Zeitschrift: Botanica Helvetica
Band: 98 (1988)
Heft: 2

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DOI: https://doi.org/10.5169/seals-68588

Botanica Helvetica 98/2, 1988

0253-1453/88/020271-07 $ 1.50+0.20/0

Surface glycosyl receptors of *Phytophthora megasperma* f. sp. *glycinea* and its soybean host

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Manuscript accepted September 15, 1988

Abstract

Hohl H. R., and Balsiger S. 1988. Surface glycosyl receptors of *Phytophthora megasperma* f. sp. *glycinea* and its soybean host. Bot. Helv. 98: 271–277.

We demonstrate the presence of galactose (aminocaproyl-galactosamine) and fucose binding sites on the outer surface of germinated cysts of the plant pathogens *Phytophthora megasperma* f. sp. *glycinea,* and *P. infestans. P. palmivora* carries fucose but no demonstrable galactose binding sites. Protoplasts of soybean hypocotyls have a glucosyl receptor exposed at their surface, probably one of the widely distributed β-lectins. A likely lectin-ligand type bond responsible at least in part for the adhesion of the pathogen to the plasma membrane of the host occurs between the terminal glucosyl residues present on the former and the glycosyl receptors of the latter.

Introduction

Although the mechanisms are still poorly understood, carbohydrates and their receptors appear to participate in cell surface interactions between fungal pathogens and their hosts (Albersheim and Anderson-Prouty 1975, Callow 1984, Ralton et al. 1986, 1987). These involve soluble glucan elicitors from the former and glucan receptors from the latter (Peters et al. 1978, Yoshikawa et al. 1983, Darvill and Albersheim 1984, Schmidt and Ebel 1987). There is also some evidence for elicitation of responses from pathogen and host by direct contact between the surface of the pathogen and the plasma membrane of the host (Furuichi et al. 1980, Odermatt et al. 1988). These and other contacts between host and parasite might be mediated by carbohydrate ligands and lectins present on the respective surfaces (Hinch and Clarke 1980, Hargreaves and Keon 1983, Hohl and Balsiger 1986a).

In a previous paper (Hohl and Balsiger 1986a) we presented evidence that adhesion of germ tubes of *Phytophthora megasperma* f. sp. *glycinea* to the plasma membrane of its soybean host involves lectin-ligand type bonds. In a subsequent study (Hohl and Balsiger 1986b) mannose/glucose and galactose/N-Acetyl-galactosamine residues were shown to be exposed on the surface of the host plasma membrane. Mannose/glucose residues were the only carbohydrates detected on the surface of the germinated cysts of the pathogen.

To establish more clearly the presence of lectin-sugar type interactions between pathogen and host it was necessary to demonstrate the presence of specific sugar recep-

tors on the corresponding surfaces of the two partners. The present paper describes an approach to gain this information. We used an agglutination method involving agarose beads coated with specific sugar residues which were mixed with either host-derived protoplasts or germinated cysts of the pathogen. Additional information was obtained using the Yariv reagent (Jermyn and Yeow 1975, Fincher et al. 1983). These are synthetic compounds with terminal glycosyl moieties [1,3,5-tris(4-β-D-glucopyranosyloxyphenylazo)-2,4,6-trihydroxy-benzene, where the terminal glucopyranosyl residues may be replaced by other sugars such as α-galactose] capable of precipitating material carrying specific sugar receptors.

Material and methods

Host plants. – Soybean (*Glycine max* (L.) Merr.) seedlings of cv. Harosoy and Harosoy 63 were grown for 5 to 6 d at 23–25 °C in the dark, and protoplasts prepared from their hypocotyls as described before (Hohl and Balsiger 1986 b).

Growth of fungi and production of cysts. *Phytophthora megasperma* f. sp. *glycinea* Kuan & Erwin (isolates 80 = race 1, 83 = race 6, and 241 = race 3), *P. infestans* (Mont.) de Bary strain 191, and *P. palmivora* (Butler) Butler, strains P113, 74 and 76, were grown and cysts prepared as described earlier (Hohl and Balsiger 1986 b).

