Aflatoxin-Induced Alteration in Soybean Membrane Protein

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ABSTRACT

Isolates of aflatoxin-producing strains of *Aspergillus* can grow on *Glycine max* beans. Furthermore, both mixed aflatoxins and aflatoxin B_1 inhibit the elongation of both attached and excised *G. max* roots. In addition, the toxin inhibits the capacity of the excised roots to take up [^{14}C]leucine. This suggests that the toxin may inhibit the activity of an uptake system responsible for uptake of low-molecular-weight (MW) compounds by possibly altering membrane structure and/or function. In this connection, incubation of excised roots in media containing either mixed aflatoxins or aflatoxin B_1 results in a diminution of acid-insoluble protein, but neither sterol nor lipid phosphorus levels, of an 80,000 × g pellet (purported "crude" plasmalemma fraction). To provide preliminary evidence that mixed aflatoxins can decrease the amount of a specific plasmalemma protein (which might regulate the uptake of low-MW compounds), we incubated roots with and without mixed aflatoxins and then gel-filtrated integral proteins which were released by detergents from 80,000 × g pellets that had been obtained by differential centrifugation of Miracloth filtrates. The released proteins were gel-filtrated on Sephadex G-100 columns. Sodium dodecyl sulfate, Triton X-100 and Tween 20 each solubilized greater than 85% of the pellet's protein. Gel filtration yielded 280 nm absorbing void volume and retarded peaks for substances which were either precipitated by trichloroacetic acid (total protein) or solubilized by detergents (integral protein) from pellets that were derived from aflatoxin-treated and nontreated roots. The amounts of protein which were recovered within column void volumes following gel filtration of 80,000 × g, acid-precipitable protein were not significantly different for treated and nontreated roots, suggesting that incubation of roots with aflatoxins does not reduce the pellet's content of an acid-precipitable, high-MW (100,000 or more) protein. However, this conclusion is highly tentative as less than 15% of the acid-insoluble protein which was layered onto the column was recovered. The amplitude of the void volume peaks for detergent-released proteins from treated roots was consistently less than that for nontreated roots, suggesting that treatment of roots with aflatoxins diminishes the "crude" plasmalemma fraction's content of high-MW protein(s). This suggestion was supported through calculation of the amounts of protein which were recovered within both the void volume and retarded peaks and comparing these to the total protein levels that were recovered from the columns. The amount of protein which was found within the void volume peak following gel filtration of either Triton X-100 or Tween 20-released proteins from pellets that were derived from treated roots was less than that for nontreated roots.

INTRODUCTION

Isolates of aflatoxin-producing strains of *Aspergillus* grow on *Glycine max* beans (1-8). Both mixed aflatoxins and (aflatoxin B_1) AFB_1 inhibit the elongation of attached (9,10) and excised (11) *G. max* roots. In addition, the toxin inhibits the ability of the excised roots to take up [^{14}C]-leucine and to incorporate it into acid-insoluble, cytoplasmic protein (11). This suggests that the toxin may inhibit an uptake system regulating the uptake of low-molecular-weight (MW) substances by possibly altering membrane structure and/or function. In this connection, Kunimoto et al. (12) reported that AFB_1 inhibited both uridine and thymidine uptake by HeLa and LS178Y cells.

Danley et al. (10,13) reported that incubation of excised *G. max* roots in media containing either mixed aflatoxins or AFB_1 resulted in a reduction of acid-insoluble protein, but not sterol or lipid phosphorus levels, in a presumed "crude" plasmalemma fraction (14,15).

This communication reports our preliminary attempts to determine whether mixed aflatoxins reduce the amount of a specific plasmalemma protein.

EXPERIMENTAL PROCEDURES

Germination Conditions and Incubation of Excised Roots

Glycine max, cv. Essex (Southern States Cooperative, Inc., Richmond, VA, 1977 crop) seeds were imbibed for 3 days and the resulting roots excised and incubated according to Chao and Dashek (16) and Young et al. (11), except that 50 μg/mL chloramphenicol and 30 μg/mL AFB_1 or 65.64 μg/mL combined aflatoxins (AFB_1 [30.00 μg/mL], AFB_2 [1.04 μg/mL], AFG_1 (33.60 μg/mL] and AFG_2 [1.00 μg/mL]), were added to the incubation medium. The mixture of AFB_1, AFB_2, AFG_1 and AFG_2 was prepared using strain NRRL 2999 of *Aspergillus parasiticus*.

