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Monoclonal Antibodies Against Fusicoccin with Binding Characteristics Similar to the Putative Fusicoccin Receptor of Higher Plants[1]

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ABSTRACT

Monoclonal antibodies were raised against fusicoccin. The toxin, linked to bovine serum albumin through its t-pentenyl moiety, served as immunogen. Hybridomas secreting anti-fusicoccin antibodies were screened by radioimmunoassay employing a novel radioactive derivative, [^{3}H]-nor-fusicoccin-alcohol of high specific activity (1.5×10^{14} Bq/mole). The two monoclonal antibodies reported here are of high apparent affinity for fusicoccin (0.71×10^{-9} molar and 1.85×10^{-9} molar). This is comparable to the apparent affinity of rabbit antiserum raised against the same type of conjugate (9.3×10^{-9} molar). A method for the single step purification of the monoclonal antibodies from ascites fluid is reported. A solid-phase immunoassay, using alkaline phosphatase as enzyme, exhibits a measuring range from 0.1 to 1.5 picomoles (about 70 picograms to 1 nanogram) of fusicoccin. The displacement of [^{3}H]-nor-fusicoccin-alcohol from the antibodies by compounds structurally related to fusicoccin exhibits similar selectivity as a microsomal binding assay with the same tracer as radiolabeled probe.

The wilt-inducing *Fusicoccum amygdali* toxin, FC,[2] has proven a valuable tool in plant physiology in the study of regulation of membrane energization and transport processes (15). FC stimulates electrogenic proton extrusion across the plasmalemma and possibly acts on the plasmalemma-located, vanadate-sensitive H^+-ATPase (15, 22) by a mechanism still unknown. Effects of FC on H^+ extrusion or growth have been found widespread among higher and lower plants, but no effects of FC on bacteria, fungi, or animals were so far reported (15). There is some evidence for the presence of membrane associated FC-binding proteins in plants (2, 18, 19, 24). These can be separated from the ATPase (24) and might function in FC reception and action. In addition, the widespread occurrence of FC effects on growth and H^+ extrusion in plants suggests that the mechanism by which FC acts might also function in the nonpathological situation, *e.g.* in the regulation of membrane energization of plant cells. However, compounds with structural similarity to FC, which might act as endogenous ligands for the FC binding sites, are not known.

Antibodies have been used both for the localization and isolation of receptors (12) as well as for the identification and isolation of compounds from complex biological fluids (6).

If the structure of a receptor-occupying ligand is known, it should theoretically be possible to generate a monoclonal antibody with binding characteristics quite similar to the receptor site. Such an antibody would be a helpful aid in the search for unknown ligands with related structures. In addition, the generation of anti-idiotypic antibodies against the idiotypic epitopes of this ligand-binding antibody would provide a probe carrying an internal image of the ligand (3). Such anti-idiotypic antibodies have been used for receptor isolation and/or localization (12). We describe here the production, characteristics, and use in sensitive immunoassays, of monoclonal antibodies against FC, which exhibit specificities similar to those of the FC binding sites in microsomal vesicles of higher plant cells.

MATERIALS AND METHODS

Chemicals. Compounds (1) to (4) and (6) to (9)(see Fig. 4) were kindly provided by Professor A. Ballio, Rome, Italy. FC was also purchased from Italchemia, Segrate, Italy. Tritiated sodium borohydride (2.7×10^{15} Bq mol^{-1}) was purchased from NEN Chemicals, Dreieich, FRG, and alkaline phosphatase, fetal calf serum, HAT- and HT-stock solutions were from Boehringer, Mannheim, FRG. Complete Freund's adjuvant was from Difco Laboratories. RPMI 1640 medium and penicillin-streptomycin solution were purchased from Gibco. Polyethylene glycol 1500 (No. 9727) was from Roth, Karlsruhe, FRG, whereas most of the plasticware used was bought from NUNC, Roskilde, Denmark and Falcon, Grenoble, France. All other chemicals and reagents were of the highest purity available.

Immunization Procedures. Rabbits (12–16 weeks old) were immunized with FC-BSA conjugates in PBS (1 mg ml^{-1}), emulsified in an equal volume of Freund's complete adjuvant as described (25). Six week old female Balb/c mice were immunized intraperitoneally with 150 μg FC-BSA conjugate in PBS, emulsified with 150 μl complete Freund's adjuvant. On d 7, 14, 18, 19, 20, 21, the animals were boosted with 100 μg immunogen in 100 μl PBS. Blood was collected from the tail vein. Mice were bled the day of fusion, before spleen cells were prepared.

Cell Fusion and Cloning. Cell fusions were performed (9) using 50% PEG for the fusion of 0.9 to 10×10^8 mouse splenocytes with 5 to 25×10^7 myeloma cells on d 22 after the start of the immunization procedure. Hybrids were selected in RPMI 1640 medium containing HAT and 20% fetal calf serum. Hybridoma antibody production was screened by RIA (25) and the antibody-

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[2] Abbreviations: FC, fusicoccin, CR, cross reaction; DTT, dithiothreitol; EDTA, ethylenediamine tetraacetic acid; ELISA, enzyme linked immunosorbent assay; FPLC, fast protein liquid chromatography; MAB, monoclonal antibodies; PMSF, phenylmethylsulfonyl fluoride; RIA, radioimmunoassay; R_t, retention time.

producing cells were cloned at least 3 times by limiting dilution.

