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Isolation of lipoxygenase isoforms from Glycine max embryo axes based on apparent cross-reactivity with anti-myosin antibodies

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Abstract

Three lipoxygenase isoforms were isolated from Glycine max embryo axes. A number of proteins around 97 kDa cross-reacted with several anti-actin and anti-myosin antibodies and these were used to follow their purification through gel filtration, hydroxyapatite and anion exchange columns. The 97-kDa cross-reactive material eluted in the unbound fractions of the last anion exchange column, and displayed two components of p*I*'s 6.2 and 6.3. Further phase partition of this fraction in TX-114 yielded a hydrophobic 97 kDa protein. Additionally, a 95-kDa protein was retained and eluted from this last column. Partial peptide sequences indicated that the 95 kDa protein was soybean lipoxygenase-1, the first 97 kDa protein was lypoxygenase-3, and the hydrophobic 97 kDa protein was lipoxygenase-2. Several possible reasons for the cross-reactivity with the antibodies are discussed. To our knowledge, this is the first example of individual lipoxygenase isoforms isolated from soybean embryo axes.

Keywords: Embryo; Lipoxidase; Lipoxygenase; Purification; Seed; Glycine max

1. Introduction

Lipoxygenases are ubiquitous enzymes in eukaryotes and are involved in a number of reactions of hydroperoxidation of polyunsaturated fatty acids [1]. Their products are either intermediate or final in the pathway and have been found in relatively high levels in plants [2], where they are involved in the synthesis of compounds with growth regulator activity and signaling molecules such as jasmonic acid, traumatin, and β-ionone [3,4]. Additionally, their expression increases during pathogen infection, thus implicating them in the synthesis of compounds important for the defense response or signaling events within this pathway [3,4].

Lipoxygenases were initially separated from soybean cotyledons as four distinct isoforms designated lipoxygenases-1, -2, -3a, and -3b [5]; however, lipoxygenases-3a and -3b are thought to arise from postranslational modification from a protein coded by the same gene [5]. Accordingly, at least eight lipoxygenase genes have been identified in soybean (Glycine max), but only three of these are mainly expressed in seeds [6]. Thus, it is generally agreed that soybean cotyledons contain three isozymes termed lipoxygenases-1, -2, and -3. These three isoforms accumulate in the soybean seed during maturation [7] and they may do so in preparation for processes needed during the development of the shoot. For example, during soybean embryo axis development, lipoxygenases-1, -2 and -3 were present at low levels at early stages, but at later stages, their expression changed. At these stages, lipoxygenase-1 increased continuously during axis elongation up to 13-fold. On the other hand, lipoxygenases-2 and -3 had a more moderate increase which was not continuous. Lipoxygenases-1 and -3 were the main isoforms responsible for the increase in activity [3,8]. Although the presence of the enzymes in the mature embryo axis could not be determined and their analysis was carried out on the separate axes only after the shoot reached 5 mm, their expression was higher in these axes than in cotyledons,

Abbreviations: ATP, adenosine triphosphate; DTT, dithiothreitol; EGTA, ethylene glycol-bis(2-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; PIPES, piperazine-*N*,*N*′-bis(2-ethanesulfonic acid)

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suggesting that they might be important for the processes occurring at this point in development [3].

Lipoxygenases have been isolated from soybean cotyledons but none from the mature seed embryo axis and, consequently, it is not known which isoforms exist in this tissue, and what expression pattern they undergo during early germination stages. In this work, we used these mature soybean embryo axes as source for the isolation of lipoxygenases-1, -2 and -3. We report the unusual immunochemical basis of the isolation procedure, their biochemical characterization, and partial peptide sequence analysis.

2. Materials and methods

2.1. Plant material

Glycine max dry seeds were sterilized with 10% bleach, rinsed extensively in tap water and washed 10 min with double-distilled water. The embryo axes were then excised from these seeds with a sterile knife, dried on a paper towel, and then finely ground to a powder.

