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Lectin Receptors on the Plasma Membrane of Soybean Cells. Binding and Lateral Diffusion of Lectins†

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ABSTRACT: Protoplasts prepared from suspension cultures of root cells of *Glycine max* (SB-1 cell line) bound soybean agglutinin (SBA), concanavalin A (Con A), and wheat germ agglutinin (WGA). Binding studies carried out with ^{125}I-labeled SBA, Con A, and WGA showed that these interactions were saturable and specific. Fluorescence microscopy demonstrated uniform membrane labeling. The mobility of the lectin–receptor complexes was measured by fluorescence redistribution after photobleaching. The diffusion constants (D) for SBA and Con A were 5×10^{-11} and 7×10^{-11} cm^2/s, respectively. In contrast, WGA yielded a diffusion constant of 3×10^{-10} cm^2/s. Pretreatment of the protoplasts with either SBA or Con A resulted in a 6-fold reduction in the mobility of WGA ($D \simeq 5 \times 10^{-11}$ cm^2/s). The results suggest that the binding of SBA or Con A may lead to alterations of the soybean plasma membrane which, in turn, may restrict the mobility of other receptors.

The measurement of the dynamics of plasma membrane receptors has catalyzed research into the role of lateral mobility as an important component of transmembrane signaling mechanisms (Cherry, 1979; Edidin, 1981; Peters, 1981). From these investigations, two different schemes have emerged for the motion of membrane components: (a) lateral mobility as a consequence of Brownian movement (Frye & Edidin, 1970; Saffman & Delbruck, 1975; Schlessinger et al., 1976) and (b) directional flow on the cell surface (Taylor et al., 1971; Koppel et al., 1982). Lateral mobility based on diffusional fluxes in a two-dimensional continuum has been characterized for a great number of membrane proteins (Peters, 1981), and to a lesser extent for a few varieties of lipid (Peters, 1981) and glycolipid (Wolf et al., 1977; Schindler et al., 1980b). In general, these measurements have been performed by using the technique of fluorescence redistribution after photobleaching (FRAP;[1] Koppel, 1979; Peters, 1981) and have yielded values of from 10^{-8}–10^{-12} cm^2/s for membrane proteins to 10^{-8}–10^{-9} cm^2/s for phospholipids and glycolipids in the same membranes. Of particular interest was the observation that a particular diffusing species of protein could have an immobile component (Peters, 1981). A number of theories have been presented to explain this type of protein mobility in the context of intraplasma membrane and cytoskeletal interactions with the diffusing molecule (Edelman, 1976; Schindler et al., 1980b; Koppel et al., 1981).

The other type of movement for cell membrane components observed was a directional flow ultimately leading to cap formation on lymphoid cells (Taylor et al., 1971) and the movement of a concanavalin A (Con A)–receptor complex during late anaphase or telophase to the developing cleavage furrow in J7742 mouse macrophages (Koppel et al., 1982). This type of protein mobility has been ascribed to cross-linking of membrane receptors that normally occurs only when exogenously added ligands bind to the receptors. One receptor movement mechanism need not exclude the other, since receptors cross-linked by specific ligands and undergoing directed

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[1] Abbreviations: SBA, soybean agglutinin; Con A, concanavalin A; WGA, wheat germ agglutinin; FRAP, fluorescence redistribution after photobleaching; MGB, modified Gamborg buffer; MGB–N_3–BSA, modified Gamborg buffer containing 0.01 M NaN_3 and 0.2% (w/v) bovine serum albumin (BSA).

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motion, e.g., capping, should not interfere with other receptors randomly diffusing through the plane of the membrane.

Although the use of reagents such as the lectin Con A to induce and modulate receptor redistribution provided much of the initial information on receptor mobility of both types, relatively little attention has been focused on whether the plant-derived lectins have similar interactions with receptors of plant cells. In the present study, we have prepared protoplasts from a suspension culture of soybean (*Glycine max*) cells and investigated the binding and lateral diffusion of lectins bound to the plasma membrane of these cells. FRAP measurements performed on a series of soybean protoplasts demonstrated a slower mobility and higher recovery for Con A and soybean agglutinin (SBA) than observed in animal cells. Moreover, a significant decrease was observed for the mobility of wheat germ agglutinin (WGA) receptors in the presence of SBA and Con A.

Materials and Methods

Cell Culture and Protoplast Isolation. The SB-1 line of soybean (*Glycine max*) cells was kindly provided by Dr. F. Constabel (Prairie Regional Laboratory, Saskatoon, Saskatchewan, Canada) and was grown in the dark. Protoplasts were prepared by a modified procedure of Constabel (1975). Actively growing SB-1 cells (24–48 h after transfer) were digested with an equal volume (20 mL) of enzyme solution containing 400 mg of cellulysin (Calbiochem, La Jolla, CA), 200 mg of pectinase (Sigma, St. Louis, MO), and 2 g of D-sorbitol (Sigma), pH 5.5. After 2 h, the protoplast suspension was filtered through a 48-μm nylon filter and pelleted by centrifugation in a clinical centrifuge for 4 min at 460*g*. The pelleted protoplasts were washed by gentle resuspension and centrifugation by using 5 mL of protoplast medium (Constabel, 1975), which was modified by substituting 30 g of D-sorbitol for glucose (modified Gamborg buffer, MGB). After three washes, the protoplasts were resuspended in 5 mL of MGB.

