Characterization and partial purification of an oligopeptide elicitor receptor from parsley (*Petroselinum crispum*)

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Abstract Parsley cells recognize the fungal phytopathogen *Phytophthora sojae* through a plasma membrane receptor. A 13 amino acid oligopeptide fragment (Pep-13) of a 42 kDa fungal cell wall glycoprotein was shown to bind to the receptor and stimulate a complex defense response in cultured parsley cells. The Pep-13 binding site solubilized from parsley microsomal membranes by non-ionic detergents exhibited the same ligand affinity and ligand specificity as the membrane-bound receptor. Chemical crosslinking and photoaffinity labeling assays with [^{125}I]Pep-13 revealed that a monomeric 100 kDa integral plasma membrane protein is sufficient for ligand binding and may thus constitute the ligand binding domain of the receptor. Ligand affinity chromatography of solubilized microsomal membrane protein on immobilized Pep-13 yielded a 5000-fold enrichment of specific receptor activity.

Key words: Ligand affinity chromatography; Phytoalexin; *Phytophthora sojae*; Signal transduction

1. Introduction

Plant receptors for pathogen-derived elicitors are instrumental to pathogen recognition and subsequent activation of the plant's surveillance system [1–3]. A number of receptors for fungal elicitors have been reported to reside in the plasma membrane of plant cells. Ligand binding is believed to mediate generation of an intracellular signal and subsequent stimulation of plant defense reactions through activation of an intracellular signal transduction cascade. Kinetic properties of elicitor binding proteins, such as high affinity, saturability, and reversibility of ligand binding, together with a direct correlation between the binding affinities and the elicitor activities of the respective ligands indicate that such proteins function as physiological receptors.

Insight into the precise molecular mechanisms underlying non-self recognition and intracellular signal generation requires isolation of elicitor receptors and the encoding genes. In addition, these genes are considered to be most valuable tools for fungal disease control in transgenic crop plants. Isolation of a number of these genes is therefore attempted worldwide. Unfortunately, low abundance of elicitor receptors and the apparent lack of specialized cell types in which elicitor receptors are strongly expressed have severely hampered isolation of these proteins by means of ligand affinity chromatography and other chromatographic techniques.

Very recently, purification of a 75 kDa soybean plasma membrane protein was reported [4,5]. This protein interacts with elicitors of phytoalexin production such as a *Phytophthora sojae*-derived mixture of structural isomers of β-glucans or a synthetic hepta-β-glucoside. A cDNA encoding this protein was isolated and used for production of recombinant receptor in *Escherichia coli* [5]. This protein recognized large glucan fragments with high affinity, but only barely interacted with the hepta-β-glucan elicitor.

An oligopeptide fragment (Pep-13) from a cell wall protein of the same fungus, *P. sojae*, induces transcriptional activation of defense-related genes and phytoalexin production in cultured parsley cells and protoplasts [6]. Recognition of the elicitor by its plasma membrane receptor rapidly stimulates large, transient influxes of H^+ and Ca^{2+}, effluxes of K^+ and Cl^-, production of reactive oxygen species and activation of a MAP-kinase pathway [6–10]. Pharmacological studies and use of structural derivatives of Pep-13 with differing elicitor activities revealed that induction of macroscopic ion fluxes and, subsequently, superoxide anions are necessary for defense-related gene activation and phytoalexin production in elicitor-treated parsley cells. Thus, recognition of Pep-13 by its receptor initiates a signaling cascade through which activation of plant defense responses is mediated. Here we report the molecular characterization of the Pep-13 receptor and partial purification of the functionally intact receptor protein by ligand affinity chromatography.