2.2. Protein fractionation

One gram of embryo powder was homogenized with 5 ml of microtubule stabilizing buffer (0.1 M PIPES, 2 mM EGTA, 1 mM MgSO4, pH 6.9) [9], modified by adding 30% sucrose, 1 mM DTT, and one tablet of protease inhibitor cocktail (Complete™ Boehringer-Mannheim). This mixture was centrifuged at 16 000 × *g*, 10 min. The supernatant was loaded onto a gel filtration column packed with Sephacryl S-300 (Sigma, St. Louis MO). Fractions were collected and then analyzed by Western blot with a polyclonal antiserum raised against calf thymus actin [10], which also had cross-reactivity with myosin heavy chains (see below). The immunoreactive fractions of a 97-kDa protein were pooled, concentrated, and loaded on a second column of hydroxyapatite (BioRad, Hercules, CA) equilibrated in 5 mM KH_2PO_4, 5 mM K_2HPO_4 pH 7.0, 50 mM KCl, 2 mM DTT and one tablet of protease inhibitor cocktail (Complete, Boehringer-Mannheim). The column was washed with the same buffer, and eluted with a 5–250 mM phosphate gradient. The immunoreactive samples were pooled, concentrated and loaded onto an anionic exchange column of DEAE-Sephacel (Sigma), equilibrated in 5 mM DTT, 1 mM ATP in 160 mM potassium phosphate buffer, pH 7. The column was washed with the same buffer and fractions from the flowthrough were tested by Western blot. The immunoreactive samples were pooled, the phosphate lowered to 25 mM, and loaded onto a DEAE-Sephacel column equilibrated with 25 mM potassium phosphate buffer pH 7.0, added with 2 mM DTT. The fractions in the flowthrough, with the immunoreactive anti-(calf thymus actin) 97 kDa band were pooled and combined with 1 mM ATP, 1 mM EGTA, 1 mM EDTA, 5 mM DTT, one tablet of Complete™, 20% glycerol, and stored at −20 °C until further analyses. The bound 95 kDa protein was eluted with a linear gradient of 0–1.5 M KCl in 25 mM potassium phosphate, 2 mM DTT, pH 7.0, and stored as above until further analyses. The preparation containing the 97 kDa polypeptide was also subjected to hydrophobic phase partition in TX-114 [11]. Briefly, the protein solution (15 μg) was mixed with Triton X-114 (1–1.2% final concentration) at 4 °C, and then incubated at 25 °C followed by centrifugation. The two phases were recovered and partitioned again. The Triton X-114 phase was recovered separately and washed repeatedly with 50 mM Tris–HCl, 2 mM $MgCl_2$, pH 7.0. The protein that remained in the detergent phase was analyzed.

2.3. Lipoxygenase assay

The lipoxygenase activity assay was adapted from Smith et al. [12]. Briefly, 8% polyacrylamide native gels were loaded with protein samples in Laemmli's buffer [13] without β-mercaptoethanol (β-ME) and SDS. The gels were incubated with 13 mg ortho-dianisidine and 25 μl of 1 M linolenic acid in 25 ml 0.1 M phosphate buffer pH 6.6, with gentle agitation in the dark until bands appeared.

2.4. Antibodies and reagents

Anti-(calf thymus actin) and anti-(chicken gizzard myosin) antisera were a kind gift of Dr. John L. Wang (Michigan State University, East Lansing, MI). Commercial anti-myosin antibodies and secondary antibodies were purchased from Zymed Laboratories (San Francisco, CA). Anti-(soybean lipoxygenase) antibodies were a kind gift of Dr. James Siedow (Duke University, Durham, NC) and have been shown to immunolocalize successfully common bean lipoxygenase [14]. Soybean lipoxygenase and rabbit muscle myosin were from Sigma.

2.5. SDS-polyacrylamide gel electrophoresis (SDS-PAGE), two-dimensional SDS-PAGE (2-D PAGE), and immunoblotting

Proteins were analyzed by SDS-PAGE according to Laemmli [13]. In some cases, proteins were analyzed by 2-D PAGE using isoelectric focusing gels in the first dimension, and SDS-PAGE on the second dimension [15]. The gels, either one- or two-dimensional, were stained for protein or electrotransferred for immunoblotting [16]. Electrotransferred membranes were blocked overnight at 4 °C, and incubated with the appropriate primary antibody in PBS-T (0.5% Triton X-100 in 0.14 M NaCl, 0.01 M NaH_2PO_4/Na_2HPO_4, pH 7.5). Membranes were washed with PBS-T and then incubated with the appropriate alkaline phosphatase-conjugated secondary antibodies. After washing, alkaline phosphatase activity was developed with nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate according to the manufacturer (Boehringer-Mannheim).

