Arch. Microbiol. 116, 119–124 (1978)

Archives of
Microbiology

Studies on Growth Inhibition by Lectins of Penicillia and Aspergilli

RIVKA BARKAI-GOLAN*, DAVID MIRELMAN, and NATHAN SHARON**
Department of Biophysics, The Weizmann Institute of Science, Rehovoth, Israel

Abstract. It has previously been shown in our laboratory that wheat germ agglutinin (WGA) binds to *Trichoderma viride* and inhibits growth of this fungus. Here we report on the effect of WGA, soybean agglutinin (SBA) and peanut agglutinin (PNA) on Penicillia and Aspergilli. Binding of the lectins to the fungi was examined with the aid of their fluorescein isothiocyanate (FITC) conjugated derivatives. FITC-WGA bound to young hyphal walls of all species, in particular to the hyphal tips and septa, in agreement with the chitinous composition of the cell walls of the two genera. Hyphae of all species examined were labelled, though in different patterns, by FITC-SBA and FITC-PNA, suggesting the presence of galactose residues on their surfaces. Young conidiophores, metulae (of the Penicillia), vesicles (of the Aspergilli), sterigmata and young spores, were also labelled. The three lectins inhibited incorporation of [^{3}H]acetate, *N*-acetyl-D-[^{3}H]glucosamine and D-[^{14}C]galactose into young hyphae of *Aspergillus ochraceus*, indicating interference with fungal growth. Inhibition of spore germination by the three lectins was also observed. Preincubation of the lectins with their specific saccharide inhibitors prevented binding and the inhibitory effects. We conclude that lectins are useful tools for the study of fungal cell surfaces, and may also serve as an important aid in fungal classification. The present findings also support the suggestion that one role of lectins in plants is protection against fungal pathogens.

Key words: Lectins – Fluorescin-conjugated lectins – Fungal cell walls – Inhibition of fungal growth – *Penicillium italicum* – *Aspergillus niger* – *Aspergillus flavus* – *Aspergillus ochraceus* – *Stemphylium botryosum* – Role of lectins in plants.

Lectins, a class of sugar-binding proteins that are widely distributed in nature, primarily in plants, are being used to an increasing extent in the investigation of the surfaces of animal cells (Sharon and Lis, 1972; Lis and Sharon, 1977). Their application to the study of fungi has, however, been very limited. Interactions of lectins with fungal hyphae were first demonstrated by Mirelman et al. (1975), who found that wheat germ agglutinin (WGA), a lectin specific for chitin oligosaccharides, binds to hyphal tips and hyphal septa of *Trichoderma viride* and inhibits hyphal growth and spore germination of this chitin fungus. Based on these findings, it was suggested that WGA has a role in the protection of wheat seedlings against chitin-containing phytopathogens. It was further postulated that lectins with sugar specificities different from those of WGA may function similarly, as natural inhibitors of the growth of fungi, the surfaces of which are covered by other polysaccharides. Subsequently, Galun et al. (1976) examined the binding of WGA, as well as five other lectins with different sugar specificities, to three mycobionts isolated from lichens. From the binding characteristics they concluded that chitin is a mycobiont hyphal wall component, and have suggested that lectins may be useful in studies of the chemical composition of hyphal wall surfaces.

To assess to what extent the binding of lectins to fungi and their growth inhibiting effects are indeed general phenomena, and to obtain further information on the possibility of applying lectins to probe fungal surfaces, we investigated the interaction of

Abbreviations. Con A = concanavalin A; PNA = peanut agglutinin; SBA = soybean agglutinin; WGA = wheat germ agglutinin; FITC = fluorescein isothiocyanate; GlcNAc = *N*-acetyl-D-glucosamine; GalNAc = *N*-acetyl-D-galactosamine

* *Permanent address and address for offprint requests:* Department of Fruit and Vegetable Storage Research. The Volcani Center Agricultural Research Organization, Beth-Dagan, Israel
** Established Investigator of the Chief Scientist's Bureau of the Israel Ministry of Health

0302-8933/78/0116/0119/$ 01.20

several lectins with a large number of fungi belonging to different taxonomic groups. The lectins used in this study were mainly: soybean agglutinin (SBA), specific for D-galactose and *N*-acetyl-D-galactosamine (GalNAc); peanut agglutinin (PNA), specific for D-galactose; WGA, specific for *N*-acetyl-D-glucosamine (GlcNAc).

