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Effect of Chitosan on Membrane Permeability of Suspension-Cultured *Glycine max* and *Phaseolus vulgaris* Cells[1]

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DAVID H. YOUNG[2], HARALD KÖHLE, AND HEINRICH KAUSS
Fachbereich Biologie der Universität, Postfach 3049, D-6750 Kaiserslautern, Federal Republic of Germany

ABSTRACT

Treatment of suspension-cultured *Glycine max* cv Harosoy 63 cells with soluble chitosan (20–500 micrograms per milliliter) increased membrane permeability as shown by leakage of electrolytes, protein, and UV absorbing material. Severe damage to the cell membrane by chitosan (100 and 500 μg/ml) was also indicated by reduced staining with fluorescein diacetate and the leakage of fluorescein from preloaded cells. Other basic polymers (poly-L-lysine, histone, DEAE-dextran, protamine sulfate, and glycol chitosan) also increased permeability, whereas the basic monomers L-lysine and D-glucosamine, and acidic or neutral polymers were not active. Chitosan-induced leakage was inhibited by divalent cations, the order of effectiveness being $Ba^{2+} > Ca^{2+} > Sr^{2+} > Mg^{2+}$. Na polygalacturonate and Na poly-L-aspartate also reduced polycation-induced leakage, probably by formation of polycation-polyanion complexes. A chitosan-polygalacturonate complex precipitated on mixing solutions of the two polymers containing approximately equal numbers of galacturonate and glucosamine residues, but not with either polymer in excess. A similar concentration-dependent precipitation of chitosan by Na poly-L-aspartate was found. Leakage from *Phaseolus vulgaris* cv Grandessa cells was also induced by chitosan, and was inhibited by Ca^{2+} and Na polygalacturonate.

Chitosan (β-1,4-linked glucosamine) is a major component of the fungal cell wall in the Zygomycetes (3), and is probably formed by enzymic deacetylation of chitin (2). It is apparently not restricted to the Zygomycetes since recent reports have suggested its production at the host-pathogen interface during infection of pea by *Fusarium solani* (10, 11) and of wheat by *Puccinia striiformis* (12). Moreover, a chitin deacetylase is produced by the plant pathogen *Colletotrichum lindemuthianum* (H. Kauss, W. Jeblick, and D. H. Young, in preparation). The abilities of chitosan to elicit phytoalexin synthesis in peas (10), to induce the lignification response in wounded wheat leaves (22), and to inhibit the growth of a wide range of fungi (1) indicate its potential importance as a component of host-fungal interactions.

Synthetic polycations such as homopolymers of basic amino acids and DEAE-dextran, or basic proteins interfere with a wide variety of membrane functions in animal, plant, and bacterial cells (7, 24), and also cause leakage of material from plant tissue (5, 18, 25). In this study, we demonstrate that chitosan, a naturally-occurring polycation, increases the membrane permeability of plant cells due to its polycationic nature, and also binds to polygalacturonate, a component of plant cell walls.

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[2] Present address: Department of Plant Pathology, University of Wisconsin, Madison WI 53706.

MATERIALS AND METHODS

Cell Cultures. The cell suspension culture of *Glycine max* cv Harosoy 63 was a gift from J. Ebel, Freiburg University, Germany and that of *Phaseolus vulgaris* cv Grandessa (seeds from Bruno Nebelung, Münster, Germany) was initiated by inoculation of callus derived from sterile hypocotyl explants of 6-d-old seedlings. Suspension cultures were grown at 26°C in the dark on a 1.5-cm radius rotary shaker at 120 rpm in Erlenmeyer flasks containing B5 medium (9) which was modified by using 50 μM $FeSO_4$-EDTA as the source of iron (8), and subcultured at 6- and 12-d intervals, respectively.

