Characterization of the Cryptogein Binding Sites on Plant Plasma Membranes*

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Cryptogein is a 98-amino acid proteinaceous elicitor of tobacco defense reactions. Specific binding of cryptogein to high affinity binding sites on tobacco plasma membranes has been previously reported (Kd = 2 nM; number of binding sites: 220 fmol/mg of protein). In this study, biochemical characterization of cryptogein binding sites reveals that they correspond to a plasma membrane glycoprotein(s) with an N-linked carbohydrate moiety, which is involved in cryptogein binding. Radiation inactivation experiments performed on tobacco plasma membrane preparations indicated that cryptogein bound specifically to a plasma membrane component with an apparent functional molecular mass of 193 kDa. Moreover, using the homobifunctional cross-linking reagent disuccinimidyl suberate and tobacco plasma membranes incubated with 125I-cryptogein, we identified, after SDS-polyacrylamide gel electrophoresis and autoradiography, two 125I-cryptogein linked N-glycoproteins of about 162 and 50 kDa. Similar results were obtained using Arabidopsis thaliana and Acor pseudoplatanus plasma membrane preparations, whereas cryptogein did not induce any effects on the corresponding cell suspensions. These results suggest that either cryptogein binds to nonfunctional binding sites, homologues to those present in tobacco plasma membranes, or that a protein involved in signal transduction after cryptogein recognition is absent or inactive in both A. pseudoplatanus and A. thaliana.

Most often, the interaction between plants and microorganisms results in an incompatible interaction characterized by multicomponent defense responses such as phytoalexin production or reinforcement of plant cell walls and is sometimes associated with the collapse of the challenged plant cells in the so-called hypersensitive response (1). Such reactions are induced by elicitors, which either originate from the pathogens or are produced from plant cell walls by fungal hydrolytic enzymes (2).

Pathogen recognition in cultivar-specific interaction can often be determined by a gene-for-gene relation. Race-specific elicitors, which are encoded directly or indirectly by pathogen avirulence genes, induce resistance in plants carrying the corresponding dominant R genes, whose products were assumed to be receptors for avirulence gene products (3, 4). Many such R genes have been identified (for review, see Ref. 5). Most of them encode cytoplasmic proteins that contain a central nucleotide binding site and a C-terminal domain with a variable number of leucine-rich repeats (5). The N-terminal region contains either leucine zipper motifs or shows homology with the cytoplasmic domain of the Drosophila Toll and mammalian interleukin-1 receptors (6). These nucleotide binding site leucine-rich repeats containing proteins show homologies to proteins regulating apoptosis in mammals and worms (7). Taken together, these results suggest that the corresponding proteins may be involved in a conserved pathway in the response of eukaryotes to pathogens (8), including the hypersensitive response. Nevertheless, there is no clear evidence that R gene products function as receptors for elicitors. For example, the tomato Cf-9 gene, which confers resistance to the race of Cladosporium fulvum carrying the correspondingavr9 gene, encodes an extracellular membrane-anchored glycoprotein consisting predominantly of leucine-rich repeats (9) that did not directly interact with the AVR9 elicitor (10). Among at least 14 identified R genes, only 1 R gene product has been shown to interact with the corresponding Avr elicitor, namely the product of the tomato Pto gene, which confers resistance to the race of Pseudomonas syringae expressing avrPto gene and which encodes a cytoplasmic serine/threonine protein kinase (11, 12) whose interaction with avrPto was demonstrated using the yeast two-hybrid system (13). Thus, the molecular basis of plant pathogen recognition remains to be elucidated. Besides these plant/pathogen-specific interactions, many elicitors do not induce defense responses in a cultivar- or species-specific manner, although their recognition is likely to be mediated by high affinity receptors.

Results have reported the presence of high affinity binding sites for different kinds of elicitors including glucans (14, 15), glycoproteins (16–18), proteins (10, 19, 20), and syringolide 1, a glycolipid elicitor produced by P. syringae pv. tomato strains carrying the avrD gene (21). High affinity binding sites for these elicitors have been identified in membrane-enriched fractions, with the exception of syringolide 1, whose binding sites were found in a soluble protein fraction from soybean leaves (21).