Roots were excised into a pH 6.0 incubation medium consisting of 1% sucrose, 0.5 mM sodium citrate, 20 μg/mL streptomycin and 10 μg/mL 2-4 dichlorophenoxyacetic acid (2-4, D) (16). Excised roots were blotted gently with Kimwipes and their wet wt recorded prior to incubation. Roots were shaken for 18 hr at 24 ± 2 C in 125-1,000-mL Erlenmeyer flasks containing 20-200 mL of the medium just described at 100-150 rpm in a New Brunswick gyrotory shaker. Roots were incubated at the ratio of 1 g wet wt/20 mL medium.

Preparation of "Crude" Plasmalemma Fractions

Following an 18-hr incubation, excised roots were homogenized into a medium consisting of 250 mM sucrose, 2 mM EDTA, 25 mM pH 7.2 Tris (17), except that Tris-HCl was used. Roots were homogenized on ice with a mortar and pestle at a ratio of 4 g wet wt/mL homogenization medium. Homogenates were filtered through Miracloth and the filtrate was centrifuged for 15 min at 1,000 × g. The 1,000 × g supernatant was centrifuged at 40,000 × g for 30 min and the resulting supernatant at 80,000 × g for 30 min (Fig. 1). The 80,000 × g centrifugation was performed at 4 C in an International Model-B35 Ultracentrifuge whereas the 1,000 and 40,000 × g centrifugations were done at 4 C in a Sorvall RC-2B preparative centrifuge.

Precipitation or Release of "Crude" Plasmalemma Proteins

Membrane proteins were released by treatment with detergents as in Table I. Protein was precipitated from the 80,000 × g pellet through the addition of cold 10% trichloroacetic acid (TCA) (23) with the final TCA pellet being resuspended in 0.5 N NaOH or 0.15 M NaCl prior to protein quantification.

Protein Assay

Total protein was quantified by absorption at 280 nm or,

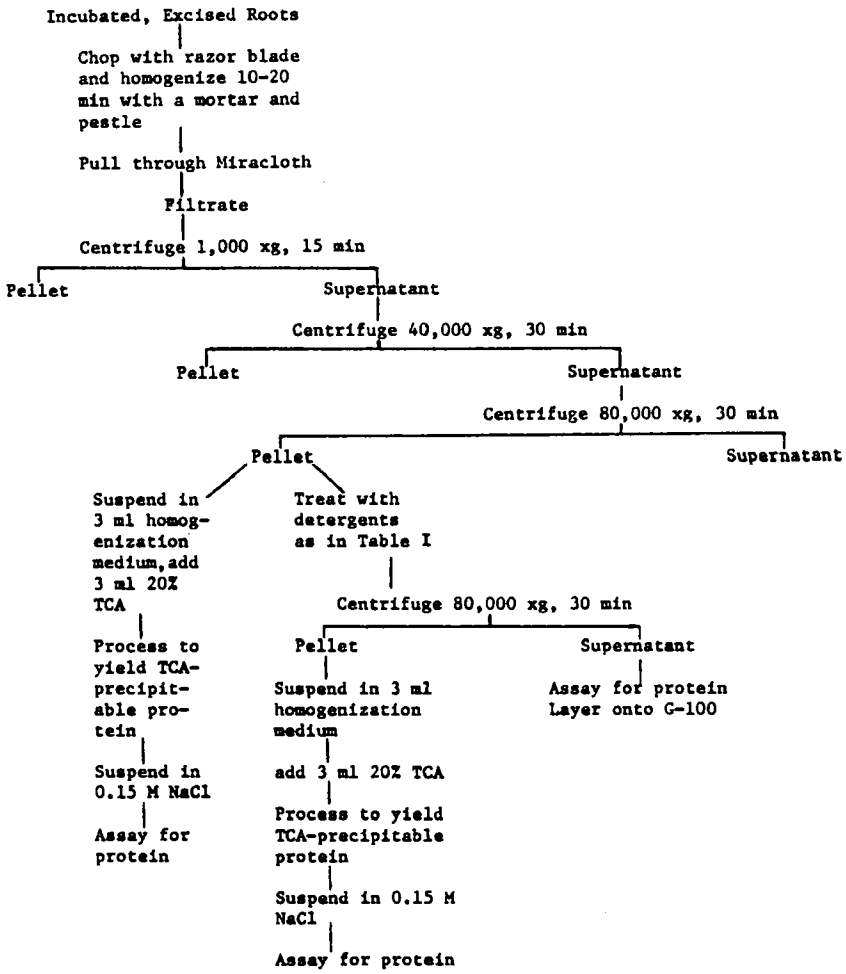

FIG. 1. Flow chart for isolation and detergent-treatment of "crude" plasmalemma fractions.

occasionally, colorimetrically with Coomassie blue (24) and bovine serum albumin (crystalline, Pfaltz and Bauer, Stamford, CT) as a standard.

