Partial Purification and Characterization of Lipoxygenase of Canola Seed (*Brassica napus* var. Westar)

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Lipoxygenase was extracted from canola seeds (*Brassica napus* var. Westar) and partially purified by precipitation with solid ammonium sulfate at 20–50% of saturation. Further purification of the enzyme was performed by conventional ion-exchange chromatography on DEAE-cellulose. The partially purified enzyme demonstrated the presence of a major and two minor protein fractions which were also found in a commercial soybean lipoxygenase (Sigma Chemical Co.); however, the lipoxygenase activity profile indicates the presence of a single enzymatic active fraction in both canola seed extract and commercial soybean lipoxygenase. The pH for optimum activity was 7.5. The apparent K_m value calculated from the best straight line was 2.0×10^{-4} M. The effect of cyanide on the enzyme activity was investigated. The partially purified lipoxygenase from canola seed showed higher specificity for linoleic acid used as substrate as compared to linolenic acid. The activity of the canola lipoxygenase extract was considerably greater with linoleic acid as substrate when compared with linoleic acid esters; the observed order of activity was as follows: linoleic acid > monolinolein > dilinolein > trilinolein. However, the enzyme showed higher specificity for the canola lipid extract than for linoleic acid and its esters. A chromogenic reaction on polyacrylamide disc gel electrophoresis demonstrated that the enzyme activity in the canola lipoxygenase is associated with the major band.

INTRODUCTION

Lipoxygenase (EC 1.13.11.12) is a dioxygenase that catalyzes, as its primary activity, the hydroperoxidation by molecular oxygen of linoleic acid and other polyunsaturated lipids containing a *cis,cis*-1,4-pentadiene moiety (Axelrod et al., 1981). Lipoxygenase activity has been demonstrated in a wide range of plant tissues (Pinsky et al., 1971). Lipoxygenase from soybean has been isolated and studied extensively; however, the fragility of the enzyme, the complexity of its isolation and purification procedures, and its mode of action are some of the factors that contribute to the variation in the results reported in the literature (Galliard and Chan, 1980).

Interest in lipoxygenase is related to its action on endogenous unsaturated fatty acids resulting in the production of hydroperoxides. These hydroperoxides contribute to the formation of many aldehydes and alcohols which are responsible either for the desirable, fresh-vegetable flavors associated with normal metabolism of growing plants or for undesirable off-flavors which occur after harvesting and during storage or processing of many foods (Sessa, 1979).

The lipoxygenase activity in beans is characterized by a wide range of optimum pH varying from 6.0 to 9.0 and becoming inactive at pH greater than 11.0 (Galliard and Phillips, 1971). Tappel (1961) reported that soybean lipoxygenase activity was affected significantly by modification of the pH of the medium. The substrate selectivity for soybean lipoxygenase (Verhue and Franke, 1972). French bean lipoxygenase (Kermasha and Metche, 1986), and asparagus lipoxygenase (Ganthavorn and Powers, 1989) has been studied. Cyanide ion is a well-known inhibitor of heme proteins such as peroxidase; however, the addition of cyanide at a certain concentration resulted in an apparent increase in lipoxygenase activity (Kermasha and Metche, 1986). This apparent increase in activity could be explained by the fact that the partially purified extract may contain other competing enzymes such as catalase and peroxidases, which are also known to be inhibited by cyanide (Siddiqi and Tappel, 1956). Cyanide has been used to distinguish between heme protein and lipoxygenase-catalyzed oxidation (Ganthavorn and Powers, 1989).