Information on the biochemical properties of these putative receptors mainly concerns their apparent molecular masses. For instance, the binding site for the Pep-13 elicitor, a peptide derived from a 42-kDa glycoprotein from Phytophthora sojae, corresponds to a 91-kDa nonglycosylated plasma membrane protein identified after cross-linking with 125I-Pep-13 (22). A 75-kDa binding protein for the N-acetylcysteine-oligosaccharide

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eliciton was identified by photoaffinity labeling and cross-linking in plasma membrane preparations of rice cells (23). The binding site for a β-glucan elicitor derived from Phytophthora megasperma forma sp. glycinea was identified as a 70-kDa soybean plasma membrane protein by photolabeling using a 125I-derivatized β-glucan (24). A β-glucan binding protein was purified from soybean root microsomes, and cDNA cloning indicated that it encodes a protein with an apparent molecular mass of 74 kDa, which has no clear membrane-spanning domain, suggesting that the protein may require other components for localization in the plasma membrane (25).

In this study, we have characterized the cryptogein plant plasma membrane binding sites. Cryptogein, a proteinaceous elicitor of tobacco defense responses, that was isolated from Phytophthora cryptogaea, belongs to a family of 10-kDa holoproteins called elicinins (26). Elicitins cause hypersensitive response and induce systemic acquired resistance of tobacco plants (Nicotiana tabacum cv. Xanthi) against fungal and bacterial pathogens (27). Using tobacco cell suspensions, we previously established that cryptogein induces the following early events: protein phosphorylation (28), large calcium influx (29), chloride and potassium efflux, plasma membrane depolarization, activation of a plasma membrane NADPH oxidase (30), activation of mitogen-activated protein kinases (31, 32), and defense gene activation (33, 34). These events are thought to occur after specific binding of cryptogein to high affinity binding sites on tobacco plasma membranes (19). These binding sites conform to criteria defined for receptors: high affinity, saturaibility, reversibility, and drug displacement and could correspond to the putative receptor of cryptogein. Using three other elicins, we have shown that they bound to the same site with similar affinities and induced the same events on tobacco cells (20). Nevertheless, the intensity of these events (calcium influx, extracellular medium alkalization, and active oxygen species production) differed from one elicitin to the other.

Results obtained in this present work have defined the biochemical nature of these binding sites, the molecular mass of the potential cryptogein receptor, and the subunit organization. Interestingly, similar binding sites were also detected in other cryptogein-insensitive plant species.

EXPERIMENTAL PROCEDURES

Materials—Tobacco plants (N. tabacum cv. Xanthi) were grown in a greenhouse for two months. Tobacco cells (N. tabacum cv. Xanthi) were cultivated in Chandler medium (35) on a rotary shaker (150 rpm, 24 °C) under continuous light (photon flux rate 30–40 μmol m−2 s−1) and used during the exponential growth phase. A. pseudoplatanus cells were cultivated as described previously (36). Cryptogein was purified according to Bonnet et al. (27).

Plasma Membrane Preparation—Tobacco plasma membrane-enriched fractions were obtained as described previously (20). Plasma membrane-enriched fractions from A. pseudoplatanus cells were prepared according to the same protocol with some modifications. The aqueous polymer two-phase system was 6.4% (w/w) dextran (Mr 500,000) and 6.4% (w/w) polyethylene glycol (Mr 3350) in a 5 mM phosphate buffer, pH 7.8, containing 0.3 mM sucrose and 3 mM KCl. The protein content of plasma membrane preparations was measured according to Lowry et al. (37) using bovine serum albumin as a standard.

Radiodination of Cryptogein—Iodination of cryptogein was performed as described previously (20). Briefly, 50 μg of cryptogein were incubated for 20 min at 20 °C with 185 MBq of Na125I (Amersham Pharmacia Biotech) in a 50 mM phosphate buffer, pH 7.5, using Iodogen (Pierce) as the catalyst. Radiolabeled cryptogein (125I-cryptogein) was purified by gel filtration on a G-25 Sephadex (Amersham Pharmacia Biotech) column equilibrated with 50 mM phosphate buffer, pH 7.5.

