BARLEY FAMILY FIVE
PATHOGENESIS-RELATED
PROTEINS

by

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ABSTRACT

Pathogenesis-related (PR) proteins are expressed at particular stages of normal plant growth and development, but are expressed at high levels in response to pathogen attack in tissues where they are otherwise absent. PR proteins of family five (PR-5 proteins) are a sub-class of the PR group of proteins. PR-5 proteins have demonstrated antifungal activity in vitro, and have been associated with delayed development of fungal disease symptoms in transgenic plants carrying genes encoding PR-5 proteins.

In previous studies, PR-5 proteins have been associated with osmotic stress, the hydrolysis of fungal cell-wall polysaccharides, antifreeze activity and bifunctional α-amylase/trypsin inhibition, but the major mechanism of PR-5 protein activity has been thought to be permeabilisation of fungal plasma membranes. However, the crystal structures of PR-5 proteins do not have features normally associated with direct pore formation. Furthermore, the PR-5 protein activity is specific towards particular fungal species, which suggests that they might recognise specific structures at the fungal cell surface before membrane damage occurs.

An unexpected function for PR-5 proteins was suggested by the observation that PR-5 proteins is crude extracts of germinated barley bound to an alkaline-treated pachyman sample. Linkage analysis of the treated pachyman revealed that several different polysaccharides were present in the sample. The constituents of the pachyman sample were tested individually for their ability to bind barley PR-5 proteins, and the binding agent was shown to be insoluble (1→3)-β-glucan.

To further investigate the interaction between barley PR-5 proteins and (1→3)-β-glucans, PR-5 proteins were isolated from barley. The PR-5 proteins were
purified using a combination of ammonium sulphate fractional precipitation, anion exchange and cation exchange chromatography, and gel filtration chromatography. Using these methods, two isoforms were purified from barley, and designated HvPR5\textsubscript{b} and HvPR5\textsubscript{c}. HvPR5\textsubscript{b} and HvPR5\textsubscript{c} have molecular weights of 21,846 Da and 21,365 Da, respectively, as determined by electrospray mass spectrometry. They have amino acid sequences that are approximately 60% identical, and are cleanly the products of separate genes.

Purified HvPR5\textsubscript{b} and HvPR5\textsubscript{c} were used in binding studies. HvPR5\textsubscript{c} was found to interact with insoluble (1→3)-β-glucan, but not cellulose, pustulan, mannann, xylan or chitin. Binding was greatest to linear (1→3)-β-glucan, but lower if (1→6)-β-linked branches or glucosyl substituents were present. Binding was maximal at approximately pH 5.0, and was unaffected by high NaCl concentrations, Mg\textsuperscript{2+}, Ca\textsuperscript{2+} or Zn\textsuperscript{2+}, or by the addition of EDTA. The binding data was fitted to the hyperbolic Langmuir isotherm function, and kinetic parameters associated with the interaction were determined. HvPR5\textsubscript{b} did not show significant binding to any of the polysaccharides tested, suggesting that only specific PR-5 proteins may interact with (1→3)-β-glucans.

Several cDNAs and a gene encoding proteins closely related to HvPR5\textsubscript{c} were isolated. The cDNAs all encoded proteins of approximately 21 kDa with a potential N-glycosylation site near the COOH-terminus. One cDNA, designated HvPR5\textsubscript{c}2, carried a short COOH-terminal extension that might be involved in the vacuolar targeting of that particular isoform. The isolated gene carried an AGCCGCCC motif that is conserved in several PR protein genes, and has been linked with ethylene responsiveness, and some elements associated with gibberellin response. Southern blot analysis revealed the presence of between 4 and 6 genes encoding HvPR5\textsubscript{c}-like
proteins in the barley genome. Using wheat-barley addition lines and RFLP mapping, some genes were linked to the short arm of barley chromosome 5H.

Molecular models of HvPR5b and HvPR5c were constructed. The models were compared with the known structures of several PR-5 proteins, and examined for features that may be associated with (1→3)-β-glucan binding. A (1→3)-β-glucan binding domain was located on the surface of HvPR5c, but no obvious feature that would prevent HvPR5b binding was located. HvPR5b was particularly proline-rich in the proposed binding domain, a property that might reduce the flexibility of this region and somehow impede binding. All known PR-5 proteins that bind (1→3)-β-glucans have a conserved N-glycosylation site, which is absent in all PR-5 proteins that did not bind (1→3)-β-glucans.