Antibody Production and Purification. Antibody-secreting cells were either cultivated in RPMI 1640 medium, containing 10% newborn calf serum, 100 μg/ml streptomycin and penicillin, and 50 μM β-mercaptoethanol in 150 to 1000 ml glass Erlenmeyer flasks or injected intraperitoneally into Balb/c mice to produce ascites tumors. Antibodies were precipitated from ascites or cell culture supernatants with 50% saturated, neutral $(NH_4)_2SO_4$, redissolved in PBS and dialyzed against distilled water. They were then lyophilized and stored at −18°C. For the production of purified monoclonal antibody, crude ascites fluid was diluted 1:1 in buffer A (10 mM sodium phosphate, pH 6.8), centrifuged for 10 min at 100,000×*g*, and loaded onto a Biogel HTP (Biorad Labs.) column (100 × 10 mm i.d.). The flow rate was 1 ml min^{-1} at 4°C, using a Pharmacia FPLC-System. The column was eluted with a linear gradient of sodium phosphate (pH 6.8) from 10 to 300 mM in 100 min (flow rate 1 ml/min). The active fractions were dialyzed and lyophilized. The purity of the obtained immunoglobulin fraction was checked by SDS-PAGE using 4% stacking gels and 11% separation gels (14).

Synthesis of [^{3}H]-Nor-Fusicoccin-alcohol. FC (5 mg, 7.35 μmol), dissolved in 1 ml tetrahydrofuran, was added to 1 ml H_2O containing 7.35 μmol OsO_4 and 54 μmol $NaIO_4$, stirred in the dark for 12 h at 20°C and, after removal of the solvent *in vacuo*, loaded onto a SepPak C_{18} cartridge (Waters Ass., Eschborn, FRG). After rinsing with 5 ml H_2O, the column was eluted with 1.0 ml methanol, and the product analyzed by TLC using $CHCl_3$:isopropanol (9:1, v/v) as solvent system. The aldehyde was recognized by spraying the plates with a 2.5% solution of 4-amino-3-hydrazino-5-mercapto-1,2,4-triazole in 1 N NaOH (8). FC and its derivatives were visualized by spraying the plates with 5% H_2SO_4 in methanol and heating for 5 min at 120°C. Nor-FC-aldehyde (3 mg, 4.4 μmol) was then dissolved in 0.02 ml methanol, and added to 0.48 ml of ice-cold sodium carbonate buffer (pH 9.5) (1 mol L^{-1}). [^{3}H]-$NaBH_4$, (0.052 mg dissolved in 0.12 ml 0.1 N NaOH [3.67×10^9 Bq] was then added and incubation proceeded at 0°C for 10 min. The aqueous phase was extracted twice with 0.5 ml ethyl acetate, evaporated and subjected to TLC using the same solvent as above. The compound was obtained radiochemically pure and stored in 10% CH_3CN in ethanol at −20°C.

Synthesis of FC-Alkaline Phosphatase and FC-BSA Conjugates. Nor-FC-aldehyde (0.68 mg, 1 μmol) in 0.02 ml methanol was added to 1.0 mg alkaline phosphatase in 0.1 ml 3 M NaCl, 1 mM $MgCl_2$, 1 mM $ZnCl_2$, 3 mM triethanolamine (pH 7.6). After 1 h stirring on ice, 0.5 mg $NaBH_4$ were added, and incubation continued for 10 min, followed by the addition of 0.3 ml 0.1 M acetic acid. After dialysis against 20 mM citrate buffer (pH 6.5) the uncoupled low mol wt phase was checked on TLC, revealing only one spot co-migrating with the nor-FC-alcohol. The dialyzed enzyme conjugate was stored at −20°C in 50% glycerol. BSA (50 mg), dissolved in 1 ml $NaHCO_3$ (0.1 M, pH 9.6), was added to 2.5 mg (3.67 μmol) nor-FC-aldehyde in 0.2 ml methanol and the pH adjusted to 9.2 with 1 M KH_2PO_4 (0.12 ml). After incubation for 1 h on ice, 1 mg $NaBH_4$ was added and the pH adjusted to 8.2 with 1 M KH_2PO_4 (0.1 ml), followed by a 10-min incubation on ice. Then, 0.14 ml 0.1 N formic acid were added, and incubation continued for 15 min. The product was dialyzed against water and lyophilized (yield: 46 mg protein).

HPLC. Purification of FC-derivatives was conducted on an HPLC-system using a Waters dual pump system with a U6K injector, a model 810 programmer, and a Lambda max-UV-detector. The column was packed with Shandon hypersil ODS 10 μm (250 × 4 mm with precolumn 40 × 4 mm), and the separation performed at 50°C column temperature at a flow rate of 1.5 ml min^{-1} in 50% aqueous methanol (isocratic). The FC-derivatives were detected at 210 nm. R_t: FC 15.2 min, monodeacetyl-FC = 10.3 min, dideacetyl-FC = 7.5 min, and nor-FC-alcohol = 6.1 min.