Binding Experiments—Binding experiments were performed as described previously (20). Plasma membrane preparations (50 μg protein) were incubated in a 10 mM HEPES-KOH, pH 7.0, 0.1 mM sucrose, 5 mM MgCl2, binding buffer (final volume of 100 μl) with 1 to 30 nM 125I-cryptogein. Binding was performed for 90 min on ice. Nonspecific binding was determined in the presence of 10 μM unlabeled cryptogein. Bound ligand was separated from free ligand by filtration under a vacuum through GF/B filters (Whatman) presoaked in 1% bovine serum albumin. After 3 washes, each with 5 ml ice-cold binding buffer containing 0.1% bovine serum albumin, radioactivity remaining on the filters was measured in a Beckman LS 600 TA scintillation counter. Control experiments with plasma membranes prepared in the absence of NaIO4 could result in decreased specific binding was not detected in plasma membranes incubated in binding buffer for 16 h at 37 °C.

Protease Treatments—Plasma membrane preparations (5 mg of protein/ml) were incubated in binding buffer for 30 min at 37 °C on a rotary shaker (80 rpm) with appropriate protease concentrations: 100 μg/ml Pronase, 25 μg/ml XVIII protease (Staphylococcus aureus) (Sigma). Protease treatments were terminated using an antiprotease mixture consisting of 100 μg/ml phenylmethylsulfonfluor fluoride, trypsin inhibitor (trypsin/trypsin inhibitor = 1:2, w/w), 1 μg/ml pepstatin, and 1 μg/ml leupeptin. Plasma membrane preparations were then used for 125I-cryptogein binding assays. In the binding medium, proteases did not lead to 125I-cryptogein proteolysis during the binding step as verified by SDS-PAGE1 and autoradiography. Control assays contained the same antiprotease mixture and proteases inactivated by heating at 100 °C for 15 min before plasma membrane treatments.

Metaperiodate and Glycosidase Treatments—Plasma membrane preparations (5 mg of protein/ml) were incubated at 4 °C for 16 h in the dark in binding buffer without sucrose but supplemented with 5 mM NaIO4. 125I-cryptogein was then used in binding experiments. In other experiments, the sodium concentration of NaIO4 was added to plasma membrane preparations 30 s before initiation of 125I-cryptogein binding, which lasted 90 min. When treated with glycodiesases, plasma membrane preparations were incubated in binding buffer without sucrose for 16 h at 37 °C but with either O-glycosidase (10 units/ml), which hydrolyzes the bond between serine/threonine residues and the carbohydrate chain, or with N-glycosidase F (20 units/ml) (Roche Molecular Biochemicals), which cleaves the N-glycan linkage between asparagine and the carbohydrate chain. Protease activity was not detected in either the commercial glycosidase preparations (Roche Molecular Biochemicals) or the plasma membrane preparation when incubated during 16 h at 37 °C in the binding buffer without sucrose. In control assays, plasma membranes were incubated in the presence of NaIO4 with heat-inactivated glycodiesases (100 °C for 15 min). Plasma membranes were then stored on ice before 125I-cryptogein binding assays.

125I-Cryptogein Chemical Cross-Linking—After 125I-cryptogein binding, cross-linking reagents were added to the ligand binding mixture at the appropriate concentrations: 0.5 mM dithiobis(sulfosuccinimidyl) carbonate (DSS), 0.5 mM bis(sulfosuccinimidyl) carbonate (BS3), 0.5 mM 3,3'-dithiobis(sulfosuccinimidylpropionate) (DTSSP) (Pierce), or 2.5% glutaraldehyde (Sigma). Cross-linking reactions were performed on ice for 30 min and terminated by the addition of 100 mM Tris-HCl, pH 8.0. When the photoactivatable cross-linker sulfosuccinimidyl-2-(p-azidosalicylamido)-ethyl-1,3'-dithiopropionate (SASD) was used, it was first bound to an NH2-containing amino acid of the 125I-cryptogein. Then the derivatized elicitor was used in a binding experiment, and photoactivation was performed for 2 min at 320 nm. Cross-linked plasma membrane proteins were pelleted by centrifugation (30 min at 100,000 × g), solubilized in a modified Laemmli buffer with or without 50 mM dithiothreitol, and subjected to SDS-PAGE analysis in both reducing and nonreducing conditions. Gels were dried and subsequently analyzed by autoradiography.

