were read 30 min after addition of erythrocytes. [B] Agglutinins eluted from Sepharose. [C] Agglutinins from Sepharose separated on Sephadex G100 (Nicolson and Blaustein 1972). [D] Neukom and Markwalder (1975). [E] Fincher *et al.* (1974). [F] Mares and Stone (1973*a*, 1973*b*). [G] Anderson *et al.* (1977).

of *Cerianthus*-induced haemagglutination by wheat galactan was only marginally better than the inhibition observed with wheat arabinogalactan (Table 2). The pattern of reactivity observed with the wheat preparations was largely reflected in the results obtained with the ryegrass preparations except that the *Lolium* arabinogalactan proved quite active against the tridacnin, *Axinella* and *Cerianthus* agglutinins. Wheat arabinoxylan weakly inhibited tridacnin but proved inactive in haemagglutination inhibition with the other four agglutinin preparations (Table 2).

Gel Precipitation and Immunoelectrophoresis Studies with Tridacnin and Myeloma Protein J539

Gel immunodiffusion experiments using tridacnin with wheat and ryegrass preparations revealed that whereas both the *Lolium* preparations and the wheat galactan

precipitated, the wheat arabinogalactan and arabinoxylan preparations did not. These results are clearly shown in Figs 1*a–d*. The mouse anti-galactan serum J539 precipitated with both the wheat and ryegrass arabinogalactans and the galactans prepared from these native preparations. No precipitin lines were observed with the wheat arabinoxylan.