2.6. Protein quantitation and amino acid analysis

Protein was quantitated with the commercial Bradford [17] dye-binding assay (BioRad). For amino acid sequence analysis, protein bands were excised from 10% polyacrylamide gels. Some proteins were digested according to Medeiros et al. [18] and peptides sequenced at the Protein/Peptide Sequencing Facility of the National Institutes of Health, Bethesda, MD, by either Edman degradation, or mass spectrometry. Hydrophobic 97 kDa protein was trypsin-digested and sequenced at the Harvard Microchemistry Facility of Harvard University (Harvard, MA), by microcapillary reverse-phase HPLC nano-electrospray tandem mass spectrometry (μLC/MS/MS) on a Finnigan LCQ DECA quadrupole mass spectrometer.

3. Results

3.1. Antibodies against calf thymus actin and chicken gizzard myosin cross-react with a 97-kDa polypeptide in the soybean embryo axis extract

Anti-(calf thymus actin) antiserum immunostained the 42 kDa actin polypeptide (Fig. 1A, lane 1, lower right arrow), the 212 kDa band corresponding to myosin heavy chain (Fig. 1A, lane 1, left arrow), and a couple of bands around 90 kDa (Fig. 1A, lane 1), of chicken muscle extracts. The same antibody was able to immunostain a 42 kDa actin polypeptide from soybean embryo axis, and bovine smooth muscle extracts (Fig. 1A, lanes 2 and 3, respectively, lower right arrow). Interestingly, a 97 kDa polypeptide was strongly immunostained in the soybean embryo axis extract (Fig. 1A, lane 2, upper right arrow). This same protein was also immunostained, albeit to a lesser extent, with anti-(chicken gizzard myosin) antibodies (Fig. 1B lane 1, left arrow). This antibody also immunostained chicken muscle myosin (Fig. 1B, lane 2, right arrow), but again, to a lesser extent. Based on the criteria of cross-reaction with these antibodies, a purification scheme for the 97 kDa protein from embryo axes was designed.

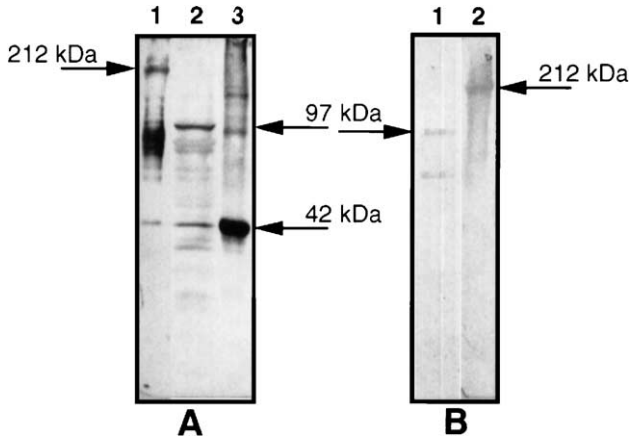

Fig. 1. Western blot analysis of Glycine max embryo axes proteins with anti-(calf thymus actin) (A) and anti-(chicken gizzard myosin) (B) antibodies. Dry embryo axes were extracted as described in Materials and methods and run on 10% polyacrylamide denaturing gels, followed by transfer and immunodetection. (A) Two prominent bands from the embryo extract cross-reacted with anti-(calf thymus actin) antibodies (lane 2), actin (42 kDa) and a 97 kDa protein. This antibody also cross-reacted with the 212 kDa chicken muscle myosin heavy chain and actin (lane 1), and bovine actin (lane 3). (B) The same 97 kDa protein in embryo extracts was detected with anti-(chicken gizzard myosin) antibodies (lane 1). The antibody also immunostained the 212 kDa chicken muscle myosin heavy chain (lane 2). The uppermost arrows point towards the position of migration of the 212 kDa polypeptide; the middle arrows towards the 97 kDa polypeptide; and the lower arrow towards the 42 kDa polypeptide.