Antibody Characterization. The antibody subclass was determined by double immunodiffusion (17), using subclass-specific anti-immunglobulin antibodies purchased from Nordic, Tilburg, Netherlands.

Radio- and Enzyme Immunoassay. Unless otherwise stated, the assay conditions corresponded to those reported (25).

Microsomal Binding Assay. Microsomal vesicles were prepared from 3 week old *Vicia faba* L. cv Osnabruecker Markt leaves. Leaf tissue (100 g) was homogenized in 0.25 M sucrose, 3 mM EDTA, 3 mM DTT, and 10 mM ascorbate in 25 mM Tris/Mes-buffer (pH 7.2), and, after filtration through a cotton layer, the filtrate was centrifuged for 10 min at 4°C (13,000*g*). A crude membrane fraction was obtained from the supernatant by sedimentation at 80,000*g* for 30 min at 4°C. The pellets were homogenized in 5 ml binding buffer (10 mM Tris/Mes, pH 5.5, 1 mM $MgSO_4$, 1 mM EDTA, 2.6 mM DTT, 1 mM $CaCl_2$, 0.2 mM PMSF). Protein was determined by the method of Bradford (4). In the standard binding assay, 100 μg protein was incubated in 1.0 ml binding buffer, in the presence of 10 nM [^{3}H]-nor-FC-alcohol (1540 Bq) and varying amounts of the competitor. After incubation for 60 min at 20°C, 0.5 ml ice-cold binding buffer was added and the samples centrifuged at 80,000*g*. The resuspended pellets were then analyzed for radioactivity in a scintillation counter. The relative reactivity of a competitor in the binding assay was calculated at 50% inhibition of [^{3}H]-nor-FC-alcohol binding from competition curves run for each compound, assigning FC the relative value of 100%. The same approach was used to calculate immunological cross-reactivity.

RESULTS AND DISCUSSION

Radio- and Immunochemicals. Since FC is a low mol wt compound (mol wt = 680 D), an immune response can be elicited against the molecule only after coupling to an immunogenic carrier. Pini *et al.* (20) obtained such FC-conjugates by coupling periodate oxidized dideacetyl-FC to BSA. In this case, both the glucosyl moiety and the aglycone are modified and although the antibodies raised against this conjugate are able to recognize FC and were found useful for the RIA of FC (11), we attempted to minimize structural alterations during immunogen synthesis. Furthermore, the coupling site was chosen so as to leave the physiologically significant structural features unchanged. The biological activity of de-*t*-pentenyl FC was found comparable to that of FC in a number of bioassays (1, 2, 18, 21), indicating that the *t*-pentenyl-substituent at C-6′ of the glucosyl residue might be a suitable coupling site.

Periodate oxidation of FC after incubation with osmiumtetroxide resulted in complete reaction after 12 h. Dihydro-FC was not converted under these conditions indicating that the oxidation took place at the *t*-pentenyl moiety. The reaction product (aldehyde positive, R_f = 0.78 in $CHCl_3$:isopropanol = 9:1 [v/v], yield: 95%) was convertable by $NaBH_4$ to the corresponding nor-FC-alcohol (R_f: 0.65, FC: R_f = 0.82, HPLC: Rt = 6.1 min on Shandon hypersil ODS, FC: R_t = 15.2 min). The alcohol, in turn, gave two major deacetylation products upon alkaline hydrolysis (R_f = 0.46 and 0.23 in $CHCl_3$:isopropanol = 8:2, R_f, nor-FC-alcohol = 0.76), confirming the presence of both ester links in the aldehyde intermediate. Nor-FC-aldehyde coupling to alkaline phosphatase was carried out at near neutral pH to avoid ester hydrolysis. This was proven by the absence of nor-FC-alcohol deacetylation products in the low mol wt fraction of reaction mixtures after coupling was completed. Conjugates of nor-FC-aldehyde and BSA (FC-BSA) were consistently obtained with coupling ratios of about 3 mol of FC per mol of protein, as determined by spectroscopic analysis in concentrated H_2SO_4 (λ_{max} = 386 nm for FC and nor-FC-alcohol, calculations based

on ϵ_{386} (FC) = 8.2×10^6 cm^2 mol^{-1}). The shelf life of FC-alkaline phosphatase conjugates, based on immunological reactivity, exceeded 6 months when stored in 50% glycerol in 10 mM citrate buffer (pH 6.5) at −18°C.

