GENE NOTE

RS2: a sugar beet gene related to the latex allergen Hev b 5 family

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Abstract

A novel gene (RS2) has been isolated from a Beta vulgaris (cv. Regina) cDNA library. The expression of this gene was enhanced in the mature storage organ as compared to leaf tissue. The protein encoded by this gene was found to be alanine- and glutamic acid-rich and it resembles members of the latex allergen Hev b 5 family.

Key words: Beta vulgaris, storage organ, latex allergen, parenchymatous cells.

Sugar beet (Beta vulgaris) stores sucrose in a large storage organ that develops from root and hypocotyl tissue. This storage organ displays an anomalous form of secondary thickening from a series of supernumerary cambia arranged in concentric rings. These cambia are responsible for the production of alternating zones of vascular tissue and parenchyma cells in which the sugar is stored. In order to obtain information about gene expression in the various cell types of the storage root and to isolate storage organ enhanced genes a cDNA library (cDNA synthesis kit and Agt10 cloning kit, Amersham) was created. For this purpose RNA was isolated from a mature sugar beet (cv. Regina) storage organ (Slater, 1988). RS2 was obtained by differential screening of the library with photobiotin-labelled root and leaf RNA (McInnes et al., 1987). Root-enhanced sequences were selected on the basis that their plaques gave a positive signal with a root RNA probe, but not with the leaf probe. The sequence obtained for RS2 was shown to be 816 nucleotides long. It contained an open reading frame of 474 nucleotides (158aa) coding for a protein with a predicted molecular mass of 16.098 kDa. The protein was rich in alanine (42aa, 26.6%) and also, being rich in glutamic acid (40aa, 25.3%), it had a predicted pI of 3.72. The glutamic acid was arranged in a significant number of the repeated motif XEEX (where X was often alanine) throughout the protein, but particularly in the carboxy terminal region. RS2 showed homology with other proteins of a similar size, pI and containing this arrangement of glutamic acid (Table 1). The proteins included the major latex allergen Hev b 5 (Slater et al., 1996; Akasawa et al., 1996), a putative allergen protein from Prunus armeniaca (unpublished results), the kiwi fruit protein pKIWI501 (Ledger and Gardner, 1994), and TUB8 from Solanum tuberosum (Taylor et al., 1992). RS2 also showed some homology with a grape-ripening related protein, GRIP68 (unpublished results), and a buckwheat seed protein (unpublished results). Hev b 5 is one of over a dozen major allergenic proteins found in latex. The others include an endochitinase and several proteins that are involved in rubber biosynthesis. These allergens are responsible for natural rubber latex (NRL) allergy (Niggemann and Breiteneder, 2000) and persons with NRL are often sensitized against various fruits that are believed to have proteins with similar epitopes. This cross-reaction is also seen in the reverse direction and the phenomenon has become known as the latex-fruit syndrome. The apparent structural relationship of the predicted protein pKIWI501, with Hev b 5, led to the suggestion that it might be the corresponding fruit protein involved in the latex-Kiwi fruit cross-reaction (Slater et al., 1996; Akasawa et al., 1996). The functions of RS2 and the other proteins listed in Table 1 remain unknown, but, they are all believed to be cytoplasmic structural proteins. The similarity of RS2 with these proteins raised the question whether it too had the potential to elicit a cross-reaction within the context of the latex-fruit syndrome. Hypersensitivity to beet is not a common disease, very few reports have been made of allergies related to beet components (Hohenleutner et al., 1996). However, examination of the RS2 protein sequence indicated that there were

Table 1. Summary of RS2 homologues

<table>
<thead>
<tr>
<th>Organism</th>
<th>Gene</th>
<th>Accession number</th>
<th>Identity (DDBJ blastn)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beta vulgaris</td>
<td>RS2</td>
<td>AJ278989</td>
<td>100%</td>
</tr>
<tr>
<td>Vitis vinifera</td>
<td>Grip68</td>
<td>AJ237987</td>
<td>46% (72/155)</td>
</tr>
<tr>
<td>Prunus armeniaca</td>
<td>Putative allergen protein</td>
<td>AF134731</td>
<td>46% (65/135)</td>
</tr>
<tr>
<td>Hevea brasiliensis</td>
<td>Hev b 5</td>
<td>U51631</td>
<td>43% (65/150)</td>
</tr>
<tr>
<td>Solanum tuberosum</td>
<td>TUB8</td>
<td>Z11679</td>
<td>47% (61/169)</td>
</tr>
<tr>
<td>Actinidia delicosa</td>
<td>pKIWI501</td>
<td>L27810</td>
<td>41% (60/146)</td>
</tr>
<tr>
<td>Fagopyrum esculentum</td>
<td>Seed protein</td>
<td>D87983</td>
<td>49% (58/118)</td>
</tr>
</tbody>
</table>

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possible sites of interaction with antibodies to Hev b 5. Six human IgE-binding regions have been identified in Hev b 5, two of which contained the sequence EEXXA, where X was P, T or K (Beezholt et al., 1999). It was interesting to find that the RS2 predicted protein sequence contained several examples of this motif (positions 46–50; 103–107).

RS2 was isolated because it was identified as being storage root enhanced during the library screening. This was confirmed by using Northern blot hybridization (Fig. 1a). In sugar beet seedlings the gene was expressed in root, hypocotyl and cotyledons in equal amounts. In older plants the relative amount of expression in the developing storage root increased in comparison with the leaf. The explanation for this enhancement was uncovered by the use of whole mount in situ hybridization with tissue from a beetroot storage organ (WISH, de Almeida Engler et al., 1994). This showed that RS2 was expressed in the large number of parenchymatous storage cells that were produced in the mature storage organ (Fig. 1b). Although it is not root specific, this pattern of expression is likely to make the RS2 promoter useful for targeting to the storage organ.

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References