3.2. The 97 kDa protein is composed of several isoforms

We followed the fractionation of the anti-(calf thymus actin) cross-reactive material through a series of columns. The first step was gel filtration on Sephacryl S-300, in which the cross-reactive material concentrated to a few fractions with a complex protein composition (not shown). The immunoreactive material was loaded on a hydroxyapatite column and eluted with a phosphate gradient. The immunoreactive proteins which eluted at ~ 160–180 mM phosphate were pooled and loaded onto a DEAE-Sephacel column previously equilibrated with 160 mM phosphate. Under these conditions, the proteins were recovered in the flowthrough (Fig. 2A and E) and co-eluted with actin (Fig. 2E, lower left arrow) similar to the hydroxyapatite eluted fractions (not shown). The protein sample was dialyzed against 25 mM phosphate and loaded again on the column pre-equilibrated in this lower ionic strength buffer. Under these conditions, the 97 kDa protein was recovered in the flowthrough (Fig. 2B and F, lanes 1–5 and 1–3, respectively). The protein pattern in this last fraction was mainly composed of the 97 kDa protein (Fig. 2B, lanes 1–4). After eluting the column with a 0–1.5 M KCl gradient, two main proteins of slightly lower molecular weight than 97 kDa were observed to elute at 22 mM KCl (Fig. 2C and G, KCl, lower right and right arrows, respectively). The 95 kDa protein was also less reactive with the anti-(calf thymus actin) antibodies (Fig. 2G, right arrow). This was a first indication that the 95 and 97 kDa proteins were different isoforms. In addition, a high-purity 97 kDa protein could be recovered in the hydrophobic phase of Triton X-114 after phase partition and was observed as a single band by silver staining (Fig. 2D, TX-114, upper right arrow) This feature indicated that this was also a different isoform of the protein. Immunostaining of the enriched proteins on two-dimensional gels revealed two isoforms of the 97 kDa protein of p*I*'s 6.2 and 6.3 (Fig. 3A, arrows), whereas the 95 kDa species displayed two isoforms of p*I*'s 5.8 and 5.9 (Fig. 3B, arrows). These data

