Isolation of a cDNA clone for pea (*Pisum sativum*) seed lipoxygenase

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1. A lipoxygenase cDNA clone, pCD45, was identified in a *Pisum sativum* L. (pea) seed mRNA cDNA library by hybrid-release/translation followed by immunoprecipitation with antiserum raised against lipoxygenase from *Glycine max* L. (soya bean). 2. pCD45 hybrid-selected an mRNA encoding the larger of the two polypeptides of $M_r \sim 95000$ that were immunoprecipitated from cell-free translation products of pea seed poly(A)-containing RNA by the *G. max* anti-lipoxygenase. 3. 'Northern'-blot analysis showed the mRNA that hybridized to pCD45 to be ~ 3000 nucleotides in length. 4. Three to five copies of the lipoxygenase gene corresponding to pCD45 were estimated to be present per haploid *Pisum* genome; hybridization of the cDNA insert from pCD45 to *G. max* DNA was also detected.

INTRODUCTION

Lipoxygenases (linoleate: oxygen oxidoreductase, EC 1.13.11.12) are a group of enzymes that catalyse the oxygenation of fatty acids with a *cis,cis*-penta-1,4-diene system. Although lipoxygenase activity is widespread throughout the plant kingdom, the physiological role of the enzyme is not understood. Lipoxygenases are important commercially because their action destroys essential polyunsaturated fatty acids and gives rise to compounds that can produce either desirable or undesirable tastes and odours (for a review, see Galliard & Chan, 1980). The enzyme from wheat (*Triticum aestivum*) also has many functions in bread-making (Nicolas *et al.*, 1982). Legume seeds have particularly high levels of lipoxygenase activity (Pinsky *et al.*, 1971). The enzyme has been implicated in spoilage and off-flavour in soya-bean (*Glycine max*) protein products (probably through the production of short-chain-length volatile carbonyl compounds that bind to the soya protein) and in the development of rancid off-flavour in unblanched vegetables, including peas (*Pisum sativum* L.) (Bengtsson & Bosund, 1966) and lentils (*Lens culinaris* L.) (Eskin & Henderson, 1977).

Lipoxygenases have been identified in the seeds of many legumes, but the most comprehensively studied are those from soya bean and pea. Soya bean contains the enzymes of type 1 and type 2 [see Galliard & Chan (1980) for a review of lipoxygenase nomenclature], of which the latter probably comprises lipoxygenases-2 and -3 (Reynolds & Klein, 1982). Lipoxygenase-1, the so-called 'Theorell enzyme' (Theorell *et al.*, 1947), is heat-stable, has a different substrate preference, and has a higher pH optimum for maximal activity than have lipoxygenases-2 and -3 (Christopher *et al.*, 1970, 1972; Hildebrandt & Kito, 1984) and is apparently present in peas at much

lower concentrations than are lipoxygenases-2 and -3 (Yoon & Klein, 1979).

Recent measurements of the M_r values for pea seed lipoxygenases and their polypeptides suggest that lipoxygenases-2 and -3 are both monomeric proteins with polypeptides of M_r 94000–95000 (Reynolds & Klein, 1982), a value that is in good agreement with those reported for soya-bean lipoxygenases-1, -2 and -3 (Haydar *et al.*, 1975; Diel & Stan, 1978), which all have similar, but distinguishable, apparent M_r values on SDS/polyacrylamide-gel electrophoresis (Kitamura *et al.*, 1983b; Kitamura, 1984). Apart from measurements of the apparent polypeptide M_r and the isoelectric points of pea seed lipoxygenases-2 and -3 (Spaapen *et al.*, 1977; Yoon & Klein, 1979), virtually nothing is known of the physical biochemistry of pea seed lipoxygenases; early amino acid analyses produced conflicting results for the aspartic acid and half-cystine contents of the enzyme: one study used a mixture of isoenzymes (Eriksson & Svenssen, 1970), whereas another used purified lipoxygenase-2 (Arens *et al.*, 1973).

