Biochem. J. (1989) 264, 929–932 (Printed in Great Britain)

The cDNA cloning of a pea (*Pisum sativum*) seed lipoxygenase

Sequence comparisons of the two major pea seed lipoxygenase isoforms

Paul M. EALING*† and Rod CASEY
The John Innes Institute and A.F.R.C. Institute of Plant Science Research, Colney Lane, Norwich NR4 7UH, U.K.

Cloning and sequencing of two cDNAs from mRNA of maturing pea (*Pisum sativum*) seeds allowed the deduction of the complete amino acid sequence of a lipoxygenase polypeptide which is most similar to that of soya-bean lipoxygenase 2. The predicted M_r of this polypeptide is 97134, and its sequence permits comparisons between the lox2-type and the lox3-type lipoxygenase isoforms from pea and soya bean (*Glycine max*).

INTRODUCTION

Lipoxygenases are a class of enzymes which catalyse the hydroperoxidation of *cis-cis*-pentadiene moieties in polyunsaturated fatty acids [1]. In soya-bean (*Glycine max*) seeds three major lipoxygenase isoforms have been identified: lipoxygenases 1 (lox1), 2 (lox2) and 3 (lox3), which can be distinguished by their substrate- and regio-specificity and pH optima [2]. In pea (*Pisum sativum*) seeds, one minor (lox1-like) and two major (lox2 and 3-like) activities have been demonstrated. We have previously reported the sequence of a near-full-length cDNA from pea seeds, the deduced amino acid sequence of which is most similar to that of soya-bean lox3 [3]. Here we report the sequence of the second major pea seed lipoxygenase polypeptide, and make comparisons with other known lipoxygenase sequences.

MATERIALS AND METHODS

The preparation and size fractionation of poly-adenylated RNA from developing pea (*Pisum sativum* cv. Birte) and hybrid selection, translation *in vitro* and examination of translation products by SDS/PAGE and fluorography were carried out as previously described [4]. cDNA cloning, sequencing and primer-extension methods were as described in [3]. All other reagents and enzymes were obtained as reported elsewhere [4].

Secondary-structure predictions and alignments were made by using the University of Wisconsin Genetics Computer Group sequence analysis software package [5].

RESULTS AND DISCUSSION

Isolation of cDNA sequences

A cDNA library from size-fractionated mRNA [3] was screened with a partial-length cDNA probe corresponding to a pea seed lipoxygenase [4]. Two cDNAs were identified that hybridized to the probe only under conditions of reduced stringency, one of which (pPE923) contained an insert of approx. 3 kb, the size expected of a full-length cDNA corresponding to the probe [4]. Hybrid-selection/translation experiments with pPE923 showed it to correspond to a polypeptide of apparent M_r approx. 90000 on 10%-(w/v)-polyacrylamide/SDS gels (Fig. 1). This suggests that pPE1036 [3] and pPE923 correspond to the two major polypeptides immuno-precipitated from total Birte pea seed protein [4]. The insert of pPE923 was completely sequenced in both directions and found to encode an open reading frame of 846 amino acids with 91% similarity (BESTFIT alignment [5]) to the deduced amino acid sequence of soya-bean lox2 [6] (Table 1). Primer-extension analysis using an oligonucleotide chosen for lack of similarity with pPE1036 showed that pPE923 is approx. 73–79 bp short of full length (Fig. 2).

Another cDNA library was screened with the insert of pPE923, and a longer clone, pPE320, was selected. Approx. 1.6 kb of this clone was sequenced in both directions. The sequence obtained was identical with that of pPE923 and included 400 bp at the 5′ end and 260 bp at the 3′ end; the remainder was primed with oligo-nucleotides complementary to pPE923. The length of pPE320 is only 3 bp short of the theoretical maximum predicted by the primer-extension results. Thus pPE320 might be a near-full-length version of pPE923 or it may correspond to a transcript from a closely related member of the gene family, since copy-number reconstruction analyses with genomic Southern blots indicate approx. four or five copies of the gene (results not shown). The combined DNA sequence predicted from these cDNAs is shown in Fig. 3, together with the predicted amino acid sequence.

Sequence comparisons with other lipoxygenases

Deduced amino acid sequences of all the major soya-bean and pea seed lipoxygenase isoforms have now been reported [3,6,7,8], as well as sequences from human [9,10] and rabbit [11]. A number of authors have pointed out the existence of two areas of relative invariance within

* To whom correspondence and reprint requests should be addressed.
† Current address: D.S.I.R., Grasslands Division, Palmerston North, New Zealand.
These sequence data have been submitted to the EMBL/GenBank/DDBJ Nucleotide Sequence Databases under the accession number X17061.