Metaperiodate and N-glycosidase treatments after cross-linking were performed as described. 125I-Cryptogein-cross-linked plasma membrane proteins, solubilized in Laemmli buffer (10 μl), were diluted in 10% (w/v) Nonidet P-40 containing 0.25 units/ml N-glycosidase F or 5 mM NaIO4 (final volume, 50 μl) and incubated for 16 h at 37 °C or 4 °C respectively, before analysis by SDS-PAGE. In these conditions, no protease activity in the glycosidase preparation was detected (assayed on pure labeled cryptogein and bovine serum albumin).

Radiation Inactivation Assays—Decrease in biological activity due to radiation exposure of gamma rays is an exponential function of the absorbed dose and is related to target size or molecular mass. The decrease of 125I-cryptogein-specific binding was compared with the decrease of the glucose-6-phosphate dehydrogenase (6PDH) activity, which was used as an internal standard, and to the decrease of plasma

1 The abbreviations used are: PAGE, polyacrylamide gel electrophoresis; DSS, disuccinimidyl suberate; BSA, bis(sulfosuccinimidyl) carbonate; DTSSP, 3,3'-dithiobis(sulfosuccinimidyl propionate); SASD, sulfosuccinimidyl-2-(p-azidosalicylamido)ethyl-1,3'-dithiopropionate; 6PDH, glucose-6-phosphate dehydrogenase.
membrane ATPase intrinsic activity as a control. A 5-ml solution containing 1 mg of plasma membrane proteins in 20 ml HEPES-KOH, pH 7.0, 0.2 M sucrose, 10 mM MgCl2 buffer with 25 units of G6PDH type XXIII from Leuconostoc mesenteroides (Sigma) was distributed in 10 microcentrifuge tubes (0.5 ml/tube). Samples were flushed under nitrogen and arranged in a circle in a Thermos full of dry ice. The Thermos was placed near a 60Co source (30 TBq) and revolved (2 rpm) to ensure isodoses for each sample. Samples were maintained in dry ice throughout the radiation exposure. Every 2 days, one sample was removed and stored at −80 °C. A control sample was stored at −50 °C without radiation. At the end of the experiment, samples were thawed, and 125I-cryptogein binding, G6PDH, and ATPase activities were measured.

RESULTS

Biochemical Properties of Cryptogein Binding Sites—We have previously shown that radiolabeling of cryptogein (9.25 TBq/mmol−1) or iodination with a large excess of nonradioactive iodine did not reduce cryptogein effects on tobacco cell suspensions (20). Binding characteristics of 125I-cryptogein were previously established on plasma membranes prepared from tobacco leaves (19, 20).

To verify whether the specific cryptogein binding sites corresponded to a protein, tobacco plasma membrane preparations were treated with various proteases: trypsin, Pronase, and protease XVIIB. These treatments reduced 125I-cryptogein-specific binding in tobacco plasma membrane preparations by 90 to 100%, whereas treatments with heat-inactivated proteases were ineffective (Table I). Interestingly, a heat treatment of plasma membranes at 100 °C for 15 min led to only a 15% decrease in cryptogein-specific binding (Table I).

To determine whether cryptogein-specific binding involved a carbohydrate moiety, plasma membranes were treated with metaperiodate, which oxidizes glycosides to form aldehydes, or with N-linked carbohydrates and N-glycosidase treatments induced a strong decrease in 125I-cryptogein-specific binding (Table II), whereas O-glycosidase treatment did not.