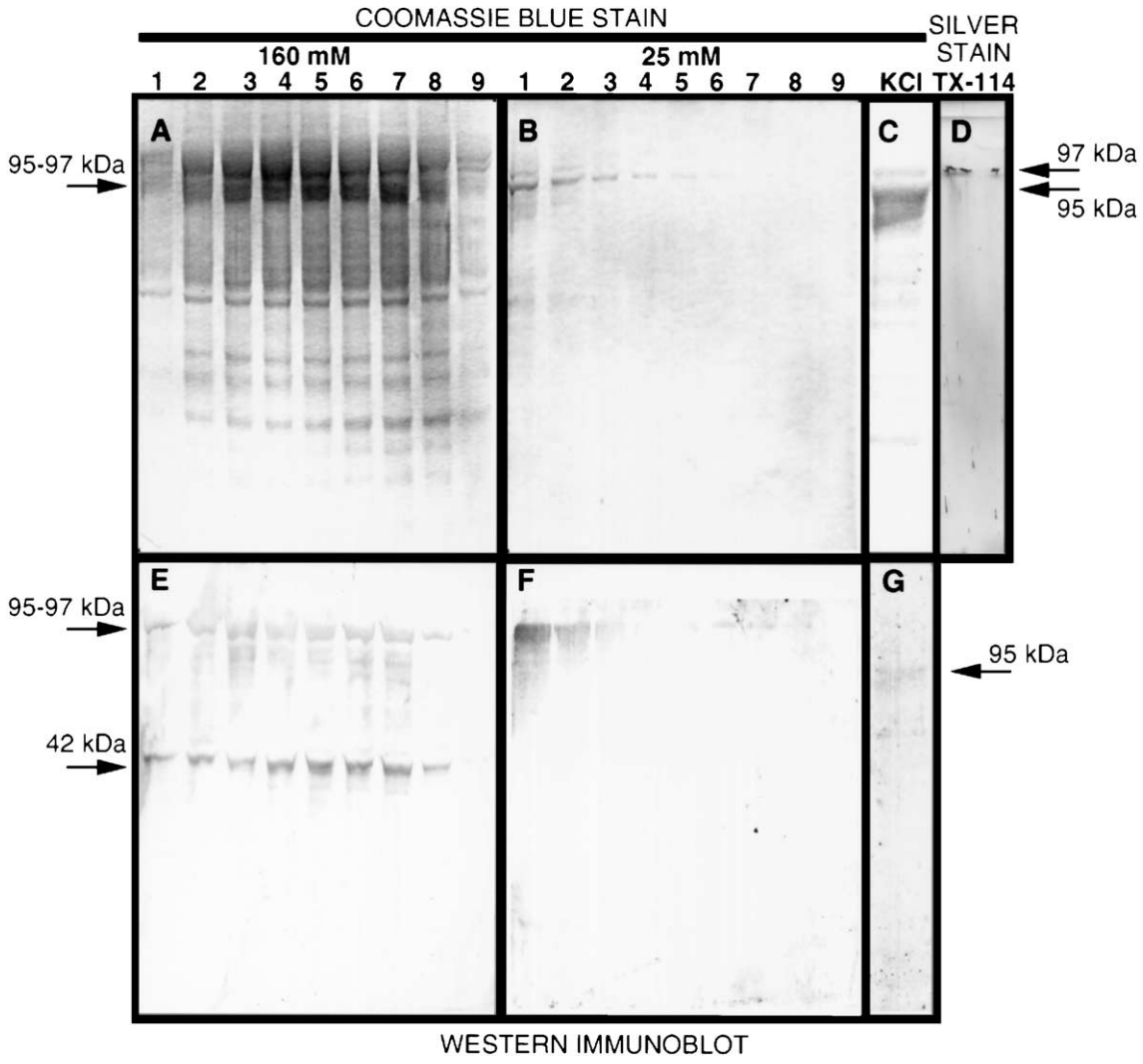

Fig. 2. Coomassie blue or silver-stained gels (top) and Western immunoblots (bottom) of fractions from further chromatographic steps after phosphate elution from the hydroxyapatite column. (A, E) Fractions loaded onto a DEAE-Sephacel column equilibrated in 160 mM phosphate as described in Materials and methods. The flowthrough fractions were analyzed and the cross-reactive protein was observed throughout the unbound material (E, lanes 1–9). The presence of soybean embryo axis actin is also observed in these fractions (42 kDa in E, lanes 1–9). (B, F) This material was concentrated, the phosphate lowered to 25 mM, and loaded onto a second DEAE-Sephacel column equilibrated in 25 mM phosphate. The cross-reactive proteins were observed throughout the unbound material (F, lanes 1–3). This column was then eluted with a KCl gradient and a main 95 kDa protein band and corresponding cross-reactive material were obtained in the fractions eluting at 22 mM KCl (C and G, KCl). The cross-reactive material from (B) was subjected to hydrophobic phase partition and the hydrophobic phase analyzed by SDS-PAGE and silver stain (D, TX-114). A single 97 kDa polypeptide was observed (D). The upper arrows in (A), (D), (E) and (G) point towards the position of migration of the 95–97 polypeptides. The lower arrow in (E) points towards the position of migration of the 42 kDa polypeptide.

further supported the existence of various isoforms of the proteins in the different preparations.

3.3. Sequence analysis of peptides and activity assays indicated that the proteins are lipoxygenases

Peptide sequence analysis of the purified 95 kDa protein showed perfect matches to lipoxygenase-1 from soybean (Table 1), and strong similarity to other lipoxygenases (not shown). The 97 kDa protein showed perfect matches to lipoxygenase-3 from soybean (Table 1), and strong similarity to other plant lipoxygenases (not shown). Furthermore, the hydrophobic 97 kDa protein showed numerous perfect amino acid matches to soybean lipoxygenase-2 (Table 1), and to a lesser extent, to other lipoxygenases (not shown). In addition, native gel activity assays showed strong lipoxygenase activity in a number of fractions along the purification starting with the crude extract (Fig. 4, lane 1). Activity was present in the Sephacryl S-300 (Fig. 4, lane 2), as well as the hydroxyapatite (Fig. 4, lane 3) immunoreactive fractions. The activity was then recovered in the flow-through fraction from the first DEAE-Sephacel column