Sephadex Gel Filtration of Acid-Insoluble or Detergent-Released Proteins

The TCA-precipitable, 80,000 × g, pellet protein was suspended in 0.15 M NaCl and then either layered directly onto 1.0 × 121 or 2.2 × 122 cm Sephadex G-100 columns or lyophilized and the residue reconstituted in 2 mL of 50 mM, pH 6.5, phosphate buffer prior to layering. Detergent-released proteins were layered onto the column without prior lyophilization. Aliquots of acid-insoluble or detergent-released membrane protein were assayed for total protein before layering. The columns, which were calibrated with Pharmacia Blue Dextran 2000 and bovine serum albumin, were eluted with phosphate buffer. Collected fractions were assayed for protein by either absorption at 280 nm, or colorimetrically, with Coomassie blue.

RESULTS AND DISCUSSION

Does the 80,000 × g Pellet Contain the Plasmalemma?

The validity of employing the 80,000 × g pellet from homogenates of developing *G. max* roots as a source of nonenriched plasmalemma has been reported by Travis and his coworkers (14,15,25,26). The data in Table II provide additional evidence that the 80,000 × g pellet can be used as a "crude" plasmalemma fraction because it contains the plasmalemma marker enzyme, pH 6, K^+-stimulated ATPase (17). However, a total balance sheet relating the activity of ATPase present within each fraction (Fig. 1) to that within the total root homogenate following its filtration through Miracloth was not performed. Thus, we cannot state whether the 80,000 × g pellet contains the bulk of the ATPase which is present within the total root homogenate. This analysis, together with an electron microscopical examination (counterstaining with the plasmalemma mar-

TABLE I

Summary of Detergent Methods for Release of Membrane Proteins

Treatment	Reference
5-10 mg membrane (protein basis)/mL of 1% Triton X-100 containing 15% w/v sucrose at 4 C, overnight	Strobel and Hess (18) Louvard et al. (19)
1-2 mg membrane (protein basis)/mL of 1% (w/v) sodium dodecyl sulfate and 10% (w/v) sucrose in 0.1 M Tris-HCl (pH 8.5), 4 C, overnight	Dulaney and Touster (20)
1-2 mg membrane (protein basis)/mL of 0.1% deoxycholate in 1.5 M KCl, 0.25 M sucrose and 0.01 M Tris (pH 7.6), 4 C, overnight	Franke et al. (21)
30 mg membrane (protein basis)/mL of pH 8.0, 0.10 M Tris-HCl buffer containing 5.0% Tween 20, 2 hr at room temperature	Hjerten and Johansson (22)

TABLE II

Enzymatic Activities of Subcellular Fractions Obtained by Differential Centrifugation of Homogenates of Excised Roots Cultured in Medium Containing or Lacking Aflatoxins

Fraction	K^+-stimulated ATPase activity (mg Pi released/hr/mg protein)		NADH-cytochrome c reductase activity (OD/min/mg protein)		Inosine diphosphatase activity (mg Pi release/hr/mg protein)	
	Plus aflatoxin	Minus aflatoxin	Plus aflatoxin	Minus aflatoxin	Plus aflatoxin	Minus aflatoxin
1,000 X g pellet			1.11 ± 0.36	1.03 ± 0.16	0.00 ± 0.00	0.00 ± 0.00
40,000 X g pellet			0.00 ± 0.00	0.00 ± 0.00	9.97 ± 3.16	9.65 ± 3.21
pH 6.0	3.19 ± 2.20	2.60 ± 1.37				
pH 9.0	2.81 ± 1.94	2.85 ± 1.66				
80,000 X g pellet			0.45 ± 0.07	0.41 ± 0.11	12.48 ± 3.88	11.71 ± 4.37
pH 6.0	3.42 ± 1.22	3.59 ± 1.26				
pH 9.0	3.21 ± 0.99	3.50 ± 1.22				
80,000 X g supernatant			0.45 ± 0.04	0.41 ± 0.19	–	–