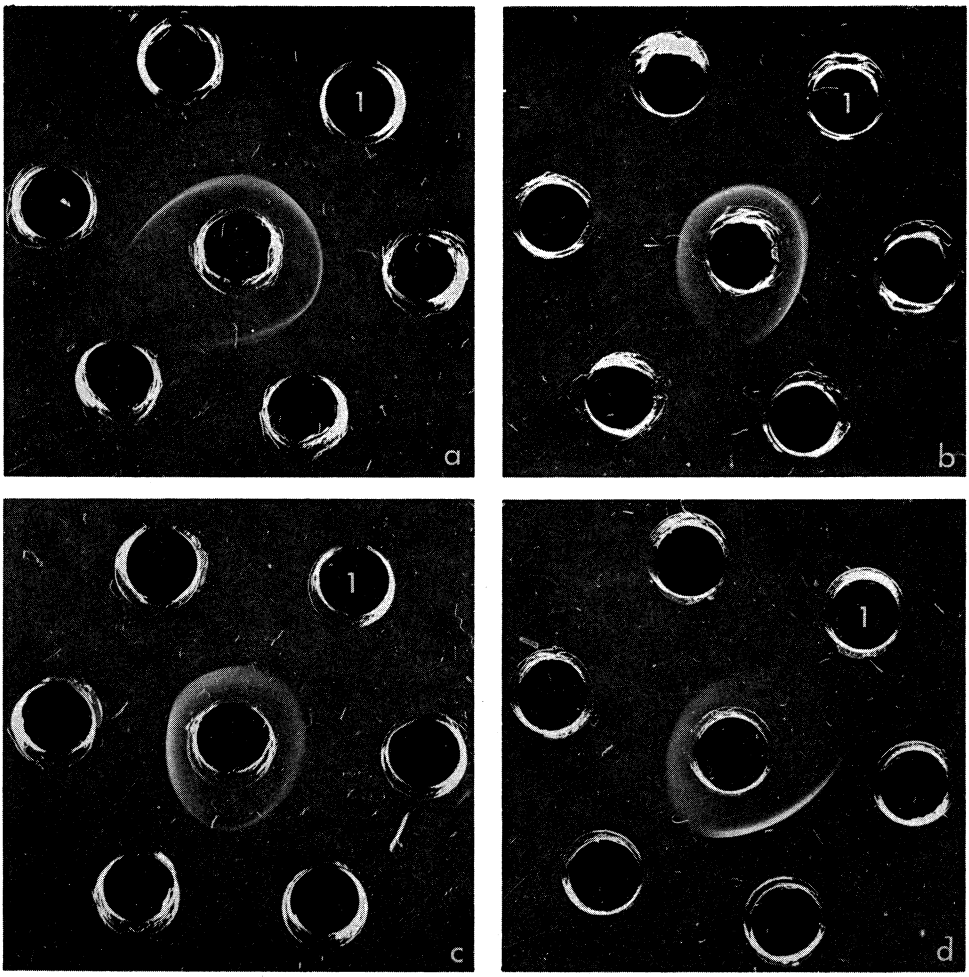


Fig. 1. Agar gel immunodiffusion patterns formed from the reactions of tridacnin with wheat and ryegrass (*L. multiflorum*) arabinogalactan and galactan preparations (see Materials and Methods). Unless otherwise indicated all wheat and *Lolium* preparations examined were prepared according to Fincher *et al.* (1974) or Fincher and Stone (1974). Centre wells: tridacnin 4 mg/ml. Peripheral wells (wells are numbered clockwise from 1): (*a*) *1* and *6*, *Lolium* galactan–protein 1 mg/ml; *2* and *3*, *Lolium* arabinogalactan–protein 1·2 mg/ml; *4* and *5*, wheat arabinogalactan–peptide 2 mg/ml. (*b*) *1* and *2*, wheat galactan–peptide (Neukom and Markwalder 1975) 1·1 mg/ml; *3* and *4*, wheat arabinogalactan–peptide 2 mg/ml; *5* and *6*, wheat galactan–peptide 1·8 mg/ml. (*c*) *1* and *2*, *Lolium* galactan–protein 0·8 mg/ml; *3* and *4*, *Lolium* arabinogalactan–protein 1 mg/ml; *5* and *6*, wheat galactan–peptide 1·8 mg/ml. (*d*) *1* and *6*, wheat arabinoxylan 1·2 mg/ml; *2*, empty; *3*, *Lolium* galactan–protein 1 mg/ml; *4*, *Lolium* arabinogalactan–protein 1·2 mg/ml; *5*, wheat galactan–peptide 1·8 mg/ml.

Homogeneity and electrophoretic behaviour in agar gel of the wheat galactan was investigated by examining the wheat preparation using immunoelectrophoresis in agar at pH 8·6. A single, clearly defined precipitin arc showing β–γ mobility formed when diffusion was allowed to occur with both tridacnin and serum J539 (Fig. 2).

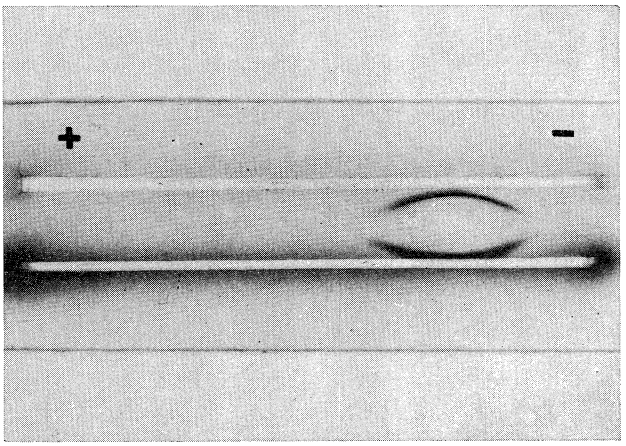


Fig. 2. Immunoelectrophoretic examination of wheat galactan–peptide in agar gel at pH 8·6. Well: wheat galactan–peptide (Neukom and Markwalder 1975) 0·6 mg/ml. Upper trough: tridacnin 2 mg/ml. Lower trough: mouse myeloma anti-galactan 290 μg N/ml.