Canola, an improved cultivar of rapeseed (*Brassica* sp.), is one of the principal commercial oilseeds in the world. Franke and Freshe (1954) reported the absence of lipoxygenase activity in rapeseed, while Bronisz et al. (1958) found a small degree of lipoxygenase activity in rapeseed extracts. St. Angelo et al. (1979) did not confirm the presence of lipoxygenase in rapeseed. Appleqvist (1972) suggested that the enzyme was probably present in rapeseed but could not be detected because of the presence of an inhibitor. Christophersen and Bremer (1972) reported that erucic acid inhibited fatty acid oxidation in mitochondria from rat heart and liver; since erucic acid is the most abundant fatty acid (40% of total fatty acid) in rapeseed, it was suggested that this acid could have an inhibitory effect on lipoxygenase activity. In addition, St. Angelo et al. (1979) reported that erucic acid inhibited the activity of lipoxygenase enzymes from soybean and peanut. Phenolic compounds are also known to form several types of bonds with proteins, e.g., hydrogen, covalent, ionic, and hydrophobic (Loomis, 1974). The mechanism of inhibition of lipoxygenase and the type of binding to lipoxygenase by erucic acid and by phenolic compounds have not yet been determined (Sessa, 1979). The fact that canola seeds contain less than 5% erucic acid (of total fatty acid) while rapeseed contains approximately 40% erucic acid suggests that the lipoxygenase activity of the two types of *Brassica* could be entirely different.

As far as we are aware, the lipoxygenase of canola has not been studied either in terms of isolation or in terms of its physicochemical characteristics. The aim of this work was to prepare an active lipoxygenase extract, to purify this extract, and to characterize the enzyme activity.

MATERIALS AND METHODS

Certified seeds of canola (*B. napus* cv. Westar), obtained from Agriculture Canada, Saskatoon, Saskatchewan, Canada, were

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used in this study. The seeds were cooled in a dry ice bath (−30 °C), homogenized to a fine powder, and successively defatted with acetone and diethyl ether according to the procedure described previously (Kermasha and Metche, 1986).

Preparation of Crude Extract. Lipoxygenase was extracted from the ground seed by using a modification of the procedure described by Kermasha and Metche (1986). The defatted powder was suspended in 0.1 M Tris-HCl buffer solution containing 0.1% Triton X-100 (ICN Biochemicals, Inc.) at pH 7.3 (1:3 w/v) with mechanical stirring for 16 h. All procedures were performed at 4 °C unless otherwise indicated. The resulting suspension was centrifuged for 30 min at 48000*g*, the pellet discarded, and the supernatant subjected to two additional centrifugation steps (30 min, 48000*g*). The supernatant was then subjected to three repeated ultracentrifugations at 200000*g* for 3 h. The pellet was discarded, and the supernatant (fraction I) was subjected to further purification.

Ammonium Sulfate Fractionation. The supernatant (fraction I) was treated with solid ammonium sulfate at 20% of saturation and allowed to stand for 30 min. The precipitate (fraction II) was obtained by centrifugation at 48000*g* for 1 h; the resulting supernatant was treated further with ammonium sulfate at 50% of saturation. The precipitate (fraction III) was obtained by centrifugation at 48000*g* for 1 h, and the supernatant was treated further with ammonium sulfate at 100% of saturation. The precipitate (fraction IV) was obtained by centrifugation at 48000*g* for 1 h. Fractions II–IV were resuspended in a minimal amount of Tris-HCl buffer solution (0.1 M, pH 7.3) and dialyzed against the same buffer, and any resulting insoluble material was removed by further centrifugation (1 h, 48000*g*). The active enzymatic fraction was dialyzed against the same buffer by using successive large volumes of Tris-HCl buffer in a relatively short period of time (4 h) to minimize any lose in enzyme activity. The dialyzed enzymatic fraction was then lyophilized and subjected to kinetic studies.

DEAE-Cellulose Chromatography. Fraction III was applied to a 1.6 cm × 30 cm column of DEAE-cellulose ion exchange (Sigma Chemical Co.) equilibrated with 0.5 M sodium phosphate buffer at pH 7.4 (buffer A). A linear gradient elution was used for the elution of active protein fraction; a mixture of buffer A and buffer B (buffer A containing 0.5 M sodium chloride) was used for this purpose. Fractions (2 mL) of eluent were collected at the rate of 30 mL/h. The absorbance of the eluted fractions was measured at 280 nm.