2. Materials and methods

2.1. Materials

Solid-phase peptide synthesis was performed on Economy Peptide Synthesizer EPS 221 (ABIMED, Langenfeld, Germany) according to the manufacturer's instructions. Synthesized peptides were purified to homogeneity as described [6]. N-terminal acetylation of Pep-13 was achieved prior to cleavage from the solid phase by incubation with a 10-fold molar excess of 98% acetic acid for 1 h. Radioiodination of Pep-13 and its derivatives (specific radioactivity 2200 Ci/mmol) was performed by Anawa AG (Wangen, Switzerland). Bis(sulfosuccinimidyl) suberate (BS^3), *N*-hydroxysulfosuccinimidyl-4-azidobenzoate (S-HSAB), sulfosuccinimidyl-6-(biotinamido)hexanoate (NHS-LC-Biotin), UltraLink AB1, and the BCA reagent for determination of protein concentrations were from Pierce (Oud Beijerland, The Netherlands). Detergents were obtained from Calbiochem (Bad Soden, Germany). Sepharose 4B and avidin agarose were from Pharmacia (Freiburg, Germany) and Toyopearl AF-Tresyl-650M from Tosohaas (Stuttgart, Germany).

2.2. Preparation and solubilization of parsley microsomal membranes

Parsley (*Petroselinum crispum*) microsomal membranes were prepared as described [6]. Solubilization of microsomal membranes (5 mg/ml protein) was performed on ice by consecutively adding detergent stock solutions (150 mg/ml) up to final detergent concentrations given in the text. After 60 min insoluble material was removed by ultracentrifugation (140 000 × *g*, 1 h, 4°C) and the supernatant was used without intermittent storage.

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2.3. Ligand binding assay, chemical crosslinking and photoaffinity labeling

Ligand binding assays, BS^3-mediated chemical crosslinking of [^{125}I]Pep-13, and analysis of crosslinked proteins were performed as described [11] with the following modifications. Whatman GF/B glass fiber filters saturated with 0.3% polyethyleneimine were utilized for ligand binding assays with solubilized protein. Chemical crosslinking of [^{125}I]Pep-13 to solubilized proteins was achieved by directly adding the crosslinker to the reaction mixture.

For photoaffinity labeling 5 pmol of N-terminally acetylated [^{125}I]Pep-13 (Nac-[^{125}I]Pep-13) was incubated with 1 mM S-HSAB for 60 min at room temperature (RT). Excess S-HSAB was inactivated by addition of 1 M Tris/HCl, pH 8.0 (12 h, RT). 200 fmol of photoaffinity-labeled Nac-[^{125}I]Pep-13 was incubated with 400 μg microsomal membrane protein under ligand binding assay conditions. Subsequently, microsomes were pelleted (90 s at 10 000 × *g*), washed (1 ml PBS, pH 8.0), and made up to the original volume with PBS, pH 8.0. Samples were irradiated for 15 min with UV light (254 nm). Labeled proteins were analyzed by SDS-PAGE/autoradiography as described before. In contrast to our previous work [11] radiolabeled broad-range molecular size markers from Bio-Rad (Munich, Germany) were utilized for formula weight determination. This may explain the discrepancy in the size of the Pep-13 receptor determined in this and the previous study.

2.4. Ligand affinity chromatography

For immobilization 10 μmol Pep-13 was dissolved in 2 ml PBS (pH 8.0) and incubated with either 1 ml Sepharose 4B, UltraLink AB1 or Toyopearl AF-Tresyl-650M for 1 h at RT with continuous stirring. In the case of Sepharose 4B the matrix was washed with 100 ml 10 mM HCl before coupling. After immobilization any remaining reactive groups on the matrix were inactivated with 10 ml 3 M ethanolamine, pH 9.0. The amount of immobilized peptide was estimated by determination of the difference between integrated HPLC peaks corresponding to Pep-13 [6] before and after coupling.

Detergent-solubilized microsomal membranes (1 ml) were incubated with 50 μl Pep-13 matrix for 3 h at 4°C. The matrix was washed with 5 × 1 ml 100 mM Tris-HCl, pH 8.0, 500 mM NaCl, 1% octyl glucoside, and Pep-13 binding proteins eluted by incubating the matrix in 200 μl of the same buffer containing 10 mg/ml Pep-13/A2 for 3 h at 4°C. For ligand binding assays Pep-13/A2 was removed by addition of 20% PEG-4000 (1 h, 4°C), proteins were pelleted by ultracentrifugation (1 h, 140 000 × *g*, 4°C) and redissolved in ligand binding assay buffer.