Quantitative Precipitin Studies

Tube precipitin examinations were carried out in order to obtain a quantitative assessment of the reactions between the wheat and ryegrass arabinogalactans and galactans, and the tridacnin, *Axinella*, *Abrus* and *Ricinus* lectin preparations. *Cerianthus* agglutinins were not available in sufficient quantity to permit their use in precipitation studies. Results with tridacnin supported the immunodiffusion results described above. Little or no precipitation occurred with wheat arabinogalactan or with wheat arabinoxylan. Wheat galactan and both *Lolium* preparations showed strong precipitation (Fig. 3*a*). These results were reflected when supernatants from each precipitin tube were examined (Fig. 3*b*). Supernatants from the tubes containing the wheat galactan preparations showed no haemagglutinating activity but no reduction in the haemagglutinating activity was found with supernatants from tubes containing wheat arabinogalactan or wheat arabinoxylan. Incomplete removal of the tridacnin haemagglutinins was observed in the tubes containing the smallest amount of *Lolium* galactan and a more gradual decline in activity was seen with supernatants from the tubes containing native *Lolium* arabinogalactan (Fig. 3*b*).

Precipitation studies in which the wheat preparations were used with *Axinella* and *Abrus* reagents supported the haemagglutination inhibition results obtained with these reagents. When arabinogalactan was used with *Axinella* extract (Fig. 4), maximum precipitation (approximately 0·75 μg N) was produced with 50–60 μg of peptido-glycan. By contrast, only 10 μg of the wheat galactan was needed to precipitate almost 3 μg N. The difference in the amount of nitrogen precipitated by the two

wheat preparations was even more striking with the *Abrus* reagent. In this case no precipitate was detected with up to 69 μg of native arabinogalactan, whereas the galactan readily precipitated with the *Abrus* lectin, producing maximum precipitation of 9–10 μg N with 50–80 μg of galactan (Fig. 5).

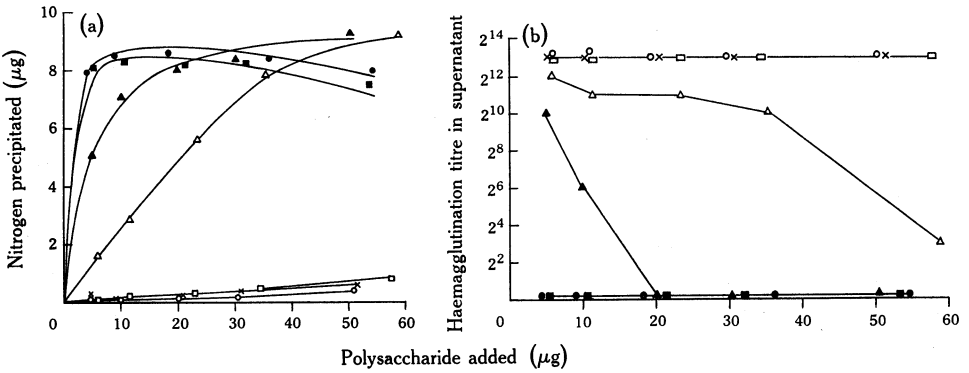
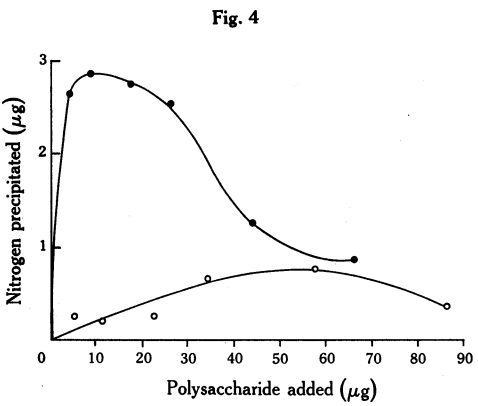


Fig. 3. (*a*) Quantitative precipitin examinations using tridacnin (9·9 μg N per tube) with wheat arabinogalactan–peptide (○) (Fincher *et al.* 1974), wheat galactan–peptide (●) (Fincher *et al.* 1974), wheat arabinogalactan–peptide (□) (Neukom and Markwalder 1975), wheat galactan–peptide (■) (Neukom and Markwalder 1975), *Lolium* arabinogalactan–protein (△) (Fincher *et al.* 1974), *Lolium* galactan–protein (▲) (Fincher and Stone 1974), and wheat arabinoxylan (×) (Mares and Stone 1973*a*, 1973*b*). Total volume in each tube, 125 μl. (*b*) Haemagglutination titres against human group O erythrocytes found in quantitative precipitin supernatants after reaction of tridacnin with the peptidoglycans in (*a*). Symbols as in (*a*). Haemagglutination titre in control tubes (saline and tridacnin only) = 2^{13}.



