Structure and Kinetic Thermal Stability Studies of the Interaction of Monohydric Alcohols with Lipoxygenase 1 from Soybeans (*Glycine max*)[†]

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Monohydric alcohols inhibited the activity of lipoxygenase 1 from soybean. The inhibition was reversible and of mixed type. Monohydric alcohols increased the Michaelis constant (K_m) and decreased the velocity maximum (V_{max}). At very low concentrations of alcohols, no structural changes were observed in the enzyme molecule, implicating that the inhibition by monohydric alcohols could be due to the binding of alcohols at the substrate binding/catalytic site. Monohydric alcohols at higher concentrations induced more ordered structure in the enzyme. The equilibrium constants obtained for native to helical intermediate(s) transitions are in the millimolar range and increased with the carbon chain length of alcohols. The free energy changes for the transitions are small but positive in nature. The environment around aromatic amino acid residues became more hydrophobic in these alcohols. Alcohols decreased the thermal stability of lipoxygenase. The activational enthalpy (ΔH^*) and activational entropy (ΔS^*) of thermal inactivation increased in the presence of alcohols.

Keywords: *Lipoxygenase; monohydric alcohols; stability; structure*

Lipoxygenase (linoleate:oxygen 13-oxidoreductase, EC 1.13.11.12) from soybeans is a single polypeptide chain of 839 amino acid residues consisting of two domains. The two domains are the N-terminal domain consisting of 146 residues in an eight-stranded antiparallel β-barrel and the major C-terminal domain consisting of 693 residues containing 23 helices and two antiparallel β-sheets (Boyington et al., 1993). It is an iron-containing enzyme devoid of any disulfide linkages, with a relatively greater proportion of hydrophobic amino acids (Shibata et al., 1987). The enzyme catalyzes the oxidation of linoleic acid and other polyunsaturated fatty acids containing one or more *cis,cis*-1,4-pentadiene systems (Axelrod et al., 1981). The enzyme which acts on long chain fatty acids with long hydrophobic tails has a hydrophobic substrate binding site located in the C-terminal domain of the enzyme (Sloane et al., 1991; Boyington et al., 1993).

The native globular structure of proteins are stabilized predominantly by noncovalent interactions (Jaenicke, 1991). Hydrophobic interactions play a dominant role in maintaining the structure and stability of proteins (Baldwin and Eisenberg, 1987). Alcohols such as methanol and trifluoroethanol are known to increase the alpha-helical structure of proteins at high concentrations (Singer, 1962). At low concentrations, alcohols are known to stabilize the enzymes at low temperatures (Brandts and Hunt, 1967; Shifrin and Parrott, 1975). The effects of alcohols (ranging from C_1 to C_{12}) and their thiol derivatives on kinetic properties and thermodynamics of lipoxygenase-catalyzed reactions have been reported from various laboratories. The mixed type of inhibition by monohydric alcohols with an increase in the carbon chain length (from C_1 to C_4) has been reported (Mitsuda et al., 1967). Recently, inhibition of

lipoxygenase by higher alcohols is shown to be of a competitive type in nature (Kuninori et al., 1992). On the basis of NMR spectroscopy measurements, Slappendel et al. (1982) have concluded alcohols bind in the vicinity of iron on the yellow form of lipoxygenase L1.

Here we present the data on the inhibition, structure, and thermal stability of lipoxygenase as affected by monohydric alcohols. Based on the measurements presented, a scheme is proposed to account for the interaction of monohydric alcohols with lipoxygenase 1 (LOX1).

MATERIALS AND METHODS

Soybean lipoxygenase (LOX1) was purified essentially by the method of Axelrod et al. (1981) with an additional step of molecular sieve chromatography on Sephadex G-75 (90 cm × 2.5 cm) using Braggs variety of soybean seeds after dehulling and defatting by hexane. This additional step of molecular sieve chromatography enhanced the specific activity from 110 to 140 units/mg of protein. The purity of the enzyme was ascertained by SDS–PAGE. The major band corresponding to a molecular weight of 97 000 accounted for more than 95% of the protein.

The following chemicals were used, linoleic acid (>99% purity) from Nucheck Prep Inc., Elysian, MN; SDS from Sigma Chemical Co., St. Louis, MO; DEAE Sephadex A50 and Sephadex G-75 from Pharmacia Fine Chemicals, Uppsala, Sweden; ultrapure acrylamide from LKB Biotechnology, Sweden, and used without further purification. Methanol, ethanol, *n*-propanol, and *n*-butanol were of analytical grade reagents from Sarabhai Merck, Baroda (India); potassium iodide was an analytical grade reagent from E-Merck, Bombay (India). Freshly distilled alcohols were used for all the experiments.

Enzyme Assay. The enzyme was assayed according to the method of Axelrod et al. (1981) by following the absorption changes at 234 nm due to the formation of conjugated dienes using Tween 20-solubilized substrate. One unit of enzyme activity is defined as the amount of enzyme required to form 1 μmol of product/min at 25 °C under the assay conditions. The enzyme concentration was determined by measuring optical density at 280 nm and using a value of $E_{1cm}^{1\%}$ at 280 nm = 14.0. The relative activity is expressed as a percentage ratio of enzyme activity in the presence of various alcohols to

the enzyme activity in the absence of alcohol at the end of 3 min. Kinetic constants were calculated using both the non-linear regression of Duggleby (1981) and the least squares analysis as described earlier (Srinivasulu and Rao, 1993).