Agglutination experiments. – The following carbohydrate-coated agarose beads were purchased from SIGMA Chemical Comp. (St. Louis, USA): N-Acetyl-D-galactosamine-agarose (galNAc-agarose), N-Acetyl-D-glucosamine-agarose (glcNAc-agarose), L-fucose-agarose (fuc-agarose), β-D-glucose-agarose (glc-agarose) D-mannose-agarose (man-agarose), α-methyl-D-mannoside-

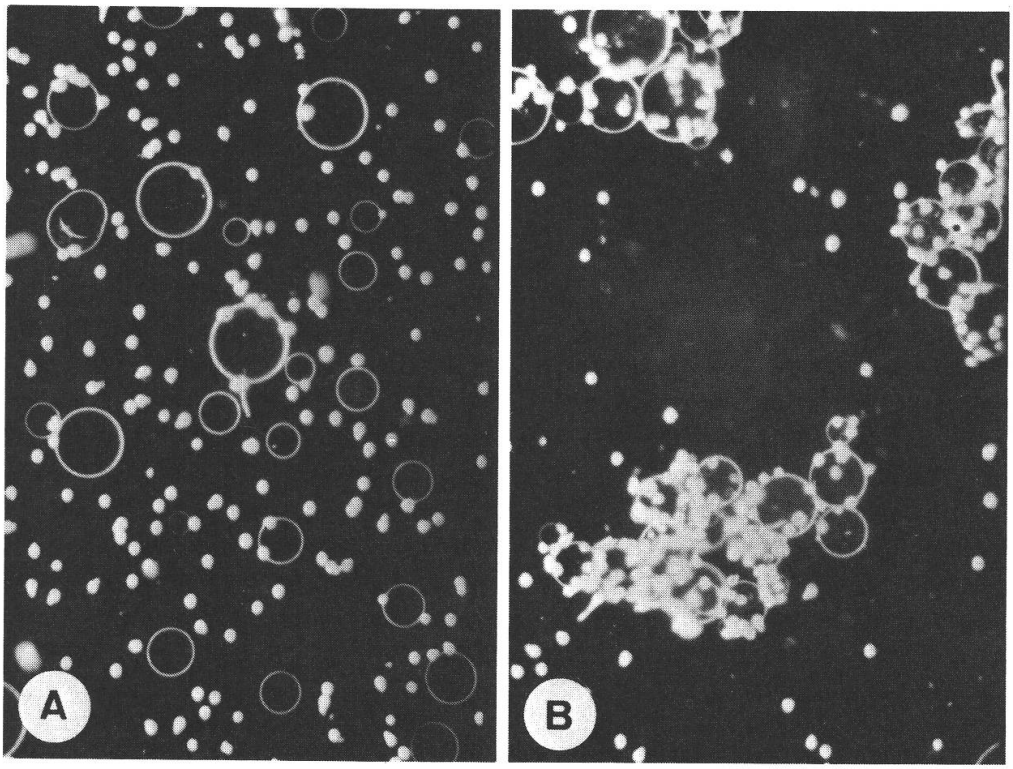

Fig. 1. Agglutination test. Mixed incubation of germinating cysts of *Phytophthora megasperma* f. sp. *glycinea* and glycosyl-coated agarose beads. In (A) the beads are coated with glucose residues (no agglutination), in (B) with galactosamine residues (strong agglutination).

agarose (met-man-agarose), and α-lactose-agarose (lac-agarose). Caproyl-galactosamine-agarose (galNCap-agarose) was purchased from Pharmacia.

For agglutination 20 µl of beads in distilled water or PBS (phosphate buffer solution, in g/liter: NaCl 8.0, $Na_2HPO_4 \cdot 2H_2O$ 1.7, KH_2PO_4 0.4) were pipetted onto depression slides. Approximately 10^5 cysts pregerminated for 1 h were then mixed in with the beads to give a final volume of 100 µl, and agglutination was checked after 30 min. For controls cysts were preincubated for 60 min and maintained during the test in 0.1–1 M of the corresponding sugar.

For testing protoplasts they together with the agarose beads were suspended in a near-isotonic KCl-buffer (KCl 0.2 M, MES 10 mM, and EDTA 2 mM, adjusted to pH 7.0 with 1 N NaOH). 2500 protoplasts in 25 µl were mixed with the beads on depression slides and the agglutination checked between 15 and 120 min. Controls were performed in the presence of 0.1 M of the appropriate sugar.