Pep-13 (10 mg) was biotinylated with NHS-LC-Biotin according to the supplier's instructions, and subsequently purified to homogeneity by HPLC [6]. Upon radioiodination 2 nM of biotinylated [^{125}I]Pep-13 was crosslinked to microsomes with 10 mM BS^3 as described before. Triton X-100-solubilized material (2%, 1 h) was cleared by centrifugation (1 h, 10 000 × *g*, 4°C) and 1 ml of the supernatant incubated with 100 μl avidin agarose for 30 min at RT. The matrix was washed with 3 × 1 ml 100 mM Tris-HCl, pH 6.8, 100 mM NaCl, 2% Triton X-100 and bound proteins eluted with 10 × 50 μl 100 mM Tris-HCl, pH 6.8, 5% glycerol, 5% SDS, 100 mM biotin. Eluted proteins were analyzed by chemical crosslinking/SDS-PAGE/autoradiography as described before.

3. Results

Solubilization in a functionally active form is a prerequisite for the purification of membrane receptors. We have tested a series of structurally diverse detergents for their ability to release the Pep-13 receptor from parsley microsomal membranes. As shown in Table 1, non-ionic detergents such as *n*-octyl-β-D-glucopyranoside (octyl glucoside), Triton X-100, Lubrol PX, MEGA-10, and digitonin were most efficient, while zwitterionic (CHAPS, Zwittergent 3-12) and ionic detergents (cholic acid, deoxycholic acid) were much less efficient or failed to solubilize this protein. Treatment of microsomal membranes with increasing concentrations of NaCl (up to 1.5 M) removed 20% of the total membrane protein, but did not solubilize the Pep-13 binding site. Thus, the Pep-13 receptor appears to be an integral component of the membrane rather than merely attached to it. Using octyl glucoside (1.5%), Triton X-100 (1.5%) or digitonin (1%), 80% of the total binding activity could be solubilized by either detergent. A detergent-to-protein ratio of about 6:1 (w/w) was determined to be necessary for optimum recovery of the elicitor binding site by octyl glucoside (not shown). Maximum amounts of elicitor binding protein were solubilized with detergent concentrations corresponding to the critical micelle concentration (CMC) of the particular detergent or higher concentrations. Routinely, solubilization of membrane protein by either detergent resulted in a two-fold enrichment of specific Pep-13 binding activity. The solubilized receptor could be stored at −20°C for 5 days without any significant loss of binding activity. However, storage at 4°C reduced binding activity by 30% within 24 h, and after 72 h elicitor binding activity was no longer detectable. Receptor activity could only be maintained at CMC or higher detergent concentrations suggesting that

Table 1
Solubilization of the Pep-13 receptor from parsley microsomal membranes with various detergents

Detergent	Detergent concentration (%)	Specific binding solubilized (%)	Protein solubilized (%)
None	0	0	0
Lubrol PX	0.5, 1, 1.5, 2 [v/v]	40–60	45
Triton X-100	0.5, 1, 1.5, 2 [v/v]	50–80	59
Tween 20	1.5 [v/v]	6	29
Digitonin	0.5, 1, 1.5, 2 [w/v]	50–70	38
n-Hexyl-β-D-glucopyranoside	1.5 [w/v]	0	21
n-Heptyl-β-D-glucopyranoside	1.5 [w/v]	0	28
n-Octyl-β-D-glucopyranoside	0.5, 1, 1.5, 2 [w/v]	50–80	40
n-Octyl-β-D-thioglucopyranoside	1.5 [w/v]	80	n.d.
n-Octanoyl sucrose	1.5 [w/v]	1	n.d.
MEGA-8	1.5 [w/v]	5	19
MEGA-9	1.5 [w/v]	60	n.d.
MEGA-10	1.5 [w/v]	70	35
Cholic acid	1.5 [w/v]	16	45
Deoxycholic acid	1.5 [w/v]	15	42
CHAPS	1.5 [w/v]	5	37
Zwittergent 3-12	1.5 [w/v]	10	61
Zwittergent 3-08	1.5 [w/v]	0	10