Lipoxygenases are of interest in terms of developmental regulation of gene expression. Hildebrandt & Hymowitz (1983) have shown that, in soya bean, the activities of lipoxygenases-1, -2 and -3 are maximal over a period of about 5–20 days before seed physiological maturity, but whereas the activities of lipoxygenases-2 and -3 increased again between 4 and 7 days of germination, that of lipoxygenase-1 decreased on germination. Genetic variants of soya bean have been described that lack lipoxygenase-1 (Hildebrandt & Hymowitz, 1981), lipoxygenase-2 (Kitamura *et al.*, 1983a) or lipoxygenase-3 (Kitamura *et al.*, 1983b). Independent segregation of the genes for soya-bean lipoxygenases-1 and -3 has been reported, whereas the genes for lipoxygenases-1 and -2 are tightly linked; the absence of lipoxygenase isoenzymes

Abbreviations used: bp, nucleotide base-pairs; DMSO, dimethyl sulphoxide; kb(p), kilobase(-pair)s; SDS, sodium dodecyl sulphate; DNAase, deoxyribonuclease; RNAase, ribonuclease; $1 \times$ SSC, 0.15 M-NaCl/0.015 M-sodium citrate.

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has been shown to be due to single recessive alleles (Hildebrandt & Hymowitz, 1982; Kitamura *et al.*, 1983a,b; Davies & Nielsen, 1984). Early investigations of lipoxygenases in different pea tissues (Anstis & Friend, 1974) indicated that the isoenzyme distribution was tissue-dependent. This suggests that expression of the genes for pea lipoxygenases might be regulated in a tissue-specific fashion.

Studies of lipoxygenase structure and gene expression will be greatly facilitated by the availability of cloned DNA species. We report here the cloning and characterization of a pea seed lipoxygenase cDNA.

MATERIALS AND METHODS

Chemicals and enzymes

Chemicals were of AnalaR grade, or of highest available purity, from Sigma, Poole, Dorset, U.K., or BDH, Dagenham, Essex, U.K. Oligo(dT)-cellulose, nuclease-free sucrose and restriction enzymes *EcoRV*, *BamHI*, *HindIII* and *Sall* were from Bethesda Research Laboratories (U.K.), Cambridge, U.K. Restriction enzyme *EcoRI* was from Boehringer, Lewes, East Sussex, U.K. DMSO was redistilled (b.p. 191–193 °C), and formamide and glyoxal were deionized, before use. RNAase-free DNAase I was a gift from Ms. S. Curson of the John Innes Institute. All other reagents and enzymes were as described elsewhere (Domoney & Casey, 1983, 1984).

Plant material

Developing seeds of *Pisum sativum* cvs. Birte and Dark Skinned Perfection (hereafter called 'DSP') at the later stages of development (Domoney *et al.*, 1980; Domoney, 1981) were used for the preparation of total or polyribosomal RNA. Seeds were frozen in liquid N₂ at harvest and stored at –70 °C. Leaves were harvested from five genotypes of *Pisum sativum*: the round-seeded and wrinkled-seeded near-isogenic lines BC1/4R and BC1/4W, the cvs. DSP and Birte and JI 497; from three genotypes of *Pisum fulvum*: JI 224 Sp, JI 224 Br and JI 849; and from *Glycine max* cv. Maple Amber plants, and stored at –70 °C. Mature, dry seeds of the same *Pisum* genotypes and the *Glycine max* cultivar were used for protein extraction.

Preparation of poly(A)-containing RNA

Unless otherwise stated, all procedures were carried out at 0–4 °C. Membrane-bound polyribosomes were prepared from developing seeds as previously described (Domoney & Casey, 1983). Total RNA was extracted from developing seeds as described by Covey & Grierson (1976), except that solid sodium diethyl dithiocarbamate was added to the homogenizing medium to a final concentration of 0.1 M, immediately before blending. After three successive extractions with an equal volume of phenol/chloroform (1:1, v/v) followed by ethanol precipitation (at –20 °C), total nucleic acids were washed with aq. 80% (v/v) ethanol, drained, redissolved in water (0.2 ml/g of starting material) and RNA was precipitated in 3 M-sodium acetate, pH 6.0, for 30 min. The precipitate was washed once with 3 M-sodium acetate, pH 6.0, dissolved in water, ethanol-precipitated at –20 °C, washed with aq. 80% (v/v) ethanol, drained and dissolved in 50 mM-Tris/HCl/5 mM-MgCl₂, pH 7.5

(0.2 ml/g of starting material). RNAase-free DNAase was added to 20 µg/ml and the solution incubated for 30 min at room temperature. After addition of NaCl and Tris/HCl, pH 8.5, to a final concentration of 0.1 M, the mixture was extracted twice with phenol/chloroform at room temperature and then the RNA was ethanol-precipitated at –20 °C.

Poly(A)-containing RNA was selected from total RNA or from membrane-bound polysomes by chromatography on oligo(dT)-cellulose as described previously (Domoney & Casey, 1983).