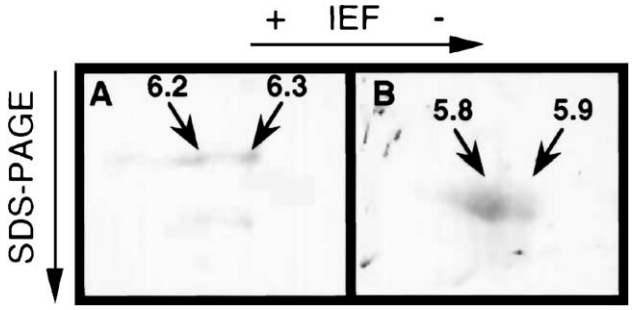

Fig. 3. Western blotting analysis of the 97 (A) and 95 (B) kDa protein fractions run on a two-dimensional gel, electrotransferred and immunostained with anti-(calf thymus actin) antibodies. The 97 kDa protein fraction appears as two spots of p*I*'s ~ 6.2 and 6.3 (A, arrows). The 95 kDa protein fraction appears as two spots of p*I*'s ~ 5.8 and 5.9 (B, arrows).

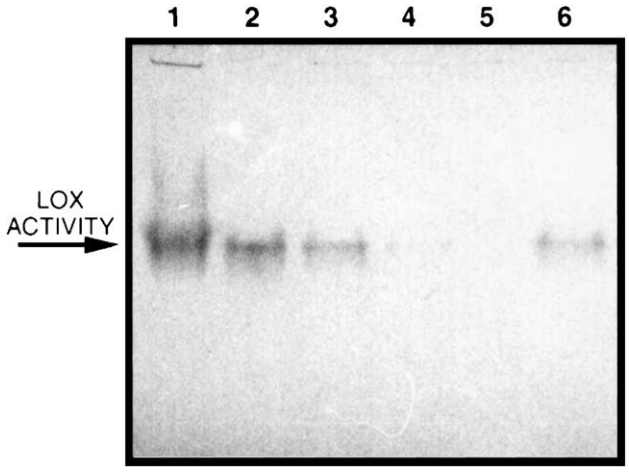

Fig. 4. Native gel activity assays for lipoxygenases. An 8% polyacrylamide gel was run under native conditions and the lipoxygenase activity was detected as described in Materials and methods. This activity was detected in: the total extract prior to the columns (1); the Sephacryl S-300 fractions containing anti calf-thymus actin cross-reactive material (2); the hydroxyapatite bound and phosphate eluted cross-reactive material (3); and in the unbound material from the DEAE-Sephacel column equilibrated in 160 mM phosphate (4). The activity was retained in the DEAE-Sephacel column equilibrated in 25 mM phosphate since no activity was detected in the flowthrough (5), and it was recovered in the KCl eluted material from the same column (6). The arrow indicates the bands displaying lipoxygenase activity (LOX activity).

(Fig. 4, lane 4). Finally, this activity could be observed in the fractions corresponding to the 95 kDa protein which eluted from the last DEAE-Sephacel column at 22 mM KCl (Fig. 4, lane 6). Surprisingly, the 97 kDa protein in the flowthrough fraction did not have any activity in similar assays (Fig. 4, lane 5), nor was any activity detected associated with the 97 kDa hydrophobic protein (not shown), suggesting that these are stored as inactive forms in the embryo axis tissue.

Table 1
Peptide sequences of the exact matches yielded by the mass spectrometric analysis of the digested 95 and 97 kDa protein gel bands with soybean lipoxygenases-1 and -2, respectively (NCBI protein accession Nos. CAA47717 and P09439, respectively).

Protein	Sequence	Amino acid position
95 kDa	PVLGGSSTFPYPR	204–217
(LOX-1)	GPTVTDPNTEK	223–233
	FPQPHVVQVSQSAWMTDEEFAR	327–348
	QINQLNSAK	419–427
	QSLINANGIIETTFLPSK	544–561
	NWVFTDQALPADLIKGVAIKDPSTPHGVR	573–602
	DDDVKNDSELQHWWK	636–650
	DNPHWTSDSK	768–777
	NNDPSLQGNR	800–809
97 kDa	KNVLDFNSVADLTK	28–41
(LOX-2)	SLTLEDVPNQGTIR	141–154
	SAWMTDEEFAR	367–377
	GLQEFPPKSNLDPTIYGEQTSK	390–411
	QSLINADGIIEK	572–583
	HSVEMSSAVYK	590–600
	GVAIKDPSAPHGLR	617–630
	RLLPEKGTPEYEEMVK	741–756
	HASDEVYLGQR	785–795
97 kDa	DEAFGHLK	261–268
(LOX-3)	HASDEVYLGER	776–786
	ALEAFK	797–802