1-5 g fresh weight excised roots were incubated, harvested and organelles prepared as in Fig. 1; for assay of K^+-stimulated ATPase activity, organelles were suspended in 2% sucrose, 1 mM $MgSO_4$ and 1 mM Tris, pH 7.2 to yield 10 μg/100 μL protein and were then assayed for enzyme activity according to Hodges and Leonard (17); to insure that the reaction was enzymatic, 10, 20, 30 and 50 μg of organellar protein were used for assaying ATPase activity; for determining NADH-cytochrome-c-reductase activity, isolated organelles were resuspended in 0.05 M Tris-HCl, pH 8.0, 0.67 M sucrose, and 0.001 M histidine to yield 1 mg/mL protein, and were then assayed for enzyme activity according to Hatefi and Rieske (27); to insure that the reaction was enzymatic, 10, 30 and 60 μg of organellar protein were used for assaying NADH-cytochrome-c-reductase activity; for determining IDPase activity, isolated organelles were resuspended in 61 mM Tris-HCl, pH 7.5, to yield 60 μg/100 μL protein, and were then assayed for enzyme activity according to Dauwalder et al. (28); to insure that the reaction was enzymatic, 15, 7.5, 3.7, 3.0, 1.5 and 0.75 μg organellar protein were used for assaying IDPase activity.

ker stain, periodic acid-chromic acid-phosphotungstic acid), of the pellet is in progress. Table II reveals that the fraction appears to be cross-contaminated with endoplasmic reticulum and dictyosomes as indicated by the presence of NADH-cytochrome-c-reductase and inosine diphosphatase activities, marker enzymes for the endoplasmic reticulum (27) and dictyosomes (28), respectively. In this connection, Travis and coworkers acknowledge that the 80,000 x g pellet is a cross-contaminated source from which an enriched plasmalemma fraction can be obtained.

Does Incubation of Excised Roots in Medium Containing Aflatoxin Reduce the Total or Specific Protein Contents of "Crude" Plasmalemma Fractions?

Danley et al. (10,13) reported that an 18-hr incubation of excised *G. max*, cv. Essex roots in a medium containing either 30 μg/mL pure AFB_1 or 33.28 μg/mL mixed aflatoxins (5.0 μg/mL AFG_1, 0.2 μg/mL AFB_2, 27.5 μg/mL AFG_1, 0.58 μg/mL AFG_2) resulted in a low (27%) but statistically significant reduction in acid-insoluble protein from "crude" plasmalemma preparations. This reduction could have resulted from a diminution of each protein within the plasmalemma preparation or, alternatively, in a specific protein which might constitute a disproportionate amount of the protein within the fraction. Because aflatoxins can impair the uptake of thymidine and uridine into HeLa and LS178Y cells, as well as of leucine into *Cucumis* cotyledonary discs, it is possible that aflatoxins may inhibit the synthesis and/or insertion of a membrane-associated protein responsible for the uptake of low-MW compounds. To partially test this, we compared the Sephadex G-100 gel filtration profiles of detergent-released proteins of "crude" plasmalemma fractions which were obtained from homogenates of toxin-treated and nontreated roots.

Table III compares the capabilities of 3 detergents to release integral proteins from the membranous organelles which compose the 80,000 x g pellet. Each of the detergents released >85% of the protein which was present within the pellet. The percentages for nontreated roots were 94, 86 and 84 for SDS, Tween 20 and Triton X-100, respectively. For treated roots, the percentages for the same sequence of detergents were 90, 87 and 91, respectively. Thus, incubation of excised roots in a medium containing aflatoxins does not appear to enhance the capacity of detergents to solubilize proteins from the 80,000 x g pellet.

The finding that Triton X-100 and SDS can release proteins from a "crude" plasmalemma fraction is consistent with the results of Booz and Travis (26) who reported that both detergents could solubilize integral, membrane proteins from enriched plasmalemma fractions which were obtained from homogenates of developing *G. max* (L.) Merr., cv. Wells roots. The enriched plasmalemma fraction was derived from an 80,000 x g pellet by resuspension and

TABLE III

Comparison of Various Methods for Release of Proteins from "Crude" Plasmalemma Fractions

	Sodium dodecyl sulfate		Fresh wt Tween 20 (mg/g)		Triton X-100	
	Treated	Untreated	Treated	Untreated	Treated	Untreated
Protein remaining after detergent treatment	0.013	0.029	0.029 ± 0.025	0.037 ± 0.016	0.033 ± 0.004	0.026 ± 0.015
Total protein within the fraction	0.210 ± 0.040	0.290 ± 0.090	0.210 ± 0.040	0.290 ± 0.090	0.210 ± 0.040	0.290 ± 0.090

Glycine max, cv. Essex seeds were imbibed and the resulting roots excised and incubated with and without aflatoxins according to Young et al. (11) and Danley et al. (10,13); roots were homogenized and "crude" plasmalemma fractions were prepared as in Fig. 1; protein was solubilized from the fractions with the above detergents as in Table I; following solubilization, the membranous residue was suspended in 10% TCA to precipitate protein; the total protein of the "crude" plasmalemma fractions was obtained through addition of 10% TCA to the entire 80,000 X g fraction; TCA-precipitate protein was suspended in 2 mL of 0.15 M NaCl; aliquots of the resuspension, as well as solubilized proteins, were assayed for protein by absorption at 280 nm and/or colorimetric assay at 595 nm.

subsequent sucrose gradient centrifugation.