Chemicals. Chitosan from crab shells (Sigma) was dissolved in 90 ml 0.1 N acetic acid/g chitosan by stirring overnight, centrifuged at 27,000*g* for 20 min to remove insoluble material, then precipitated by neutralization to pH 8.0 with 5 N NaOH. The precipitate was washed extensively with distilled H_2O by centrifugation and freeze-dried. The glucosamine content of this purified preparation was estimated to be 100% by the method of Ride and Drysdale (23). Aqueous solutions of purified chitosan and glycol chitosan (Sigma) were prepared for use by dissolving 100 mg in 18 ml 0.1 N acetic acid and dialyzing four times against 2 liters of distilled H_2O. Poly-L-lysine hydrobromide (mol wt, >70,000), DEAE-dextran (approximate mol wt, 500,000), histone (calf thymus, type II), Na polygalacturonate (grade II), Na poly-L-aspartate (mol wt, 20,000–50,000), glucosamine hydrochloride, L-lysine monohydrochloride, L-aspartic acid, FDA[3], and BSA (fraction V) were from Sigma. Protamine sulfate and galacturonic acid were from Merck (Darmstadt, Germany). Solutions of aspartic acid and galacturonic acid were prepared at 2 mg/ml in incomplete medium (see below) and the pH adjusted to 5.5 with 0.1 N NaOH before use. Laminarin (Ferak, Berlin, Germany) was dissolved in incomplete medium before use by heating to 60°C.

Permeability Changes. Cultures of *G. max* and *P. vulgaris*, grown for 6 and 12 d, respectively, were centrifuged at 1500*g* for 5 min and the cells washed four times in approximately 5 volumes of an incomplete medium consisting of 1 mM NaH_2PO_4 and 2% sucrose (w/v), adjusted to pH 5.5 with 0.1 N NaOH. The cells were then allowed to settle in a measuring cylinder for 20 min and resuspended in incomplete medium to give a 4:1 ratio of total volume:settled volume (approximately 190 mg fresh weight of cells/ml). Incomplete medium was used since polycation-induced leakage was strongly inhibited by the complete growth medium used, probably due to its high salt content.

Test mixtures contained 2 ml of cell suspension, incomplete medium and the appropriate test substances in a total volume of 3 ml in 5 ml glass tubes closed with plastic caps. Polycations were added last except in polyanion inhibition experiments in which, after addition of the polycation, the tubes were mixed and the polyanion added immediately. In each experiment, control mixtures without cells were included for all treatments, and also

[3] Abbreviation: FDA, fluorescein diacetate.

controls containing cells which were heated at 100°C for 5 min to provide an estimate of the total possible leakage of electrolytes and UV absorbing material. The protein content of total cell extracts, prepared as described below, was taken as an estimate of total possible protein leakage. All tubes were mixed gently at room temperature for the appropriate period on a roller mixer (Denley Technology, Sussex, England) then allowed to stand for 1 to 2 min so that most of the cells settled out. A 1.6-ml aliquot of the supernatant was centrifuged for 2 min at 10,000*g* in an Eppendorf 3200 centrifuge to remove the few remaining cells and the A_{260}, conductivity and protein content of the supernatant were determined. Protein was determined by the method of Lowry *et al.* (20). For conductivity measurements, 1 ml of supernatant was diluted with 50 ml deionized H_2O. All values given have been corrected for this dilution, and are expressed as Siemens (mho). Electrolyte leakage was calculated by subtracting the conductivity value of controls without cells from that of the supernatant from test mixtures with cells. Since the conductivity changes resulting from polycation-induced leakage were considerably greater than the conductivity of the incomplete medium, alone (8.8×10^{-5} mho) or containing polycations in the concentration range examined, possible errors resulting from differential uptake of Na phosphate from the medium or from adsorption of polycations to the cells were small and have been ignored.

Gel Filtration of Leaked Solutes. Test mixtures (3 ml) were incubated for 3 h with chitosan (500 μg/ml), $CHCl_3$ (1 ml added to the test mixture and the aqueous phase used subsequently for gel filtration), Triton X-100 (0.1%, v/v) or toluene-ethanol (150 μl of a stock solution, consisting of 1 volume of toluene and 4 volumes of ethanol, per test mixture). Supernatants from test mixtures were obtained as described above and 15 ml from pooled replicate samples were freeze dried. To prepare total cell extracts by homogenization, the cells from pooled replicate test mixtures were washed twice in incomplete medium by centrifugation at 1500*g* then resuspended to give the original volume, and homogenized for 1 min in an Ultra-Turrax homogenizer with the sample cooled in ice. After centrifugation of the homogenate at 27,000*g* for 20 min at 4°C, 15 ml of the supernatant were freeze dried.