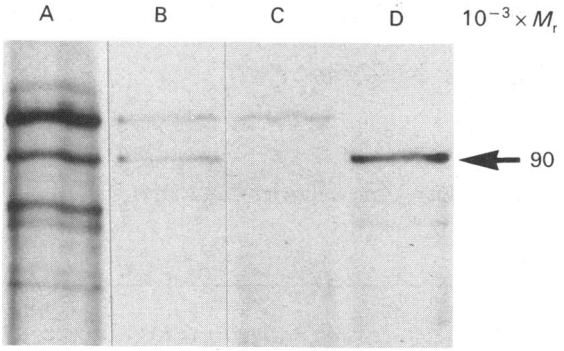

Fig. 1. Immunoprecipitation of hybrid-selected/translation products

'*In vitro*' translation products were examined by SDS/10%-PAGE and fluorography; lane A, 'in vitro' translation products of the size-selected mRNA; lane B, immunoprecipitate of the translation products in lane A; lane C, immunoprecipitate of the '*in vitro*' translation products of mRNA hybrid selected with the cDNA clone pPE1046 [3]; lane D, immunoprecipitate of the '*in vitro*' translation products of mRNA hybrid selected with the cDNA clone pPE923. Immunoprecipitations were performed by using anti-(soya-bean lipoxygenase) serum [4]. The arrow marks the position expected of a polypeptide of apparent M_r 90000.

these aligned sequences which might be important to the activity of these enzymes [9,12]. These are also conserved in the sequence predicted by pPE923.

We have observed differences among the predicted *N*-terminal sequences of the soya-bean and pea isoforms which may possibly relate to the differences between lox2- and lox3-like activities. The lox2-type isoforms of both pea and soya bean appear to have five additional amino acids which do not appear in the sequence of either the pea or soya-bean lox4-type isoform; soya-bean lox1 lacks a large number of amino

Table 1. Percentage similarities of soya-bean lipoxygenase (lox1, 2 and 3) sequences at the amino acid and DNA (cDNA) levels with the predicted amino acid sequences and cDNA sequences of pPE923/320 and pPE1036

Pair-wise comparisons of sequences were made by the program BESTFIT [5]. For protein sequences, this uses a symbol-comparison table with mismatches scored according to the evolutionary distance between amino acids [15]. The first value (before the solidus) is the amino acid similarity; the value after the solidus in the DNA similarity.

		Soya bean		
		lox1	lox2	lox3
Pea	pPE923/320	87/79	91/81	86/75
	pPE1036	83/73	87/75	93/83

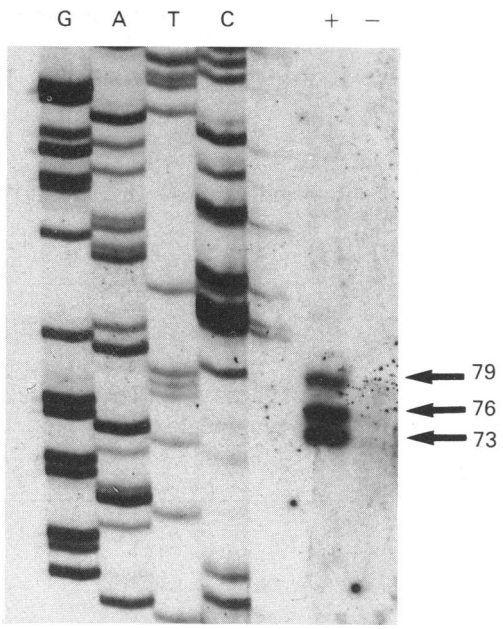

Fig. 2. Primer extension analysis of pPE923

An oligonucleotide primer complementary to nucleotide residues 142–158 of the cDNA was synthesized, annealed to size-fractionated mRNA and extended with reverse transcriptase. The lane marked '+' shows the extended primer fragment sizes after electrophoresis on a 6%-polyacrylamide sequencing gel with an M13 dideoxy sequence ladder (lanes G, A, T and C) as size markers. The approximate extent (number of bp) by which each fragment extends beyond the 5′ end of the cDNA insert of pPE923 is indicated on the right-hand side. The lane marked '−' is a no-RNA control.

acids in this region (Fig. 4). When the DNA sequence of pPE9023 is aligned with that of pPE1036 [3], a 15 bp 'insert' corresponding to the five additional amino acids (bp 129–143) is seen, flanked by a 3 bp direct repeat [i.e. TGG(TGGTGGAAACGTCCA)TGG]. In the sequence of pPE1036 [3], only the flanking sequence is seen (i.e. ...TGGTGG...) in the corresponding position. When the cDNA sequences for soya-bean lox2 and lox3 are aligned, however, it is apparent that they are related by the 'insertion' of 15 bp of DNA in a different position, without analogous direct repeats.