Protein Determination. The different fractions (I–IV) obtained during the purification procedures by ammonium sulfate precipitation were assayed for protein content by using a modification of the Lowry method (Hartree, 1972).

Substrate Preparation. Linoleic acid (*cis*-9,*cis*-12-octadecadienoic acid), linoleic acid (*cis*-9,*cis*-12,*cis*-15-octadecatrienoic acid), monolinolein (1-mono[(*cis,cis*)-9,12-octadecadienoyl]-*rac*-glycerol), dilinolein (1,3-di[(*cis,cis*)-9,12-octadecenoyl]-*rac*-glycerol), and trilinolein (1,2,3-tri[(*cis,cis,cis*)-9,12,15-octadecadienoyl]-*rac*-glycerol) as well as canola lipid extract were used as substrates for measurement of lipoxygenase activity. The substrates were prepared in Tris-HCl buffer solution (0.1 M, pH 7.3) according to the method described previously (Kermasha and Metche, 1986). The final concentration of each substrate in the solution was 4×10^{-3} M.

Canola lipid extract, used as endogenous substrate for the determination of lipoxygenase activity, was obtained as residual lipid fractions from the successive defatting process as described above and according to the procedure described previously (Kermasha and Metche, 1986). The extracting solvents, acetone and diethyl ether, were removed by flash evaporation under nitrogen atmosphere, and the lipid extract was recovered and used for the enzymatic assay.

Enzyme Assay. Enzyme assay was performed according to the procedure described previously (Kermasha and Metche, 1986). Lipoxygenase activity was measured by the increase in absorbance (A) at 234 nm (Surrey, 1964), using a Beckman DU-65 spectrophotometer. The specific activity of lipoxygenase was expressed as the increase in A (mg of protein)$^{-1}$ min^{-1}, where A is equal to 0.001 absorbance at 234 nm (Ali Asbi et al., 1989; Ganthavorn and Powers, 1989; Klein, 1976; Shastry and Rao, 1975).

Enzyme Substrate Selectivity. The substrate selectivity of canola seed lipoxygenase was assyed with linoleic acid, linolenic acid, monolinolein, dilinolein, trilinolein (Sigma) and canola lipid extract as substrates. The enzymatic assay was performed, under a gentle air stream in a 500-mL double-layered flask at 25 °C, according to the procedure described previously (Kermasha and Metche, 1986). The formation of hydroperoxides was measured by an increase in absorbance at 234 nm. The specific activity of lipoxygenase was expressed as the increase of A (mg of protein)$^{-1}$ min^{-1}, where A is equal to 0.001 absorbance at 234 nm.

Electrophoresis. Polyacrylamide disc gel electrophoresis (PAGE) was performed as described by Davis (1964) using Tris-glycine buffer (5 mM, pH 8.3). Sodium dodecyl sulfate (SDS)–PAGE was performed according to the method described by Weber et al. (1972). A chromogenic reaction for detecting lipoxygenase activity on the gels was performed according to the procedure described by Guss et al. (1967). The gels were incubated for 30 min at 25 °C in a reaction medium containing the substrate linoleic acid (2.5×10^{-3} M) in Tris-HCl buffer (0.1 M, pH 7.3). After enzymatic reaction, the gels were treated with 5% potassium iodide in 15% acetic acid. The lipoxygenase bands developed a brown-to-blue color within minutes. All electrophoresis experiments were performed with series of controls containing all components but without protein fraction. For the chromogenic experiment, an additional control trial using all components including substrate, but without enzyme extract, was also performed.