The trace amounts of (1→3)-β-glucan hydrolase activity present in preparations of barley PR-5 proteins, but none was observed using isolated fungal cell walls. There was no increase in hydrolysis observed when combinations of barley PR-5 proteins and (1→3)-β-glucanases were incubated with insoluble (1→3)-β-glucans. Furthermore, no hydrolysis of Rhynehosporium secalis cell walls was observed using combinations of barley PR-5 proteins, (1→3)-β-glucanases or chitinases. Finally, purified HvPR5b and HvPR5c were assessed for antifungal activity against several fungal isolates. Both HvPR5b and HvPR5c significantly inhibited the growth of Fusarium graminearum. The effect of the barley PR-5 proteins on F. graminearum plasma membranes was assessed using a novel confocal microscopy technique. Barley PR-5 proteins were found to induce the hyperpolarisation of the Fusarium membrane, which was quantified at approximately -30 mV.
# TABLE OF CONTENTS

## CHAPTER 1  
**General Introduction**

<table>
<thead>
<tr>
<th>Section</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1 General Plant Defence Mechanisms</td>
<td>1</td>
</tr>
<tr>
<td>1.1.1 Defence strategies of the plant</td>
<td>1</td>
</tr>
<tr>
<td>1.1.2 Recognition of invading microorganisms</td>
<td>2</td>
</tr>
<tr>
<td>1.2 Pathogenesis-Related Proteins</td>
<td>3</td>
</tr>
<tr>
<td>1.2.1 PR-1 Proteins</td>
<td>5</td>
</tr>
<tr>
<td>1.2.2 PR-2 Proteins</td>
<td>6</td>
</tr>
<tr>
<td>1.2.3 PR-3 Proteins</td>
<td>8</td>
</tr>
<tr>
<td>1.2.4 Plant transformation with chitinases and (1→3)-β-glucanases</td>
<td>10</td>
</tr>
<tr>
<td>1.2.5 PR-4 Proteins</td>
<td>12</td>
</tr>
<tr>
<td>1.2.6 PR-5 Proteins</td>
<td>12</td>
</tr>
<tr>
<td>1.3 Thaumatin</td>
<td>13</td>
</tr>
<tr>
<td>1.4 PR-5 Proteins in Higher Plants</td>
<td>14</td>
</tr>
<tr>
<td>1.4.1 Acidic PR-5 proteins</td>
<td>14</td>
</tr>
<tr>
<td>1.4.2 Basic PR-5 proteins</td>
<td>15</td>
</tr>
<tr>
<td>1.5 Regulation of PR-5 Proteins</td>
<td>16</td>
</tr>
<tr>
<td>1.5.1 Hormonal regulation of PR-5 gene expression</td>
<td>17</td>
</tr>
<tr>
<td>1.5.2 Tissue-specificity of PR-5 gene expression</td>
<td>17</td>
</tr>
<tr>
<td>1.5.3 Induction of PR-5 proteins in response to pathogen attack</td>
<td>18</td>
</tr>
<tr>
<td>1.6 Antifungal Activity of PR-5 Proteins</td>
<td>19</td>
</tr>
<tr>
<td>1.6.1 The molecular mechanism of PR-5 protein antifungal activity</td>
<td>19</td>
</tr>
<tr>
<td>1.6.2 Plant lectins</td>
<td>20</td>
</tr>
<tr>
<td>1.6.3 Evidence that PR-5 proteins have lectin-like activity</td>
<td>22</td>
</tr>
<tr>
<td>1.7 Aims of the Present Study</td>
<td>23</td>
</tr>
</tbody>
</table>

## CHAPTER 2  
**Purification and Characterisation of Barley PR-5 proteins**

<table>
<thead>
<tr>
<th>Section</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1 Introduction</td>
<td>24</td>
</tr>
<tr>
<td>2.2 Materials and Methods</td>
<td>25</td>
</tr>
</tbody>
</table>
2.2.1 Materials
2.2.2 Germination of barley (Hordeum vulgare var. Clipper)
2.2.3 Extraction of protein from germinated grain
2.2.4 DEAE-cellulose chromatography
2.2.5 CM-Sepharose chromatography
2.2.6 BioGel P-30 gel-filtration chromatography
2.2.7 Final CM-Sepharose chromatography
2.2.8 Protein determination
2.2.9 Polyacrylamide gel electrophoresis
2.2.10 Detection of barley PR-5 proteins by Western analysis
2.2.11 NH2-terminal sequence analysis and electrospray mass spectrometry

2.3 Results and Discussion
2.3.1 Extraction of proteins from barley germinated for 5 days, ammonium sulphate fractional precipitation and DEAE-cellulose chromatography
2.3.2 CM-Sepharose ion-exchange chromatography
2.3.3 BioGel P-30 gel-filtration chromatography
2.3.4 Second CM-Sepharose chromatography
2.3.5 Characterisation of HvPR5b and HvPR5c