The nor-FC-aldehyde intermediate was converted to the tritiated nor-FC-alcohol by reaction with sodium borotritide as described and afforded the radiochemically pure tritiated alcohol (purity checked by TLC and HPLC in the systems given), which served as RIA-tracer but also proved an efficient and easily accessible FC-derivative for plant FC-receptor binding studies (M Feyerabend, EW Weiler, unpublished data). The specific activity, determined spectroscopically in H_2SO_4 and by scintillation counting, of the synthesized [^{3}H]-nor-FC-alcohol, was 1.5×10^{14} Bq mol^{-1}. Stored in dilute solution in 10% acetonitrile in ethanol at −20°C, decomposition was less than 5% per year

Antibody Characteristics and Purification. The FC-BSA conjugates elicited a strong immune response, detected as the presence in serum of antibodies binding [^{3}H]-nor-FC-alcohol in all animals (rabbits, Balb/c mice) immunized as in Weiler (25). The response developed after the first boost and was persistent during prolonged immunization in both species. For the generation of MAB, a short-term immunization schedule was selected to improve the possibility to elicit antibodies recognizing the glucosyl-moiety. The hybridoma lines characterized here (cell line codes: 19-VI-A5 and 20-VII-C5) were obtained from two different fusions and proved stable after three recloning steps by limiting dilution. Since they are derived from X63 Ag8.653, a nonproducer myeloma (13), hybrid-MAB secretion by the hybridoma clones is excluded. Both cell lines secrete IgG_1 and can be used for MAB production in cell culture and via ascites fluid. A prerequisite for the use of MAB for the immunoaffinity chromatography of FC-like metabolites as well as for the generation of anti-idiotypic antibodies is the availability of a highly pure MAB preparation. Various techniques for MAB purification have been developed (5, 7, 23), of which liquid chromatography on hydroxylapatite has proven particularly useful for IgG type antibodies (23). As Figure 1 shows, the chromatographic process

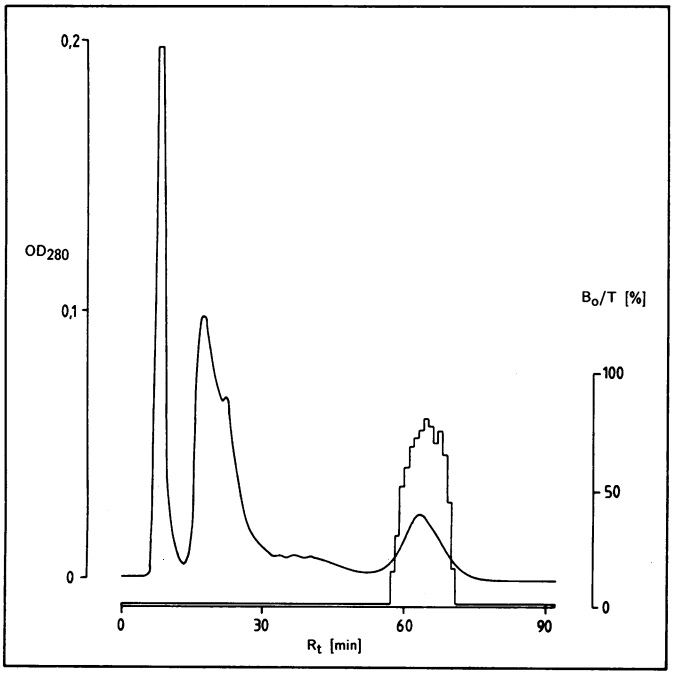

FIG. 1. Purification of MAB 19-VI-A5 by FPLC. Cleared ascites fluid was applied onto a Biogel HTP (100 × 10 mm) column and eluted with a linear gradient from 10 to 300 mM NaP_i (pH 6.8) (flow 1 ml min^{-1}). Immunoreactivity was analyzed by RIA.

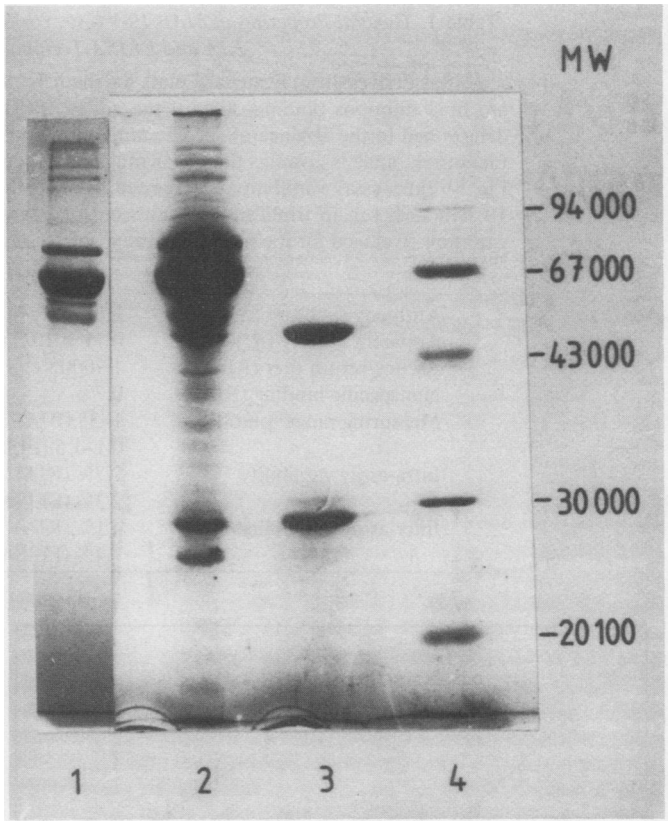

FIG. 2. SDS-PAGE of MAB 19-VI-A5. Crude ascites (lane 2), low-salt eluate (combined fraction from experiment shown in Fig. 1, Rt = 17–30 min, lane 1), high-salt eluate (combined fraction from experiment shown in Fig. 1, Rt = 57–75 min, lane 3). Lane 4: marker proteins. The gel was stained with Coomassie brilliant blue.