Table I. Specific binding at time 0 min was measured using a nonradioactive ligand in comparison to the decrease of the activity of exogenous enzyme of known molecular mass, G6PDH ($M_r = 104$ kDa). We verified the reliability of this method by estimating the functional molecular mass of the endogenous plasma membrane ATPase at the same time. Fig. 1 shows that the activity decay obeys a single exponential function of the absorbed dose. The functional molecular mass of ATPase was estimated to be 196 ± 5 kDa (average of three independent experiments), which is in agreement with published data showing that the enzyme corresponds to a 100-kDa polypeptide (39), which functions as a dimer (40). In the same experiment the functional molecular mass deduced for the cryptogein binding site was 193 ± 9 kDa.

125I-Cryptogein Chemical Cross-linking—The homobifunctional cross-linking reagent DSS was used to covalently bind 125I-cryptogein to its tobacco plasma membrane binding site. 125I-Cryptogein-cross-linked plasma membrane proteins were then subjected to SDS-PAGE under reducing conditions and
revealed by autoradiography. We detected two bands corresponding to cryptogein-linked proteins with apparent molecular masses of 172 ± 15 and 60 ± 4 kDa (based on the average of 10 independent experiments) (Fig. 2A, lane 1). In 20% of the experiments, the band corresponding to the 60-kDa cryptogein-linked protein was not detected (data not shown). Labeling of plasma membrane proteins was abolished when binding assays were performed in the presence of 10 μM unlabeled cryptogein (Fig. 2, lanes 2) or when other elicitors (10 μM) were used as competitors in binding experiments (data not shown). Assuming that cryptogein is cross-linked to these polypeptides in a 1:1 ratio, the molecular masses of these polypeptides should be 162 and 50 kDa respectively. With the assumption that both proteins are associated, thus forming a complex, one would expect a band around 222 kDa corresponding to a tripartite complex that includes the 10-kDa-labeled cryptogein. However, the generally low efficiency of cross-linkers (only 1 to 5% of the labeled cryptogein was cross-linked) did not allow detection of such a band, which would have resulted from a double-cross-linking event.

When 125I-cryptogein-cross-linked plasma membrane proteins were subjected to SDS-PAGE under nonreducing conditions, both bands corresponding to the 172- and 60-kDa cross-linked proteins were also detected (Fig. 2B, lane 1), suggesting that these polypeptides are not linked together or to another polypeptide by disulfide bonds. Cross-linking assays performed with BS3, a water-soluble DSS analog, or with DTSSP were less efficient and mainly revealed a band corresponding to the 172-kDa-cryptogein cross-linked protein, whereas the band corresponding to the 60-kDa cryptogein-linked polypeptide was barely detectable (data not shown). 125I-Cryptogein cross-linking was not observed with the photoactivable cross-linker SASD or with 2.5% DSS analog, or with DTSSP were less efficient and mainly revealed a band corresponding to the 172-kDa-cryptogein cross-linked protein.

DISCUSSION

In the present study, the high affinity binding sites of cryptogein previously identified in tobacco plasma membrane were characterized (19, 20). We first determined the chemical nature of these binding sites by treating plasma membrane preparations with proteases or glycosidases. Proteases reduced the cryptogein-specific binding by at least 80%. Metaperiodate and N-glycosidase, but not O-glycosidase treatments, resulted in a strong reduction of cryptogein-specific binding (73 and 81%, respectively). Taken together, these results suggest that cryptogein binds to plasma membrane glycoprotein(s) with an N-linked carbohydrate moiety involved in the binding of the elicitor. Radiation inactivation experiments were performed to determine the functional molecular mass of the putative cryptogein receptor in tobacco plasma membranes. This method has been successfully used to determine functional molecular masses of numerous enzymes or receptors (41). For example, radiation inactivation experiments have allowed determination of the functional size of the insulin receptor in liver membranes and to show that the receptor corresponds to a heterodimer (42). On tobacco plasma membranes, the functional molecular mass of the endogenous plasma membrane ATPase was estimated to be 196 ± 5 kDa, a value that fits well with the reported functional molecular mass of this enzyme (40). In the same assays, the functional molecular mass of the cryptogein receptor was estimated to be 193 ± 9 kDa.
Cryptogein binding characteristics on A. pseudoplatanus and A. thaliana plasma membrane preparations