Freeze dried samples were redissolved in 3 ml of 20 mM Na phosphate, pH 6.5, containing 0.02% NaN_3 and chromatographed on a 2.5 × 20 cm column of Sephadex G-25 in the same buffer at a flow rate of 20 ml/h. The eluates were monitored for *A* at 260 and 280 nm, and for protein.

FDA Staining. To 10 μl of cell suspension on a glass slide were added 10 μl of FDA solution prepared by diluting a stock solution (0.05% in acetone) 10-fold with incomplete medium. After 5 min incubation at room temperature, the cells were examined under incident illumination from a Ploemopak 2 UV light source with a Leitz Ortholux II fluorescence microscope using filter block E2 (transmission filter BP 436/7 and suppression filter LP 490).

Preloading of cells was achieved by incubating 20 ml cell suspension, prepared as described above, with 1 ml of FDA solution for 5 min, then washing the cells five times with incomplete medium by centrifugation at 1500*g* and finally resuspending them in 15 ml incomplete medium. Equal volumes of cell suspension and chitosan solutions at different concentrations in incomplete medium were mixed on glass slides and the release of fluorescence from the cells with time was followed under the fluorescence microscope.

Precipitation of Chitosan by Polyanions. To 0.5 ml of chitosan (200 μg/ml) in 1 mM Na phosphate, pH 5.5, in plastic tubes was added 0.5 ml of Na polygalacturonate or Na poly-L-aspartate at a series of concentrations in the same buffer. The tubes were mixed, and aliquots removed immediately for determination of the total polygalacturonate using the naphthoresorcinol assay for uronic acids (4), and total chitosan (23). After 1 h at room temperature, the tubes were centrifuged at 10,000*g* for 2 min in an Eppendorf 3200 centrifuge and the chitosan and polygalacturonate contents of the supernatants were measured.

RESULTS

Polycation-Induced Leakage. Treatment of *G. max* cells with chitosan induced the leakage of electrolytes, UV absorbing material, and protein into the medium (Fig. 1). The UV absorption spectrum of the leaked solutes showed a peak at 260 nm and was very similar to that of the total cellular solutes released by heating the cells at 100°C. In terms of total cell content, electrolyte leakage exceeded that of UV absorbing material and protein; a 3-h treatment with chitosan at 100 μg/ml induced the leakage of 72.8% of the total electrolytes, 35.6% of the total UV absorbing material, and 11.5% of the total extractable protein. The basic polymers poly-L-lysine, histone, DEAE-dextran, protamine sulfate, and glycol chitosan also induced leakage (Table II). In contrast, the monomeric bases D-glucosamine and L-lysine, the polyanions Na polygalacturonate and Na poly-L-aspartate, as well as BSA and laminarin which have little or no net charge at pH 5.5, showed no effect at concentrations up to 500 μg/ml.

Gel Filtration of Leaked Solutes. On Sephadex G-25 chromatography of the solutes which leaked from *G. max* cells treated with chitosan at 500 μg/ml for 3 h (Fig. 2A), the UV absorbing material was not excluded from the gel and is therefore of low mol wt (<5,000 D). The elution profiles of absorption at 260 and 280 nm indicate that it is heterogeneous. The protein which leaked from the cells consisted of both low and high mol wt material. $CHCl_3$ released 2- to 3-fold more protein than the chitosan treat-