Figure 2 presents G-100 elution profiles of acid-insoluble protein (a measure of total protein) of the 80,000 x g pellet which were obtained from homogenates of aflatoxin-treated and nontreated excised roots. Two 280-nm absorbing peaks were observed for both treated and nontreated roots. One of the peaks eluted within the void volume and the other was retarded.

Although there appears to be a peak shift for protein which was precipitated from the 80,000 x g pellet of aflatoxin-treated roots, this could well be artifactual because the acid-insoluble protein which was precipitated from the 80,000 x g pellet derived from nontreated roots eluted prior to the blue dextran marker. The total amounts of protein which were recovered within the void volumes that were obtained by gel filtrating proteins from treated and nontreated roots were similar, suggesting that incubation of roots with aflatoxins did not alter the acid-precipitable, high-MW protein content of the 80,000 x g pellet. However, less than 12% of the protein which was layered onto the column was recovered, thereby rendering conclusions regarding aflatoxin-induced changes in the acid-insoluble protein content of the membranes composing the 80,000 x g fraction highly tentative at best.

The following Sephadex elution profiles are concerned with assessing whether incubating roots with aflatoxins alters the 80,000 x g pellet's complement of integral membrane proteins.

The Sephadex G-100 elution profiles of proteins which were solubilized by Triton X-100 from 80,000 x g pellets that were derived from homogenates of (A) nontreated and (B) treated roots are depicted in Figure 3. Void volumes and broad, retarded peaks were seen for both treated and nontreated roots. When column eluates were assayed for the occurrence of protein by absorption at 280 nm, it appeared that the amplitude of the void volume peak for

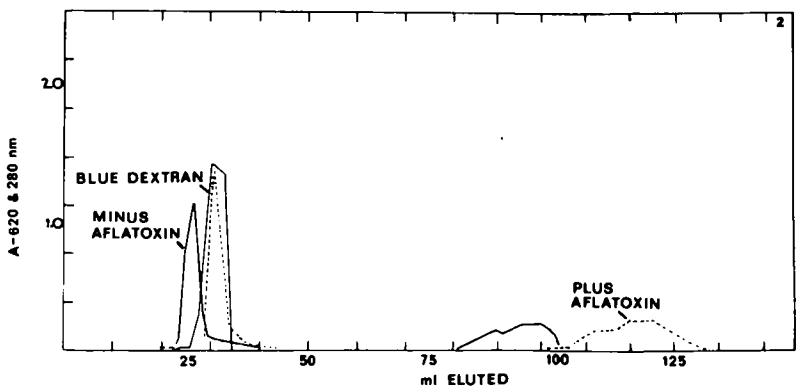

FIG. 2. Sephadex G-100 elution profiles of trichloroacetic-acid-insoluble proteins of "crude" plasmalemma fractions from cells of excised *Glycine max*, cv. Essex roots incubated with and without aflatoxins.