2.4 Conclusions

CHAPTER 3
Barley PR-5 Protein-Polysaccharide Interactions
3.1 Introduction
3.2 Materials and Methods
3.2.1 Materials
3.2.2 Generation of antibodies against HvPR5b
3.2.3 Purification of polyclonal antibodies using protein A affinity
3.2.4 Biotinylation of antibodies and barley PR-5 proteins
3.2.5 ELISA using biotinylated antibodies
3.2.6 Preparation of pachyman
3.2.7 Preparation of other polysaccharides
3.2.8 PR-5 protein adsorption on insoluble polysaccharides
3.2.9 Preparation of BLACore sensor chip surfaces

3.2.10 Binding of soluble polysaccharides to immobilised PR-5 proteins

3.3 Results and Discussion

3.3.1 Generation of polyclonal antibodies

3.3.2 Purification and bioanalysis of polyclonal antibodies

3.3.3 Determination of conditions for ELISA assays

3.3.4 Preparation of polysaccharides for binding studies

3.3.5 Identification of (1→3)-β-glucan as the polysaccharide bound by PR-5 proteins

3.3.6 Preparation of branched and debranched (1→3)-β-glucan fractions

3.3.7 Binding studies on insoluble (1→3)-β-glucans

3.3.8 Kinetic parameters of PR-5 protein binding to pachymann

3.3.9 Barley PR-5 protein binding to soluble (1→3)-β-glucans

3.4 Conclusions

CHAPTER 4

Isolation and Characterisation of cDNAs and a Gene Encoding Barley PR-5 Proteins

4.1 Introduction

4.2 Materials and Methods:

4.2.1 Materials

4.2.1.1 Commercially obtained oligonucleotides

4.2.2 Isolation of a PR-5 probe by PCR

4.2.3 Preparation of [32P]-radiolabeled PCR probes

4.2.4 Screening of the barley cDNA libraries

4.2.5 Screening the barley genomic library

4.2.6 Rescue of clones from λZAPII into pBluescript

4.2.7 Isolation of bacteriophage DNA

4.2.8 Subcloning DNA fragments into pBluescript

4.2.9 Transformation of pBluescript into E. coli

4.2.10 Plasmid DNA mini-preparations

4.2.11 Southern hybridisation analysis of barley genomic DNA

4.2.12 Mapping and chromosomal location of barley PR-5 genes
CHAPTER 5
Molecular Modelling of PR-5 Proteins

5.1 Introduction

5.2 Materials and Methods

5.2.1 Generation of HvPR5b fragments for amino acid sequencing

5.2.2 Modelling PR-5 proteins

5.2.3 Docking a (1→3)-β-glucan into the HvPR5c model

5.2.4 Hydrophobic cluster analysis

5.3 Results and Discussion

5.3.1 Deduction of the primary sequence of HvPR5b

5.3.2 Comparative modelling of PR-5 proteins

5.3.3 Three-dimensional structure of PR-5 proteins

5.3.3.1 Surface charges of PR-5 proteins

5.3.3.2 Post-translational modification of PR-5 proteins

5.3.3.3 Hydrophobic cluster analysis

5.3.4 Determination of a polysaccharide binding site

5.3.5 Docking a linear (1→3)-β-glucan in the cleft of HvPR5c

5.3.6 Comparison of the (1→3)-β-glucan binding domain of HvPR5c with other PR-5 proteins

5.4 Conclusions
CHAPTER 6

Functional Analysis of PR-5 Proteins

5.1 Introduction 99
5.2 Materials and Methods 100
6.2.1 Materials 100
6.2.2 Assay for inhibition of fungal growth 101
6.2.3 Assay for inhibition of yeast growth 101
6.2.4 Confocal microscopy 102
6.2.5 Hydrolysis of fungal cell-wall polysaccharides 102
5.3 Results and Discussion 104
6.3.1 Barley PR-5 protein inhibition of yeasts 104
6.3.2 Barley PR-5 protein inhibition of filamentous fungi 105
6.3.3 Hyperpolarisation across the Fusarium graminearum plasma membrane 106
6.3.4 Hydrolysis of fungal cell-wall polysaccharides by barley PR-5 protein 109
5.4 Conclusions 111

CHAPTER 7

Summary and Future Directions

7.1 Summary of experimental results 112
7.2 Amino acids involved in (1→3)-β-glucan binding 115
7.3 The importance of (1→3)-β-glucan binding to antifungal activity 116
7.4 Crystallisation of barley PR-5 proteins 117
7.5 Production of transgenic plants carrying constructs encoding PR-5 proteins 118

REFERENCES 120