used in this study allows the single step FPLC purification of MAB 19-VI-A5 from crude ascites fluid (cleared by centrifugation prior to loading). The antibody elutes (at 180 mM NaP_i) as a single peak. This is shown by the immunoreactivity profile (Fig. 1) obtained by RIA and further by SDS-PAGE analysis (Fig. 2). As compared to the crude ascites (Fig. 2, lane 2), in which the IgG protein is a minor constituent, the immunoreactive protein (Fig. 1; Fig. 2, lane 3) is nearly pure IgG (heavy and light chain) with only trace contaminations of other proteins in the range from 25 to 32 kD. In contrast, the peak eluting at low NaP_i (17–30 min, Fig. 1) is devoid of immunoglobulin and contains the albumin, transferrin, and other ascites proteins (lane 1, Fig. 2). The procedure described here allows rapid preparation in milligram amounts of highly pure, immunologically active MAB 19-VI-A5 in a single step from crude ascites fluid.

Radio- and Enzyme Immunoassay. The general parameters of the optimized RIA and ELISA techniques are given in Table I and the standard curves are shown in Figure 3. As compared to a serum-based RIA for FC described earlier (11), the techniques reported here are considerably more sensitive (*e.g.* ELISA using MAB 19-VI-A5, is more sensitive by a factor of 50) allowing the quantitation of FC in fmol amounts. The apparent affinity for FC, as obtained from Scatchard analysis of the heterologous ligand system [^{3}H]-nor-FC-alcohol and FC, is in the 10^{-9} M range for all MABs and by a factor of about 10 lower (10^{-8} M) for rabbit antiserum. The high affinity of the MAB described here is not accidental. In employing RIA for hybridoma screening, low (about 10 nM) levels of radiolabeled ligand are used as tracer. The detection of low-affinity MAB is thus practically excluded.

Antibody Selectivity. For a cross-reaction analysis, increasing

Table I. *General Properties of MAB 19-VI-A5, 20-VII-C5, and Rabbit Serum 478 and Assay Parameters of RIA and ELISA Techniques Based on the Antibodies*

K_a was derived from Scatchard plots for the heterologous system [^{3}H]-nor-FC-alcohol/FC. The titers given are final dilutions binding 30% of the added tracer under standard conditions. Nonspecific binding was determined in the absence of specific antibodies and is expressed in % of total activity added per tube. The measuring range is given as the linear range of standard curves when logit B/Bo is plotted *versus* log FC (see Fig. 3). Intra-assay variability is expressed as % coefficient of variation, averaged for the measuring range, of 10 standards run in triplicate in 5 consecutive assays. Interassay variability is expressed in % coefficient of variation, averaged for the measuring range, of 10 standard means obtained from 5 consecutive assays.

	MAB 19-VI-A5	MAB 20-VII-C5	Serum 478
Antibody subclass	IgG_1	IgG_1	polyclonal
Apparent affinity (K_a)	0.71×10^{-9} M	1.85×10^{-9} M	0.93×10^{-8} M
Ascites/serum titer (RIA)	1:9000	1:13500	1:1080
Nonspecific binding (RIA)	0.7%	0.7%	1.0%
Measuring range (pmol)	1–35 (RIA) 0.1–1.5 (ELISA)	1.5–40 (RIA)	2–70 (RIA)
Intra-assay variability	2.7% (RIA) 2.3% (ELISA)	2.5% (RIA)	3.0% (RIA)
Inter-assay variability	4.1% (RIA) 7.4% (ELISA)	5.2% (RIA)	7.3% (RIA)

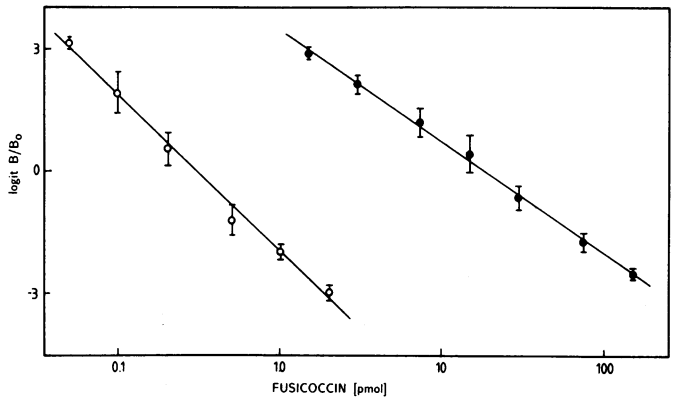

FIG. 3. Standard curves for the RIA (●) and ELISA (○) of FC using MAB 19-VI-A5. The curves shown are averaged from $n = 6$ (ELISA) and $n = 7$ (RIA) individual standard curves. The bars represent ± SD of the standard-average of each day's experiment. Logit B/Bo = ln (B/Bo)/(100-B/Bo).

