A hybridoma cell line that produces a monoclonal antibody specific for indole-3-acetic acid (IAA) was prepared. The DNA fragments coding the variable regions of the light and the heavy chains of the antibody were prepared by PCR using the cDNA of the antibody as a template. A chimera DNA for a single chain variable fragment (scFv) was constructed, and expressed in Escherichia coli. The scFv antibody expressed in E. coli as well as the original monoclonal antibody showed a specific binding to IAA.

Key words: single chain variable fragment (scFv); indole-3-acetic acid (IAA); enzyme-linked immunosorbent assay (ELISA)

Immunological methods have been used as powerful and useful techniques in many aspects of plant science. The successful production of antibodies in plant cells offers a variety of possibilities not only for large-scale production of antibodies as diagnostic reagents, but also for the protection of plants from the attack of pathogens and for immunomodulation of plant growth.

A single-chain variable fragment (scFv) antibody consists of the variable domains of the heavy and the light chains of an immunoglobulin and a flexible linker peptide. A variety of scFv antibodies have been expressed and showed activities in plants successfully. Cytoplasmic expression of a scFv against artichoke mottled crinkle virus has been shown to delay infection in transgenic tobacco. Transgenic tobacco producing scFv against beet necrotic yellow vein virus (BNYVV) was partially protected from the pathogenic effects exerted by the virus. The expression of scFv against abscisic acid (ABA) expressed in the endoplasmic reticulum (ER) of tobacco induced a wilty phenotype of the transgenic plants, and seed specific production of the scFv modulated the levels and the effect of ABA leads in a tissue- or time-specific manner. Shimada et al. reported on the expression of a scFv against gibberellin A19/24 (GA19/24) in tobacco. GA19 is a biosynthetic precursor of GA1, and GA24 of GA4. The anti-GA19/24-scFv reduced plant height by interfering with the conversion of GA19/24 to GA1/4. These reports suggest that the expression of anti-phytohormone antibodies in plants can be used for immunomodulation of plant growth. If an active scFv specific to a target phytohormone is available, it is convenient to express it in plants to modulate plant growth by disturbing endogenous plant hormone levels, because it is not necessary to expend as much effort for assembling as is required when the heavy and the light chains of an antibody are expressed separately.

The plant hormone auxin, mainly indole-3-acetic acid (IAA), in higher plants plays a critical role in regulating various plant growth processes. There have been several reports about antibodies against IAA. To raise an antibody against IAA, it must be coupled to a carrier protein. The nitrogen in the indole ring (IAA-N), the carboxyl group (IAA-C), and the hetero atoms introduced on a benzene ring of IAA (IAA-H) are often used for the conjugation. The antibodies raised against IAA-C showed higher and wider cross-reactivity to IAA conjugates than the antibodies against IAA-N or IAA-H, and required methylation of samples for the analysis of free IAA in plants. In this study, we constructed a scFv gene from the hybridoma producing monoclonal antibodies against IAA-N. The scFv was expressed in E. coli and its binding activity to IAA was investigated.

A hybridoma cell line which produced a monoclonal antibody against IAA-N-bovine serum albumin (BSA), in which the indole nitrogen was linked with BSA by the Mannich reaction, was prepared. This antibody was isotyped and found to be an IgG1 chain with a κ light chain. The typical logit-log standard curve with this monoclonal antibody for enzyme-linked immunosorbent
ELISA was performed by the method of Atzorn and Weiler.\(^{16}\) IAA-N-alkaline phosphatase was used as an enzyme tracer.

Calculation of the exchange from B/B\(_0\) to logit is shown below.

\[
\text{Logit} = \ln \left( \frac{B/B_0}{(100 - B/B_0)} \right)
\]

Table 1. Comparison of Cross-reactivities of Antibodies against IAA-N

<table>
<thead>
<tr>
<th>Compound</th>
<th>Cross-reactivities</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>IAA</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>1-NAA</td>
<td>164</td>
<td>29</td>
<td>25</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>2-NAA</td>
<td>17</td>
<td>8</td>
<td>5</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>2,4-D</td>
<td>0.7</td>
<td>&lt;1</td>
<td>0.4</td>
<td>0.09</td>
<td></td>
</tr>
<tr>
<td>phenylacetic acid</td>
<td>0.01</td>
<td>10</td>
<td>0.3</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>indole-3-pyruvic acid</td>
<td>5</td>
<td>8</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>indole-3-propionic acid</td>
<td>0.7</td>
<td>4</td>
<td>0.3</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>indole-3-acetone</td>
<td>0.2</td>
<td>&lt;1</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>indole-3-acetonitrile</td>
<td>0.1</td>
<td>5</td>
<td>&lt;0.04</td>
<td>&lt;0.1</td>
<td></td>
</tr>
<tr>
<td>indole-3-acetylglucine</td>
<td>0.03</td>
<td>1</td>
<td>—</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>indole-3-acetamide</td>
<td>0.03</td>
<td>5</td>
<td>—</td>
<td>&lt;0.1</td>
<td></td>
</tr>
<tr>
<td>tryptophan</td>
<td>&lt;0.01</td>
<td>0</td>
<td>&lt;0.05</td>
<td>&lt;0.1</td>
<td></td>
</tr>
<tr>
<td>tryptamine</td>
<td>0.04</td>
<td>0</td>
<td>&lt;0.04</td>
<td>&lt;0.1</td>
<td></td>
</tr>
</tbody>
</table>

The values are expressed as % of the reactivity to IAA on a molar basis. A, monoclonal antibody in this study; B, monoclonal antibody,\(^9\) C, polyclonal antibody,\(^10\) D, polyclonal antibody.\(^11\)

The antibody in this study has not been measured for the use of IAA levels in plants, this specificity and sensitivity offer advantages for measuring free IAA in the presence of natural IAA conjugates.

