Fatty Acid Biosynthesis: Desaturation

Isolation of two putative acyl-acyl carrier protein desaturase enzymes from Kochia scoparia

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Abstract

5-Hexadecenoic acid can be used to produce a semiochemical method to control the disease-carrying mosquito Culex quinquefasciatus. This unusual fatty acid is produced in the seed of Kochia scoparia. We have isolated two acyl-acyl carrier protein desaturases from this species and expressed them in Escherichia coli to facilitate functional characterisation.

Introduction

In tropical and sub-tropical urban areas the mosquito Culex quinquefasciatus is the major vector species of the filarial worm Wuchereria bancrofti, which causes bancroftian filariasis in man. It is estimated that more than 15 million people are currently infected with filariasis. This mosquito also transmits arboviruses, which cause encephalitis in both man and horses. Cases of St. Louis encephalitis, spread by Culex mosquitoes, were reported recently in New York, resulting in applications of insecticidal sprays to some districts in an attempt to control the population of the vector.

This mosquito species has very specific requirements for its larval habitats and, given the right conditions, maturing eggs secrete an ovipositioning pheromone that is the main olfactory cue to gravid female mosquitoes of the same species to lay their eggs in the same body of water. Combined with a juvenile-hormone-type insecticide this semiochemical could be used as an effective control method of this disease vector [1]. Recently it was observed that the mono-unsaturated fatty acid 16:1 Δ5 (5-hexadecenoic acid) could be used as a precursor from which the biologically active enantiomer can be synthesized in a two-step process. A naturally occurring source of this unusual fatty acid was found in Kochia scoparia, a member of the Chenopodiaceae.

5-Hexadecenoic acid is a seed-specific fatty acid in K. scoparia, and constitutes around 5% of the total seed lipids in this plant [2]. Initial results indicated that there are two distinct acyl-ACP (acyl carrier protein) desaturases present in the seed and only one form present in leaf tissue, suggesting that 16:1 Δ5 may be the product of desaturation by a variant form of acyl-ACP desaturase.

Experimental

K. scoparia fatty acid analysis of tissue

Methylated total fatty acid samples from the seeds and leaves of K. scoparia were run on GC and GC-MS and compared with standards.

Western analysis of acyl-ACP desaturase expression in K. scoparia

12% SDS/PAGE was used to separate total protein extracts from seed and leaf tissue of K. scoparia. This was then blotted on to nitrocellulose and probed with antibodies raised in rabbit against...
Table I

Fatty acid profile of *K*. *scoparia* seeds

<table>
<thead>
<tr>
<th>Component</th>
<th>% of total FAMEs extracted</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:0</td>
<td>9.4</td>
</tr>
<tr>
<td>16:1 Δ^5</td>
<td>5.45</td>
</tr>
<tr>
<td>16:1 Δ^9</td>
<td>0.652</td>
</tr>
<tr>
<td>18:0</td>
<td>2.8</td>
</tr>
<tr>
<td>18:1 Δ^5</td>
<td>1.389</td>
</tr>
<tr>
<td>18:1 Δ^9</td>
<td>22</td>
</tr>
<tr>
<td>18:2 Δ^5,9,12</td>
<td>0.3</td>
</tr>
<tr>
<td>18:2 Δ^5,9,12</td>
<td>44</td>
</tr>
<tr>
<td>18:3 Δ^5,9,12</td>
<td>1.733</td>
</tr>
<tr>
<td>18:3 Δ^5,9,12,15</td>
<td>5.1</td>
</tr>
<tr>
<td>18:3 Δ^5,9,12,15*</td>
<td>3.6</td>
</tr>
<tr>
<td>Other</td>
<td>3.6</td>
</tr>
</tbody>
</table>

*Tentative identification based on previous work by Kleiman et al [2].

the avocado Δ^9-18:0-ACP desaturase using methods described previously [3–5].

Amplification of the coding sequences of two putative acyl-ACP desaturases from first-stand cDNA

A PCR-based strategy was used to amplify ACP-desaturase isoforms, with two distinct (putative) acyl-ACP desaturases being successfully cloned and sequenced. These full-length genes, given the designations pK9 and pKN, were cloned into the *Escherichia coli* expression vector pET9a (Stratagene). The plasmids pETpK9 and pETpKN were then transformed into *E. coli* and the transgene expressed. The proteins produced by this bacterial expression were analysed by Western analysis as described above.

Results and discussion

Comparison of the FAMEs (fatty acid methyl esters) from *K. scoparia* seed samples with the retention times of standards on GC and GC-MS allowed the tentative identification of fatty acids present (Table 1). By using degenerate primers to initially amplify possible acyl-ACP desaturases, fragments of two possible isoforms were identified. The full-length sequences of both isoforms were compared with each other as well as with other characterized acyl-ACP desaturases available on databases. Both forms appear to have a plastid transit-peptide sequence at the N-terminus and have ≈68% identity at the nucleotide level, pK9 has >80% identity with the spinach Δ^9-18:0-ACP desaturase, while pKN appears to be more divergent. When modelled, the areas of possibly interesting difference occur around the entrance to the catalytic channel that runs through the centre of these enzymes [6].

Western analysis of *K. scoparia* protein samples

Blots indicated the presence of two distinct acyl-ACP desaturases of slightly different masses (around 45 and 49 kDa) present in seed, while only the 49 kDa form appears to be present in leaf tissue. This pattern is similar to that found in other plant species expressing diverged acyl-ACP desaturases [4,5].

Western analysis of transformed *E. coli* proteins

Antigenic cross-reaction with the Δ^9-18:0-ACP desaturase antibody occurred with the protein samples obtained from bacterial expression of both pETpK9 and pETpKN. The proteins produced appear to have different molecular masses, of ≈43 and 49 kDa. Further functional analysis of both these putative desaturases is being carried out.

Conclusion

It would appear that there are two distinct isoforms of acyl-ACP desaturase present in the seed tissue of *K. scoparia*. The full-length genes of two putative acyl-ACP desaturases have been cloned from this tissue and are undergoing functional characterization.

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References


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