Parsley microsomal membranes were solubilized as described in Section 2 with the detergent concentrations indicated. Specific binding of [^{125}I]Pep-13 to and protein content of the solubilized material is given in percent corresponding to a 100% binding and protein content determined in microsomal membranes in the presence of detergent. n.d., not determined.

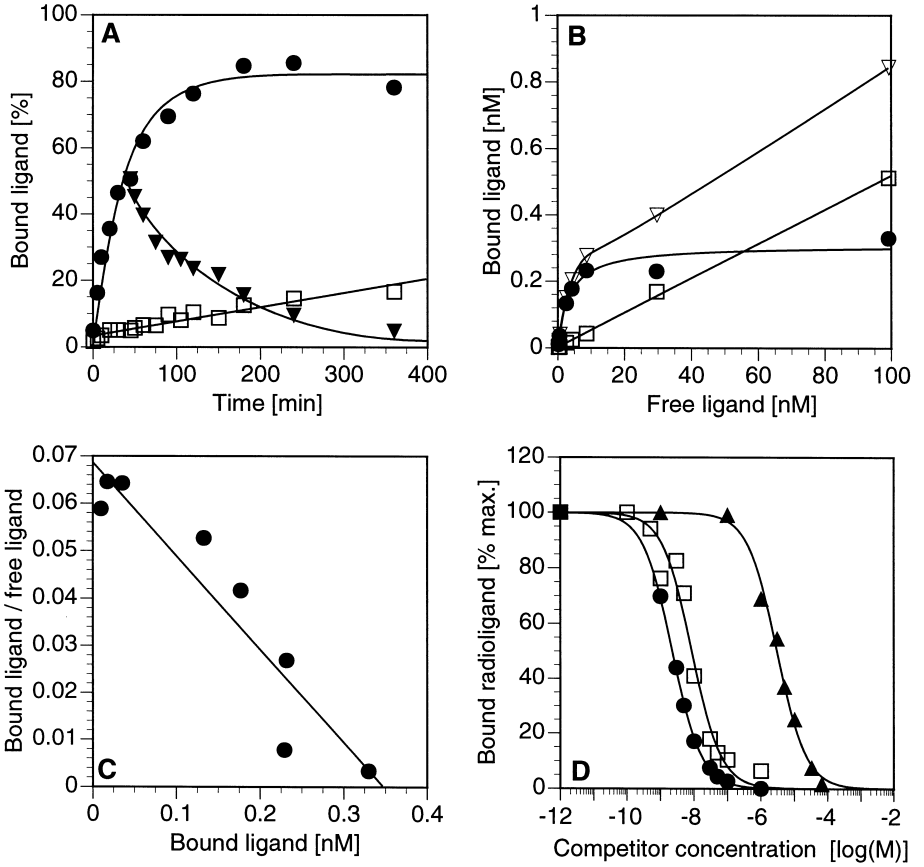

Fig. 1. Binding of [^{125}I]Pep-13 to detergent-solubilized parsley microsomal membrane proteins. Parsley microsomal membranes were solubilized with 1.5% octyl glucoside and solubilized material was used in binding assays as described in Section 2. A: Kinetics of specific binding (circles), non-specific binding (squares) and displacement (triangles) of [^{125}I]Pep-13. Specific binding represents the difference between total binding and non-specific binding determined in the presence of 15 μM Pep-13. Displacement of [^{125}I]Pep-13 was initiated by adding 15 μM Pep-13 to the assay mixture 40 min after addition of the radioligand. B: Saturability of [^{125}I]Pep-13 binding. Increasing concentrations of [^{125}I]Pep-13 were incubated with solubilized parsley microsomal membrane protein, and total binding (triangles), specific binding (circles) and non-specific binding (squares) was determined. C: Scatchard plot of specific binding from B. D: Competitive inhibition of binding of [^{125}I]Pep-13 to solubilized parsley microsomal membrane protein by Pep-13 structural derivatives. Binding assays were performed with increasing concentrations of Pep-13 (circles), Pep-13/A12 (squares) or Pep-13/A2 (triangles), and specific binding of [^{125}I]Pep-13 was determined. See text for details on Pep-13/A12 and Pep-13/A2.

incorporation into micelle structure is essential for biological activity of the protein.

Since solubilization of receptors can alter kinetic properties of the ligand/receptor interaction ligand binding assays with [^{125}I]Pep-13 and solubilized parsley microsomal membrane protein as receptor source were performed. Kinetic analysis of elicitor binding demonstrated that half-maximal association of the radioligand and octyl glucoside-solubilized receptor