Binding of Fluorescent Lectins. The binding of various lectins to protoplasts was assayed by fluorescence microscopy following the representative protocol detailed below: (a) protoplasts (5×10^5/mL) were incubated with lectin solution (50 μg/mL), SBA (Allen & Neuberger, 1975), Con A (Cunningham et al., 1972), or fluorescein-conjugated WGA (Miles, Elkhart, IN) for 20 min at 23 °C; (b) protoplasts were washed by centrifugation and resuspension in MGB containing 0.01 M NaN_3 and 0.2% (w/v) bovine serum albumin (MGB–N_3–BSA); (c) in cases where SBA or Con A was used, the protoplasts were further incubated for 15 min with 100 μg/mL rabbit antibodies directed against SBA or Con A, followed by fluorescein-conjugated (Difco, Detroit, MI) or rhodamine-conjugated (Cappel, West Chester, PA) goat antibodies directed against rabbit immunoglobulin; (d) after the protoplasts were washed, they were suspended in a final volume of 50 μL of MGB–N_3–BSA and observed under a Leitz fluorescence microscope, equipped with a Leitz KP490 dichroic mirror. Micrographs were taken with Kodak Tri-X film, which was pushed to ASA 1600. Staining of protoplasts with Calcofluor White (American Cyanamid, Wayne, NJ) was done as described by Constabel (1975).

The demonstration of sugar specificity with respect to lectin binding was accomplished by incubating the lectin and protoplasts together in the presence of 0.2 M sugar for 60 min at room temperature (*N*-acetyl-D-galactosamine for SBA, methyl α-D-mannoside for Con A, *N*-acetyl-D-glucosamine for WGA).

Binding of ^{125}I-Labeled Lectins. Con A was labeled with radioactive iodine (^{125}I; Amersham, Arlington Heights, IL) by using the iodogen 1,3,4,6-tetrachloro-3a,6a-diphenylglycoluril (Fraker & Speck, 1978). SBA and WGA were iodinated according to the method of Greenwood et al. (1963). Protoplasts (5×10^5/mL) were incubated with ^{125}I-labeled lectins for 60 min at room temperature. After three washes by centrifugation and resuspension of the cells in MGB–N_3–BSA, the protoplasts were transferred to γ counting vials, and the radioactivity was determined. Nonspecific binding was determined by incubating the radiolabeled lectin in the presence of the competitive saccharide. The values for specifically bound lectin were obtained by subtracting the amount of nonspecifically bound lectin from the total bound.

Fluorescence Recovery after Photobleaching. Lectins directly conjugated with fluorescein or rhodamine were obtained from Vector Laboratories (Burlingame, CA) and were used in all the fluorescence photobleaching experiments. Protoplasts were prepared for photobleaching by the following procedure: (a) protoplasts (5×10^5/mL) were incubated with fluorescently derivatized lectin at 5, 50, or 250 μg/mL for 20 min at 23 °C; (b) the protoplasts were washed by centrifugation and resuspension in 1 mL of MGB–N_3–BSA; (c) after the protoplasts were washed, they were suspended in a final volume of 50 μL of MGB–N_3–BSA. For experiments involving the use of the inhibitor of cell wall regeneration, coumarin (Aldrich, Milwaukee, WI), MGB containing 1.4 mM coumarin was used instead of MGB–N_3–BSA.

The sequential labeling of protoplasts with two different lectins was done by pretreatment of the protoplasts (5×10^5/mL) with 50 μg/mL SBA or Con A for 20 min at 23 °C. After the cells were washed by centrifugation and resuspension in MGB, they were labeled with 50 μg/mL rhodamine-derivatized WGA as described above.

Prewashed slides were placed in phosphate-buffered saline for at least 1 min, rinsed with distilled water, and dried prior to use. Cover slips were rinsed in distilled water and dried. A drop of protoplast suspension was placed on a washed slide, mounted with a coverslip, sealed with warm paraffin wax, and subjected to FRAP.

The lateral diffusion coefficients of fluorescently labeled lectins bound to the plasma membrane of protoplasts were measured by the FRAP method as previously described (Koppel et al., 1980). The experimental optics and electronics have been described in detail elsewhere (Koppel, 1979). Briefly, a Leitz Ortholux II fluorescence microscope, equipped for incident illumination, is used to focus an argon laser beam (diameter 1 μm) onto the sample and collect the fluorescence for detection and subsequent processing by photon-counting electronics. The angular orientation of the incident laser beam, and hence the location on the sample of the focused spot along the scan axis, is controlled by a servo-activated galvanometric optical scanning mirror. Fluorescein emission was monitored with an incident wavelength of 476.5 nm in combination with a Leitz TK510 dichroic mirror and a K530 barrier filter. For rhodamine emission, the incident wavelength was 514.0 nm, and a Leitz TK580 dichroic mirror and K570 barrier filter were used. All measurements were done with a X40/0.65 NA dry objective. The redistribution of the fluorescence, following a localized photobleaching pulse, was analyzed with a normal-mode analysis, following the approach of Koppel et al. (1980).