levels of competitor were incubated with the antibody in the presence of the standard level of radio- or enzyme-labeled FC derivative. The results for MAB 19-VI-A5, MAB 20-VII-C5, and serum 478 are given in Table II and the structures are shown in Figure 4. In addition, the following compounds were checked and showed no detectable inhibition at the highest level tested (corresponding to CR ≪ 0.01%): gibberellin A_3, abscisic acid, indole-3-acetic acid, trans zeatin, hepta-β-glucoside elicitor, 22β,23β-brassinolide, plant leaf movement factor 1, and *t*-cinnamic acid.

The cross-reaction analysis allows the following conclusions: (a) The MABs are generally somewhat more selective than the (polyclonal) rabbit antiserum although the overall pattern of selectivity in all assay systems and of all antibodies is similar. Such behavior was earlier observed for other hapten-specific antibodies (16) and was explained as indicating the presence, in antisera, of low-affinity, low-selectivity antibody subpopulations (16), and/or mixed antibody populations recognizing somewhat different epitopes of the hapten.

(b)The selectivity of MAB 19-VI-A5 is, at least in the two assays used here, assay-independent with the notable exception of the cross-reaction of nor-FC-alcohol itself. The compound competed more effectively with the FC-enzyme conjugate than with the homologous [^{3}H]-nor-FC-alcohol in the RIA. This is explained as reflecting the lower affinity of the FC-alkaline phosphatase conjugate to MAB 19-VI-A5 as compared to [^{3}H]-nor-FC-alcohol.

(c) There are some notable differences in selectivity of the antibodies reported here and those reported earlier (11, 20). Antisera raised against periodate-oxidized dideacetyl-FC protein conjugates (20) exhibited a high affinity against the aglycone and apparently very little affinity for the α-D-glucosyl moiety of FC. This reflects the drastic changes in the glucosyl part introduced by the oxidative cleavage employed. In contrast, the FC aglycone is almost unreactive with the antibodies reported here, suggesting a stronger influence of the glycosyl moiety on antigen binding in

Table II. *Cross-Reactivities of FC-Related Compounds in Immunological Assays Compared to Relative Reactivities of the Compounds in Two Microsomal FC-Binding Assays*

In all cases, relative reactivity and cross-reaction were obtained from competition analysis using increasing levels of the respective competitor to inhibit the binding of [^{3}H]-nor-FC-alcohol in the RIA and the *Vicia* microsomal assay, and FC-alkaline phosphatase in the ELISA as described in "Materials and Methods." The value given was calculated from the I_{50}s as: cross-reaction (%), relative activity (%) = 100 × (I_{50} [FC]/I_{50}[competitor]). The data for the maize microsomal assay were calculated from the I_{50} values given in (2).

Compound[a]	Cross-Reaction				Relative Activity	
	Radioimmunoassay			ELISA 19-VI-A5	In microsomal binding assay	
	478	20-VII-C5	19-VI-A5		*Vicia*	Maize
			%			
(1)	100	100	100	100	100	100
(2)	103	103	70	102	107	100
(3)	67	98	101	94	98	51
(4)	30	70	61	74	51	60
(5)	101	64	63	64	92	
(6)	29	0.6	0.1	0.7	4.2	1.4
(7)	17	0.4	0.2	0.5	0.2	0.4
(8)	0.4	<0.1	0.2	0.2	37	128
(9)	0.9	0.1	0.1	0.2	12	13

[a] For structures of compounds, see Fig. 4.