The same exact matches yielded by Edman degradation analysis are shown for the other 97 kDa protein band, with soybean lipoxygenase-3 (NCBI protein accession No. AAB41272). The underlined letters show an EAF sequence that is found twice in lipoxygenase-3 and once in most myosins.

3.4. A variety of anti-myosin antibodies cross-react with the 97 kDa preparation and at the same time, anti-lipoxygenase antibodies recognize a 97 kDa protein in myosin preparations

We compared the reaction of a commercial anti-myosin antibody preparation, the anti-(chicken gizzard myosin), and the anti-(calf thymus actin) antibodies (Fig. 5, lanes 1, 2 and 3, respectively), against a purified 97 kDa preparation containing both lipoxygenases-2 and -3 (Fig. 5, lane 4). We observed that all of these three antibody preparations cross-reacted with the 97 kDa polypeptide, and the anti-(calf thymus actin) antibody yielded the strongest response (Fig. 5, lane 3). As expected, anti-(soybean lipoxygenase) antibodies reacted strongly against the same protein (Fig. 5, lane 5). We suspected that a similar 97 kDa protein was a contaminant of myosin preparations and was the one responsible for the elicitation of a particular set of antibodies. Thus, we tested anti-(soybean lipoxygenase) against two samples containing myosin. First, we observed that anti-(soybean lipoxygenase) antibodies were able to immunostain a 97 kDa polypeptide on a commercial myosin preparation (Fig. 5, lane 6). Second, a similar reaction was observed with the same antibodies on a chicken muscle extract (Fig. 5, lane 7). This polypeptide was also recognized by the same antibodies in an MSB-extracted supernatant from embryo axes (Fig. 5, lane 8). Finally, commercially purified soybean lipoxygenase was immunostained by

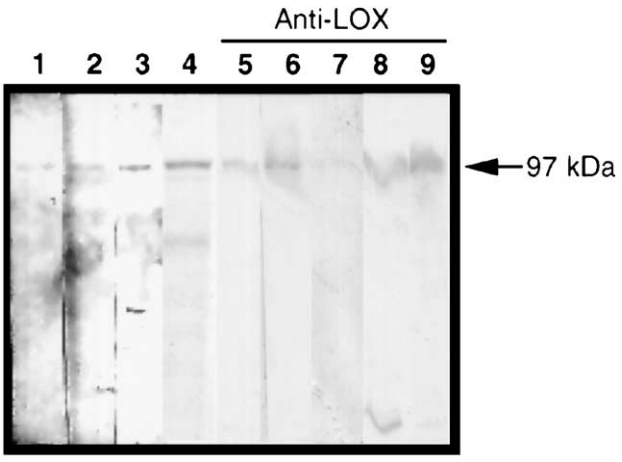

Fig. 5. Western immunoblotting analysis of anti-myosin and anti-lipoxygenase antibodies against the 97-kDa protein (lanes 1–3, 5), myosin preparations (lanes 6, 7) and embryo axis extract (lane 8). Two micrograms of the purified 97 kDa protein preparation showed mainly the 97 kDa polypeptide (lane 4). This same amount of protein was immunostained by: a commercial (Zymed) polyclonal anti-myosin antibody (lane 1); an anti-myosin antibody raised against chicken gizzard actin (lane 2); the anti-(calf thymus actin) antibody (lane 3); and an anti-(soybean lipoxygenase) antibody (lane 5). Anti-(soybean lipoxygenase) antibodies cross-reacted significantly with a 97 kDa polypeptide present in: 14 μg of commercially purified myosin (lane 6); 2.4 μg of chicken muscle extract (lane 7); ~ 75 μg of total soybean embryo axis extract (lane 8); and 0.16 μg of commercially purified soybean lipoxygenase (lane 9). The arrow points towards the position of migration of the 97 kDa polypeptide.