Scanning Electron Microscopy. Freshly isolated protoplasts were also prepared for scanning electron microscopy by fixation in 2% (v/v) glutaraldehyde as described by Williamson et al. (1976). The cells were postfixed in 2% (w/v) osmium tetraoxide at 4 °C for 30 min. After the cells were washed, they

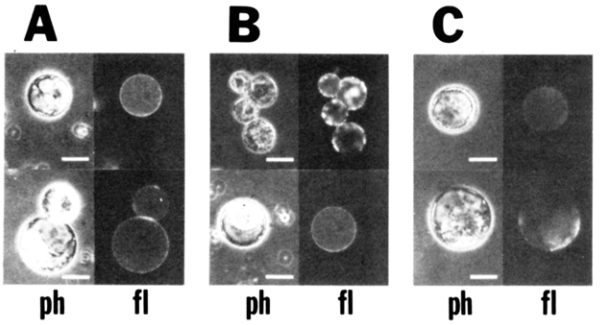

FIGURE 1: Fluorescence staining patterns of soybean protoplasts treated for 20 min at 23 °C with various lectins. (A) Soybean agglutinin (50 μg/mL); (B) concanavalin A (50 μg/mL); (C) wheat germ agglutinin (50 μg/mL). ph, phase contrast microscopy; fl, fluorescence microscopy; magnification 130×; bar = 25 μm.

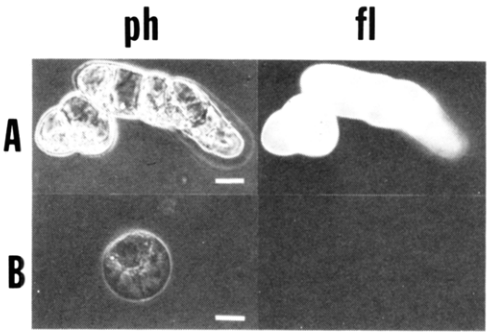

FIGURE 2: Fluorescence pattern of soybean cells and soybean protoplasts treated with Calcofluor (0.05% w/v). (A) Undigested cells (magnification 80×, bar = 40 μm); (B) protoplasts (magnification 28×, bar = 25 μm). ph, phase contrast mieroscopy; fl, fluorescence microscopy.

were applied to a glass cover slip coated with polylysine, dehydrated in alcohol, critical point dried, and coated with carbon followed by gold. The specimens were examined on a JEOL JSM 35C at an acceleration voltage of 15 kV. Photographs were taken with Polaroid 665 film.

Results

Binding of Lectins to Soybean Protoplasts. Protoplasts prepared from suspension cultures of soybean root cells bound the lectins SBA, Con A, and WGA. Typical fluorescence microscopy results, obtained after incubation for 20 min at 23 °C are shown in Figure 1. The general labeling patterns of the cells showed a diffuse ringlike stain, suggesting that most of the receptors were uniformly distributed in the cell membrane (Figure 1). However, in a number of protoplasts (accounting for about 15% of the population), the fluorescence was localized in patches spread over one hemisphere of the cell or completely segregated over one pole of the cell (Figure 1, B top, C bottom). Both of these patterns were observed for all lectins tested in our study. The proportion of cells showing patches or polar distribution of fluorescence did not change with different times of incubation. Finally, the binding of the lectins to the protoplasts resulted in agglutination of some of these cells (Figure 1, A bottom, B top).

The results of control experiments demonstrated that the observed fluorescence patterns were due to binding of the lectin to carbohydrate structures on the plasma membrane. First, protoplasts prepared from the SB-1 cell line did not exhibit autofluorescence. Second, the majority (>95%) of the protoplasts used in our studies did not stain with the fluorescent dye Calcofluor which is commonly used to demonstrate the presence of cell wall material (Nagata & Takebe, 1970). Figure 2A is an example of the fluorescent staining due to the binding of Calcofluor to the cell wall of intact soybean cells. In marked contrast, a similarly stained protoplast, shown in Figure 2B, exhibits no fluorescence. Third, when freshly prepared protoplasts were fixed in glutaraldehyde, followed by osmium tetraoxide, and examined by scanning electron microscopy, the plasma membrane was devoid of cellulose microfibrils, as shown in Figure 3C,D. The cellulose microfibrils of the cell wall are readily visualized on untreated cells (Figure 3A,B).