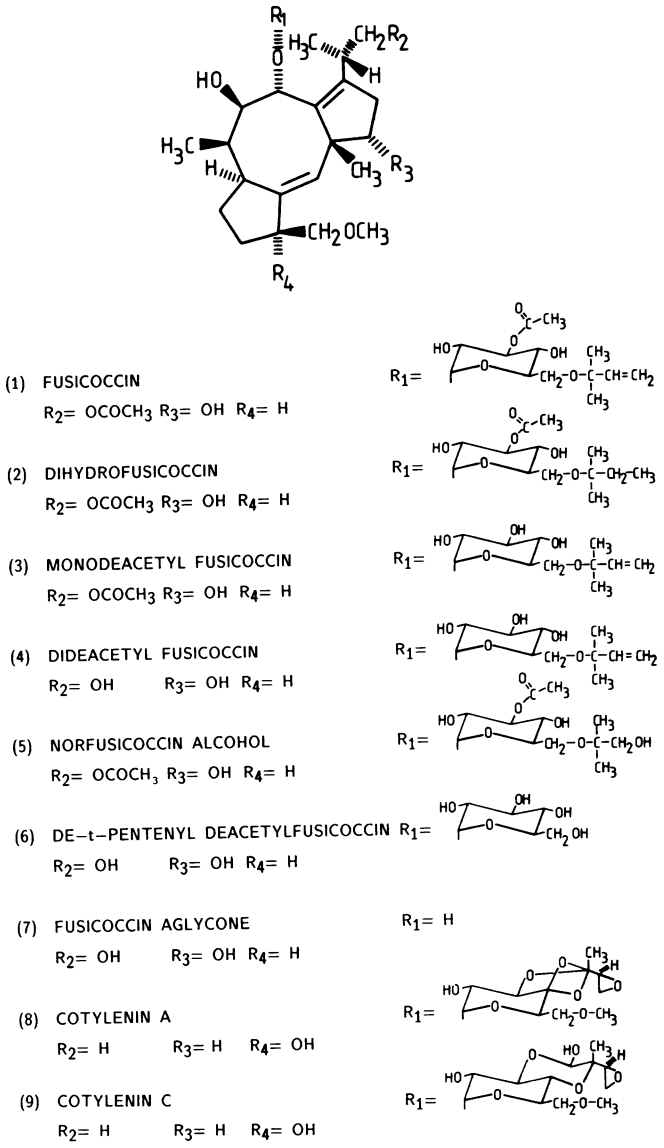

FIG. 4. Structures of FC and related compounds used in this study.

our case. Deacetylation (compounds [3] and [4]) and modifications in the *t*-pentenyl moiety (compounds [2] and [5]) had very little effect on immunoreactivity, but the complete removal of the substituent at C-6′ (compound [6]) results in an ineffective competitor in assay systems based on MAB. Although more structurally related compounds would have to be tested to reach a final conclusion, these results may indicate that the conformation of the glucosyl moiety together with the link at C-9 make an important contribution to immunoreactivity.

Comparison of Immunological and Biological Activity of FC Derivatives and Related Compounds. In a number of studies, high affinity binding sites for FC were identified in microsomal preparations of several plant species including maize and oat (2, 18, 24). It is probable that these sites are located at the plasmalemma of plant cells (24) and the comparison of characteristics of the binding sites obtained from different sources reveals a great degree of similarity. This is also true for FC-binding sites which we have characterized in plasmalemma-rich microsomal vesicles from broadbean leaf and epidermal tissue (M Feyerabend, EW Weiler, unpublished data; see also Table II). Although the function of FC-binding sites is not known, these sites are prime candidates for FC receptors.

When compared in terms of selectivity, microsomal FC-binding sites from maize coleoptiles (Table II) and *Vicia faba* L. leaf tissue (Table II) behave quite similarly. FC, dihydro-FC, as well as the dideacetyl FC derivatives are highly reactive, as is the nor-FC-alcohol. The aglycone of FC and de-*t*-pentenyldideactyl FC are very weakly active. Within the FC derivative series, there is a close correlation between the reactivity of the compound in both microsomal assays and the immunological cross-reactivity with MAB 19-VI-A5 and 20-VII-C5 (Table II) independent of the assay format (*i.e.* RIA *versus* ELISA). Within this group of compounds, the structural requirements for binding to the MABs and to the microsomal vesicles are very similar. The cotylenins tested, in contrast reacted only weakly with the MABs while being significantly competitive with both [^{3}H]-dihydro-FC in the maize microsomal assay and with [^{3}H]-nor-FC-alcohol in the broadbean assay. Apparently, the structural requirements for antibody binding are more stringent than those for competition in the microsomal assay. The data in Table II suggest, however, that MAB 19-VI-A5 and MAB 20-VII-C5 are useful tools for the search for endogenous ligands with structural homologies to that part of the FC molecule which is relevant for physiological activity.

CONCLUSION

We report the properties of MAB against FC exhibiting binding affinities and characteristics similar to FC binding sites found in membranes of higher plants. These results demonstrate the feasibility to raise hapten-sepcific MAB which share certain binding characteristics with endogenous, ligand-recognizing binding sites of plants. MAB can be produced in highly purified form in almost any desired quantity. Thus, they should have several interesting applications in receptor research: (a) they will allow localization of ligands such as plant hormones (10), and quantitation of them by RIA or ELISA. Ultrasensitive assays which are now being developed (MJ Harris, WH Outlaw Jr, R Mertens, EW Weiler, unpublished data) operate in the attomol range and allow determination of hormones at the cellular level; (b) they will be usefull as primary antibodies to elicit anti-idiotypic antibodies, *i.e.* second-generation antibodies carrying internal images of the original ligand which are useful to isolate and localize receptors or to probe their ligand-binding site (12); (c) they may be used to detect and affinity-purify as yet unknown ligands with physiological importance. Work is now in progress to identify endogenous FC-like metabolites in plant tissues.