The β-glucosyl and α-galactosyl Yariv reagents were purchased from Biosupplies (P.O. Box 835, Parkville, Victoria, Australia 3052). The basic solution consisted of 1 mg/ml of Yariv reagent dissolved in 0.15 M NaCl. For the test this solution was diluted 1 : 20 to give a final concentration of 0.05 mg/ml. The dilution was carried out with distilled water when working with germinated cysts and with the KCl buffer when tests were made with protoplasts. Germinated cysts and protoplasts in concentrations equalling those described for the agarose bead test were used and mixed appr. 1 : 1 with the Yariv reagent on depression slides. Agglutination was checked over a period of 2 h. For controls protoplasts were preincubated for 1 h with the appropriate sugar at 0.1 M. For the glucosyl Yariv reagent which gave positive results, these sugars were glcNAc, man, and glc.

Results

Agglutinations with agarose beads

The results of the agglutination experiments are shown in Table 1. Germinated cysts of all phytophthoras tested formed agglutinates (Fig. 1) with galNCap-agarose beads. *P. megasperma* f. sp. *glycinea, P. infestans* but not the three strains of *P. palmivora* tested agglutinated in the presence of beads coated with fucose residues. From the experiments it cannot be precisely determined whether these receptors are located on the cyst or germ tube wall. Preliminary evidence suggests that they are located on both of these structures.

Only beads coated with galNCap residues but not those with galNAc residues agglutained cysts. The former is attached to the agarose through the aminocaproyl residue, the latter through the 6-hydroxy group of the sugar moiety. This possibly explains the difference in agglutination obtained with these two compounds.

Control experiments showed that agglutination is inhibited in the presence of the appropriate sugars. Rather high concentrations were needed to achieve this inhibition. There was some inhibition at sugar concentrations of 0.1 M. In order to obtain dissolution of aggregates up to 1 M concentrations were necessary, however.

None of the protoplasts agglutinated in the presence of any of the agarose beads tested. This might indicate that no corresponding receptors occurred on the protoplast surfaces in sufficient amounts or with sufficient affinity to lead to agglutination. From our observations, however, it seems more likely that the test did not work properly with protoplasts as they settled rapidly while the beads remained more or less afloat. Also no close contact between protoplast and beads could be observed indicating a possible static repulsion between the two types of particles. Several attempts to overcome this problem were unsuccessful.

Agglutination of germinated cysts and protoplasts with the Yariv reagent

Germinated cysts did not agglutinate in the presence of either the glucosyl or galactosyl Yariv reagent. Protoplasts of Harosoy and Harosoy 63, however, were agglutinated by the glucosylated but not the galactosylated reagent. End titer titration showed a

Table 1. Agglutination of germinated cysts of *Phytophthora*, and of soybean protoplasts by agarose beads surface-coated with specific sugar residues

	agarose beads coated with							
	glc	glcNAC	man	met-man	lac	fuc	galNAc	galNCap
Phytophthora								
infestans 191	−	−	−	−	−	+	−	+
megasperma								
f. sp. *glycinea* 80	−	−	−	−	−	+	−	+
f. sp. *glycinea* 241	−	−	−	−	−	+	−	+
f. sp. *glycinea* 83	−	−	−	−	−	+	−	+
palmivora 74	−	−	−	−	−	−	−	nt
palmivora 76	−	−	−	−	−	−	−	nt
palmivora P113	−	−	−	−	−	−	−	+
soybean protoplasts								
Harosoy	−	−	−	−	−	−	−	−
Harosoy 63	−	−	−	−	−	−	−	−

For abbreviations of glycosyl residues see "Material and methods".
nt not tested.

dilution of 1 : 320 of the original (1 mg/ml) Yariv reagent solution to represent the lower limit for its agglutination capacity. Control experiments in the presence of appropriate sugars (see material and methods) were negative after 30 min when agglutination normally was completed. After 1 to 2 h a slight agglutination was observed in the controls, too.

Discussion

The results obtained from this study demonstrate the presence of fucosyl and aminogalactosyl receptors on the surface of germinated cysts of *P. megasperma* f. sp. *glycinea* and other phytophthoras. Some specificty is apparent as no fucosyl receptors could be demonstrated on *P. palmivora*, a pathogen of diverse tropical plants.