We have also found that the binding of SBA, Con A, and WGA was inhibited by the monosaccharides *N*-acetylgalactosamine, methyl α-D-mannoside, and *N*-acetylglucosamine, respectively. Finally, incubation of protoplasts with fluorescein-labeled or rhodamine-labeled goat antibodies directed against rabbit immunoglobulin in the absence of lectins did not show any fluorescence. All of these results strongly

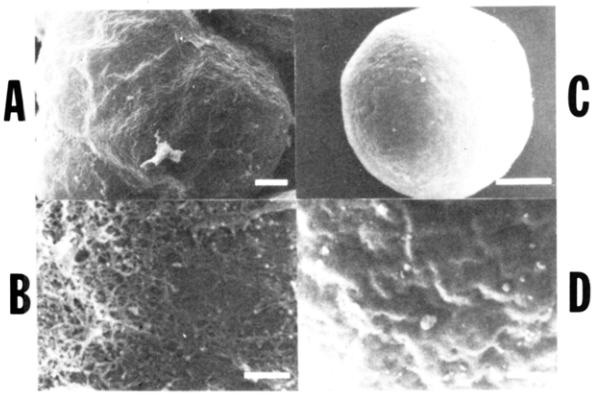

FIGURE 3: Scanning electron micrographs of undigested soybean cells and protoplasts. (A) Undigested soybean cells showing cell wall (magnification 600×, bar = 5 μm); (B) undigested soybean cell showing cellulose microfibrils of cell wall (magnification 4600×, bar = 1 μm); (C) freshly isolated protoplast (magnification 1184×, bar = 4 μm); (D) high magnification of freshly isolated protoplast showing no cellulose microfibrils on plasma membrane (magnification 4500×, bar = 1 μm).

suggest that the observed staining patterns for each of the lectins could be ascribed to the binding of the ligand to heterosaccharide structures of the plasma membrane.

The binding of the lectins SBA, Con A, and WGA to soybean protoplasts was dependent on the concentration of the ligand added (Figure 4). This was demonstrated by incubating ^{125}I-labeled lectin with soybean protoplasts for 1 h at 23 °C. Under these conditions, the binding of each one of the lectins can be saturated. At 50 μg/mL, the concentration used for many of the FRAP and fluorescence microscopy studies, there are approximately 5×10^7, 2×10^8, and 4×10^7 subunits of SBA, Con A, and WGA bound per cell, respectively.

Lateral Mobility of Lectins Bound to Soybean Protoplasts. The mobility of fluorescently labeled lectins bound on protoplasts at 23 °C was determined by using the FRAP method. Photobleaching experiments were done on individual, nonagglutinated cells. Representative data from an experiment using SBA (50 μg/mL) are shown in Figure 5. This graph shows a semilogarithmic plot of the time course of the first normal mode of fluorophore distribution (Koppel et al., 1980) after a photobleaching pulse. Each point represents a complete fluorescence scan across the protoplast. The inset presents a typical scan across the protoplast prior to the photobleaching pulse. The peaks indicate that the fluorescent lectin is associated predominantly with the membrane, giving more intense fluorescence at the edges of the cell. The data from this and similar experiments did not show significant heterogeneity in

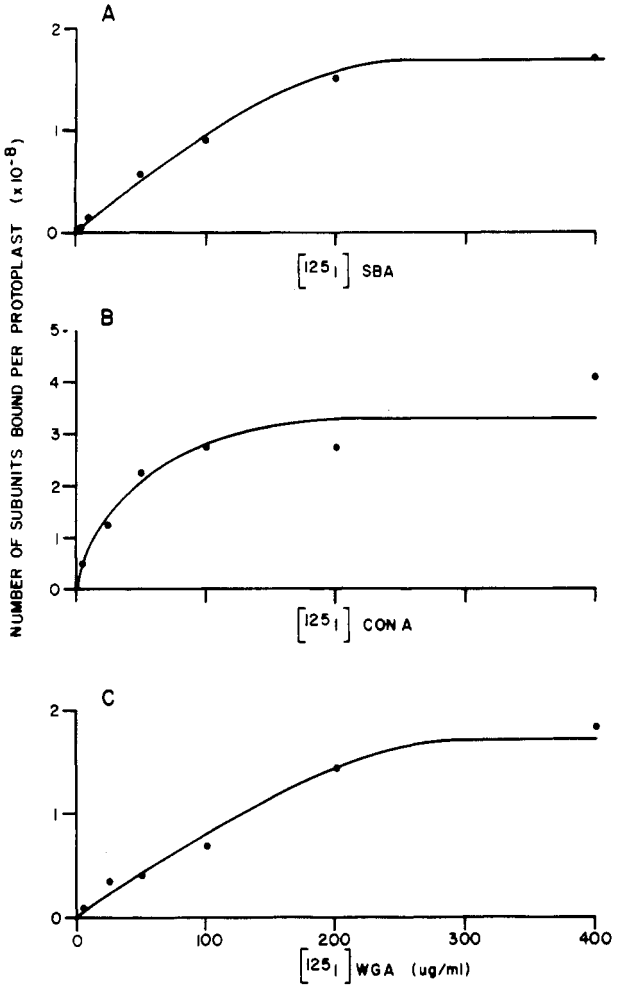

FIGURE 4: Dose–response curve of the binding of ^{125}I-labeled lectin to soybean protoplasts. ^{125}I-Labeled lectin was incubated with 5 × 10^5 protoplasts/mL for 1 h at 23 °C in duplicate. Nonspecific binding of ^{125}I-labeled lectin was determined by incubating ^{125}I-lectin with the protoplasts in the presence of the competing sugar and subtracted from total ^{125}I-lectin bound for each concentration. The subunit molecular weights used in the calculations were 3 × 10^4, 2.6 × 10^4, and 1.8 × 10^4 for soybean agglutinin, concanavalin A, and wheat germ agglutinin, respectively.