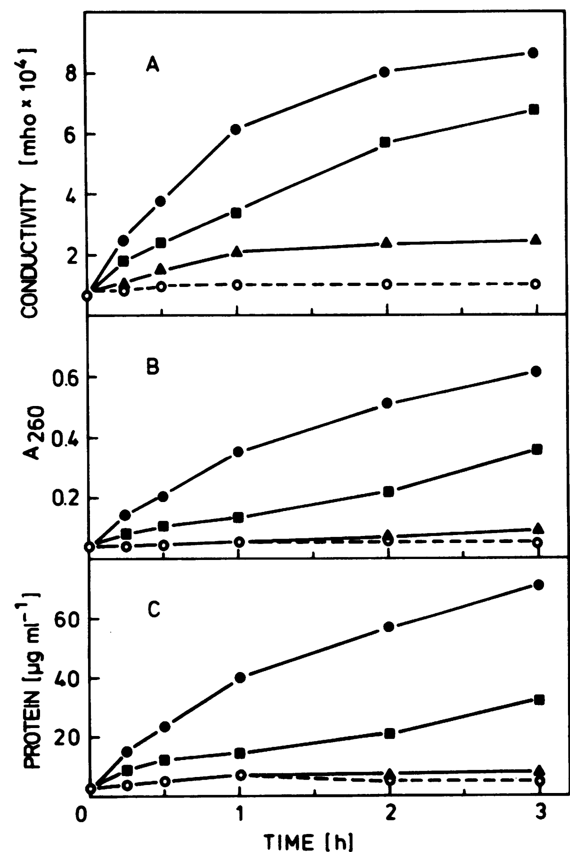

FIG. 1. Leakage from *G. max* cells treated with chitosan at 20 μg/ml (▲), 100 μg/ml (■), and 500 μg/ml (●) as a function of time. A, Conductivity; B, A_{260}; C, protein; (○), values for controls without chitosan. The values for conductivity, A_{260}, and protein representing total leakage were 9.2×10^{-4} mho, 1.01, and 2.78 μg/ml, respectively.

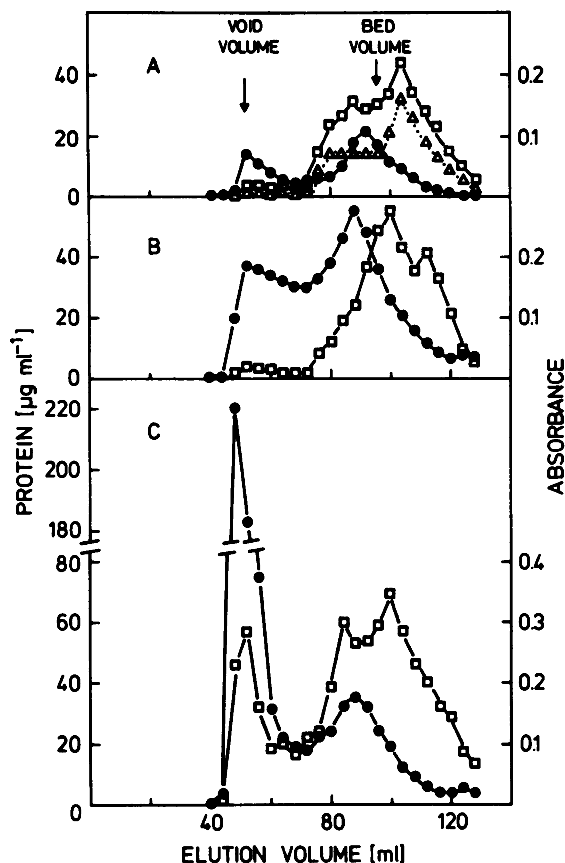

FIG. 2. Sephadex G-25 elution profiles of solutes leaked from *G. max* cells by treatment with chitosan at 500 µg/ml for 3 h (A), by $CHCl_3$ treatment for 3 h (B), and of total cell extract (C) prepared from the same amount of cells as in A and B. The void and bed volumes of the column were determined by chromatography of BSA and glucose, respectively. (●), protein; (□), A_{260}; (△), A_{280}.

ment; however, on gel filtration, the relative proportions of high- and low-mol-wt protein were approximately the same as those for chitosan (Fig. 2B). Homogenization released approximately twice as much protein as treatment with $CHCl_3$, and this additional protein was of high mol wt (Fig. 2C). Triton X-100 and toluene-ethanol treatments were similar to the chloroform treatment in terms of the amounts of protein released and its mol wt. The release of some high-mol-wt protein by toluene-ethanol is in contrast to a recent report that toluene-ethanol induces the formation of relatively small pores in plant cell membranes (17).