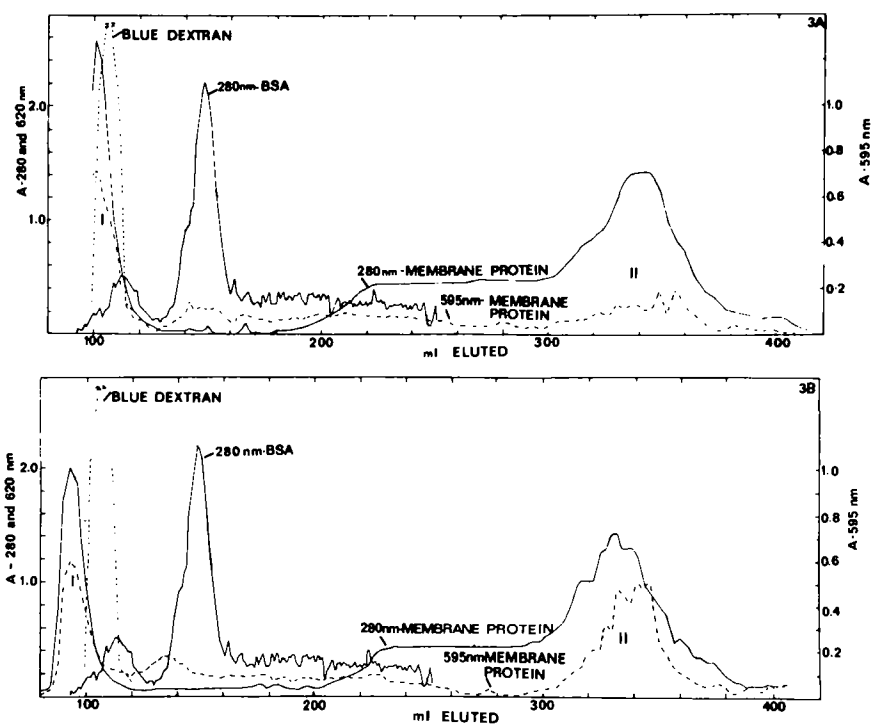

FIG. 3. Sephadex G-100 elution profiles of Triton X-100-released proteins of "crude" plasmalemma fractions from cells of excised *G. max* roots incubated (A) without and (B) with aflatoxins.

TABLE IV

Distribution of Protein in Sephadex Peaks Following Gel Filtration of Acid-Insoluble or Detergent-Released Proteins of 80,000 × g Pellets Obtained from Homogenates of Aflatoxin-Treated and Untreated Roots

Treatment	Protein release procedure	Peak I	Peak II	Protein recovered of that layered onto column (%)
Plus AFB_1	TCA	54.5	43.4	7.9
Minus AFB_1		53.9	33.8	13.8
Plus AFB_1	Triton X-100	19.9	71.9	
Minus AFB_1		41.5	52.6	
Plus AFB_1	Tween 20	43.8	44.0	44
Minus AFB_1		66.3	28.9	39
Plus AFB_1	Sodium dodecyl sulfate	61.9	26.9	81
Minus AFB_1		71.5	17.4	>100

Triton X-100-solubilized membrane protein(s) from treated roots was less than that for detergent-released membrane protein(s) from nontreated roots. Furthermore, when column eluates were assayed colorimetrically with Coomassie blue for total protein, we observed an increase in the amplitude of the retarded peak for roots which had been incubated with aflatoxins.

When the total amounts of protein which were found within peaks I and II are related to those which were recovered from the column (Table IV), it is apparent that incubation of roots with aflatoxin results in a lower recovery of Triton X-100-solubilized protein within peak I than that for nontreated roots.

Figure 3 also demonstrates that the broad, retarded peak eluted subsequent to bovine serum albumin and, therefore, possessed an MW <68,000.

The elution profiles of proteins which were solubilized by Tween 20 from 80,000 × g pellets that were centrifuged from homogenates of (A) treated and (B) nontreated roots are presented in Figure 4. As in the case of Triton X-100, the amplitude of the void volume peak was reduced for protein solubilized by Tween 20 from the 80,000 × g pellet of treated roots compared to that for the void volume peak of solubilized protein from nontreated roots. This reduction was also reflected in the amounts of protein which were recovered within peaks I and II. Table IV demonstrates that the amounts of protein within peaks I and II which were solubilized by Tween 20 from an 80,000 × g pellet that had been derived from homogenates of treated roots were identical. In contrast, 2.3 times more protein was seen within peak I than within peak II from nontreated roots. However, only 39% (nontreated) and 44% (treated) of the proteins which were layered onto the column were recovered.

Figure 5 presents the Sephadex G-100 elution profiles for proteins which were solubilized by SDS from 80,000 × g pellets that were obtained from homogenates of treated and nontreated roots. In contrast to the Triton X-100 and Tween 20 results, it appears that the elution patterns for treated and nontreated roots do not coincide. However, we have not performed sufficient replications to verify this. Nevertheless, as in the case of Triton X-100 and Tween 20, both the amplitude and the amount of protein which was present within peak I for gel-filtrated proteins obtained from treated roots was less than that for nontreated roots.

The similarity in elution profiles between acid-insoluble (total) and detergent-released (integral) protein is surprising and suggests that the bulk of the protein within the membranes of the 80,000 × g pellet is composed of integral proteins.

The void volumes most likely represent a heterogenous mixture of proteins possessing MW of 100,000 or more. The void volume peak should be subjected to ion exchange chromatography to separate the various proteins which compose the peak. Such separated proteins could then be subjected to polyacrylamide gel electrophoresis. Combined gel filtration, ion exchange chromatography and gel electrophoresis may reveal any existing differences in the protein compositions of 80,000 × g pellets of toxin-treated and nontreated, excised roots. One of the positive attributes of the findings reported herein is the removal of low-MW peptides (peak II) which would complicate ion exchange chromatography and gel electrophoresis.