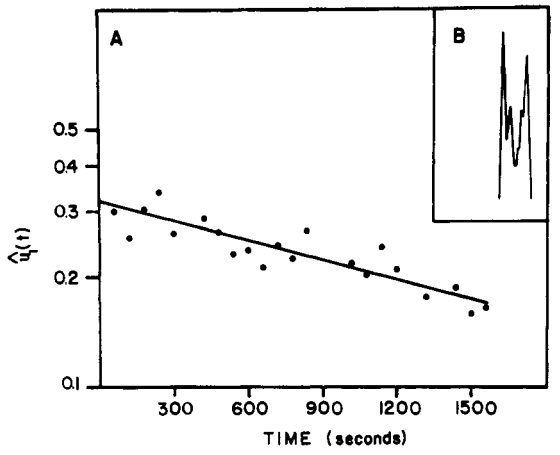

FIGURE 5: (A) Semilogarithmic plot of $\hat{\mu}_1(t)$ (the experimental estimate of the normalized first moment of the fluorophore concentration distribution) as a function of time after photobleaching on a soybean protoplast labeled with fluorescein-derivatized soybean agglutinin. D = 3.6 × 10^{-11} cm^2/s. (B) A typical scan across the protoplast membrane prior to the photobleaching pulse.

diffusion rates in terms of deviations from a single exponential decay. Therefore, diffusion coefficients (D) were determined

Table I: Diffusion Coefficients of Soybean Agglutinin Bound to Soybean Protoplasts at 23 °C

soybean agglutinin concn (μg/mL)	treatment	D^a (×10^{-11} cm^2/s)	% recovery^a
5		5.8 ± 4.6	83 ± 11
50		4.6 ± 2.1	91 ± 6
250		6.2 ± 2.6	84 ± 8
50	no NaN_3–BSA	6.9 ± 0.4	83 ± 8
50	1.4 mM coumarin	2.1 ± 1.8	79 ± 10

^{a} Values are expressed as mean ± standard deviation.

Table II: Diffusion Coefficients of Concanavalin A and Its Succinyl Derivative Bound to Soybean Protoplast at 23 °C

probe	concn (μg/mL)	D^a (×10^{-10} cm^2/s)	% recovery^a
Con A	5	0.41 ± 0.09	86 ± 8
	50	0.72 ± 0.52	78 ± 11
	250	1.4 ± 0.09	83 ± 16
succinyl-Con A	5	1.5 ± 1.4	93 ± 4
	50	1.0 ± 0.6	90 ± 9
	250	3.8 ± 2.8	92 ± 7

^{a} Values are expressed as mean ± standard deviation.

Table III: Diffusion Coefficients of Wheat Germ Agglutinin Bound to Soybean Protoplasts at 23 °C

probe	concn (μg/mL)	treatment	D^a (×10^{-10} cm^2/s)	% recovery^a
WGA	5		1.5 ± 0.2	86 ± 7
	50		3.0 ± 1.8	84 ± 13
	250		4.3 ± 2.8	80 ± 9
WGA	50	pretreat with 50 μg/mL SBA	0.46 ± 0.17	34 ± 11
WGA	50	pretreat with 50 μg/mL Con A	0.55 ± 0.32	41 ± 14

^{a} Values are expressed as mean ± standard deviation.

from the initial slopes of the semilogarithmic plots and correspond to the ensemble average D values.

The experiment shown in Figure 5 yielded a value of 3.6 × 10^{-11} cm^2/s for the diffusion coefficient of SBA (50 μg/mL) at 23 °C. D values of this magnitude are indicative that the component, though mobile in the membrane, has very slow lateral mobility. Furthermore, we did not obtain any significant change in the diffusion coefficient (Table I) when the photobleaching experiments were repeated with either very low (5 μg/mL) or very high (250 μg/mL) concentrations of SBA (see Figure 4). The molecular weight of SBA under these conditions was estimated to be 120 000 by gel filtration studies. Therefore, the aggregation of SBA into high molecular weight complexes (Lotan et al., 1974) is not involved in the slow diffusion constant.