the anti-(soybean lipoxygenase) antibodies (Fig. 5, lane 9). All anti-myosin and anti-lipoxygenase antibodies immunostained the same 97 kDa band in all the samples. The specificity of these anti-(soybean lipoxygenase) antibodies has been previously demonstrated [14]. In addition, the same antibodies did not immunostain bovine serum albumin or molecular weight markers run on parallel blots (data not shown). These data strongly suggested that this cross-reaction was due to a contaminant animal lipoxygenase in the original actin and myosin preparations used for the corresponding antibody generation. Although this seems to be the reason for the cross-reactivity, we cannot rule out the possibility of the existence of common epitopes in both proteins (see below).

4. Discussion

Lipoxygenases are enzymes involved in the synthesis of signaling molecules and compounds with growth regulator activity [2,4]. Lipoxygenases from soybean have been previously isolated from the seed cotyledon [5], but their presence in embryo axes has been only marginally characterized. We designed a purification procedure based on conventional chromatography using this starting material, and obtained three distinct isoforms of lipoxygenases, one 95- and two 97-kDa proteins. Analysis of the lipoxygenase isovariant distribution by 2-D PAGE revealed at least two spots for the 95 kDa, and two others for the 97 kDa proteins. Some of these may be postranslationally modified proteins as reported previously [5]. Separate amino acid analysis of the purified proteins revealed that of these three isoforms, the 95 kDa protein corresponded to lipoxygenase-1, and the 97 kDa proteins corresponded to lipoxygenases-2 and -3. (Table 1). Interestingly, lipoxygenase-2 was in very low levels and it was only detected when concentrated in a Triton X-114 detergent phase suggesting that, in our hands, at least one of these 97-kDa proteins exists as a hydrophobic isoform. Hildebrand et al. [3] found that this isoform was also present at lower levels when compared to the other two in developing embryo axes.

We found that the 95 kDa lipoxygenase-1 isoform was the only one with detectable activity on native gels. The lack of activity of the 97 kDa lipoxygenases-2 or -3 in the dry embryo may be real and perhaps they are later activated during seed development. For example, Hildebrand et al. [3] were able to observe activity of all three isoforms at different developmental stages of the embryonic axes, but they were unable to detect activity in dry embryos. Nevertheless, we cannot rule out that the activity is lost during the purification process, although it could be argued that this hypothetical loss was not uniform for all three isoforms.

We purified the proteins based on cross-reactivity with antibodies against calf thymus actin [10]. The anti-(calf thymus actin) antibody revealed cross-reactivity against chicken muscle myosin heavy chain (Fig. 1, lane 1) and thus, due to the molecular weight of the cross-reactive protein in the soybean extracts, it was initially believed that these proteins corresponded to some heavy chain of a soybean myosin-like protein or its degradation product. As stated above, these turned out to be lipoxygenase isoenzymes. The reason for the cross-reactivity appeared to be a lipoxygenase contamination, which remains closely associated with myosin preparations (Fig. 5, lanes 6 and 7). If this was the case, this association may be similar to that of human 5-lipoxygenase with Grb2 and cytoskeletal proteins [19]. Alternatively, the existence of common or related epitopes in the myosin and soybean lipoxygenases cannot be ruled out. Although alignment of amino acid sequences do not reveal any significant similarity, a thorough characterization and determination of antibody binding sites on both proteins may reveal significant findings.

Our procedure to isolate lipoxygenases from soybean embryo axes is based on anti-myosin antibody cross-reaction with these proteins. Although this purification process can be monitored by enzymatic activity, this latter strategy would result in the loss of inactive lipoxygenase isoforms such as the lipoxygenases-2 and -3 reported here. The antibody cross-reactivity provides an alternative to this problem, although it would also be more accurate to use isoform-specific anti-lipoxygenase antibodies. Nevertheless, our purification procedure provides an alternative tool for the isolation and characterization of these enzymes from embryo axis tissue, and its application to other tissues is

possible. The excellent recent review by Loiseau et al. [4] further supports our data as a first report of lipoxygenase isoforms isolated from soybean embryo axes, and it will be useful for the comparison of their biochemical features in the latent soybean embryo axis, with those of subsequent developmental stages to assess possible activity switches and effects on differential expression.

Acknowledgements

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