As a first step, the ability of the various fungi to bind the fluorescein isothiocyanate (FITC) derivatives of the lectins was examined under the fluorescent microscope. Of the fungi that exhibited binding of all the lectins tested, representative species of *Penicillium* and *Aspergillus* were selected for detailed examination of the effect of lectins on their growth and uptake of various nutrients. The results obtained, in addition to showing that lectins can provide further insight into the structure of the fungal surface, show that both SBA and PNA inhibit growth of certain Penicillia and Aspergilli, and thus support the suggestion that lectins function as part of the defence mechanisms of plants against fungal pathogens.

MATERIALS AND METHODS

Lectins and Sugars

SBA (from *Glycine max*, Gordon et al., 1972), PNA (from *Arachis hypogaea*, Lotan et al., 1975) and WGA [from *Tritium vulgaris* (*T. aestivum*), Lotan et al., 1973] were prepared by affinity chromatography according to methods developed in this laboratory. Concanavalin A (Con A) from jack bean (*Canavalia ensiformis*) was purchased from Miles-Yeda (Rehovoth). The lectins were conjugated with FITC in the presence of their specific sugar inhibitors (usually 0.2 M), according to the method of Clark and Shepard (1963). The FITC derivatives obtained were purified by affinity chromatography as above; FITC-Con A was purified on Sephadex G-25. Sugars were purchased from Pfanstiehl, except chitotriose [$(GlcNAc)_3$] which was prepared from chitin according to the method of Rupley (1964).

Fungal Organisms

Unless otherwise stated, all organisms used were isolated from post-harvest fruit rots or from stored grains. They were identified by standard methods and kept in the collection of the Department of Fruit and Vegetable Storage Research, The Volcani Center.

Lectin Binding

Young colonies were obtained by growth for 40–48 h at 25° C on microscope slides coated with minimal medium (Galun, 1972), which had been inoculated with 0.05 ml of a 10^5/ml spore suspension in distilled water. Labelling of colonies by FITC-lectins (0.5–2 mg/ml) in buffered saline pH 7.4, was according to the procedure of Mirelman et al. (1975). Parallel preparations were treated by FITC-conjugated lectins preincubated for 30 min at 25° C with their specific inhibitors [0.4 M D-galactose for SBA and PNA, 0.4 M methyl α-D-glucoside for ConA and 2 mM $(GlcNAc)_3$ for WGA]. The preparations were then observed with a standard RA Zeiss fluorescence microscope using a BG-12 exciter filter and No. 53 barrier filter.

Effect of Lectins on the Incorporation of Precursors into Fungal Cells

The following radioactive precursors were used: sodium [^{3}H]acetate (250 mCi/mmol), *N*-acetyl-D-[1-^{3}H]glucosamine (5 Ci/mmol) and D-[1-^{14}C]galactose (60 mCi/mmol), all purchased from The Radiochemical Centre, Amersham. Suspensions of young hyphae were obtained after 24 h by shaking spores in minimal medium at 25° C. Aliquots (2 ml; 0.5 mg dry weight/ml), to which the radioactive precursors were added (5–10 µl, 1–10 µCi/ml) were mixed with the lectins (2 ml, 0.02–2 mg/ml) and incubated for 15 min. In control experiments the lectins were preincubated (30 min at 25° C) with their specific inhibitors [0.4 M GalNAc for SBA, 0.4 M D-galactose for PNA, and 2 mM chitotriose $(GlcNAc)_3$ for WGA] and the solutions containing both lectin and inhibitor (2 ml) were added to the spore suspensions containing the different precursors. Incorporation was measured as previously described (Mirelman et al., 1975). Extent of inhibition by the lectins was calculated from the ratio of radioactivity incorporated in the presence of lectins to that incorporated in their absence.

Inhibition of Spore Germination

Spore suspensions of 10^5/ml of potato-dextrose broth were prepared from 10-day old single spore cultures of the fungi on potato-dextrose agar at 25° C. The suspensions were deposited on a microscopic slide and incubated with the lectin (0.05 ml, final concentration of 0.5–15 mg/ml) in a moist chamber at 25° C for up to 20 h. Germination was assessed by occasional examination of the slide under the microscope. Rate of germination of spores incubated in the absence of lectins was taken as 100%.

RESULTS AND DISCUSSION

Lectin Binding

Table 1 presents the results of binding studies of different fungi with four FITC-labelled lectins. In all cases where binding was observed, it was shown to be sugar-specific, as evidenced by the fact that no fluorescence was observed in the presence of both lectin and its inhibitory sugar.