was achieved within 30 min, while equilibrium between association and dissociation was reached after 100 min (Fig. 1A). Addition of a 10 000-fold molar excess of unlabeled elicitor 40 min after addition of the radioligand resulted in a marked decline of bound radioactivity, demonstrating that interaction between Pep-13 and its binding site was fully reversible. In saturation analyses, octyl glucoside-solubilized membrane protein was incubated with increasing concentrations (0.1–100 nM) of the radioligand (Fig. 1B). Saturation of specific binding of Pep-13 was achieved at a ligand concentration of approximately 20 nM. Scatchard analysis of these data (Fig. 1C) suggested the presence of a single class of binding sites with an affinity constant (K_d) of 5.0 nM and an apparent receptor concentration of 100 fmol/mg solubilized membrane protein. A Hill plot of the data shown in Fig. 1B yielded a Hill coefficient of 0.98, excluding cooperativity in binding of Pep-13 to its receptor (not shown). Both ligand binding kinetics and affinity constant closely correspond to those obtained from experiments with microsomal membranes and intact protoplasts (K_d = 2.4 nM and 11.4 nM, respectively [6]). Thus, solubilization does not negatively affect ligand binding properties of the Pep-13 receptor. To examine whether the solubilized Pep-13 binding site exhibited the same ligand specificity as the membrane-localized binding site, structural derivatives of Pep-13 were tested in ligand competition assays. Binding of [^{125}I]Pep-13 could be efficiently inhibited by those analogs that were previously shown to efficiently compete for binding of the radioligand to parsley microsomal membranes (Fig. 1D) as well as to strongly activate macroscopic Ca^{2+} influx, to enhance open probability of a Ca^{2+}-permeable plasma membrane ion channel, and to stimulate oxidative burst and phytoalexin production in parsley cells [6,9,10]. A structural derivative of Pep-13 in which the tyrosine residue at position 12 was replaced by alanine (Pep-13/A12) retained its competitor activity in assays with detergent-solubilized membrane protein, and also efficiently stimulated all four responses