Specific binding was obtained by subtracting nonspecific binding from total binding. Data were obtained from Scatchard plots deduced from saturation curves and are the average of three independent experiments ± S.D., except for tobacco cell plasma membrane preparations (two assays).

| Plant species          | $K_d$ (nM) | Number of specific binding sites (fmol/mg of plasma membrane proteins) |
|------------------------|------------|------------------------------------------------------------------------|
| Tobacco leaves         | 10.3 ± 1.8 | 318 ± 37                                                               |
| Tobacco cells          | 17         | 650                                                                    |
| A. thaliana (leaves)   | 10.7 ± 6.5 | 201 ± 85                                                               |
| A. pseudoplatanus (cells) | 5.2 ± 0.8 | 198 ± 33                                                               |

![Graph showing radiation inactivation curve](image)

**Fig. 5.** Functional molecular mass determination of the cryptogein binding site and of the H^+-ATPase in A. pseudoplatanus plasma membrane preparations by radiation inactivation as described under “Experimental Procedures.” Specific binding was calculated as mentioned in Table 1, and functional molecular masses were calculated as described in Fig. 1. Specific binding at time 0 min was measured using a nonirradiated thawed sample. Data in the figure represent the mean values of three independent radiation inactivation experiments, the standard deviations between the three experiments not exceeding 7.4, 8.8, and 9% for G6PDH activity, ATPase activity, and cryptogein binding, respectively. Data were analyzed by linear regression; equations for G6PDH (●), ATPase (○), and receptor (×) inactivation profiles were $y = 2.014 - 6.439 \times 10^{-4} \times (r = 0.991); y = 1.985 - 1.175 \times 10^{-3} \times (r = 0.989); y = 1.984 - 1.133 \times 10^{-3} \times (r = 0.961)$, respectively.

In animal cells, cross-linking strategies have been widely used to determine the molecular mass of receptors such as the insulin receptor (43), the receptor of galanin, a 29-amino acid neuropeptide (44), and the interferon γ receptor (45). As reported in the introduction, high affinity binding proteins for elicitors have also been detected in plant cells by cross-linking strategies (22–24). These strategies were applied in this study to determine the molecular mass of the cryptogein-binding proteins. The hydrophobic, homobifunctional cross-linking agent DSS was successfully used to covalently bind $^{125}$I-cryptogein to its binding site. SDS-PAGE and autoradiography of $^{125}$I-cryptogein cross-linked plasma membrane proteins revealed the presence of two major bands with molecular masses of 172 ± 15 and 60 ± 4 kDa, corresponding to 162-kDa and 50-kDa polypeptides cross-linked to the 10-kDa cryptogein. When cross-linked proteins were subjected to SDS-PAGE under nonreducing conditions, both bands were present, suggesting that these polypeptides, which could be putative subunits of a multimeric structure, are not linked by disulfide bonds. Labeling of both bands disappeared in the presence of a large excess of unlabeled cryptogein or other elicitors, namely cinnamomin, capsicaine, and parasiticein. This is in agreement with competition experiments showing that these elicitors interact with the same binding sites (20). This result confirms that the 162-kDa and/or the 50-kDa polypeptide identified in cross-linking experiments could correspond to the elicitin high affinity binding site(s). Plasma membrane treatments with metaperiodate or N-glycosidase after cross-linking resulted in a decrease of the labeling of both bands (metaperiodate treatment) or in the disappearance of the $^{125}$I-cryptogein-labeled bands (N-glycosidase treatment). This suggested that the cross-link was established between a lysine residue of cryptogein and an amino-containing glycosidic motif such as N-acetylglucosamine of the receptor.

BS3, a water-soluble DSS analog, and DTSSP, a water-soluble thiol-cleavable cross-linker, cross-linked the 162-kDa polypeptide but did not allow efficient cross-linkage of the 50-kDa polypeptide. Other cross-linkers, i.e. the photoactivable cross-linker (SASD) or glutaraldehyde, were unable to cross-link $^{125}$I-cryptogein to the 50- and 162-kDa polypeptides.