FITC-WGA bound to all the fungi examined, except the chitin-less *Phytophthora citrophthora*, as previously demonstrated by Galun et al. (1976). The binding of FITC-Con A is more difficult to account for, since this lectin reacts only poorly, if at all, with β-linked glucans and with chitin (Sharon and Lis, 1972). It is possible, however, that a positive reaction with FITC-Con A indicates the presence of small quantities of α-linked D-glucose (or D-mannose) residues on the fungal surface. Interestingly, most of the fungi with dark coloured hyphae, such as *Alternaria tenuis*, *Stemphylium botryosum* and *Cladosporium herbarum* did not bind FITC-Con A, possibly because in these organisms the presumed glucose residues are covered by melanin. Indeed, very young hyphae of *S. botryosum*, or its perfect stage *Pleospora herbarum*, which are colourless, exhibited binding of

Table 1. Binding of FITC-lectins to fungi

Fungus species and taxonomic group	Chemical category[a]	Lectin and main specificity			
		WGA[b] $(GlcNAc)_3$	SBA GalNAc > D-Gal	PNA D-Gal	Con A α-D-Man, α-D-Glc
Phycomycetes					
Zygomycetes	Chitosan-chitin				
Rhizopus stolonifer Ehr.		+	−	−	+
Mucor sp.		+	−	−	+
Oomycetes	Cellulose-β-glucan				
Phytophthora citrophthora (Sm. et Sm.) Leon.		−	±	−	+
Ascomycetes	Chitin-β-glucan				
Pleospora herbarum (Pers.) Raben.		+	−	−	−[c]
Sclerotinia sclerotiorum (Lib.) de By.		+	±	−	+
Basidiomycetes	Chitin-β-glucan				
Rhizoctonia solani Kuhn		+	−	−	−
Sclerotium rolfsii Sacc.[d]		+	+	+	+
Deuteromycetes	Chitin-β-glucan				
Penicillium italicum Wehm.		+	±	±	+
P. expansum Link		+	±	±	+
P. citrinum Thom		+	±	±	+
P. digitatum Sacc.		+	±	±	±
Aspergillus niger V. Tiegh.		+	±	±	+
A. flavus Link		+	±	±	+
A. ochraceus Wilhelm		+	+	+	+
Geotrichum candidum Link		+	+	−	+
Botrytis cinerea Pers.		+	±	−	+
Fusarium moniliforme Sheld.		+	±	−	+
Trichoderma viride Pers. ex Fries		+	−	−	+
Diplodia natalensis P.E.		+	−	−	±
Stemphylium botryosum Wall.		+	−	−	−[c]
Alternaria tenuis Nees		+	−	−	−
Cladosporium herbarum (Pres.) Link		+	−	−	−

+ = marked binding; ± = moderate to weak binding; − = no binding

[a] As proposed by Bartnicki-Garcia (1968)

[b] Results with WGA, except for the Penicillia and Aspergilli, are from Margalith Galun (private communication)

[c] Binding confined to thin hyaline hyphae

[d] Received from I. Chet; isolated from soil

FITC-Con A. FITC-SBA and FITC-PNA binding to varying extents was observed to all the Penicillia and Aspergilli examined, strongly suggesting the presence on their surfaces of polysaccharides containing D-galactose (and possibly also of *N*-acetyl-D-galactosamine). This is in agreement with the reports on the presence of galactose in cell walls of various Penicillia and Aspergilli examined, for example of *P. digitatum* and *P. italicum* (Grisaro et al., 1968), *A. niger* (Bardalaye and Hordin, 1976) and *A. oryzae* (of the *A. flavus* group, Horikoshi and Iida, 1964).

The data in Table 1 also show that D-galactose residues are of much more limited occurrence in hyphal surfaces than those of *N*-acetyl-D-glucosamine or D-glucose. The binding patterns of lectins to several species of *Penicillium* and *Aspergillus* tested were examined in more detail. Both FITC-SBA and FITC-PNA gave a patchy pattern of binding along the hyphal walls with no pronounced binding at the tips and septa, indicating that both lectins bind preferentially to the relatively mature regions of the hyphae. The pattern of FITC-WGA binding was markedly different since this lectin bound mainly to hyphal tips and septa. This pattern of binding is similar to that previously observed in *T. viride* (Mirelman et al., 1975). It is noteworthy that FITC-WGA binds to those regions of the hyphae in which chitin is actively synthesized, also indicating that this chitin is not covered by other substances (e.g. other polysaccharides or melanins).