Two further experiments were conducted to ascertain the validity of the D values obtained for SBA. First, the presence of sodium azide (NaN_3–BSA) in the protoplast medium was shown to have no effect on the mobility of SBA bound to the protoplasts (Table I). Second, in order to prevent the deposition of newly synthesized cell wall material on the surface of the protoplast plasma membrane, we conducted a series of photobleaching experiments in the presence of coumarin, an inhibitor of cell wall synthesis (Meyer & Herth, 1978). The resulting diffusion coefficient (2.1 × 10^{-11} cm^2/s) showed no significant difference from previous data. Thus, the slow lateral mobility of SBA bound to protoplasts is not due to the

regeneration of cell wall components and is not affected by the lack of metabolic energy.

The diffusion constants of other lectins bound on their receptors of the soybean protoplast are summarized in Tables II and III. At a concentration of 50 μg/mL, Con A yielded a diffusion constant of 7.2×10^{-11} cm^2/s, similar to that obtained for SBA (see Table I). The diffusion constant for succinyl-Con A, a chemical derivative of Con A (Gunther et al., 1973), was approximately 1×10^{-10} cm^2/s (Table II). In contrast, WGA gave D values of $\sim 3 \times 10^{-10}$ cm^2/s (Table III). Therefore, it was apparent that lectins bound to soybean membranes exhibited two (rather arbitrary) classes of lateral mobility: (a) relatively fast ($D \simeq 3 \times 10^{-10}$ cm^2/s) and (b) relatively slow ($D \simeq 5 \times 10^{-11}$ cm^2/s).

Modulation of WGA–Receptor Mobility by SBA and Con A. The effect of the binding of unlabeled SBA or unlabeled Con A on the mobility of the more rapidly diffusing rhodamine-conjugated WGA was studied. In both cases, the D values of the labeled lectin were decreased by 6-fold (Table III). Therefore, WGA yielded diffusion constants of 4.6×10^{-11} cm^2/s in the presence of SBA and 5.5×10^{-11} cm^2/s in the presence of Con A. Both of these values approached the diffusion coefficients of the unlabeled lectin (Tables I and II).

Using ^{125}I-labeled WGA, we have determined that pretreatment of soybean protoplasts with unlabeled SBA (50 μg/mL) did not alter the number of molecules of WGA bound to the cells. There were approximately 4×10^7 WGA subunits bound per protoplast at a concentration of 50 μg/mL ^{125}I-WGA. This value is comparable to that obtained in the absence of SBA (Figure 4C). Therefore, the decrease in D values of WGA in the presence of SBA most probably cannot be ascribed to changes in the number of receptors (either the disappearance of a population of rapidly diffusing receptors or the appearance of a population of relatively immobile receptors).

Because many lectins are themselves glycoproteins, it was important to establish that WGA did not interact with SBA or Con A. Using double-immunodiffusion assays, we found no evidence of interaction in the two pairs of lectins. We have also carried out gel filtration studies of ^{125}I-labeled WGA in the presence and absence of unlabeled SBA. The positions of elution for ^{125}I-WGA under both conditions were essentially identical. It appears, therefore, that the modulation of the mobility of WGA bound on its receptors was not due to cross-linking of the mobile lectin to another lectin anchored on a set of slow-moving receptors.

Discussion

Plant lectins have been shown to bind and agglutinate protoplasts prepared from carrot (Glimelius et al., 1974), grapevine (Burgess & Linstead, 1976), soybean (Williamson et al., 1976), tobacco (Burgess & Linstead, 1976, 1977; Larkin, 1978), broad bean (Larkin, 1978), and leek (Williamson, 1979) cells. Our present results confirm the observations of Williamson and co-workers on soybean cells (Williamson et al., 1976). Calcofluor staining and scanning electron microscopy show neither the characteristic fluorescence indicative of cell wall material (Nagata & Takebe, 1970) nor cellulose microfibrils (Burgess et al., 1977), respectively. Although small amounts of residual cell wall components may still be present, the specific saturable binding of lectins observed is best interpreted in the context of lectin binding to heterosaccharide structures that are plasma membrane components in a manner analogous to animal cells.

These considerations are particularly important in interpreting the results of experiments aimed at assessing the lateral mobility of lectins bound to the plasma membrane of protoplasts. The key observations of the present study include the following: (a) the diffusion coefficient of SBA bound on soybean membrane is 5×10^{-11} cm^2/s; this value is not significantly affected by ligand concentration or by the presence of sodium azide, a metabolic inhibitor, or coumarin, an inhibitor of cell wall regeneration (Meyer & Herth, 1978); (b) the diffusion coefficient of Con A bound to protoplasts is similar to that observed for SBA ($D \simeq 7 \times 10^{-11}$ cm^2/s); (c) in contrast, WGA showed much higher lateral mobility on the protoplasts, yielding diffusion coefficients of 3×10^{-10} cm^2/s; (d) the binding of SBA or Con A to soybean protoplasts can trigger a reduction in the mobility of WGA ($D \simeq 5 \times 10^{-11}$ cm^2/s).