Size fractionation of poly(A)-containing RNA

Poly(A)-containing RNA (70–100 µg) was heat-denatured at 65 °C for 5 min in 100 µl of 10% (v/v) DMSO/10 mM-Tris/HCl (pH 7.5)/10 mM-LiCl/1 mM-EDTA, rapidly cooled on ice/water and layered over a 10 ml linear 5–20% (w/v)-sucrose gradient in 95% (v/v) DMSO/4% (v/v) formamide/10 mM-Tris/HCl (pH 7.5)/10 mM-LiCl/1 mM-EDTA (Bishop *et al.*, 1969). After centrifugation for 48 h at 150 000 *g*_{av} and 25 °C, RNA was recovered from 200 µl gradient fractions by ethanol precipitation at –20 °C in the presence of 0.15 M-sodium acetate, pH 4.8, and 3 µg of calf liver tRNA per fraction. The precipitated RNA was washed with aq. 80% ethanol, dried, and dissolved in water (10 µl/fraction).

Identification of pea lipoxygenase mRNA

The mRNA species that encoded specific seed proteins were located in the gradient fractions. For this purpose, 0.5 µl of the redissolved RNA from each gradient fraction was translated in a reticulocyte-lysate system that contained [³H]leucine. Translation was followed by immunoprecipitation and SDS/polyacrylamide-gel electrophoresis/fluorography, as described by Domoney & Casey (1983).

The antibodies against purified pea seed proteins and against soya-bean lipoxygenase were raised in rabbits and in goats respectively (Domoney & Casey, 1983; Yabuuchi *et al.*, 1982). The soya-bean antibodies gave a strong reaction to soya-bean lipoxygenase-1, but also had some cross-reaction with soya-bean lipoxygenases-2 and -3 (Yabuuchi *et al.*, 1982). The antiserum to lipoxygenase reacted strongly with extracts of both soya-bean and pea seeds in rocket immunoelectrophoresis and double-immunodiffusion experiments. Antibody to soya-bean urease was a gift from Dr. J. Polacco, University of Missouri, Columbia, MI 68212, U.S.A.

Selection of a pea seed lipoxygenase cDNA clone

The construction of a cDNA library in pAT153, from poly(A)-containing RNA from *P. sativum* cv. Birte, has been described (Domoney & Casey, 1983). Recombinant cDNA clones containing inserts of more than 1 kb were selected and characterized by hybrid-release translation as described previously (Domoney & Casey, 1983), except that bound RNA was eluted in buffer containing 50% (v/v), instead of 90% (v/v), formamide. Immunoprecipitation was performed by using antibodies against pea legumin, vicilin, convicilin, as well as soya-bean lipoxygenase and urease. The total translation products of selected mRNA species and the immunoprecipitated polypeptides were analysed by SDS/polyacrylamide-gel electrophoresis/fluorography.

The mRNA species complementary to cDNA clones

were characterized by 'Northern'-blot analyses (Domoney & Casey, 1983). Filters were hybridized to nick-translated cDNA insert and washed as described below.

Gene number estimation

Leaf DNA samples (10 µg), prepared as described previously (Domoney & Casey, 1985) and digested with *EcoRV* (5 units/µg of DNA) or *EcoRI* (10 units/µg of DNA), were subjected to electrophoresis on 0.8% agarose gels overnight and transferred to nitrocellulose filters. The plasmid pCD45 was linearized with *SaII* (the cDNA insert contained *EcoRI*, *BamHI* and *HindIII* restriction sites), and amounts of linearized plasmid equivalent to 0.5, 1, 2 and 5 copies per haploid genome [4.6 pg (Murray *et al.*, 1978)] in 10 µg of pea DNA were included on each gel. Nitrocellulose filters were pre-hybridized in 0.6 M-NaCl/0.12 M-Tris/8 mM-EDTA, pH 8.0, containing 0.1% (w/v) SDS and 10 × Denhardt's (1966) solution, at 65 °C for 3 h. Hybridization was in the above solution containing 0.1% (w/v) Na₂P₂O₇ and 10% (w/v) dextran sulphate, with (1–3) × 10⁶ c.p.m. of nick-translated cDNA insert/ml of hybridization solution, at 65 °C for 18 h. Filters were washed at least twice in 0.1 × SSC/0.1% (w/v) SDS at 65 °C, dried and autoradiographed. Autoradiographs were scanned as described previously (Domoney & Casey, 1985).