From this hybridoma cell line, cDNAs were prepared and used as a template to amplify each of variable regions of light and heavy chains (\(V_L\) and \(V_H\) respectively) by polymerase chain reaction (PCR) under the following cycle conditions: 94°C for 5 min followed by 35 cycles of 94°C for 30 sec, 59°C for 30 sec, 72°C for 1 min, and 72°C for 5 min. The primers used were as follows: L-BACK:5’-GAGATTTGACATCTGTGACCCAGTCT-3’, L-FOR:5’-GTTAGATCTCTGGACGGTCTC-3’, H-BACK:5’-AGTTSMARTGCGAGSAGTCTGG-3’, and H-FOR:5’-TGAGGAGACGGTACCCAGTCTGG-3’. Primers L-BACK and H-BACK were designed to be complementary to the first-strand cDNA encoding the converted N-terminal region and primers L-FOR and H-FOR, to be complementary to the mRNA in the J regions.\(^{18}\) To add linker oligonucleotides to cloned \(V_L\) and \(V_H\) fragments, each of the fragments was re-amplified by PCR under the following cycle conditions: 94°C for 5 min followed by 35 cycles of 94°C for 1 min, 67°C for 1 min, 72°C for 1.5 min, and 72°C for 7 min with new primer sets: scL-BACK:5’-GAGATTTGACATCTGTGACCCAGTCT-3’, scL-FOR:5’-GAGGCGCGGGCGGCGGCCGCAGAACCCACACGAGACCCACCGCGAGTCGTCTGAGGACACGGTCTCGTTCACCTGTTTGCACGGGTCCCATCGCTGGGATC-3’, scH-BACK:5’-GGCCGCGCGCGGCGGCGGCGGCCGGCGGCCACCGCGACGACGAGTCGG-3’, scH-FOR:5’-TAGGGGCGGCGN(I fidelity)TGGAGGAGACGGTGACCGGTCGG-3’, primers scL-FOR and scH-BACK contain the coding sequence of the synthetic linker peptide. Each of the PCR products was cloned and used as a template to join the \(V_L\) and \(V_H\) genes by PCR with primers scL-BACK and scH-BACK under the following cycle conditions: 94°C for 5 min followed by 40 cycles of 94°C for 1 min, 68°C for 1 min, 72°C for 1.5 min, and 72°C for 7 min.

The constructed scFv gene was cloned into pGEX-4T-1 (Amersham) for expression of the scFv as a fusion protein with gultathione-S-transferase (GST). \(E.\ coli\) cells carrying the scFv gene were cultured, induced by isopropyl \(\beta\)-d-thiogalactopyranoside (IPTG), and harvested. After the cells were lysed by sonication, soluble and insoluble fractions were obtained. Since the expression of the GST-scFv fusion protein, whose molecular mass was estimated to be about 50,000 Da by deduced amino acid sequences of the \(V_L\) and \(V_H\) genes, was detected in the insoluble fraction, this fraction was solubilised by adding urea (Fig. 2, lane 5) and was refolded by excluding urea stepwise (Fig. 2, lane 7). Affinity purification was performed with a gultathione Sepharose 4B column (Amersham) and thrombin was added to the eluate from the column for the cleavage of GST and the scFv protein. Affinity purification with a gultathione Sepharose 4B column was performed again.
Purification of Anti-IAA ScFv Expressed in E. coli. The expression and affinity purification of the scFv followed the manufacturer’s protocol (Amersham). The cells cultivated were lysed by sonication and centrifuged. Lane 1, supernatant from uninduced culture; Lane 2, urea-solubilized fraction from uninduced culture; Lane 3, urea-unsolubilized fraction from uninduced culture; Lane 4, supernatant from induced culture; Lane 5, urea-solubilized fraction from induced culture; Lane 6, urea-unsolubilized fraction from induced culture; Lane 7, refolded protein fraction; Lane 8, eluate from glutathione sepharose 4B; Lane 9, purified scFv.

for the removal of GST and scFv protein was obtained (Fig. 2, lane 9).

The binding activity of the GST-scFv and the scFv to IAA was studied by ELISA (Fig. 1). In ELISA, the wells were coated with the original monoclonal antibody, the GST-scFv, and the scFv. Both the GST-scFv and the scFv as well as the original monoclonal antibody showed specific binding IAA. The concentrations required for 50% inhibition of binding (IC50) for the original monoclonal antibody, GST-scFv, and the scFv were 4.0, 0.5, 1.5 × 10−8 mol/l respectively. Though it is difficult to compare their affinity to IAA, the affinity of scFv might be slightly higher than that of the original monoclonal antibody, because no apparent difference was observed between the enzyme tracer activities in the wells without IAA. The scFv with this high sensitivity, which is less expensive to produce than monoclonal antibodies, is useful for the quantification of auxins.

Judging by these results, the scFv expressed in plant cells might bind to IAA in vivo and is expected to have effects similar to those of anti-auxins. Application of 2,3,5-triodobenzoic acid (TIBA) at the beginning of berry softening promotes the ripening of grapes. The fruit-drop ratio increased when TIBA was applied to the peduncle of citrus. If the scFv against IAA is successfully expressed during fruit ripening under control of the promoter which promotes the expression of ripening-related genes, a xyloglucan endo-transglycosylase gene of grape for example, it might trigger these phenomena by the reduction of IAA polar transport and IAA levels in the target tissues.

In addition, since this scFv is expected to show high affinity to IAA immobilized via indolic nitrogen, it might be also useful for immunohistochemical analysis of IAA after fixation of IAA in tissues with formaldehyde and gultaldehyde.

References

14) Marcussen, J., Ulvskov, P., Olse, C. E., and Rajagopal, R., Preparation and properties of antibodies against...