Difference Spectroscopy. Difference spectra measurements were made at 25 °C using tandem cells of 10 mm path length in the Shimadzu double-beam spectrophotometer UV-160A in the wavelength region 220–340 nm. Difference spectra were obtained by placing the enzyme solution in the reference cell and the enzyme containing different concentrations of alcohol solution in the sample cell. The enzyme concentration was 4.7×10^{-6} M.

Fluorescence Measurements. Fluorescence measurements were made with a Shimadzu RF 5000 automatic recording spectrofluorometer with a 5 nm band width for both excitation and emission monochromators. The emission spectra of the enzyme were recorded after excitation at 285 nm at 25 °C in the region of 300–400 nm in both the presence and absence of alcohols at pH 9.0, 0.2 M borate buffer. The enzyme concentration was 7.6×10^{-7} M.

Fluorescence-Quenching Measurements. The quenching of enzyme fluorescence by acrylamide or potassium iodide in the presence of fixed concentrations of alcohols (2.5 M for methanol, 1.7 M for ethanol, 1.35 M for n-propanol, and 0.44 M for butanol) was measured. The measurements were made in 0.2 M borate buffer, pH 9.0. Temperature was maintained by circulating water through the cell. The enzyme samples at a concentration of 7.6×10^{-7} M were excited at 285 nm, and emission at 333 nm was recorded for quenching measurements. The band widths for excitation and emission monochromator were 5 and 10 nm, respectively. The enzyme fluorescence was quenched by progressive addition of acrylamide or potassium iodide. Sodium thiosulfate (0.1 M) was added to the KI solution to prevent I^{3-} formation. Since the absorption of potassium iodide at the excitation wavelength was not detectable, no correction was given for inner filter effect. The absorption of acrylamide at 295 nm was corrected using the equation (Lehrer and Leavis, 1978)

$$F_{corr} = F_{obs}10^{A/2} \tag{1}$$

where A is the increase in absorbance at the center of the cuvette by the addition of acrylamide. The quenching data were analyzed with the Stern–Volmer equation

$$F_0/F = 1 + K_{SV}(Q) \tag{2}$$

where Q is the quencher concentration and K_{SV} is the dynamic quenching constant. To monitor the conformational changes, fluorescence quenching data were also analyzed with the modified Stern–Volmer equation (Lehrer, 1971)

$$F_0/\Delta F = 1/(f_a)K_{SV}(Q) + 1/f_a \tag{3}$$

where ΔF is equal to $F_0 - F$ and f_a is the maximum fractional accessible protein fluorescence.

Circular Dichroism Measurements. The circular dichroism (CD) spectra were recorded at 25 °C on a Jasco J-20C spectropolarimeter as described previously (Srinivasulu and Rao, 1993). Path lengths were 1 and 10 mm depending upon the concentration of protein and the wavelength range. Each spectrum was a superposition of two or more scans. Mean residue ellipticity values (deg $cm^2/dmol^{-1}$) were calculated in the usual fashion using the mean residue weight 112 reckoned from the molecular weight of lipoxygenase. CD difference spectra were obtained by subtracting the ellipticity values mathematically at each wavelength for the sample from the control. In the presence of varying concentrations of alcohols, measurements were made after allowing 20 min for equilibration. Each spectra is at least an average of three determinations. The secondary structure of lipoxygenase in different alcohol solutions was analyzed by the method of Bolotina et al. (1980) as described earlier (Srinivasulu and Rao, 1993).

Transition Temperature Measurements. The lipoxygenase was incubated at different temperatures from 27 to 55
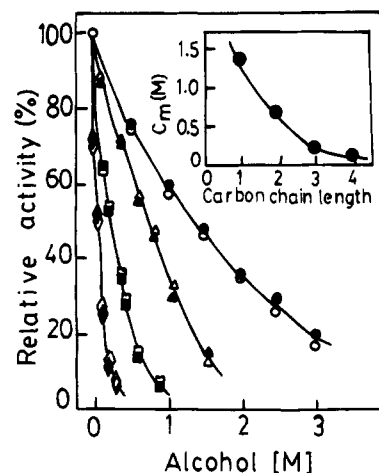

Figure 1. Effect of varying the concentrations of alcohols with an increase in carbon chain length on the relative activity of the enzyme (●, methanol; ▲, ethanol; ■, n-propanol; ♦, n-butanol). Inset shows the decrease in the midpoint of concentration (C_m) as a function of carbon chain length. Open symbols represent reversibility data.

°C for 15 min, and then the remaining activity was measured at 27 °C. From the plot of percent remaining activity versus temperature, the midpoint of transition (T_m) was calculated where 50% of the activity was diminished. The concentration of enzyme was 4.25×10^{-7} M.