Protein gel electrophoresis and 'Western' blots

Extracts of ground, dry pea or soya-bean seeds were prepared as described by Domoney & Casey (1985) and Kitamura *et al.* (1983b) respectively. SDS/polyacrylamide-gel electrophoresis was carried out as described previously (Domoney & Casey, 1983), with 10%

(w/v) or 15% (w/v) separating gels and a 5% (w/v) stacking gel. Gel electrophoresis of urea/SDS extracts under partially denaturing conditions with a 5–9% (w/v) gradient or a 7% (w/v) gel was as described by Kitamura *et al.* (1983b) and Kitamura (1984) respectively. Fractionated proteins were electrophoretically blotted on to nitrocellulose in aq. 20% (v/v) methanol/0.19 M-glycine/25 mM-Tris (50V, 15 °C). Filters were stained for protein with black Rotring ink (Hancock & Tsang, 1983). Lipoxygenase polypeptides were detected on filters with anti-lipoxygenase IgG and rabbit anti-goat IgG-alkaline phosphatase conjugate, by using the method of Blake *et al.* (1984) [modified to include 3% (w/v) bovine serum albumin in the blocking step].

RESULTS

Identification of lipoxygenase in mature seeds

'Western'-blot analysis of whole pea seed extracts on 10%-polyacrylamide/SDS gels, using anti-lipoxygenase, showed the presence of two polypeptides (apparent M_r values (~97000 and ~90000) in all pea genotypes examined (Figs. 1a and 1b). The same method detected a closely spaced doublet of polypeptides (apparent M_r ~ 94000) in soya bean. Thus the two species differ in that the pea isoenzymes separate into two well resolved major bands in the 10%-polyacrylamide gels, whereas the soya-bean isoenzymes are not well separated under these conditions.

Kitamura *et al.* (1983b) have described a method that relies on partially denaturing conditions and 5–9% gradient gels to resolve the soya-bean isoenzymes more

Fig. 1. Gel-electrophoretic/'Western'-blot analysis of lipoxygenase in pea and soya-bean seed extracts

(a, b) SDS/polyacrylamide-gel electrophoresis of extracts of *Pisum sativum*, cv. Birte (10% gel): (a) Coomassie Blue stain; (b) 'Western' blot probed with anti-lipoxygenase. (c–j) 'Western' blot of a partially denaturing 5–9% gel of *Pisum* extracts probed with anti-lipoxygenase: (c) BC1/4R; (d) BC1/4W; (e) cv. DSP; (f) cv. Birte; (g) JI 497; (h) JI 224 Sp; (i) JI 224 Br; (j) JI 849. (k–l) Coomassie Blue stain of SDS/polyacrylamide-gel electrophoretograms of *Glycine max* extracts: (k) 5–9% gradient gel; (l) 7% gel. L1, L2 and L3 are the polypeptides from soya-bean lipoxygenases-1, -2 and -3 respectively; α' and α are the polypeptides of β-conglycinin, the 7S storage protein of soya-bean seeds.

Fig. 2. Fractionation of poly(A)-containing RNA from total RNA of cv. Birte on a denaturing sucrose density gradient

○, RNA (μg/fraction) (the contribution from the carrier tRNA has been subtracted). ●, Trichloroacetic acid-precipitable ³H (c.p.m.) from the translation of 0.5 μl of each fraction in 18 μl of messenger-dependent lysate containing 10 μCi of [³H]leucine. The hatched areas represent the proportion of the incorporated [³H]leucine that was immunoprecipitated by anti-lipoxygenase. The values above the Figure indicate the positions adopted in the gradient by mRNA species of approx. 2 kb and 3 kb. The arrow represents the fraction used for the experiments depicted in Figs. 3 and 4(b).

effectively. When this procedure was used for pea extracts, the same general pattern was observed as for the 10% gels (Figs. 1c–1j). Likewise, when 7% gels were used, which are reported to give even better separations (Kitamura, 1984), no evidence for increased complexity of the pea isoenzymes was obtained (results not shown), although the separation of soya-bean lipoxygenase polypeptides was better (Figs. 1k and 1l).

Variation in the lipoxygenase polypeptide patterns among *Pisum* genotypes was observed (Fig. 1). For example, the cv. DSP had an additional polypeptide (Fig. 1e), whereas the larger lipoxygenase polypeptide was of slightly higher mobility in JI 224 Sp (Fig. 1h) than in other genotypes. These variations will be useful as markers in genetic studies to determine inheritance and linkage relationships.