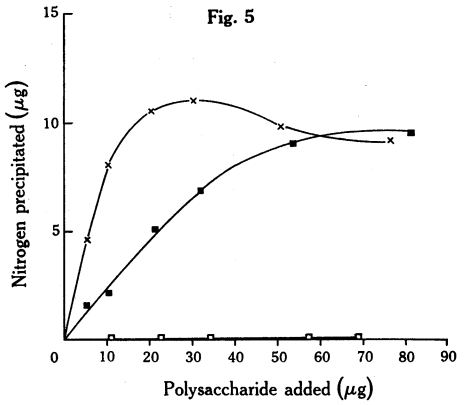
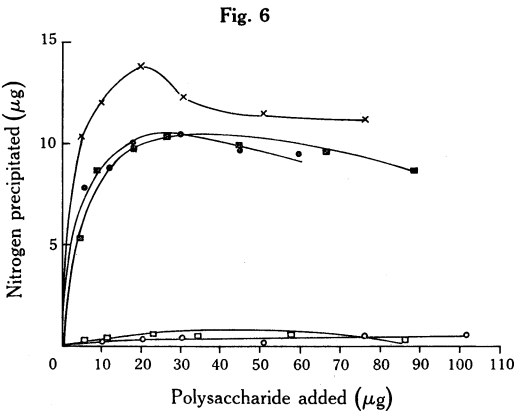


Fig. 4. Precipitation of wheat peptidoglycans using *Axinella polypoides* agglutinin extract. *Axinella* extract (100 μl, 6·3 μg N) was added to tubes containing increasing amounts of wheat arabinogalactan–peptide (○) and wheat galactan–peptide (●) (Neukom and Markwalder 1975). Total volume in each tube, 200 μl.

Fig. 5. Precipitation of wheat peptidoglycans and pneumococcus type XIV polysaccharide by *Abrus precatorius* agglutinins (13·9 μg N per tube). □ Wheat arabinogalactan–peptide (Neukom and Markwalder 1975). ■ Wheat galactan–peptide (Neukom and Markwalder 1975). × Pneumococcus type XIV polysaccharide. Total volume in each tube, 130 μl.

Although neither the wheat arabinogalactan nor the galactan inhibited *Ricinus* agglutinins eluted from Sepharose (Table 2), the galactan preparation readily precipitated with the mixed agglutinins in quantitative precipitin studies. As with the

tridacnin, *Axinella* and *Abrus* agglutinins, the native wheat arabinogalactan precipitated poorly with the mixed *Ricinus* lectins (Fig. 6). These results suggested that only one of the *Ricinus* lectins was reacting with the wheat galactan; this was confirmed using the isolated lectins RCA_I and RCA_{II} in precipitin studies with wheat galactan. Only RCA_{II} precipitated with the galactan. This finding supported the conclusion derived from haemagglutination inhibition studies with *Ricinus* agglutinins and the wheat preparations. Pneumococcus type XIV polysaccharide behaved in the expected manner with the *Abrus* and *Ricinus* precipitins (Bird 1959, 1961), producing heavy precipitation with as little as 5 μg of polysaccharide (Figs 5 and 6).