Determination of Phenolic Compounds. Phenolic compounds in the defatted canola seed, the crude enzymatic (fraction I), and the partially purified lipoxygenase (fraction III) were extracted according to the procedure described by Otsuka et al. (1989). The powdered samples were extracted successively with hexane, acetone, and a mixture of methanol/chloroform (2:1 v/v). The methanol/chloroform extract was suspended in distilled water, and the phenolic compounds were extracted with *n*-butanol. The total phenolic compounds was measured spectrophotometrically at 725 nm according to a modification of the method of Goldstein and Swain (1963). To the organic phase (0.5 mL), containing phenolic compounds, were added 7 mL of dionized water and 0.5 mL of 2 N Folin Ciocalteau solution (Fisher Scientific). The mixture was shaken and allowed to stand for 3 min before the addition of 1 mL of sodium carbonate solution (35%). The total volume was adjusted to 10 mL with deionized water. The mixture was allowed to stand in the dark for 30 min, and the absorbance was then measured at 725 nm.

Gas Chromatography. Fatty acid components of the extracted canola lipid were methylated and prepared for gas chromatography (GC) according to the method described previously by Badings and Jong (1983). GC analysis of the methylated fatty acids was carried out by using a FID-equipped Varian 3700 gas chromatograph fitted with a capillary DB 17 (0.53 mm × 30 m; Chromatographic Specialities Inc.). The initial column temperature was 120 °C, held for 2 min before being increased at 3 °C/min to a maximum of 260 °C and held for 15 min. Flow rates were set at 20 and 300 mL min for the hydrogen and air, respectively, while the carrier gas (N_2) flow rate was 0.6 mL/min. Injector and detector temperatures were 200 and 250 °C, respectively. Identification of individual fatty acids was based on retention times of purchased standards (Chromatographic Specialities), and quantitation was based on detector response relative to the concentration of each individual standard initially and use of an internal standard for the analytical runs.

RESULTS AND DISCUSSION

The properties of the fractions prepared by following purification procedures for the active lipoxygenase extract from canola are presented in Table I. Eighty-three percent of canola lipoxygenase was located in the fractions precipitated by ammonium sulfate at 0–20% and 20–50% of saturation. The low recovery of lipoxygenase activity (21%) in the 50–100% of saturation fractions may reflect the fact that the addition of ammonium sulfate at

Table I. Purification Scheme for Lipoxygenase Extract from Canola Seed

fraction	total protein,[a] mg	total act.,[b] units	sp act.[c]	recovery, %	purifn, x-fold
crude extract (fraction I)	2019	7571	3.75	100	0
0–20% ammonium sulfate precipitation (fraction II)	1743	9150	5.25	120	1.4
20–50% ammonium sulfate precipitation (fraction III)	1350	20250	15	267	4.4
50–100% ammonium sulfate precipitation (fraction IV)	986	8035	8.15	80	2.1

[a] Protein was determined according to the method of Hartree (1972), a modification of the Lowry method, using bovine serum albumin as standard. [b] Unit of activity is defined as A/min, where A is equal to 0.001 absorbance at 234 nm. [c] Specific activity is defined as A (mg of protein)$^{-1}$ min^{-1}, where A is equal to 0.001 absorbance at 234 nm.

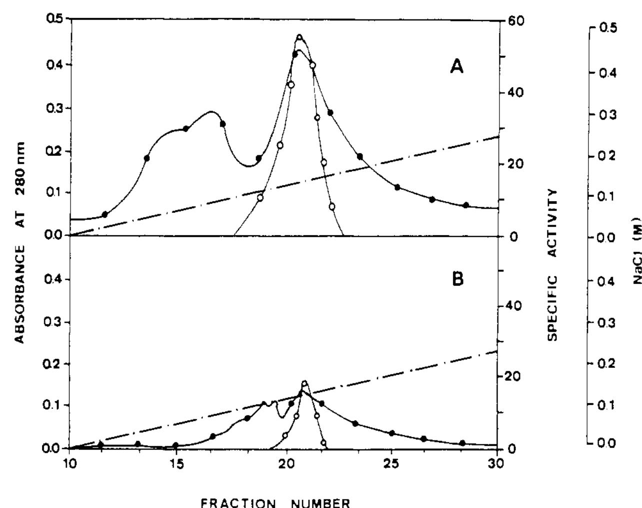

Figure 1. DEAE-cellulose gradient ion-exchange chromatograms of (A) commercial soybean lipoxygenase and (B) partially purified lipoxygenase extract (fraction III), obtained from canola seed. (●) Protein profile; (○) lipoxygenase activity profile.