Chitosan (500 µg/ml) induced the leakage of 61 µg of soluble protein/ml test mixture within 3 h, and an additional 97 µg protein/ml could be extracted from the same cells by homogenization after chitosan treatment. This total amount (158 µg/ml test mixture) was considerably less than the amount which could be extracted from cells in a control test mixture without chitosan (322 µg/ml test mixture). Presumably some protein binds to chitosan molecules attached to insoluble cell material or is precipitated by chitosan. This may reduce the amount of soluble protein found in the medium after chitosan treatment.

FDA Staining of Chitosan-Treated Cells. Staining is believed to result from the uptake of FDA and its intracellular hydrolysis by esterase to release free fluorescein, which is retained by the plasma membrane and is fluorescent (14). This technique therefore serves as an indicator of membrane integrity, and also of cell viability (14, 26).

G. max cells were treated for 1 h with chitosan at 20, 50, 100, and 500 µg/ml before staining. At 20 µg/ml, which induced only slight leakage (Fig. 1), no effect on fluorescein accumulation was observed. At the higher chitosan concentrations, fluorescein accumulation was greatly reduced and was almost totally prevented at 500 µg/ml. In parallel, increased fluorescence outside the cells was observed. Most of the cells were present in clumps of varying size and those at the edges of clumps showed a greater reduction in staining than those in the middle.

Increased permeability of the plasma membrane to fluorescein after chitosan treatment was demonstrated directly by following fluorescein release from preloaded cells. Within 15 min of adding chitosan at 500 µg/ml to preloaded cells, a clear reduction in fluorescence inside the cells and a concomitant increase outside the cells was observed, and after 1 h the cells fluoresced only weakly. Less rapid leakage was observed at 50 and 100 µg chitosan/ml, and no reduction in fluorescence of the cells could be detected within 1 h at 20 µg/ml.

Effect of Cations and Polyanions. Chitosan-induced leakage of UV absorbing material from *G. max* was strongly inhibited by divalent cations at 10 mM, the order of effectiveness being $Ba^{2+} > Ca^{2+} > Sr^{2+} > Mg^{2+}$ (Table I). The monovalent ions K^+ and Na^+ showed only very slight inhibition. Electrolyte leakage could not be measured accurately in the presence of 10 mM salts. In experiments performed at cation concentrations of 0.5 mM (results not shown), the degree of inhibition by the divalent cations was considerably less but the same order of effectiveness of the different ions was found based on measurements of both UV absorbing material and conductivity.

Leakage from chitosan-treated *G. max* cells was also reduced by Na polygalacturonate and Na poly-L-aspartate (Fig. 3). The minimum concentrations of these polyanions required to prevent leakage of electrolytes almost completely were approximately those at which the number of carboxyl groups in the polyanion equaled the number of amino groups present as chitosan (concentrations indicated by the arrows in Fig. 3). Leakage induced by other polycations was also reduced by polyanions (Table II). The monomers, D-galacturonate and L-aspartate, showed no effect on polycation-induced leakage when tested at the same concentrations on a weight basis as the polyanions.

Precipitation of Chitosan by Polyanions. Turbidity was observed in a number of mixtures of polycations with Na polygalacturonate and Na poly-L-aspartate (Table II). The possibility that this resulted from precipitation of the polymers was investigated further in the case of chitosan-polyanion mixtures by adding solutions of the polyanions at a series of concentrations to chitosan solutions at a fixed concentration (Fig. 4). As the polyanion concentration increased, chitosan began to precipitate out above a critical concentration, and was virtually completely precipitated at polyanion concentrations near to those at which the number of carboxyl groups of the polyanion in question equaled that of the amino groups in chitosan. As the polyanion concentration in-

Table I. *Effect of Cations on Leakage of UV Absorbing Material from Glycine max Cells Treated for 3 Hours with Chitosan*

The A_{260} value representing total leakage was 1.04. Cations (10 mM) were added as the chloride salts.