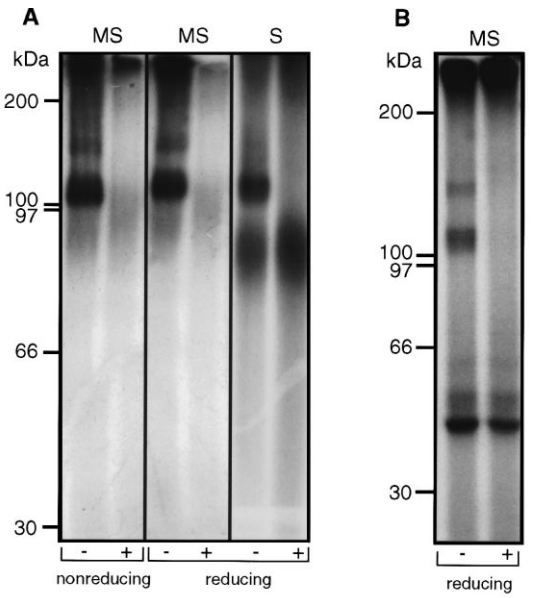

Fig. 2. Identification of the Pep-13 receptor by chemical crosslinking (A) and photoaffinity labeling (B). Parsley microsomal membranes (MS) or solubilized microsomal membranes (S) were incubated with [^{125}I]Pep-13 and subsequently subjected to BS^3-mediated chemical crosslinking. For photoaffinity labeling N-terminally acetylated [^{125}I]Pep-13 was labeled with S-HSAB, incubated with microsomal membranes and covalently attached to protein by irradiation with UV light. Binding of the radioligand was performed either in the absence (−) or in the presence (+) of 15 μM Pep-13. Radiolabeled proteins were separated by SDS-PAGE under reducing or non-reducing conditions and analyzed by autoradiography. Sizes of formula weight markers are indicated on the left.

(IC_{50}=8.6 nM with solubilized protein, IC_{50}=6.3 nM with microsomal membranes). In contrast, another single amino acid exchange within Pep-13 (tryptophan by alanine at position 2, Pep-13/A2) rendered this analog largely inactive as competitor of binding (IC_{50}=3 μM with solubilized protein, IC_{50} = 7 μM with microsomal membranes), corresponding to observed losses of stimulation of all other responses [6,10]. Taken together, ligand affinity as well as ligand specificity of the detergent-solubilized binding site remained unaltered as compared to the membrane-bound receptor.

Covalent attachment of [^{125}I]Pep-13 to its binding site in parsley microsomal membrane preparations and in octyl glucoside-solubilized membrane protein preparations was performed using either the homobifunctional chemical crosslinker, BS^3 [11], or the photoaffinity reagent, S-HSAB. For uniform photoaffinity labeling of [^{125}I]Pep-13 at the sole lysine residue N-terminally acetylated [^{125}I]Pep-13 (Nac-[^{125}I]Pep-13) was utilized. Analysis by SDS-PAGE and autoradiography of membrane proteins revealed labeling of a 100 kDa protein in either type of experiment (Fig. 2). Labeling of this protein was prevented by addition of a 10 000-fold molar excess of unlabeled Pep-13 as competitor. A second, significantly weaker band with an apparent molecular mass of 145 kDa was solely detectable in microsomal membrane preparations, but not in detergent-solubilized membrane protein preparations. No larger bands corresponding to oligomerized complexes of the receptor could be detected. In addition, electrophoretic separation of crosslinked proteins under denaturing, non-reducing conditions also revealed labeling of the 100 kDa band (Fig. 2). An oligomeric subunit structure of the receptor protein due to disulfide bridges is therefore unlikely. The 100 kDa