high concentration can result in a drop in pH even in a well-buffered system (Kermasha and Metche, 1986). The results show the presence of relatively higher enzymatic activity (54%) in the 20–50% ammonium sulfate precipitate (fraction III). The activity in the fractions precipitated with ammonium sulfate (at 20–50% of saturation) was increased 4-fold when compared to that in the crude extract. This finding is in agreement with that reported for pea lipoxygenase (Haydar and Hadziyev, 1973) and for the French bean enzyme (Kermasha and Metche, 1986).

Comparative DEAE-cellulose ion-exchange chromatograms of commercial soybean lipoxygenase (Sigma) and the partially purified enzyme (fraction III) extract of canola seed are shown in Figure 1. The results show similar ion-exchange chromatographic behavior of the two enzyme preparations. Both chromatograms demonstrate the presence of a major peak and two minor peaks; however, the lipoxygenase activity profile indicates the presence of a single enzymatically active fraction in both canola seed extract and commercial soybean lipoxygenase. The chromatograms (Figure 1) show certain similarities to those obtained with immature English pea lipoxygenase (Chen and Whitaker, 1986) and with commercial soybean (Stevens et al., 1970). On the other hand, Wallace and Wheeler (1975) demonstrated the presence of four isozymes for wheat lipoxygenase, while two isozymes of wheat germ lipoxygenase have been separated on DEAE-cellulose (Wallace and Wheeler, 1979).

Higher plants generally contain various compounds, such as phenols, which act as enzyme inhibitors (Cheetham, 1985). Because of the relatively high content of phenolic compounds in canola seed and the likelihood of interference by phenolic compounds, work was also directed at quantitating the removal of phenolic compounds during the extraction procedures (Table II). A significant decrease in the amount of phenolic compounds was found in the enzymatic extracts when compared to the canola seed. The results (Table II) show that the 20–50% ammonium sulfate precipitated protein fraction (fraction III) only one-third and one-fourth of the phenolic compounds in the defatted canola seed and half of the phenolic compounds in the defatted canola seed and crude extract (fraction I). The decrease in the amount of phenolic compounds in the partially purified enzymatic extract could be an important factor in the removal of the inhibitory effect on lipoxygenase activity, since phenolic compounds can form several types of bonds with proteins (Loomis, 1974). Repetitive centrifugation was used to remove potential inhibitors, present in canola extract, since many workers suggested that lipoxygenase was probably present in rapeseed but could not be detected because of an inhibitor (Appleqvist, 1972). Phenolic compounds, such as catechol, can interact with proteins and subsequently inhibit enzymatic activity (St. Angelo et al., 1979); however, the mechanism of inhibition is not well understood.

Table II. Total Phenolic Compounds in the Defatted Canola Seed and Lipoxygenase Enzymatic Extracts

sample	phenolic compounds	
	%[a]	%[b]
defatted canola seed	7.6	100.0
crude enzymatic extract (fraction I)	4.8	63.1
20–50% ammonium sulfate precipitation (fraction III)	2.5	32.8

[a] Percent phenolic compounds (dry weight basis). [b] Percent phenolic compounds relative to initial phenolic content in canola seed.

Canola seed lipoxygenase showed a sharp pH optimum activity at pH 7.5. This finding is identical with that reported for snap bean (Klein, 1976) and close to the optimum pH (pH 7.3) of French bean seed (Kermasha and Metche, 1986) but somewhat higher than that (6.5–7.0) for isozyme 1 of English pea (Chen and Whitaker, 1986). The optimum pH of canola seed lipoxygenase activity was also close to that of soybean lipoxygenase 2 (pH 6–7) but different from soybean lipoxygenases 1 (pH 9.0–9.5) as reported by Axelrod et al. (1981).