In spite of these reservations, the Sephadex G-100 profiles of SDS, Triton X-100 and Tween 20-solubilized 80,000 × g proteins indicating that incubation of excised roots in a medium containing mixed aflatoxins results in a diminution of total protein recovered within peak I (void volume) with a concomitant increase in the amount of lower MW peptides (peak II) suggest that incubation of roots with aflatoxins may impair either the synthesis and/or insertion of a protein(s) into the membranes which compose the 80,000 × g pellet. Another possibility is that this

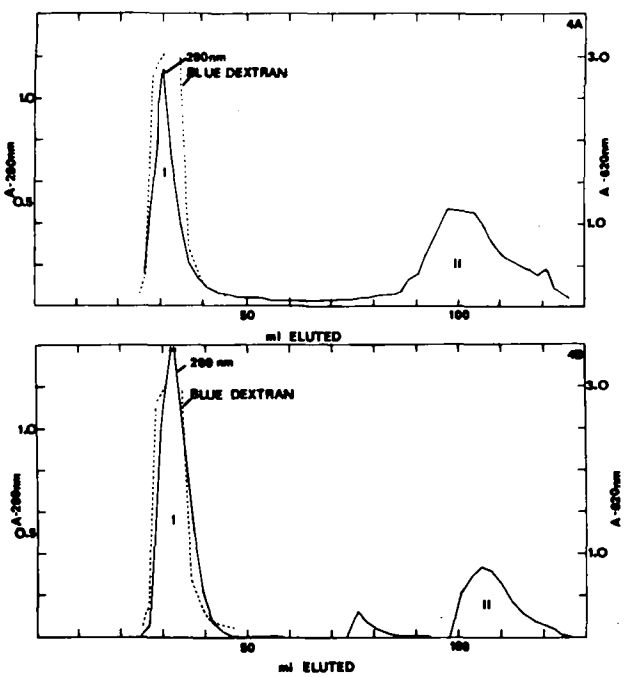

FIG. 4. Sephadex G-100 elution profiles of Tween 20-released plasmalemma proteins from excised *G. max* roots incubated (A) with and (B) without aflatoxins.

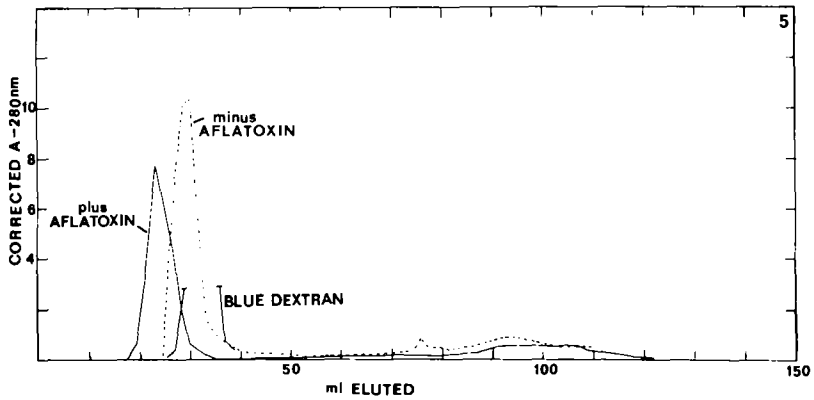

FIG. 5. Sephadex G-100 elution profiles of sodium-dodecyl-sulfate-released plasmalemma proteins from cells of aflatoxin-treated and nontreated roots.

incubation might result in degradation of an already existing high-MW membrane protein. In this connection, Booz and Travis (26) demonstrated that proteins solubilized by SDS from enriched plasmalemma fractions which were obtained from developing soybean roots could be separated by 2-dimensional slab gel electrophoresis and, furthermore, that the electrophoretograms were characteristic for a given membrane species. This observation makes possible an evaluation of whether aflatoxins do, indeed, reduce the amount of a specific plasmalemma protein. Before this can be accomplished, we must enrich our plasmalemma fraction, perhaps via the procedures of Berkowitz and Travis (14), to eliminate contaminating proteins which would obscure a plasmalemma-derived protein on gels. Recently, Danley et al. (29) reported preliminary attempts to purify (enrich) the 80,000 x g "crude" plasmalemma fraction via the procedures of Nagahashi et al. (30). Preliminary results indicate that enrichment is feasible, but that numerous technical difficulties must be overcome. The identification of the plasmalemma of *G. max* root cells can be accomplished by its reactivity with periodic acid-phosphotungstic acid-chromic acid (31) in thin sections which have been prepared for electron microscopy, its ability to bind concanavalin A-ferritin complex (25) and its pH 6, K^+-stimulated ATPase activity (30).