Secondary-structure predictions for the *N*-termini of the lox2- and lox3-type isoforms of both pea and soya bean were examined by using the algorithms of Chou & Fasman [13] and Garnier *et al.* [14], but no consistent structural features could be correlated with the differences in sequence.

This work was supported by the Agricultural and Food Research Council via a grant-in-aid to the John Innes Institute and by the Commission of European Communities (contract BAP-0063-UK).

REFERENCES

1. Vick, B. A. & Zimmerman, C. C. (1987) in The Biochemistry of Plants (Conn, E. E. & Stumpf, P. K., eds.), vol. 9, pp. 53–90, Academic Press, London and New York

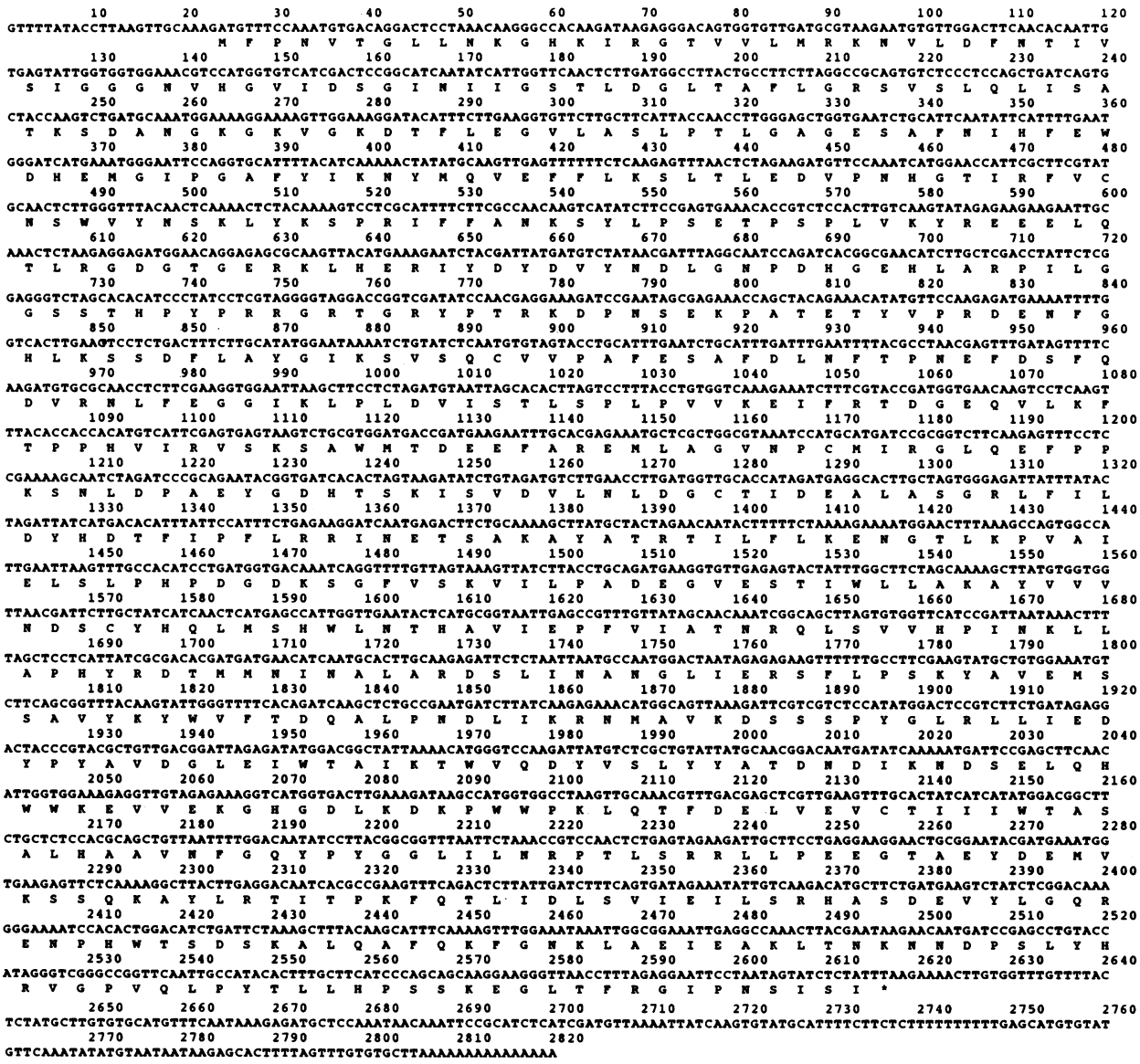

Fig. 3. Combined nucleotide and deduced amino acid sequence of the inserts of cDNA clones pPE923 and pPE320

The sequence of pPE923 starts at position 76. The sequence of pPE320 determined includes positions 1–380 (both strands), 410–590, 640–880, 1512–1673, 1690–1974 (one strand only) and 2590–2817 (both strands).