The kinetic determinations of values of K_m and V_{max} of the partially purified lipoxygenase (fraction III) were determined from a Lineweaver–Burk plot (Lineweaver and Burk, 1934). The apparent K_m value calculated from the best straight line was 2.0×10^{-4} M. This value is close to that of lupin see lipoxygenase (2.4×10^{-4} M) reported by Olias and Valle (1988) but lower than that reported (Holman, 1947) for soybean lipoxygenase (1.35×10^{-3} M). Slightly higher values have been reported for pea seed lipoxygenase, e.g., 3.08×10^{-3} M (Klein, 1976), 2.3×10^{-3} M (Haydar and Hadziev, 1973), and for immature English pea lipoxygenase, e.g., 2.0×10^{-3} M (Chen and Whitaker, 1986) and 2.6×10^{-3} M (Reynolds and Klein, 1982). This discrepancy is probably due to differences in the source of the enzymes and the assay methods (Klein, 1976).

The results (Figure 2) indicate that the addition of cyanide to the partially purified enzyme extract (fraction

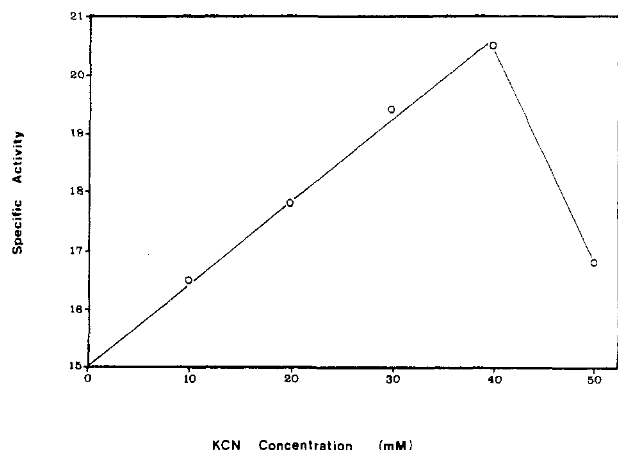

Figure 2. Effect of cyanide concentration on the partially purified lipoxygenase activity of canola seed extract (fraction III).

Table III. Fatty Acid Composition of Canola Lipid Extract

fatty acid	relative %[a] canola[b]	relative %[a] reference[c]
16:0	6.8	4.0
18:1	51.3	55.0
18:2	35.2	26.0
18:3	6.7	10.0

[a] Percent of each fatty acid relative to the total fatty acid content in the lipid fraction. [b] Fatty acid analysis of canola (*B. napus* var. Westar) used in this study. [c] Fatty acid analysis of canola (*B. napus* var. Regent), previously reported by Eskin (1987).

III) resulted in an increase in the apparent activity; however, concentrations above 40 mM led to a decrease in lipoxygenase activity. These results are similar to that reported for French bean lipoxygenase (Kermasha and Metche, 1986). Cyanide ion was reported to inhibit partially purified lipoxygenase from peanut at concentrations greater than 1 mM (Siddiqi and Tappel, 1957), while Sanders et al. (1975) reported that cyanide ion concentration below 14 mM had little effect on the activity of a purified alkaline isozyme of peanut lipoxygenase but inhibited an acid isozyme lipoxygenase from the same seed. De Lumen et al. (1978) demonstrated that 50% inhibition of green bean lipoxygenase was obtained at 20 mM final cyanide concentration. Kermasha and Metche (1986) showed that the lipoxygenase activity of a partially purified French bean extract increased 2-fold when it was incubated with 40 mM potassium cyanide; higher concentrations of cyanide resulted in a decrease in lipoxygenase activity. More recently, Ganthavorn and Powers (1989) reported that asparagus lipoxygenase, purified on (carboxymethyl)-cellulose chromatography, was 90% inhibited by 5×10^{-4} M KCN, whereas the 50% ammonium sulfate pellet was less markedly inhibited by cyanide. The apparent increase in lipoxygenase activity in the presence of low cyanide concentrations could be explained by the fact that the partially purified extract may contain other competing enzymes such as catalase and peroxidases, which are also known to be inhibited by cyanide (Siddiqi and Tappel, 1956).