Identification of lipoxygenase in cell-free translation products of poly(A)-containing RNA

Translation of selected fractions from sucrose gradients, followed by specific immunoprecipitation with antibodies to soya-bean lipoxygenase, showed (Fig. 2) that lipoxygenase mRNA is found in the fractions that contained the longest RNA from seeds at the later stages of development.

Cell-free translation and immunoprecipitation of these large RNA fractions showed that two polypeptides were specifically precipitated by anti-lipoxygenase (Fig. 3). The more intense of the two was of apparent M_r slightly

greater than that of the [³H]phosphorylase *a* marker ($M_r \sim 95000$); the other, less intense, polypeptide had an apparent M_r of less than 95000.

Immunoprecipitable cell-free products of apparently similar M_r were also identified, by the same means, in translation products of total poly(A)-containing RNA from cv. DSP. This polypeptide pattern is therefore not unique to cv. Birte. We have not examined cell-free translation products from cv. DSP on 5–9% gradient gels and do not know if an additional polypeptide, corresponding to that in the polypeptides of the mature seed (Fig. 1e), would be detected on such gels. The polypeptides are apparently being actively synthesized in seeds of cv. Birte at late stages of development, since the lipoxygenase mRNA was found in polyribosomes.

Identification of a cDNA clone for pea seed lipoxygenase

The clone pCD45 was selected from the cDNA library as the plasmid with the longest insert (1.7 kb). Hybrid-selection with poly(A)-containing RNA showed that pCD45 was homologous with an mRNA that encoded polypeptides of apparent M_r slightly greater than 95000 [Fig. 4a(ii)]. These polypeptides were specifically immunoprecipitated by anti-lipoxygenase [Fig. 4a(iii)], but not by anti-urease [Fig. 4a(iv)] or anti-legumin. The products of the hybrid-selected mRNA were equivalent to the largest polypeptides observed in cell-free translations of total poly(A)-containing RNA [Fig. 4a(i)] and

Fig. 3. An analysis, by SDS/polyacrylamide-gel electrophoresis (10% gel) and fluorography, of (a) the cell-free translation products of the gradient fraction containing large RNA species (see Fig. 2) and (b) the immunoprecipitate produced from the polypeptides shown in (a) using antiserum to soya-bean lipoxygenase

corresponded in mobility to the larger of the two classes of polypeptides shown in Fig. 3(b). The mRNA for the smaller polypeptide shown in Fig. 3(b) was not selected by hybridization to pCD45 under our standard hybrid-selection conditions.

'Northern'-blot analysis of the large, lipoxygenase-containing mRNA fractions (Fig. 4b) indicated that most of the RNA that hybridized to the insert from pCD45 had a size of about 3 kb. An mRNA species of this size would be sufficient to encode polypeptides of M_r 95 000–100 000.

Hybridization of *EcoRV*-restricted genomic DNA from eight pea genotypes with the insert from pCD45 indicated the presence of three to five copies of the lipoxygenase gene per haploid genome in seven genotypes (Figs. 5a–5l). *EcoRV*-digested DNA from JI 849 (Fig. 5h) lacked a restriction fragment which hybridized with the

Fig. 4. Hybrid selection and 'Northern'-blot analysis using pCD45

(a) An analysis, by SDS/polyacrylamide-gel electrophoresis (15% gel) and fluorography, of (i) the cell-free translation products of total poly(A)-containing RNA from developing seeds of *P. sativum* cv. Birte; (ii) the RNA that was selected by hybridization to pCD45; (iii), the immunoprecipitate produced from the polypeptides shown in (ii) using antiserum to soya-bean lipoxygenase; (iv), as (iii), but using antibody to soya-bean urease. (b) A 'Northern'-blot analysis of the RNA species used in Fig. 3. The filter was hybridized with the cDNA insert from pCD45. Numbers refer to the nucleotide lengths of the DNA markers obtained as described previously (Domoney & Casey, 1983), except that an *RsaI* fragment of pAT153 (2977 bp) was included as an additional size standard.