In conclusion, incubation of excised roots with aflatoxins may alter the protein conformation of a "crude" plasmalemma fraction.

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REFERENCES

1. Hesseltine, C.W., O.L. Shotwell, J.J. Ellis and R.D. Stubblefield, Bacteriol. Rev. 30:795 (1966).
2. Davis, N.D., and U.L. Diener, in "Proc. 1st U.S.-Japan Conference on Toxic Microorganisms," edited by M. Herzberg, U.S. Department of the Interior, Washington, DC, 1968, p. 43.
3. Bean, G.A., J.A. Schillinger and W.L. Klarman, Phytopathol. 62:745 (1972).
4. Nagarajan, V., R.V. Bhat and P.G. Tulpule, Experientia 29: 1302 (1973).
5. Gupta, S.K., and T.A. Venkitasubramanian, Appl. Microbiol. 29:834 (1975).
6. Sheretz, P.C., T. Eadie, J.W. Young and G.C. Llewellyn, J. Assoc. Off. Anal. Chem. 59:662 (1976).
7. Topsy, K., Ann. Nutr. Alim. 31:625 (1977).
8. Shotwell, O.L., E.E. Vandegraft and C.W. Hesseltine, J. Assoc. Off. Anal. Chem. 61:574 (1978).
9. Jones, H.C., J.C. Chancey, W.A. Morton, W.V. Dashek and G.C. Llewellyn, Mycopathologia 72:67 (1980).
10. Danley, J.M., H.C. Jones, G.C. Llewellyn and W.V. Dashek, in "Proc. of 4th IUPAC-Sponsored Symposium on Mycotoxins and Phycotoxins," edited by P. Krogh, Pathotox Publishers, Park Forest South, IL, 1981, 19 p.
11. Young, J.W., W.V. Dashek and G.C. Llewellyn, Mycopathologia 66:91 (1978).
12. Kunimoto, T., Y. Kurimoto, K. Aibara and K. Miyaki, Cancer Res. 34:968 (1974).
13. Danley, J.M., S. Staggers, S. Walker, A. Varner, G.C. Llewellyn and W.V. Dashek, Mycopathologia (in press).
14. Berkowitz, R.L., and R.L. Travis, Plant Physiol. 63:1191 (1979).
15. Travis, R.L., S. Geng and R.L. Berkowitz, Ibid. 63:1187 (1979).
16. Chao, Hai-Yen, and W.V. Dashek, Ann. Bot. 37:95 (1973).
17. Hodges, T.K., and R.T. Leonard, in "Methods in Enzymology," 32B, Academic Press, New York, 1974, pp. 392-406.
18. Strobel, G.A., and W.M. Hess, Proc. Natl. Acad. Sci. 71:1413 (1974).
19. Louvard, D., S. Maroux, Ch. Vannier and P. Desnuelle, Biochim. Biophys. Acta 375:236 (1975).
20. Dulaney, J.T., and O. Touster, Ibid. 196:29 (1970).
21. Franke, W.W., D.J. Morré, B. Deumling, R.D. Cheetham, J. Kartenbeck, E. Jarasch and H. Zentgraf, Z. Naturforschung. 26:1031 (1971).
22. Hjerten, S., and K.E. Johansson, Biochim. Biophys. Acta 288:312 (1972).
23. Holleman, J.M., and J.L. Key, Plant Physiol. 42:29 (1967).
24. Bradford, M.M., Anal. Biochem. 72:248 (1976).
25. Travis, R.L., and R.L. Berkowitz, Plant Physiol. 65:871 (1980).
26. Booz, M.L., and R.L. Travis, Ibid. 66:1037 (1980).
27. Hatefi, Y., and J.S. Rieske, in "Methods in Enzymology," 10, Academic Press, New York, 1973, pp. 235-239.
28. Dauwalder, M., W.G. Whaley and J.E. Kephart, J. Cell Sci. 4:455 (1969).
29. Danley, J.M., S. Staggers, A. Varner, G.C. Llewellyn and W.V. Dashek, in "Proc. of Colloque II Secretions in Plants," edited by C. Dumas, Actual. Bot. (in press).
30. Nagahashi, G., R.T. Leonard and W.W. Thomson, Plant Physiol. 61:993 (1978).
31. Roland, J.C., C.A. Lembi and D.J. Morré, Stain Technol. 47:195 (1972).