The fatty acid analysis of canola seed lipid extract, performed by gas–liquid chromatography, is summarized in Table III. The overall analysis is close to that reported in the literature (Eskin, 1987).

The enzyme substrate specificity of canola lipoxygenase extract (fraction III) was assayed for free linoleic acid, linolenic acid, monolinolein, dilinolein, trilinolein, and canola lipid extract. The results indicate that partially purified canola lipoxygenase (Table IV) has higher specificity for linoleic acid as substrate compared to linolenic acid. These findings agree with those reported for winged bean lipoxygenase (Van Den et al., 1982). On the other hand, commercial soybean lipoxygenase demonstrates enzymatic specificity with both linoleic and linolenic acids (Table IV), and these results are similar to those reported for lipoxygenase from potato tuber (Pinsky et al., 1973) and germinated sunflower seed (Leoni et al., 1985). The results (Table IV) also show that canola lipoxygenase has greater specificity for linoleic acid as substrate when compared with linoleic acid esters; the observed order of activity was as follows: linoleic acid > monolinolein > dilinolein > trilinolein. These results are similar to that reported for the partially purified lipoxygenase from French bean lipoxygenase (Kermasha and Metche, 1986). However, these results are somehow different from those reported for asparagus (Ganthavorn and Powers, 1989), which showed that the asparagus lipoxygenase was active on monolinolein as well as linoleic acid, but activity was very low on di- and trilinolein. On the other hand, canola lipoxygenase showed higher specificity for canola lipid extract compared to linoleic acid and its esters (mono-, di-, and trilinolein). The greater activity of lipoxygenase observed with the canola lipid extract compared to that with linoleic acid could be explained by the concept of preferred conformational enzyme–endogenous substrate reaction which may be related to the charge and polarity of the substrate (Bild et al., 1977); however, this higher activity was not due to the presence of free fatty acids whose content in canola lipid is relatively low (0.5–0.8%; Eskin, 1987).

Table IV. Substrate Selectivity of the Partially Purified Lipoxygenase of Canola Seed Extract (Fraction III)

source of lipoxygenase	sp act.[a] linoleate	linoleneate	monlinolein	dilinolein	trilinolein	canola oil
canola[b]	19.1	5.7	8.5	7.3	1.0	16.0
soybean[c]	58.5	51.4	28.3	25.0	4.5	8.0

[a] Specific activity is defined as A (mg of protein)$^{-1}$ min^{-1}, where A is equal to 0.001 absorbance at 234 nm. [b] Partially purified canola lipoxygenase obtained by precipitation with ammonium sulfate at 20–50% of saturation (fraction III). [c] Commercial soybean lipoxygenase (Sigma).

Electropherograms of disc electrophoresis of crude (fraction I) and partially purified lipoxygenase (fraction III) preparations from canola seed as well as commercial soybean lipoxygenase are shown in Figure 3. The crude extract shows the presence of two major and two minor bands (Figure 3a), while the partially purified enzyme extract shows the presence of one major and four minor bands (Figure 3b). The commercial soybean lipoxygenase shows the presence of four major and one minor bands (Figure 3c). SDS–PAGE (Figure 4) separated the lipoxygenase crude extract into one major and, at least, nine minor fractions (Figure 4a) and the partially purified extract into one major and three minor fractions (Figure 4b). By comparison, the commercial soybean lipoxygenase was separated into four major fractions and, at least, four minor fractions (Figure 4c). A chromogenic reaction on the polyacrylamide disc gel (Figure 5) demonstrated that the enzyme activity in the crude (Figure 5a) and the partially purified (Figure 5b) lipoxygenase extracts from canola seed (fraction III) is associated with the major band in each gel. This chromogenic reaction confirmed the presence of lipoxygenase activity in canola seed extract as well as the commercial soybean lipoxygenase (Figure 5c).