In attempting to analyze our results in the context of animal cell membrane models, which have been formulated based on a larger body of experiments, it is important to examine the membrane composition of the plant cell. In general, the phospholipid compositions of various plant tissues are not radically different from those reported for animal cells (Mazliak, 1977). One major difference that may play some role in possible differences in lateral mobility in plants is that soybean roots contain very low levels of cholesterol (Travis & Berkowitz, 1980), a membrane component implicated in a number of diffusion control mechanisms in animal cells (Owicki & McConnell, 1980; Muhlebach & Cherry, 1982). This however, is probably compensated for by high levels of β-sitosterol and stigmasterol, the major phytosterols of the plant membrane (Travis & Berkowitz, 1980).

If the lipid and sterol compositions of membranes were the dominant factors in the ability of intramembranous components to diffuse, then one would, to a first approximation, assume no real differences in lectin receptor mobility for plant and animal cells. Given our results for the diffusion constant of WGA ($D \simeq 3 \times 10^{-10}$ cm^2/s), which is comparable to that found in similar experiments in animal cells for WGA, a simple comparative analysis between membranes may be valid. However, a larger difference is observed for Con A and SBA diffusion constants on the soybean protoplast membrane when compared to the reported mobilities of Con A on animal cells. SBA has not been used in animal cell studies. Although the diffusion measurements for Con A have shown large variations (Peters, 1981), it is now generally accepted that in most animal cell systems the mobility of Con A receptors varies between 1×10^{-10} and 5×10^{-10} cm^2/s (Eldridge et al., 1980; Henis & Elson, 1981). Compared to these values, the diffusion constant of Con A on soybean protoplasts ($D = 7 \times 10^{-11}$ cm^2/s) is considerably slower than that reported for 3T3 fibroblasts, erythrocytes, and rabbit lymphocytes (Eldridge et al., 1980; Sheetz et al., 1980; Leuther et al., 1979). The behavior of SBA was similar to that of Con A, namely, $D = 5 \times 10^{-11}$ cm^2/s. Preliminary experiments indicate that SBA does not share the same receptors with Con A and WGA on the soybean protoplasts. This conclusion is based on the observation that mild trypsinization of soybean protoplasts abrogates the binding of SBA without appreciable effects on the binding of WGA and Con A (T. Metcalf, unpublished results).

A comparison of the molecular weights and saccharide-binding valencies for the various lectins and their measured diffusion constants indicates that these two parameters have a limited role in determining the mobility of lectin receptors on soybean cells. Both Con A (Wang et al., 1971) and SBA (Lotan et al., 1974) are bivalent under the conditions of the experiment, yet they both yielded considerably slower diffusion rates than tetravalent WGA (Wright, 1980; Lis & Sharon,

1981). The molecular weight of SBA in solution is approximately 120 000 while those for Con A, succinyl-Con A, and WGA are 2–4 times smaller. However, the identities of the soybean protoplast receptors for the various lectins are not known. Therefore, the actual molecular weights of the diffusing species in the membrane cannot be simply ascribed to the molecular sizes of the lectins.

Perhaps the most striking observation in the present study is the effect of SBA and Con A on the mobility of WGA receptors on the soybean plasma membrane. Pretreatment of the protoplast with either SBA or Con A, followed by incubation with fluorescently derivatized WGA, resulted in a 6-fold decrease in the diffusion coefficient of the latter lectin. This effect cannot be ascribed to the anchoring of one set of relatively mobile molecules to another set of immobile receptors through lectin–lectin interactions. This conclusion is based on double-immunodiffusion and gel filtration experiments, which suggest that WGA does not interact with SBA. Similarly, it has been shown that WGA and Con A do not interact in solution (Sela et al., 1975). Moreover, the binding of SBA does not change the amount of WGA bound to the soybean cell. Therefore, it appears that the binding of SBA and Con A results in alterations of other components of the soybean plasma membrane in such a way as to restrict the mobility of WGA receptors. To the best of our knowledge, this is the first report of an endogenously produced protein, SBA, that is capable of modulating the dynamic properties of its own membrane. Work with red blood cells has demonstrated how endogenously produced low molecular weight polyanions and cations are capable of altering erythrocyte membrane dynamics (Schindler et al., 1980a; Sheetz et al., 1982).

Although the detailed mechanism of modulation remains to be investigated, the present results may be analogous to the modulation by Con A of receptor mobility in a variety of animal cells. The binding of Con A to lymphocytes inhibits patch and cap formation of cell-surface immunoglobulin as well as many other different receptors (Yahara & Edelman, 1972). It has also been shown that Con A binding results in a 7-fold reduction in the D values of surface immunoglobulin of lymphocytes (Henis & Elson, 1981) and a 10-fold reduction in the D values of receptors on 3T3 mouse fibroblasts (Schlessinger et al., 1977). This modulation can be partially reversed by colchicine, implicating a role for microtubules in the Con A effect. If the data reported in the present paper turn out to be indeed analogous to the results obtained in animal cells, it would suggest a means by which the binding of external ligands to the plasma membrane of plant cells can alter the cytoskeletal structures of the cells. These alterations may be similar to animal cell membrane rearrangements that occur in the process of ligand–receptor-mediated endocytosis. The functional significance of this type of membrane change for a plant cell may be in the binding and subsequent transport of *Rhizobium* across the membrane of root hair cells to start the process of nodule formation.