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LITERATURE CITED

1. BALLIO A, MI DEMICHELIS, P LADO, G RANDAZZO 1981 Fusicoccin structure-activity relationships: stimulation of growth by cell enlargement and promotion of seed germination. Physiol Plant 52: 471–475
2. BALLIO A, R FEDERICO, D SCALORBI 1981 Fusicoccin structure-activity relationships: in vitro binding to microsomal preparations of maize coleoptiles. Physiol Plant 52: 476–481
3. BONA CA, H KOHLER 1984 Anti-idiotypic antibodies and internal images. *In* JC Venter, CM Fraser, J Lindstrom, eds, Monoclonal and Anti-idiotypic Antibodies. Probes for Receptor Structure and Function. Alan R. Liss, New York, pp 141–150
4. BRADFORD MM 1976 A rapid and sensitive method for the quantification of microgram guantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72: 248–254
5. BURCHIEL SW, JR BILLMAN, TR ALBER 1984 Rapid and efficient purification of mouse monoclonal antibodies from ascites fluid using high performance liquid chromatography. J Immunol Methods 69: 33–42
6. DAVIS GC, MB HEIN, DA CHAPMAN, BC NEELY, CR SHARP, RC DURLEY, DK BIEST, BR HEYDE, MG CARNES 1986 Immunoaffinity columns for the isolation and analysis of plant hormones. *In* M Bopp, ed, Plant Growth Substances 1985, Springer-Verlag, Berlin, pp 44–51

7. DESCHAMPS JR, JEK HILDRETH, D DERR, JT AUGUST 1985 A high-performance liquid chromatographic procedure for the purification of mouse monoclonal antibodies. Anal Biochem 147: 451–454
8. DICKINSON RG, NW JACOBSEN 1970 A new and specific test for the detection of aldehydes: formation of 6-mercapto-3-substituted-s-triazolo [4,3-β]-s-tetrazines. Chem Commun 1970, 1719–1720
9. EBERLE J, A ARNSCHEIDT, D KLIX, EW WEILER 1986 Monoclonal antibodies to plant growth regulators. III. Zeatinriboside and dihydrozeatinriboside. Plant Physiol 81: 516–521
10. EBERLE J, TL WANG, S COOK, B WELLS, EW WEILER 1987 Immunoassay and ultrastructural localization of isopentenyladenine and related cytokinins using monoclonal antibodies. Planta. 172: 289–297
11. FEDERICO R, P ADUCCI, M MARRA, C PINI 1981 A radioimmunoassay for fusicoccin. Z Pflanzenphysiol 104: 275–279
12. GREAVES MF, ED 1984 Monoclonal Antibodies to Receptors. Probes for Receptor Structure and Function. Chapman and Hall, London, pp 324
13. KEARNEY JF, A RADBRUCH, B LIESEGANG, K RAJEWSKII 1979 A new mouse myeloma line which has lost immunoglobulin expression of antibody in secreting hybrid cell lines. J Immunol 123: 1548–1550
14. LAEMMLI UK 1970 Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227: 680–685
15. MARRÈ E 1979 Fusicoccin: a tool in plant physiology. Annu Rev Plant Physiol 30: 273–288
16. MERTENS R, B DEUS-NEUMANN, EW WEILER 1983 Monoclonal antibodies for the detection and quantitation of the endogenous plant growth regulator, abscisic acid. FEBS Lett 160: 269–272
17. OUCHTERLONY Ö 1985 Diffusion in gel methods for immunological analysis. Progr Allergy 5: 1–78
18. PESCI P, SM COCUCCI, G RANDAZZO 1979 Characterization of fusicoccin binding to receptor sites on cell membranes of maize coleoptile tissue. Plant Cell Environ. 2: 205–209
19. PESCI P, L TOGNOLI, N BEFFAGNA, E MARRÈ 1979 Solubilization and partial purification of a fusicoccin-receptor complex from maize microsomes. Plant Sci Lett 15: 313–322
20. PINI C, G VICARI, A BALLIO, R FEDERICO, A EVIDENTE, G RANDAZZO 1979 Antibodies specific for fusicoccin. Plant Sci Lett 16: 343–353
21. RADICE M, A SCACCHI, P PESCI, N BEFFAGNA, MT MARRÈ 1981 Comparative analysis of effects of fusicoccin (FC) and of its derivative dideacetylfusicoccin (DAF) on maize leaves and roots. Physiol Plant 51: 215–221
22. RASI-CALDOGNO F, MC PUGLIARELLO 1985 Fusicoccin stimulates the H^+-ATPase of plasmalemma in isolated membrane vesicles form radish. Biochem Biophys Res Commun 133: 280–285
23. STANKER LH, M VANDERLAAN, H JUAREZ-SALINAS 1985 One-step purification of mouse monoclonal antibodies form ascites fluid by hydroxylapatite chromatography. J Immunol Methods 76: 157–169
24. STOUT R, RE CLELAND 1980 Partial characterization of fusicoccin binding to receptor sites in oat root membranes. Plant Physiol 66: 353–359
25. Weiler EW 1986 Plant hormone immunoassays based on monoclonal and polyclonal antibodies. *In* HF Linskens, JF Jackson, eds, Immunology in Plant Sciences, Modern Methods of Plant Analysis, Vol 4. Springer-Verlag, Berlin, pp 1–17