An unusual desaturase in Aquilegia vulgaris

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Abstract

Aquilegia vulgaris seed oil contains high levels of the rare fatty acid columbinic acid (18:3 Δ5.9.12), which is unusual in having the double bond at the Δ5 carbon in the trans configuration. Columbinic acid was found to be a seed-specific fatty acid not only present in the storage oil but also in membrane lipids. Several putative gene fragments have been isolated from plant RNA with sequences similar to previously characterized ‘front-end’ desaturases. Functional characterization of the Aquilegia cDNA is underway.

Introduction

In the Ranunculaceae, two species, Thalictrum [1] and columbine (Aquilegia spp.) [2], contain significant amounts of the unusual fatty acid, columbinic acid (C18:3 Δ5.9.12), and recently the presence of columbinic acid has also been noted in Isopyrum thalictroides [3]. The double bond at the Δ5
position is in the trans formation, making it an unusual occurrence in Nature. Columbinic acid was designated an essential fatty acid [4] when it was observed to reverse the effects of essential fatty acid deficiency in rats. Columbinic acid has been shown to be of use in treating a variety of ailments, such as dermatological conditions [5] and in lowering hypertension [6] and cholesterol levels [7] in rats. We hypothesize that the enzyme responsible for the synthesis of columbinic acid is a 'front-end' desaturase and hence a member of this growing family [8]. Here we describe the fatty acid content of Aquilegia vulgaris seed lipids and the cloning of 'front-end' desaturases with a cytochrome b5-fusion domain.

**Isolation of a cDNA encoding a novel desaturase**

It is assumed that columbinic acid is synthesized by the Δ^2-insertion of a double bond into linoleic acid (18:2 Δ^9,12). The enzymes responsible for this have been termed 'front-end' desaturases, since they introduce a double bond between existing double bonds and the carboxyl carbon. Functional characterization of a number of 'front-end' desaturases (such as the Δ^9- and Δ^6-fatty acid desaturases [8]) has also indicated that these enzymes contain an N-terminal cytochrome b5 domain. Moreover, the motif HX_x or 4,HX_y,31, HX_2 or 3,HHX_137,172,HX_2 or 3,HH, which is considered common to all membrane-bound desaturases and alkane hydroxylases [11], exists in variant form in 'front-end' desaturases, with a glutamine substitution for histidine in the third histidine box (QX_2 or 3,HH) [12]. Using this knowledge, primers were designed to anneal to the three histidine boxes and used to amplify fragments of possible 'front-end' desaturases from seed RNA. RNA was extracted from seeds at the same stage that lipids were extracted for the biochemical analysis. Putative candidates were further amplified using RACE (rapid amplification of cDNA ends) to amplify the entire coding sequence and sequenced in full. One open reading frame (ORF) of interest is the subject of functional characterization by expression in yeast. The deduced amino acid sequence of this ORF indicated that it encodes a 'front-end' fatty acid desaturase and a related sphingolipid long-chain base desaturase (Figure 1) indicates that all three enzymes have very similar amino acid sequences. It is noteworthy that the sphingolipid desaturase which is responsible for the Δ^2 desaturation of sphingoid long-chain bases introduces a mixed-isomer double bond, with the majority being in the trans configuration [13]. Thus, the similarity of the trans fatty acid desaturase gene sequence with the sphingolipid desaturase gene sequence might not be unexpected.

**Conclusion**

*A. vulgaris* contains high levels of columbinic acid only in seeds and not in other tissues (results not given). This unusual fatty acid is not only seed-specific but found both in storage oils
Comparison diagram of hydrophobicity plots of the ORFs of three desaturase enzymes

Hydrophobicity plots were compared for the following enzymes: (A) Aquilegia vulgaris Δ6; (B) Borago officinalis Δ6 fatty acid desaturase; and (C) Ricinus communis Δ6 sphingolipid desaturase.

and membrane lipids. A gene encoding for a ‘front-end’ desaturase, which is similar to the Borago officinalis Δ6-fatty acid desaturase and Ricinus communis Δ6-sphingolipid desaturase, has been isolated and its functional characterization will add to the growing pool of information available on enzymes that perform unusual lipid-modification reactions.

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References

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