Cation added	UV Absorption	
	−Chitosan	+Chitosan (100 µg/ml)
	A_{260}	
None	0.029	0.342
Ba^{2+}	0.027	0.058
Ca^{2+}	0.024	0.077
Sr^{2+}	0.026	0.117
Mg^{2+}	0.032	0.222
Na^+	0.030	0.280
K^+	0.030	0.327

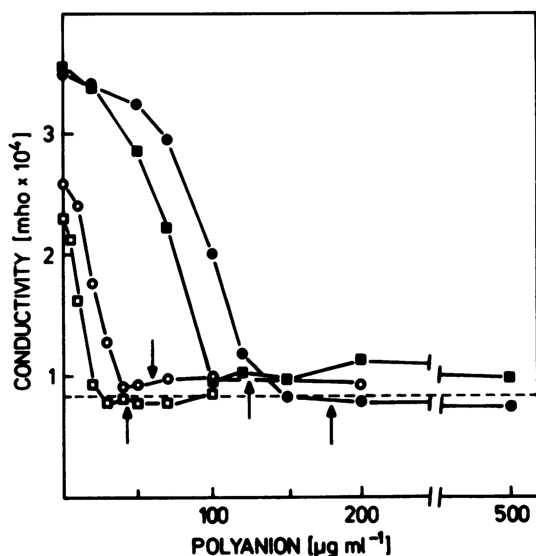

FIG. 3. Effect of Na polygalacturonate (●, ○) and Na poly-L-aspartate (■, □) on electrolyte leakage from *G. max* cells treated for 1 h with chitosan at 50 μg/ml (○, □) and 150 μg/ml (●, ■). Leakage in controls without chitosan (- - -). The arrows indicate the concentrations of Na polygalacturonate and Na poly-L-aspartate at which the number of carboxyl groups equal those of the amino groups present as chitosan. The conductivity values representing total leakage were 8.1×10^{-4} and 9.0×10^{-4} mho in the experiments with polygalacturonate and poly-L-aspartate, respectively.

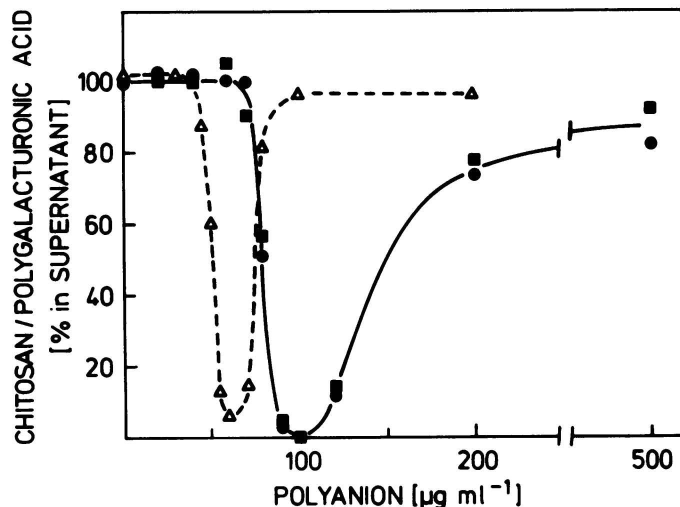

FIG. 4. Precipitation of chitosan by polyanions. Precipitation of chitosan (●) and polygalacturonate (■) in mixtures of the two polymers containing 100 μg/ml chitosan and different concentrations of Na polygalacturonate. (△), Precipitation of chitosan (100 μg/ml) in mixtures containing Na poly-L-aspartate at different concentrations.