In addition to young hyphae, young conidiophores with their metulae (in the case of the Peni-

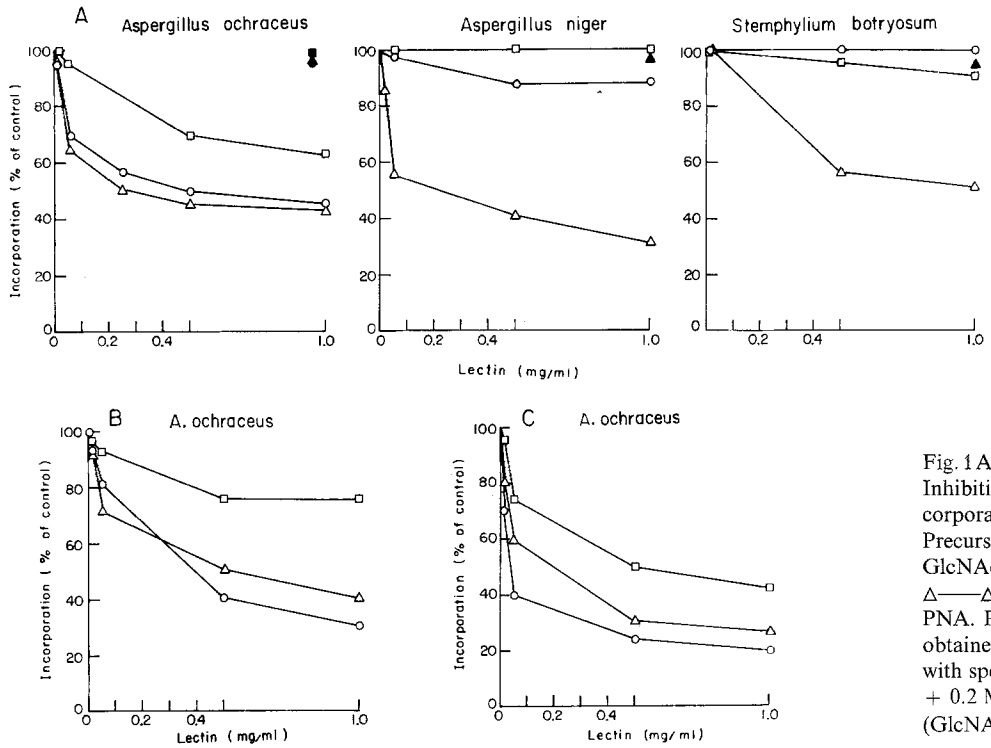

Fig. 1 A–C
Inhibition by lectins of precursor incorporation into young fungal hyphae. Precursors: (A) [^{3}H]acetate; (B) [^{3}H]-GlcNAc; (C) [^{14}C]galactose. Lectins: △——△ WGA; □——□ SBA, ○——○ PNA. Percentage of incorporation obtained when lectins preincubated with specific sugar inhibitors: ● PNA + 0.2 M D-galactose; ▲ WGA + 2 mM $(GlcNAc)_3$; ■ SBA + 0.2 M GalNAc

cillia) or vesicles (in the case of the Aspergilli) as well as sterigmata and young spores were also labelled by the three lectins. Only immature and young spores were labelled, since fluorescence could be observed always on small spores which are still attached to the sterigmata, and sometimes with the young spores in the conidial chain as well. Further evidence for this point could be drawn from examination of spores of the Aspergilli, mainly those of *A. niger*. In their first stages of development, spores of this fungus are hyaline and smooth whereas at maturity they become dark and roughened. Labelling of spores with the three lectins in this case was mostly confined to the hyaline and smooth forms.

Inhibition of Incorporation. WGA, PNA and SBA were found to inhibit the incorporation of sodium [^{3}H]-acetate, *N*-acetyl-D-[1-^{3}H]glucosamine and D-[1-^{14}C]-galactose, into young hyphae of *A. ochraceus* (Fig. 1). This may indicate interference with normal fungal growth.

Incorporation of [^{3}H]acetate into *A. ochraceus* hyphae, was inhibited by WGA and PNA, and to a lesser extent by SBA (Fig. 1 A). Incorporation was not affected when the lectins were preincubated with their specific inhibitors. With *A. niger*, however, only WGA caused a marked inhibition of [^{3}H]acetate incorporation. No inhibition of this precursor by PNA or by SBA occurred with *S. botryosum*, a chitin fungus which lacks the ability to bind either FITC-PNA or FITC-SBA (Fig. 1 A).