It was previously shown that the binding sites for cryptogein corresponded to a single class of binding sites with a Hill coefficient of 0.91 (19), which suggests that one cryptogein molecule binds to one protein or one protein complex. This binding site could correspond to either the 162-kDa protein or the 50-kDa protein or could be formed following an association of the 162- and 50-kDa proteins. Two independent proteins, both binding cryptogein, do not match a one class of binding sites, unless they have the same affinity for cryptogein. Using a radiation inactivation method in which associated proteins also contributed to the functional molecular mass (41), a functional molecular mass of 193 kDa was determined, which fits with a protein complex of 212 kDa (162-plus 50-kDa glycoproteins) or with the 162-kDa glycoprotein alone. However, the apparent molecular masses analyzed by SDS-PAGE were probably overestimated due to lower electrophoretic mobilities generally observed with glycoproteins, and therefore, a heterodimeric complex would correspond better with the estimated functional mass. The question remaining is to which protein(s) cryptogein binds? Experiments showing that heat treatment of plasma membranes that disorganizes oligomeric
structures did not diminish cryptogein binding favored the assumption that one protein in the complex is able to bind cryptogein. The absence of cross-linkage of the 50-kDa protein with BS3 and DTSSP suggests that the 162-kDa component is the protein that binds cryptogein, whereas the 50-kDa protein could be cross-linked to cryptogein in DSS experiments due its proximity to the liganded cryptogein. Thus, our results favor the hypothesis of cryptogein binding on the 162-kDa glycoprotein, the N-linked carbohydrate moiety either being directly involved in elicitor recognition or assuming a role in the conformation of the binding site.

Such multicomponent sensors of elicitors or hormones are currently reported in plants. It was suggested that auxin perception is mediated via an auxin-binding protein interacting with a transmembrane-transducing protein (46). Similarly, the binding protein for the AVR9 elicitor could be associated to the Cf-9 glycoprotein, the product of the corresponding R gene (47). Alternatively, it can be hypothesized that the 50-kDa protein corresponds to a truncated form of the 162-kDa protein produced by an alternative splicing of the 162-kDa glycoprotein mRNA, as has been demonstrated for SRK and eSRK glycoproteins, which constitute the functional SRK receptor involved in the sporophytic self-incompatibility response in Brassicaceae (48). Both 50- and 162-kDa glycoproteins could also correspond to the products of homologue genes, as in the Xa21 and Xa21D resistance genes, in which Xa21D encodes the cytoplasmic domain of the Xa21 protein (49). However, the existence of a truncated form conflicts with the absence of the 50-kDa cross-linked protein in experiments with BS3 and DTSSP. Another possibility is that the 50-kDa polypeptide could be a proteolytic product of the 162-kDa glycoprotein, although this proteolytic product (50-kDa protein) was not observed when BS3 and DTSSP were used in cross-linking experiments.

Using plasma membrane preparations from A. pseudoplatanus cell suspensions and A. thaliana leaves we showed that 125I-cryptogein binds to high affinity binding sites with binding parameters (Kd, number of binding sites) close to those obtained with tobacco leaf plasma membranes. 125I-Cryptogein cross-linking on A. pseudoplatanus plasma membrane preparations led to the detection of two bands corresponding to protein complexes of about 60 and 170 kDa. Moreover, the functional molecular masses of the cryptogein high affinity binding sites on the A. pseudoplatanus plasma membranes was estimated to be about 200 kDa by radiation inactivation experiments. All these data indicate that similar binding sites are present in plasma membrane preparations from other plant species. However, cryptogein recognition by plasma membrane components in A. pseudoplatanus and A. thaliana did not lead to signal transduction. Indeed, in the corresponding plant cell suspensions, 50 kDa cryptogein did not induce any extracellular alkalinization or active oxygen species production, two responses typical of tobacco cells treated with 25 nM cryptogein or lar alkalinization or active oxygen species production, two responses typical of tobacco cells treated with 25 nM cryptogein or lar

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