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Castanospermine Inhibits the Processing of the Oligosaccharide Portion of the Influenza Viral Hemagglutinin†

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ABSTRACT: Castanospermine (1,6,7,8-tetrahydroxyoctahydroindolizine) is a plant alkaloid that inhibits α- and β-glucosidase in fibroblast extracts [Saul, R., Chambers, J. P., Molyneux, R. J., & Elbein, A. D. (1983) *Arch. Biochem. Biophys. 221*, 593–597]. In the present study, castanospermine also proved to be a potent inhibitor of glycoprotein processing by virtue of the fact that it inhibits glucosidase I. Thus, when influenza virus was raised in the presence of castanospermine, at 10 μg/mL or higher, 80–90% of the viral glycopeptides were susceptible to the action of endoglucosaminidase H, whereas in the normal virus 70% of the glycopeptides are resistant to this enzyme. The major oligosaccharide released by endoglucosaminidase H from castanospermine-grown virus migrated like a hexose$_{10}$GlcNAc on a calibrated Bio-Gel P-4 column. This oligosaccharide was characterized as a $Glc_3Man_7GlcNAc$ on the basis of various enzymatic treatments, as well as by methylation analysis of the [2-^{3}H]-mannose-labeled or [6-^{3}H]galactose-labeled oligosaccharide. The presence of three glucose residues in the oligosaccharide was also confirmed by periodate oxidation studies of the [6-^{3}H]galactose-labeled hexose$_{10}$GlcNAc. Castanospermine did not inhibit the incorporation of [^{3}H]leucine or [^{14}C]alanine into protein in MDCK cells at levels as high as 50 μg/mL. In addition, influenza virus produced in the presence of this alkaloid were fully infective and apparently produced in similar amounts to that of control cells, as determined by plaque counts. Castanospermine did, however, cause considerable changes in cell surface properties, since MDCK cells grown in 10 μg/mL castanospermine were able to bind twice as much [^{3}H]concanavalin A as were control cells.

The biosynthesis of the oligosaccharide portion of the N-linked glycoproteins involves the participation of lipid-linked saccharide intermediates and leads ultimately to the formation of a $Glc_3Man_9GlcNAc_2$-pyrophosphoryldolichol (Elbein, 1979; Struck & Lennarz, 1980; Li & Kornfeld, 1979; Spiro et al., 1976; Robbins et al., 1977). The oligosaccharide portion of this lipid is then transferred to protein and is covalently attached to certain asparaginyl residues (Kiely et al., 1976; Czicki & Lennarz, 1977; Das & Heath, 1980; Lingappa et al., 1978; Rodriguez-Boulan et al., 1978). Following the transfer of oligosaccharide to protein, the newly formed glycoprotein undergoes a number of processing reactions, some of which occur in the endoplasmic reticulum and others in the Golgi apparatus (Turco & Robbins, 1976; Elting et al., 1980; Grinna & Robbins, 1979).

The initial processing reactions involve the removal of the three glucose residues catalyzed by two different enzymes. Glucosidase I removes the outermost 1,2-glucose unit (Kilker et al., 1981; Grinna & Robbins, 1980; Ugalde et al., 1978; Chen & Lennarz, 1978), while glucosidase II releases the next two α1,3-linked glucose residues (Grinna & Robbins, 1979; Michael & Kornfeld, 1980; Ugalde et al., 1980; Burns & Touster, 1982). These trimming reactions give a $Man_9GlcNAc_2$-protein which may be the immediate precursor of the high-mannose glycoproteins, or it may be processed further by the removal of four mannose residues by Golgi-bound α1,2-mannosidases to give a Man_5GlcNA_2-protein (Opheim & Touster, 1978; Tabas & Kornfeld, 1979; Kornfeld et al., 1978; Forsee & Schutzbach, 1981; Tulsiani et al., 1982a). A GlcNAc transferase then adds a GlcNAc residue to the α1,3-linked mannose, and this addition apparently signals another mannosidase (mannosidase II) to remove the α1,3- and α1,6-linked mannose residues (Tabas & Kornfeld, 1978, 1979; Harpaz & Schachter, 1980). Then, the other sugars of the complex chains, i.e., GlcNAc, galactose, sialic acid, and fucose, may be added sequentially to form the final complex structure (Hubbard & Ivatt, 1981; Schachter & Roseman, 1980).

Since both the high-mannose and the complex types of oligosaccharides are derived from the same intermediate, i.e., the $Glc_3Man_9GlcNAc_2$-protein, inhibitors that prevent the normal processing of these oligosaccharides should be of considerable value for studies on the roles of those oligosaccharides in glycoprotein function. We recently found that the plant alkaloid castanospermine (1,6,7,8-tetrahydroxy-

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