Table II. *Inhibition by Na-Polygalacturonate and Na Poly-L-Aspartate of Electrolyte Leakage from Glycine max Cells Treated for 1 Hour with Various Polycations*

Asterisks indicate that turbidity was observed in control polycation-polyanion mixtures without cells. The conductivity value representing total leakage was 8.9×10^{-4} mho.

Polycation Added (100 μg/ml)	Conductivity				
	Control	+Na polygalacturonate		+Na poly-L-aspartate	
		50 μg/ml	100 μg/ml	50 μg/ml	100 μg/ml
			mho × 10⁴		
None	0.95	0.96	0.94	0.96	0.93
Poly-L-lysine	6.01	5.08	1.73*	4.79	1.07
Chitosan	3.76	2.88	1.30*	1.80*	1.15
Histone	3.33	1.07*	0.97	1.02	0.93
DEAE-dextran	3.09	1.35*	0.75	0.97*	0.88
Protamine sulfate	2.22	1.51	0.80*	0.84	0.91
Glycol chitosan	1.84	1.17	0.95	0.98*	0.90

creased further, precipitation decreased again. In the mixtures of chitosan and Na polygalacturonate, the proportion of polygalacturonate precipitated was virtually identical to that of chitosan, indicating that the precipitate consists of a chitosan-polygalacturonate complex. The experiment shown in Figure 4 was performed using incomplete medium without sucrose since the latter gave a strong color in the naphthoresorcinol assay used to determine polygalacturonate. However, the same precipitation curves for chitosan were obtained in experiments in which sucrose was included. No precipitation of chitosan by D-galacturonate and L-aspartate was found when tested in the same concentration ranges as the corresponding polymers.

Chitosan-Induced Leakage from *P. vulgaris* Cells. In analogous experiments to that for *G. max* in Figure 1, chitosan induced a similar leakage of electrolytes and UV absorbing material from *P. vulgaris* cells. This leakage was also reduced by Ca^{2+} and Na polygalacturonate under the same conditions as the experiments described for *G. max* in Table I and Figure 3

DISCUSSION

Chitosan treatment greatly increased the membrane permeability of suspension-cultured cells as shown by leakage of electrolytes and other material, the inability to accumulate fluorescein in the presence of FDA, and the rapid leakage of fluorescein from preloaded cells. This effect is clearly due to the polycationic nature of chitosan since other polycations showed similar activity, whereas acidic or neutral polymers and monomeric bases were not active.

In accord with previous studies on polycation-induced permeability changes in plant cells (5, 25), the effect of chitosan was inhibited by divalent cations. It has been suggested that polycations act by displacing cations from electro-negative sites on the membrane which require coordination with cations for dimensional stability (25). The observed order of inhibitory activity in our experiments ($Ba^{2+} > Ca^{2+} > Sr^{2+} > Mg^{2+}$) may reflect the ability of the larger cations Ba^{2+}, Sr^{2+}, and Ca^{2+} to form complexes with high and usually variable coordination numbers, in contrast to Mg^{2+} which is more demanding upon its ligands and forms complexes with a fixed coordination number of 6 (13).

In previous studies on polycation-induced leakage from plant cells (5, 18, 25), only low-mol-wt material was examined. The solutes released from chitosan-treated cells also appear to be mainly of low mol wt; however, some leakage of high-mol-wt protein (>5,000 D) did occur (Fig. 2), at least at the fairly high chitosan concentration of 500 μg/ml. The relative proportions of low- and high-mol-wt protein were similar to those for protein released after drastic disruption of the membranes by $CHCl_3$ or Triton X-100. It therefore appears that quite large 'pores' can be induced in the membrane by chitosan. The observation that brief homogenization released considerably more high-mol-wt protein than treatment with $CHCl_3$ or Triton X-100 raises the possibility that leakage of high-mol-wt proteins might be limited by diffusion through the cell wall, although it seems that large polycations like

DEAE-dextran do penetrate the cell wall to interact with the plasma membrane.