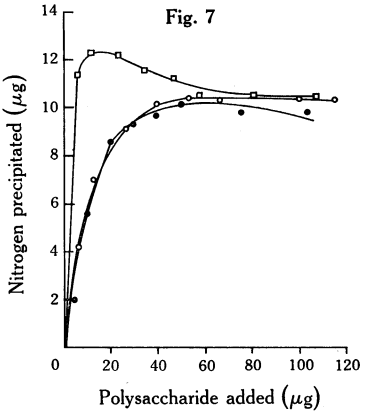


Fig. 6. Precipitation of wheat peptidoglycans and pneumococcus type XIV polysaccharide by *Ricinus communis* agglutinins eluted from Sepharose (RCA_I + RCA_{II}). *Ricinus* agglutinins (19·4 μg N) were added to tubes containing increasing amounts of wheat arabinogalactan–peptide (○) (Fincher *et al.* 1974), wheat galactan–peptide (●) (Fincher *et al.* 1974), wheat arabinogalactan–peptide (□) (Neukom and Markwalder 1975), wheat galactan–peptide (■) (Neukom and Markwalder 1975), or pneumococcus type XIV polysaccharide (×). Total volume in each tube, 125 μl.

Fig. 7. Precipitation of wheat peptidoglycans by mouse myeloma anti-galactan J539 (14·6 μg N per tube). ○ Wheat arabinogalactan–peptide (Neukom and Markwalder 1975). ● Wheat arabinogalactan–peptide (Fincher *et al.* 1974). □ Wheat galactan–peptide (Neukom and Markwalder 1975). Total volume in each tube, 125 μl.

In contrast to the invertebrate and plant precipitins, antibodies present in mouse ascites fluid J539 readily reacted with the intact wheat arabinogalactan as well as with the arabinose-'free' preparation. Fig. 7 shows, however, that for a given amount of J539 the galactan precipitated more nitrogen at an equivalence point which occurred at lower antigen concentration than was seen with the arabinogalactan.

Discussion

Anti-galactan specificity is frequently found among invertebrate lectin-like proteins (Voigtmann *et al.* 1971; Baldo and Uhlenbruck 1974; Bretting and Renwantz 1974; Gauwerky *et al.* 1974; Eichmann *et al.* 1976; Baldo *et al.* 1977*a*, 1977*b*). D-Galactose is a potent inhibitor of both *Axinella* and *Cerianthus* agglutinins and these agglutinins also demonstrate some degree of β-anomeric specificity (Baldo *et al.* 1977*a*, 1977*b*). The *Axinella* agglutinins are best inhibited by terminal non-reducing D-galactose glycosidically linked β-(1→6) (Bretting and Kabat 1976; Eichmann *et al.* 1976).

Tridacnin from the haemolymph of the clam *Tridacna maxima* is a powerful precipitating and haemagglutinating lectin. The agglutinin has been purified and shown to react with a large number of galactose-containing polysaccharides and glycoproteins from a wide variety of sources (Baldo and Uhlenbruck 1975*c*; Uhlenbruck *et al.* 1975; Baldo *et al.* 1977*a*). D-Galactose, lactose, 6-*O*-β-D-galactopyranosyl-D-galactose, D-galactosamine HCl, methyl β-D-galactoside and p-nitrophenyl-β-D-galactoside inhibited tridacnin-induced haemagglutination and precipitation, but on a molar basis *N*-acetyl-D-galactosamine was the best inhibitor (Baldo and Uhlenbruck 1975*a*, and unpublished data). The β-D-galactosides were much more active than the corresponding α-anomers indicating that the tridacnin combining sites show at least some degree of β-anomeric specificity (Baldo, Sawyer and Uhlenbruck, unpublished data).

The D-galactose specificity of plant agglutinins from *Abrus* and *Ricinus* has been well established (Pardoe *et al.* 1969, 1970; Nicolson *et al.* 1974; Baldo and Uhlenbruck, unpublished data) but for the *Ricinus* agglutinins RCA_I and RCA_{II} the best inhibitors of haemagglutination have been shown to be a β-D-galactose-(1-4)-linked disaccharide for RCA_I and *N*-acetyl-D-galactosamine for RCA_{II} (Nicolson and Blaustein 1972).