The inhibition of incorporation of various precursors into the fungal hyphae by lectins of different specificities seem to be caused by coating of the hyphal surfaces, following the binding of available receptors with their specific lectins.

Inhibition of Spore Germination

Growth inhibition by the various lectins was indicated also by their effect on fungal spore germination (Figs. 2 and 3). All lectins tested caused a marked inhibition at 5 mg lectin/ml or more, which is a much higher concentration than that needed for the inhibition of precursor incorporation (Fig. 1). It seems that the inhibition of spore germination occurs in a very early stage of the germination process, after spores have been allowed to swell and before initiation of detectable germ tubes. Inhibition of germination was mainly expressed by the prolongation of the latent period which precedes germination (Fig. 3). However, once initiated, the rate of germination appeared to be quite normal and after 18 h of incubation the percent of lectin treated spores that germinated was almost the same as that of the untreated ones.

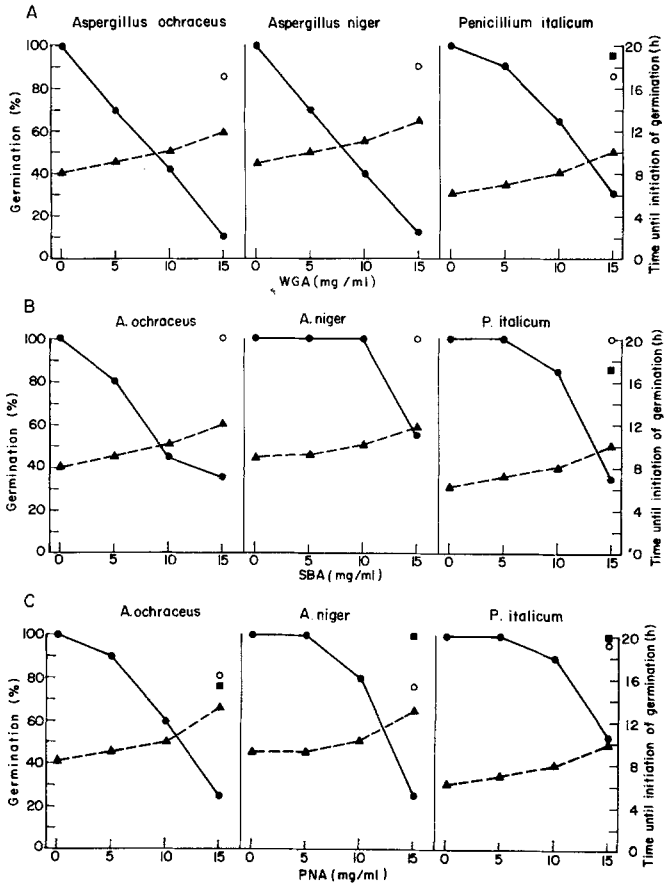

Fig. 2A–C. Effect of lectins on spore germination in potato dextrose broth at 23° C. (A) WGA; (B) SBA; (C) PNA. Percentage of germination after 14 h, ●——●; after 14 h in the presence of specific sugar inhibitor, ■; after 18 h, ○. Specific inhibitors for lectins: 2 mM $(GlcNAc)_3$ for WGA; 0.2 M GalNAc for SBA; 0.2 M D-galactose for PNA. Time of initiation of germination, ▲------▲

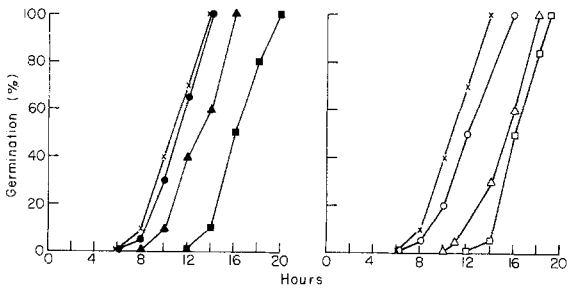

Fig. 3. Latent period of spore germination of *Aspergillus ochraceus* in the presence of PNA (●——● 5 mg/ml; ▲——▲ 10 mg/ml; ■——■ 15 mg/ml) or WGA (○——○ 5 mg/ml; △——△ 10 mg/ml; □——□ 15 mg/ml), compared with untreated spores (×——×)

CONCLUDING REMARKS

The differences in the binding patterns of lectins with different specificities to fungal hyphae indicate that there are differences in the architecture of the cell wall in different regions of the hyphae. Moreover, it seems that the chemical composition of the fungal surface changes in a characteristic manner with age. Whereas the binding of WGA is confined mainly to hyphal tips and septa, suggesting the presence of chitin in these growing areas, the binding of both PNA and SBA is more or less even along the hyphae, suggesting the presence of galactose residues on the surface of their mature parts.