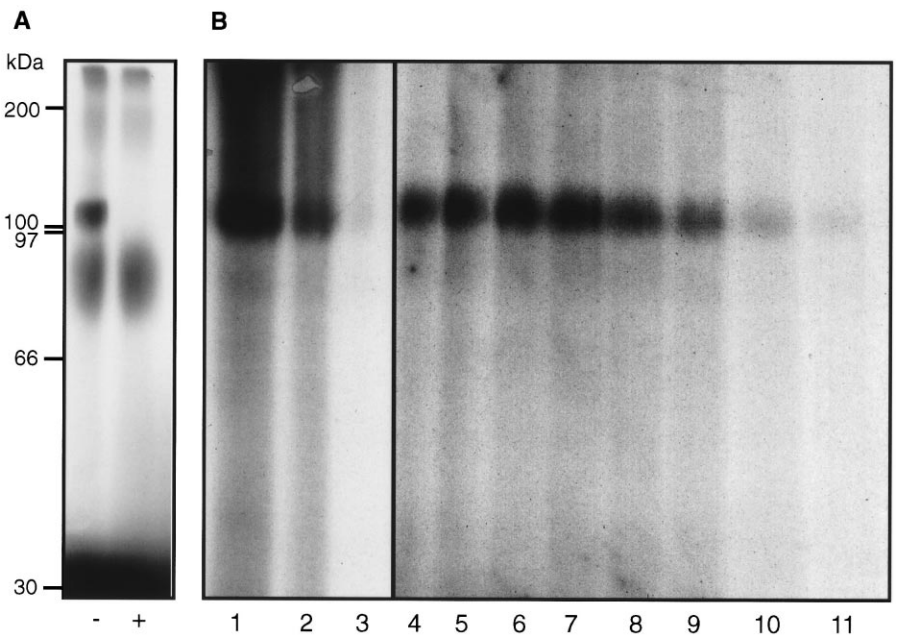

Fig. 3. Ligand affinity chromatography of detergent-solubilized parsley microsomal membrane protein. A: Octyl glucoside-solubilized parsley microsomal membrane proteins were subjected to ligand affinity chromatography on Sepharose 4B. Protein eluted from the matrix with Pep-13/A2 (for details see text) was used in chemical crosslinking assays with BS^3 and analyzed as described in legend to Fig. 2. Binding of the radioligand was performed either in the absence (−) or in the presence (+) of 15 μM Pep-13. Sizes of formula weight markers are indicated on the left. B: Biotinylated [^{125}I]Pep-13 was crosslinked to parsley microsomal membranes by BS^3. Microsomal membrane protein solubilized by 1% Triton X-100 was subjected to ligand affinity chromatography on avidin agarose as described in Section 2. Aliquots of solubilized microsomal membrane protein (lane 1), of detergent-insoluble membrane protein (lane 2), of protein which was not retained on avidin agarose (lane 3), and of protein eluted from the matrix by 100 mM biotin (lanes 4–11) were analyzed by SDS-PAGE/autoradiography. Note that aliquots analyzed in lanes 1–3 represent one fourth of the material analyzed in lanes 4–11.

polypeptide solubilized from parsley microsomal membranes may thus represent the ligand binding domain of the Pep-13 receptor.

Instability of the detergent-solubilized receptor severely confines purification protocols for this protein to only a limited number of steps. Although gel permeation and anion exchange chromatography proved successful in removing contaminating protein there was no enrichment in specific binding activity detectable in receptor-containing fractions (not shown). This was most likely due to concomitant inactivation of the receptor protein. We therefore attempted to adopt a recently reported ligand affinity chromatography-based one-step purification protocol for the soybean hepta-β-glucan receptor [4]. Three matrices (Sepharose 4B, UltraLink AB1, Toyopearl AF-Tresyl-650M) differing in hydrophobicity, spacer length, and ligand binding capacity were employed for immobilization of Pep-13. In all cases Pep-13 was coupled through its N-terminal primary amino group to the matrix. The amount of immobilized Pep-13 was approximately 6 mM regardless of the matrix used. The derivatized matrices were tested for their ability to retain the Pep-13 binding site from octyl glucoside-solubilized parsley microsomal membrane protein. Incubation of solubilized membrane protein with either matrix resulted in retention of 85–95% of the binding activity applied. Matrices without immobilized Pep-13 neither retained the binding site nor impaired binding activity of the solubilized receptor. Approximately 20% of the Pep-13 binding activity retained on the matrix could reproducibly be eluted with 6 μM Pep-13/A2, thereby yielding 5000-fold enrichment of specific receptor activity. Pep-13/A2, a structural derivative of Pep-13, is significantly less active than Pep-13 as competitor of binding of [^{125}I]Pep-13 to its receptor [6]. Unlike Pep-13 this peptide could be completely removed from eluted proteins by PEG-4000-mediated precipitation, which allowed subsequent quantification of receptor activity. Ligand binding assays performed with ligand affinity chromatography-purified receptor revealed no significant changes in the kinetics of ligand association/dissociation and IC_{50} value for Pep-13 in comparison to experiments performed with detergent-solubilized receptor. BS^3-mediated chemical crosslinking of [^{125}I]Pep-13 to proteins from fractions containing receptor activity resulted in labeling of the 100 kDa protein as viewed by autoradiography (Fig. 3A). Alternatively, biotinylated [^{125}I]Pep-13 was crosslinked to parsley microsomal membranes and membrane proteins solubilized with 1% Triton X-100 were subjected to ligand affinity chromatography on avidin agarose (Fig. 3B). After thoroughly washing the matrix bound proteins were eluted in the presence of 100 mM biotin. Again, upon autoradiographic analysis labeling of a 100kDa-band corresponding to the receptor/ligand complex could be visualized.