The failure of the native arabinogalactans to react with *Abrus* lectin and the weak reactivity observed with tridacnin, *Axinella* and *Ricinus* agglutinins is consistent with the observation that the galactan chains are substituted with arabinofuranosyl residues. These presumably mask most of the terminal D-galactosyl residues and so prevent the interactions needed to permit the formation of the three-dimensional network necessary for precipitation. The small number of terminal *D*-galactosyl units present on the native arabinogalactan appear to be insufficient to allow much cross-linking and hence precipitation to proceed. However, if sufficient arabinogalactan is used, inhibition of the lectin combining sites can be demonstrated. This interpretation is consistent with the findings with wheat galactan. Removal of terminal arabinosyl residues from the arabinogalactan, either by mild acid hydrolysis or by treatment with an arabinofuranosidase, gave rise to a product which readily reacted with each of the plant and invertebrate agglutinin preparations except *Ricinus* RCA_I. The very weak interaction of the wheat arabinoxylan in the haemagglutination inhibition experiments may arise from a slight contamination with arabinogalactan–peptide.

The observation that *Ricinus* agglutinin RCA_{II}, but not RCA_I, reacted with wheat and ryegrass galactans is difficult to interpret especially since the best inhibitors found so far for RCA_I include compounds containing D-galactose in β-anomeric linkage, for example β-lactose, methyl β-D-galactoside and β-D-galactose-(1-4)-D-mannose (Nicolson *et al.* 1974). Unlike RCA_I, RCA_{II} shows similarities to tridacnin in terms of its sugar specificity (Nicolson and Blaustein 1972; Baldo and Uhlenbruck 1975*a*, 1975*d*) and in its reactions with the wheat and ryegrass galactans.

The *Lolium* arabinogalactan, unlike the wheat arabinogalactan, readily reacted with the invertebrate agglutinins. This difference in reactivities probably relates to differences in galactose:arabinose ratios in the two polysaccharides. Thus the arabinogalactan from ryegrass endosperm cells in tissue culture had a higher galactose:arabinose ratio than either of the wheat arabinogalactan preparations, indicating that more unsubstituted galactosyl residues would be available for binding in the *Lolium* polysaccharide.

In contrast to the agglutinin preparations used, mouse myeloma protein J539 precipitated with both the wheat arabinogalactan and galactan. Once again, however,

removal of terminal arabinosyl units increased the reactivity of the wheat peptidoglycan. The explanation for the clear reactivity with wheat arabinogalactan is not immediately apparent but may be related to features of the J539 antibody combining sites recently studied by Glaudemans *et al.* (1972) and Jolley *et al.* (1974). From measurements of the binding constants of a number of derivatives of D-galactose, it was concluded that the active sites of J539 antibodies are not clefts but are in the form of a shallow extended surface area which is complementary to one side of the 6-*O*-β-D-galactopyranosyl-D-galactose molecule. It seems likely, therefore, that the presence of arabinose units on the ends of the chains does not prevent access of the J539 antibodies to the active extended site on the 1→6-linked disaccharide. The J539 mouse myeloma protein coupled to Sepharose beads (Andrews and Stone 1977) has been successfully applied to the removal of intracellular debris during the purification of ryegrass endosperm protoplasts (F. Keller and B. A. Stone, unpublished data).

Although studies with Yariv antigens indicate that the *Lolium* arabinogalactan–peptide is present in intracellular vesicles (Anderson *et al.* 1977), it has not so far been possible to show the relationship between these structures and other intracellular structures at a reasonable level of resolution. The reaction of tridacnin and *Axinella* agglutinins with *Lolium* arabinogalactan indicates that these labelled agglutinins could be useful probes for the subcellular localization of the arabinogalactan–protein in the *Lolium* cells. It would also be of interest to localize the arabinogalactan–peptide in wheat but, for this purpose, tridacnin, *Axinella*, *Abrus* and *Ricinus* agglutinins do not seem to be as promising as the mouse myeloma anti-galactan.

Acknowledgments

We thank Mrs J. R. Betts for technical assistance, Gisela Steinhausen for help with invertebrate extracts and Dr M. Potter for providing mouse myeloma ascites fluid. This work was supported by the Princess Margaret Children's Medical Research Foundation, the Wheat Industry Research Council, the Australian Research Grants Committee and the Deutsche Forschungsgemeinschaft.

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Manuscript received 30 May 1977