Fig. 5. Hybridization of nick-translated insert from pCD45 to *EcoRV*-digested DNA from BC1/4R (a), BC1/4W (b), cv. DSP (c), cv. Birte (d), JI 497 (e), JI 224 Sp (f), JI 224 Br (g) and JI 849 (h) and to *EcoRI*-digested DNA from JI 224 Sp (m), JI 224 Br (n), JI 849 (o), cv. DSP (p) and *G. max* (q)

(a)–(h) and (m)–(o) show 10 µg of restricted DNA; (p) and (q) show 5 µg of restricted DNA. The hybridization intensities given by 10 µg of *EcoRV*-digested DNA (a)–(h) can be compared with those given by amounts of *Sall*-linearized pCD45 corresponding to 0.5 (i), 1 (j), 2 (k) and 5 (l) gene copy equivalents/haploid genome per 10 µg of *Pisum* DNA. Approximate sizes of DNA markers are indicated (kbp).

lipoxygenase probe even when filters were washed at lower stringencies (0.1% SDS, 0.1 × SSC at 50 °C). At these lower-temperature washes, many additional fragments were found to hybridize in DNA from the other eight genotypes. However, when DNA from JI 849 was restricted with a number of other enzymes [e.g. *EcoRI* (Fig. 5o), *Bam*HI and *Hind*III], the hybridization pattern and gene number were the same as those for *EcoRI*-restricted DNA from the two other *P. fulvum* genotypes (Figs. 5m and 5n). It seems likely that JI 849 has lost an *EcoRV* site in or near the lipoxygenase genes, and this has resulted in large fragments (> 15 kb) that would not be transferred efficiently to nitrocellulose under the conditions used in these experiments. The *P. sativum* genotypes exhibited an identical hybridization pattern for each of the four enzymes used in restriction of genomic DNA; this pattern always differed from that obtained for the *P. fulvum* genotypes. There was no apparent difference in gene number between any of the genotypes examined. The hybridization intensities given by *EcoRI*-digested soya-bean DNA (Fig. 5q) were similar to those of *EcoRI*-digested *P. sativum* DNA (Fig. 5p).

DISCUSSION

Although the soya-bean anti-lipoxygenase serum used in this work reacts predominantly with soya-bean lipoxygenase-1, it also shows some cross-reaction with lipoxygenases-2 and -3. It is therefore impossible to assign the polypeptides detected after hybrid-release/translation experiments with pCD45 to a particular isoenzyme group on the basis of their immunoprecipitation with anti-lipoxygenase. It has been suggested (Yoon & Klein, 1979) that pea seeds contain very little lipoxygenase-1, and that the isoenzymes observed in pea seeds

correspond to soya-bean lipoxygenases-2 and -3, i.e. 'type 2' lipoxygenases. This has been confirmed by Vernooij-Gerritsen *et al.* (1982, 1984). The studies of Kitamura *et al.* (1983b) and Kitamura (1984) show that the polypeptides from the three soya-bean isoenzymes can be separated by gel electrophoresis under partially denaturing conditions and exhibit increasing mobility in the order lipoxygenase-1 > lipoxygenase-2 > lipoxygenase-3. Pea seed lipoxygenase polypeptides are more widely separated than their soya-bean counterparts under similar conditions. The different behaviour of pea and soya-bean polypeptides in these gel systems probably reflects larger variation in physical properties among the pea isoenzymes than among those of soya-bean.

The hybrid-selection by pCD45 of an mRNA that corresponds to only one of the anti-lipoxygenase immunoprecipitable polypeptides [Figs. 3b and 4a(ii)] shows that there are sequence differences between the two polypeptides. A similar result has been observed among different vicilin precursors where immunological cross-reactivity has been demonstrated, but no cross-selection in hybrid-selection experiments (Domoney & Casey, 1983). The cross-reaction of the antibody to the soya-bean enzyme with seed extracts and cell-free translation products from peas may be significant in terms of the evolution of lipoxygenases in legumes. Concanavalin A and soya-bean agglutinin share extensive sequence homologies (Hemperley & Cunningham, 1983), yet apparently are immunologically unrelated as native proteins (Shannon, 1983). It may be, then, that some elements of lipoxygenase structure have been well conserved during the evolution of the Leguminosae. This interpretation is consistent with the observation that pCD45 hybridizes to DNA from other legumes, including soya bean (Fig. 5q), French bean (*Phaseolus vulgaris*),

cowpea (*Vigna unguiculata*) and mung bean (*Phaseolus aureus*) (results not shown). If it is assumed that soya-bean and pea lipoxygenase genes have not diverged to the point where they either do not hybridize, or hybridize only poorly, with pCD45, then a similar gene number is indicated in these two species.

The lipoxygenase cDNA clone, pCD45, will facilitate the selection of lipoxygenase genes from *Pisum* genomic libraries. Sequence analyses of lipoxygenase genes will establish the primary structure of pea lipoxygenase, whereas lipoxygenase clones will prove useful in the investigations of the developmental regulation and the possible tissue-specific expression of pea lipoxygenase genes.

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