The glucosyl receptor found on the protoplast surface of the soybean host most likely corresponds to the group of compounds known as β-lectins which have been isolated from many higher and lower plants (Clarke et al. 1978). If this is the case its specificity would not be limited to glucosyl but extend to other β-glycosyl ligands. β-ligands occur mainly extracellularly but are also associated with the plasma membrane of many plants (Larkin 1977, Samson et al. 1983). The relationship if any between the glucosyl receptor and the plasma membrane receptor for fungal glucan elicitors (Yoshikawa et al. 1983, Schmidt and Ebel 1987) has not been investigated.

The glucosyl receptor was detected with the Yariv reagent specific for β-lectins but not with the agarose bead method. Thus, the bead method is not suitable for the detection of β-lectins or, perhaps unsuitable to demonstrate any lectins on plant protoplast surfaces. Our negative results obtained with the bead method must, therefore, be taken with caution.

Table 2. Summary of lectins and sugar ligands exposed on the surfaces of soybean (H and H63) protoplasts and of germinated cysts of *Phytophthora megasperma* f. sp. *glucinea* race 1

	Lectin specific for	sugar ligand(s)[c]
Protoplasts	β-glc[a]	glc/man
	gal, galNAc[b]	gal, galNAc
Germinated cysts	$galNH_2$	–
	fuc	–
	–	glc/man

Abbreviations see "Material and methods". $galNH_2$ = galactosamine.
[a] A β-lectin identified with the Yariv reagent;
[b] Soybean agglutinin (SBA) demonstrated by Ho et al. (1986);
[c] Results from Hohl and Balsiger (1986b).

Soybean lectin which has been demonstrated on protoplast surfaces of soybeans by immunological methods (Ho et al. 1986) could not be demonstrated in our study with either method. Insufficient mechanical strength of contacts or insufficient anchoring of the lectin in the plasmamembrane might account for this situation. This again demonstrates that different methods used for the demonstration of ligands and receptors may differ widely in their sensitivity and applicability.

Table 2 summarizes the glycosyl receptors (lectins) and the ligands shown to be accessible on the surfaces of germinated cysts of *P. megasperma* f. sp. *glycinea* and of soybean protoplasts. From these data several types of linkages between the two partners may be imagined. Some of the possibilities are indicated in Fig. 2.

The fucosyl receptor discovered on the surface of germinated cysts does not have a matching ligand on the host plasma membrane. It should be considered, however, that *Phytophthora* encounters additional host surfaces during its life as pathogen. Hinch and Clarke (1980) have shown that *P. cinnamomi* attaches to the root slime of Zea mays. This attachment is specific as it depends on the presence of terminal fucosyl residues of the slime. Our study shows that the corresponding fucosyl receptor is indeed present on the fungal surface. Quite likely the function of fucosyl receptors found in our study on *P. megasperma* f. sp. *glycinea,* and in *P. infestans* might well be involved in adhesion to root slimes and perhaps other root components of their corresponding hosts.

While all the components of linkage types 1 and 2 of Fig. 2 have been shown to be present, combinations 3 and 4 require a component the existence of which has not been proven. Combination 3 would need a soluble, non-attached glucose/mannose specific lectin present at the interface which could possibly form a link between the glc/man residues present on the two surfaces. This possibility is modelled to the role the lectin trifoliin is thought to be playing in the interaction between *Rhizobium trifolii* and clover (Dazzo and Truchet 1983). Combination 4 necessitates the existence of a carbohydrate component with more than one terminal galactosyl residue which could act as a linker between the soybean lectin (SBA) and the galactose-specific receptor on the fungal surface. The presence of such molecules is not unlikely but has not been directly demonstrated, yet.