The extent to which polycation-induced leakage was reduced by Na polygalacturonate and Na poly-L-aspartate (Table II) seems to depend primarily on the number of charged groups in the respective polyanion and polycation. Thus, on a weight basis, polyaspartate showed a greater effect on all polycations tested than polygalacturonate, and higher concentrations of polyanion were required to prevent leakage induced by polycations with a large number of charged groups on a weight basis such as poly-L-lysine or chitosan than by those with fewer charged groups such as histone, DEAE-dextran, or glycol chitosan.

A number of different effects caused by polycations such as the activation or inhibition of various enzymes, the retardation of blood clotting, the inactivation of viruses, the inhibition of bacterial growth and the agglutination of erythrocytes can be prevented or reversed by polyanions (reviewed in 15). A further example is the inhibition by dextran sulfate of DEAE-dextran-induced lysis of yeast spheroplasts (7). The effect of polyanions in such cases is apparently due to the formation of polycation-polyanion complexes, which under appropriate conditions may precipitate out (15, 21). Such interactions are almost certainly responsible for the effect of polyanions in the present study. Precipitate formation was observed in a number of polycation-polyanion combinations (Table II) and complex formation was demonstrated directly in the case of chitosan and Na polygalacturonate by precipitation of the two polymers in a critical concentration range when approximately equal numbers of amino and carboxyl groups were present (Fig. 4). Precipitation curves similar to those in Figure 4 have been reported for the precipitation of nucleic acids by DEAE-dextran (21). Precipitation apparently results when the oppositely charged polymers neutralize each other, reducing the net charge and decreasing the solubility. When chitosan or the polyanion was in excess (Fig. 4), precipitation was not observed, but complex formation probably occurred since chitosan-induced leakage was reduced (Fig. 3). Complexes formed under these conditions probably carry a net charge and consequently are more soluble. Monomeric galacturonate and aspartate did not affect polycation-induced leakage or precipitate chitosan. The probable explanation is that individual ionic bonds between such monomers and polycations can dissociate, whereas it is unlikely that multiple bonds between two oppositely charged polymers would break at the same instant.

The demonstration that polycations interact with polygalacturonate provides a likely explanation for reports that labeled polycations bind to the plant cell wall (6, 11). The interaction of chitosan with polygalacturonate also indicates a possible mechanism for adhesion of the fungal wall to that of the host plant during infection. The differential interaction of a factor in sweet potato consisting largely of galacturonic acid with the surface of germinated spores of different strains of *Ceratocystis fimbriata* has been implicated in determining host-pathogen specificity (16). In addition, binding of *Agrobacterium tumefaciens* to polygalacturonate of the plant cell wall may be an essential step in the initiation of crown gall tumors (19). These examples suggest the possible significance of interactions between polycations and polyanions similar to those of chitosan with polygalacturonate in host-pathogen recognition. As discussed previously (27), recognition need not involve group-specific binding such as a lectin-carbohydrate association but could be based on interactions of a less specific nature.

Recent studies using histochemical and immunochemical techniques to locate chitosan during infection of pea by *Fusarium solani* (10, 11) and of wheat by *Puccinia striiformis* (12) have suggested its presence on the outer surface of the fungal wall and, in the case of *Fusarium* infection, in the surrounding plant tissue. Based on our results, an increase in the membrane permeability of host cells would be expected in the presence of soluble chitosan, or when chitosan on the fungal wall surface makes contact with the host cell membrane. The cell wall may help to protect the plasma membrane from such effects by binding to chitosan, although its effectiveness as a natural barrier would be limited by its binding capacity. It seems possible that interactions of chitosan with membrane or cell wall components might be involved in its reported abilities to elicit phytoalexin synthesis (10), to induce lignification (22), and to inhibit fungal growth (1). In contrast to these effects which would be expected to restrict fungal growth during pathogenesis, increased permeability of host cells could facilitate infection by enhancing the flow of nutrients to the pathogen and possibly by enabling fungal products to enter host cells. More information about the location and metabolism of chitosan at the host-pathogen interface as well as its effects on plant and fungal cells will be necessary to assess more accurately the various roles which chitosan may play in host-pathogen interactions.

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