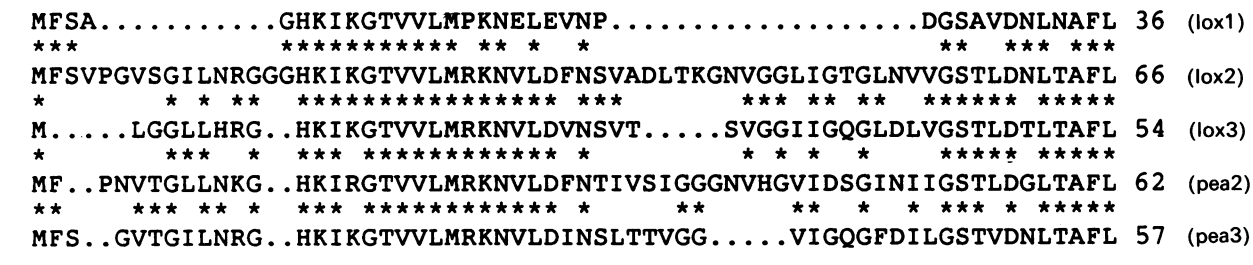

Fig. 4. Comparison of the predicted *N*-termini of the major pea and soya-bean seed lipoxygenase isoforms

Gaps in the alignments of the predicted amino acid sequences reflect the gaps found upon alignment of pair-wise cDNA nucleotide sequences within the same species; asterisks (★) show the positions of aligned identity. Pea2 is the sequence of the lox2-like pea seed lipoxygenase encoded by pPE923/320, whereas pea3 is that of the lox3-like isoform encoded by pPE1036 [3]; lox1, 2 and 3 refer to the soya-bean lipoxygenase sequences.

2. Hildebrand, D. F. (1989) Physiol. Plant. **76**, 249–253
3. Ealing, P. M. & Casey, R. (1988) Biochem. J. **253**, 915–918
4. Casey, R., Domoney, C. & Nielsen, N. C. (1985) Biochem. J. **232**, 79–85
5. Devereux, J., Haeberli, P. & Smithies, O. (1984) Nucleic Acids Res. **12**, 387–395
6. Shibata, D., Steczko, J., Dixon, J. E., Andrews, P. C., Hermodson, M. & Axelrod, B. (1988) J. Biol. Chem. **263**, 6816–6822
7. Shibata, D., Steczko, J., Dixon, J. E., Hermodson, M., Yazdanparast, R. & Axelrod, B. (1987) J. Biol. Chem. **262**, 10080–10085
8. Yenofsky, R. L., Fine, M. & Liu, C. (1988) Mol. Gen. Genet. **211**, 215–222
9. Dixon, R. A. F., Jones, R. E., Diehl, R. E., Bennett, C. D., Kargman, S. & Rouzer, C. A. (1988) Proc. Natl. Acad. Sci. U.S.A. **85**, 416–420
10. Sigal, E., Craik, C. S., Highland, E., Grunberger, D., Costello, L. L., Dixon, R. A. F. & Nadel, J. A. (1988) Biochem. Biophys. Res. Commun. **158**, 457–464
11. Fleming, J., Thiele, B. J., Chester, J., O'rey, J., Janetzki, S., Aitken, A., Anton, I. A., Rapoport, S. M. & Harrison, P. R. (1989) Gene **79**, 181–188
12. Casey, R., Domoney, C., Ealing, P. & North, H. (1989) in Proceedings of the World Congress on Vegetable Protein Utilization in Human Foods and Animal foodstuffs (Applewhite, T. H., ed.), pp. 491–496, American Oil Chemists' Society, Champaign, IL
13. Chou, P. Y. & Fasman, G. D. (1978) Adv. Enzymol. Relat. Areas Mol. Biol. **47**, 45–148
14. Garnier, J., Osguthorpe, O. J. & Robson, B. (1978) J. Mol. Biol. **120**, 97–120
15. Schwartz, R. M. & Waterman, M. S. (1978) in Atlas of Protein Sequence and Structure (Dayhoff, M. O., ed.), vol. 5 (Suppl. 3), pp. 353–358, National Biomedical Research Foundation, Washington

Received 12 September 1989/18 October 1989; accepted 24 October 1989