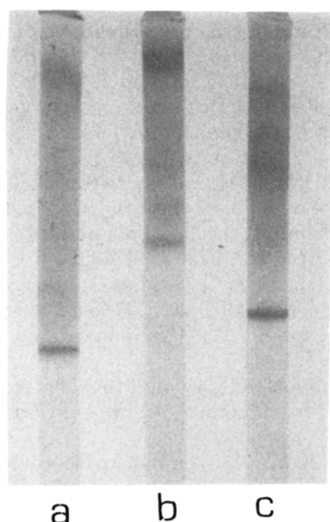

Figure 3. Polyacrylamide (PAGE) disc electropherograms: (a) crude lipoxygenase of canola seed (fraction I); (b) partially purified lipoxygenase of canola seed (fraction III); and (c) commercial soybean lipoxygenase.

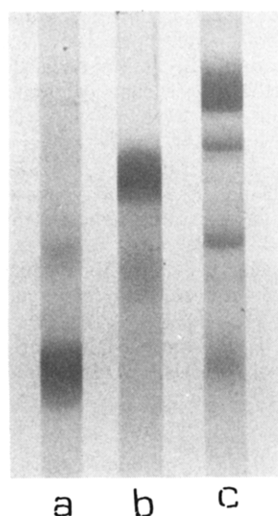

Figure 4. SDS-PAGE disc electropherograms: (a) crude lipoxygenase of canola seed (fraction I); (b) partially purified lipoxygenase of canola seed (fraction III); and (c) commercial soybean lipoxygenase.

CONCLUSION

The data gathered in this study indicate the presence of lipoxygenase activity in the seeds of canola (*B. napus* var. Westar). The partially purified enzyme showed an optimum activity at pH 7.5. The addition of cyanide to the partially purified enzyme extract (fraction III) resulted in an increase in the apparent enzyme activity; however, concentrations above 40 mM led to a decrease in lipoxygenase activity. The enzyme was further purified by conventional ion-exchange chromatography on DEAE-cellulose. The partially purified enzyme demonstrated the presence of a major and two minor protein fractions which were also found in a commercial lipoxygenase from soybean (Sigma). However, the lipoxygenase activity profile indicates the presence of a single enzymatically active fraction in both canola seed extract and commercial soybean lipoxygenase. A chromogenic reaction on polyacrylamide disc gel electrophoresis demonstrated that the enzyme activity in the canola lipoxygenase is associated with the major band compared. The partially purified lipoxygenase from canola seed showed higher specificity for linoleic acid used as substrate as compared to linolenic acid.

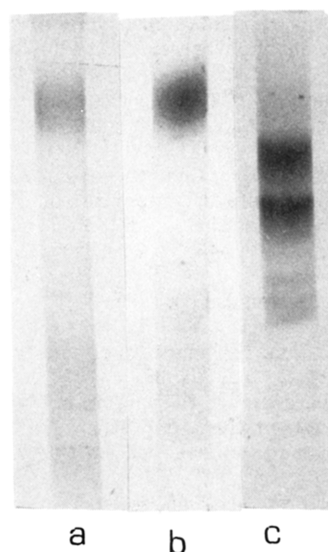

Figure 5. Chromogenic electropherograms: (a) crude lipoxygenase of canola seed (fraction I); (b) partially purified lipoxygenase of canola seed (fraction III); and (c) commercial soybean lipoxygenase, incubated with linoleic acid used as substrate.

The activity of the canola lipoxygenase extract was considerably greater with linoleic acid as substrate when compared with linoleic acid esters; the observed order of activity was as follows: linoleic acid > monolinolein > dilinolein > trilinolein. However, the enzyme showed higher specificity of the canola lipid extract then for linoleic acid and its esters.

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