Structure and expression of a cDNA encoding zeaxanthin epoxidase, isolated from a wilt-related tomato (Lycopersicon esculentum Mill.)

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Received 28 May 1997; Accepted 28 July 1997

Abstract
Zeaxanthin epoxidase (ZE) catalyses two early steps in the abscisic acid (ABA) biosynthetic pathway. The sequence of a cDNA clone encoding ZE from Nicotiana plumbaginifolia was reported in 1996 and represented the first DNA sequence data on an ABA biosynthetic enzyme. The N. plumbaginifolia cDNA has been used to provide a heterologous probe to isolate a ZE cDNA from tomato (Lycopersicon esculentum Mill.). DNA and amino acid sequence differences are considered in relation to putative functional domains within the enzyme. The results of northern analysis in tomato are discussed in relation to the effects of water stress on ZE mRNA levels.

Key words: ABA biosynthesis, zeaxanthin epoxidase, tomato.

An ABA-deficient wilt mutant of Arabidopsis (aba) is impaired in the epoxidation of zeaxanthin (Duckham et al., 1991; Rock and Zeevaart, 1991). An homologous wilt mutant of Nicotiana plumbaginifolia (aba2) was obtained from a random, non-targeted programme of transposon mutagenesis involving the Activator (Ac) element (Marin et al., 1996). DNA flanking the transposon was used to screen a cDNA library. The cDNA of N. plumbaginifolia was subsequently used to transform the Arabidopsis mutant, restoring the wild-type phenotype.

Zeaxanthin requires two epoxidation steps to convert it to violaxanthin. It was suggested that these first two steps in ABA biosynthesis were carried out by the same enzyme (Taylor, 1991). Marin et al. (1996) demonstrated this using E. coli expressing zeaxanthin epoxidase (ZE). This was the first ABA biosynthetic enzyme to be characterized at the predicted amino acid sequence level.

The present paper involves analysis of a cDNA clone for ZE isolated from the tomato (Lycopersicon esculentum Mill.). This species has a number of well-known mutants of ABA biosynthesis, but none affecting ZE (Taylor, 1991). It is important to determine which ABA biosynthesis genes are up-regulated by drought stress. The effect of a water deficit on tomato ZE mRNA levels is reported here (Table 1).

Table 1. The effect of drought treatment on ZE mRNA abundance in tomato leaves

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean log ZE mRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 2</td>
</tr>
<tr>
<td>Well-watered</td>
<td>2.947</td>
</tr>
<tr>
<td>Intermediate</td>
<td>3.184</td>
</tr>
<tr>
<td>Unwatered</td>
<td>3.329</td>
</tr>
</tbody>
</table>

The standard error of deviation was 0.180 with 28 df

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plumbaginifolia cDNA libraries were based on RNA extracted from young emboldenedj-indicates possible deletions. The amino terminus (A) appears domains (C, D) are illustrated. Sequence identity is indicated by ”, amino acids which is unique to the pepper sequence. The pepper cDNA library to be a typical plastid transit peptide (Gavel and von Heijne, 1990). The region of tomato ZE between amino acids 595 and 654; the start of this region.

The authors are grateful to Dr A Marion-Poll (INRA, France) for generously donating the N. plumbaginifolia ZE cDNA, AB was supported on the BBSRC PMBII initiative, grant number PG42,646(PMB). CJT was funded by a SERC CASE Award, Ref. No. 95052236.

Fig. 1. Comparison of the predicted amino acid composition between tomato (L.e.), pepper (C.a.) and Nicotiana (N.p) in key regions of the ZE protein sequence. Two regions (A, B) showing maximum amino acid sequence diversity, together with two highly conserved putatively functional domains (C, D) are illustrated. Sequence identity is indicated by ”, amino acid residues differing at key positions in the sequences are boxed and/or emboldened, indicates possible deletions. The amino terminus (A) appears to be a typical plastid transit peptide (Gavel and von Heijne, 1990). The tomato transit peptide much more closely resembles that of N. plumbaginifolia than it does C. annuum This is due to the presence of a region of 16 amino acids which is unique to the pepper sequence. The pepper cDNA library was prepared from a random mixture of mRNAs derived from seedlings and also from fruit at the immature green, breaker and full-red stages of ripeness (Bouvier et al., 1996). In contrast to this, both the tomato and N plumbaginifolia cDNA libraries were based on RNA extracted from young plants (Martin et al., 1996). It is possible that the unique region of the pepper transit peptide is associated with targeting the enzyme to chloroplasts to provide xanthophyll epoxide precursors of the xanthophyll cycle which contribute to the red colour of ripening pepper fruits (Bouvier et al., 1996). Bouvier et al. (1996) suggested that cleavage of the transit peptide to yield the mature epoxidase probably takes place at the sequence VKA7LEA (aa54–60) in pepper. This putative amino terminus of the mature epoxidase (B) is the most variable region between the three species. In contrast, the likely FAD binding domain (C) is highly conserved and does not break the consensus sequence (Wierenga et al., 1986; Bouvier et al., 1996). Martin et al. (1996) highlighted a region of 19 amino acids starting at amino acid 230 of the N. plumbaginifolia ZE sequence, which they described as a ‘central motif of unknown function’. (D) It should be noted that the tomato ZE sequence contains two fairly conservative amino acid substitutions at the start of this region.

and aligned using BLASTP (Altschul et al., 1990). This gave 64% identity between the Arabidopsis sequence and the region of tomato ZE between amino acids 595 and 654; the corresponding figure for N. plumbaginifolia in comparison to tomato was 88%.

Bouvier et al. (1996) reported that their xanthophyll epoxidase gene was ‘up-regulated during oxidative stress and when chloroplasts undergo differentiation into chromoplasts in pepper fruit’. In view of its role in ABA biosynthesis, tomato ZE mRNA levels were tested to investigate whether they increased in response to drought stress (Table 1). No significant effect of drought treatment on ZE mRNA was observed in this experiment, implying that water stress induced ABA accumulation is likely to be controlled at another step in the biosynthetic pathway.

Acknowledgements

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