The binding of the different lectins to young conidiophores, as well as to the metulae and sterigmata of the Penicillia and to vesicles and sterigmata of the Aspergilli, suggest that chemical surfaces of the differentiated cells (metulae and sterigmata) and the differentiated hyphae (conidiophores) are similar to those of the undifferentiated hyphae. However, spore surfaces were not always identical with that of their corresponding hyphae, as could be judged by lectin labelling. The lack of WGA binding to *Trichoderma* spores (Mirelman et al., 1975) was confirmed with other chitin fungi such as *S. botryosum* and *C. herbarum*. With species of *Penicillium* and *Aspergillus*, however, it was found that WGA as well as PNA and SBA bind also to young and immature spores. This observation is in accordance with the fact that the wall chemistry of the spores change markedly with their age. It seems that either chitin or galactose residues of the young spores surfaces are replaced or overlayered by other compounds, such as melanin, which prevent their interaction with lectins.

The inhibition by various lectins of fungal spore germination and of the incorporation of various precursors into fungal hyphae, clearly indicate their interference with normal growth. This lends further support to our previous suggestion (Mirelman et al., 1975) that lectins in plants are part of their protection system, helping them to combat attack by fungal pathogens.

Acknowledgements. We wish to thank Mrs. Ilana Harari for the preparation of the FITC-conjugated lectins.

REFERENCES

Bardalaye, P. C., Hordin, J. H.: Galactosaminogalactan from cell walls of *Aspergillus niger*. J. Bacteriol. **125**, 655–669 (1976)

Bartnicki-Garcia, S.: Cell wall chemistry, morphogenesis and taxonomy of fungi. Ann. Rev. Microbiol. **22**, 87–107 (1968)

Clark, H. F., Shepard, C. C.: A dialysis technique for preparing fluorescent antibody. Virology **20**, 642–644 (1963)

Galun, E.: Morphogenesis in *Trichoderma:* Autoradiography of intact colonies labelled by [^{3}H]*N*-acetylglucosamine as a marker of new cell wall biosynthesis. Arch. Mikrobiol. **86**, 305–314 (1972)

Galun, M., Braun, A., Frensdorff, A., Galun, E.: Hyphal walls of isolated lichen fungi. Arch. Microbiol. **108**, 9–16 (1976)

Gordon, J. A., Blumberg, S., Lis, H., Sharon, N.: Purification of soybean agglutinin by affinity chromatography on Sepharose-*N*-ε-aminocaproyl-β-D-galactopyranosylamine. FEBS Lett. **24**, 193–196 (1972)

Grisaro, V., Sharon, N., Barkai-Golan, R.: The chemical composition of the cell walls of *Penicillium digitatum* Sacc. and *Penicilium italicum* Whem. J. gen. Microbiol. **51**, 145–150 (1968)

Horikoshi, K., Iida, S.: Studies of the spore coats of fungi I. Isolation and composition of the spore coats of *Aspergillus oryzae*. Biochim. Biophys. Acta **83**, 197–203 (1964)

Lis, H., Sharon, N.: Lectins: their chemistry and application to immunology. In: The Antigens, Vol. IV (M. Sela, ed.), pp. 429–529. New York: Academic Press 1977

Lotan, R., Gussin, A. E. S., Lis, H., Sharon, N.: Purification of wheat germ agglutinin by affinity chromatography on a Sepharose-bound *N*-acetylglucosamine derivative. Biochem. Biophys. Res. Commun. **52**, 656–662 (1973)

Lotan, R., Skutelsky, E., Danon, D., Sharon, N.: The purification, composition, and specificity of the anti-T lectin from peanut (*Arachis hypogaea*). J. Biol. Chem. **250**, 8518–8523 (1975)

Mirelman, D., Galun, E., Sharon, N., Lotan, R.: Inhibition of fungal growth by wheat germ agglutinin. Nature **256**, 414–416 (1975)

Rupley, J. A.: The hydrolysis of chitin by concentrated hydrochloric acid, and the preparation of low-molecular weight substrates for lysozyme. Biochim. Biophys. Acta **83**, 243–255 (1964)

Sharon, N., Lis, H.: Lectins: cell-agglutinating and sugar-specific proteins. Science **177**, 949–959 (1972)

Received July 14, 1977