Sugars of the glucose/mannose type have been shown to inhibit adhesion between soybean protoplasts and growing germ tubes of *P. megasperma* f. sp. *glycinea* (Hohl and Balsiger 1986a). These sugars potentially inhibit formation of the bonds shown diagrammatically in combinations 2 and 3 of Fig. 2. Since combination 3 contains an unproven

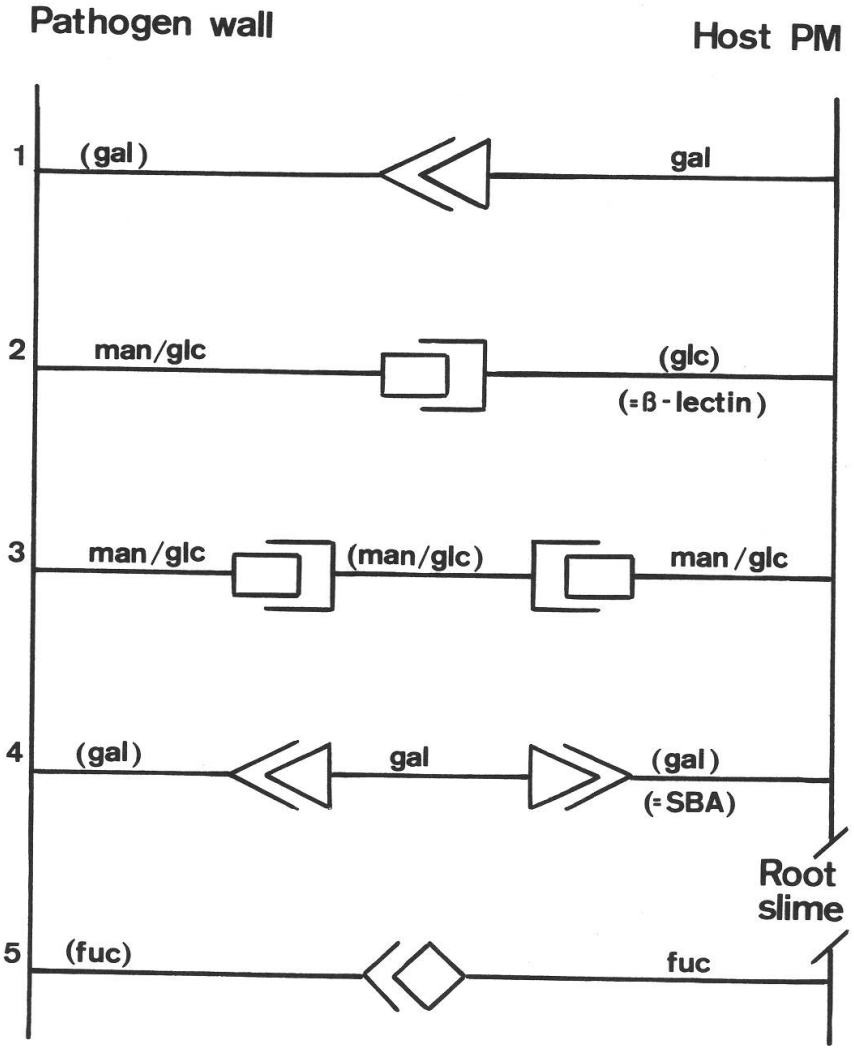

Fig. 2. Summary diagram. 1–4: Potential linkages between glycosyl receptors (lectins, with the sugar specificity shown in parentheses) and terminal glycosyl residues demonstrated on the outer surface of germinated cysts of *P. megasperma* f. sp. *glycinea* (pathogen) and on the surface of protoplasts (plasma membrane) of its soybean host. Based on experimental evidence, type 2 is the linkage most likely involved in adhesion. The presence of central, bridging linkers shown in 3 and 4, respectively, has not been demonstrated experimentally. 5: A potential link between the fucosyl-specific lectin of the pathogen and putative fucosyl residues of the root slime drawn in analogy to the situation in corn (Hinch and Clarke 1980).

element, a soluble glc/man specific lectin, combination 2 represents the type of linkage most likely to be involved in adhesion, as it best fits the results obtained so far.

While the results reported here add to our knowledge of the process of adhesion in fungal-host interactions, the lack of essential information in this area needs to be emphasized. It has not even been shown conclusively that adhesion is essential for the establishment of a compatible or incompatible situation in this system. Furthermore, adhesion refers to the purely mechanical aspects of interactions between host and parasite. Whether or not any of the bonds represented in Fig. 1 also participate in recognition, i.e. in triggering a reaction in either host or parasite (Hohl and Balsiger 1986a) remains to be determined.

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