4. Discussion

The ligand binding domain of the previously identified parsley plasma membrane elicitor receptor [6,7,11] very likely constitutes a monomeric 100 kDa integral plasma membrane protein. Solubilization of the receptor by several structurally very diverse non-ionic, amphipathic detergents, but not by high salt concentrations is indicative of integration of the protein into the membrane. Moreover, all detergents which solubilized the Pep-13 receptor form large micelles with high aggregation numbers [12,13], suggesting that receptor activity depends on integration into complex structures. This is further corroborated by the finding that activity of the solubilized receptor declined significantly at detergent concentrations below CMC. Receptors for fungal elicitors from soybean, rice and tomato have also been shown to be integral plasma membrane proteins [5,14–17].

Chemical crosslinking studies using [^{125}I]Pep-13 and parsley microsomal membranes, solubilized membrane protein, or protein eluted from immobilized Pep-13 consistently yielded labeling of a 100 kDa band. Crosslinking of biotinylated [^{125}I]Pep-13 to parsley membranes and subsequent ligand affinity chromatography of solubilized membrane protein on avidin agarose substantiated the latter experiment. Labeling of this protein band was detectable upon gel electrophoretic separation of solubilized membrane protein under either reducing or non-reducing conditions, suggesting a monomeric nature of the 100 kDa protein. Specific labeling of bands corresponding to oligomers of the 100 kDa protein could never be detected in crosslinking assays. In contrast to chemical crosslinking experiments, which may result in covalent attachment of several subunits of a receptor complex, photoaffinity labeling studies with radioligands containing one photolabile azide moiety preferentially leads to labeling of individual protein species. Since such experiments also revealed a 100 kDa protein that interacted specifically with Nac-[^{125}I]Pep-13, this polypeptide is assumed to be the monomeric ligand binding site of the Pep-13 receptor. It is yet not possible to say whether the 140 kDa band, which was detectable only in crosslinking assays with intact membranes, represents a functional subunit of a then heterooligomeric receptor complex or is merely a neighboring but functionally unrelated protein.

Ligand affinity and ligand specificity of the Pep-13 binding site remained unaffected upon detergent solubilization. Thus, the protein is amenable to purification by ligand affinity chromatography. We have developed a protocol for retention of the protein on immobilized Pep-13 and subsequent ligand-specific desorption. This protocol resulted in a 5000-fold enrichment of specific receptor activity, which is typical for this technique [18]. A 9000-fold enrichment was recently reported for the purification of the soybean hepta-β-glucan elicitor receptor by means of ligand affinity chromatography [4]. In order to purify this very low abundant receptor protein future experiments will focus on upscaling of this protocol. Information on the molecular weight and subunit structure of the ligand binding domain of this receptor will thereby facilitate direct isolation of the protein by electroelution upon gel electrophoretic separation of affinity-purified protein.

In addition, molecular characterization of the Pep-13 receptor has enabled an alternative experimental strategy for obtaining sequence information on this protein. Ligand affinity screening of mammalian COS cells transfected with size-enriched cDNA has proven successful for isolation of many receptor cDNAs [19,20], and may therefore be suitable for isolation of a cDNA encoding the 100 kDa ligand elicitor binding site from parsley.

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