Kinetics of Thermal Inactivation Measurements. The thermal stability of lipoxygenase in the presence and absence of alcohols was determined by measuring the inactivation rate constant (k_r) as a function of temperature in the range of 30–46 °C. The inactivation rate constant was obtained from the plot of the log percent remaining activity versus time. To determine activation energy values (E_a) for thermal inactivation, an Arrhenius plot was constructed. Activation parameters were then calculated as described by Moore (1962). Activation enthalpies (ΔH^*) were calculated according to

$$\Delta H^* = E_a - RT \tag{4}$$

where R is the universal gas constant and T is the absolute temperature. Activation free energy values (ΔG^*) were calculated according to

$$\Delta G^* = -RT \ln \frac{k_r h}{kT} \tag{5}$$

where k_r is the rate constant, h is the Planck constant, and k is the Boltzman constant. Activation entropy values (ΔS^*) were then calculated according to

$$\Delta G^* = \Delta H^* - T\Delta S^* \tag{6}$$

RESULTS

The effect of various monohydric alcohols of increasing carbon chain length on the relative enzyme activity is shown in Figure 1. From the plot of activity versus concentration, the midpoint concentration (C_m) at which 50% activity remained was determined and is shown in Figure 1, inset. The midpoint concentration decreased exponentially with increasing carbon chain length. From the midpoint of transition, the inhibition followed the order n-butanol > n-propanol > ethanol > methanol.

The Michaelis constant (K_m), velocity maximum (V_{max}), and V/K ratio for the kinetics of linoleic acid by enzyme were determined in the presence of different alcohols and are shown in Table 1. The monohydric alcohols increased K_m and decreased V_{max} and V/K ratio, suggesting the inhibition could be of a mixed type. How-
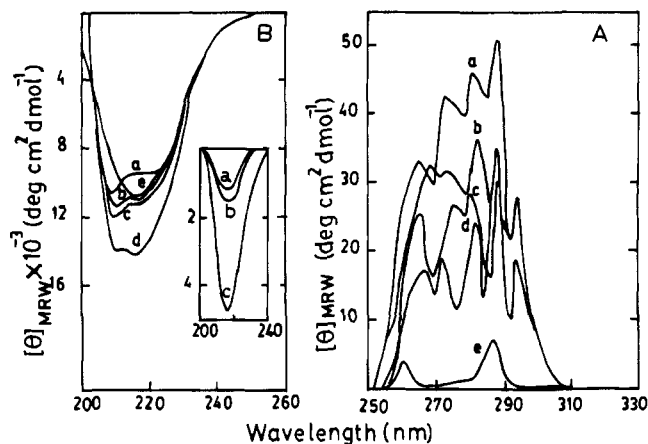
Figure 2. (A) Effect of monohydric alcohols on the near-UV CD spectra of lipoxygenase in the presence and absence of alcohols (a, control; b, 3.75 M methanol; c, 1.7 M ethanol; d, 1.34 M n-propanol; e, 0.66 M n-butanol). (B) Effect of monohydric alcohols on the far-UV CD spectrum of lipoxygenase in the presence and absence of alcohols (a, control; b, 5 M methanol; c, 3.4 M ethanol; d, 2.68 M n-propanol; e, 0.66 M n-butanol). Inset shows the difference of CD of methanol (a), ethanol (b), and n-propanol (c).

Table 1. Summary of K_m, V_{max}, and V/K in the Presence of Monohydric Alcohols

sample	concn (M)	K_m ($\times 10^{-5}$ M)	V_{max} (OD min^{-1})	V/K ($\times 10^{-5}$ min^{-1} M^{-1})
control		0.89	0.21	0.2
methanol	0.8	4.9	0.44	0.08
ethanol	0.4	2.2	0.15	0.07
n-propanol	0.2	11.2	0.50	0.05
n-butanol	0.1	12.2	1.03	0.008

ever, the alcohol concentrations used were not equimolar with respect to substrate.

Effect of Monohydric Alcohols on Enzyme Structure. In order to explain the nature of inhibition by monohydric alcohols, the effect on structure was studied at two levels of alcohol concentration, one at very low concentrations of alcohols where enzyme activity was inhibited and the other at higher concentrations of alcohols where they are known to induce formation of helical structure.

Fluorescence-Quenching Studies. To quantitate subtle changes in the conformation of the enzyme in the presence of very low concentrations of alcohols, the quenching of intrinsic fluorescence by acrylamide or potassium iodide (KI) was measured and analyzed by Stern–Volmer plots and modified Stern–Volmer plots. In the case of both acrylamide and potassium iodide, linear plots were obtained with the Stern–Volmer equation. In the case of acrylamide, the Stern–Volmer constant did not change with the addition of alcohols. The K_{SV} values were low compared to other enzymes, suggesting that fluorophores on the enzyme molecule were not easily accessible. In the case of potassium iodide, the K_{SV} values were very low, which did not change significantly with the addition of alcohols. The fractional accessibility values did not change by the addition of alcohols.

Circular Dichroism Measurements. The effect of various alcohols on the near-UV CD spectrum of lipoxygenase is shown in Figure 2A. The enzyme exhibited peaks at 293, 288, 283, 274, and 266 nm. Addition of methanol up to 2.5 M concentration did not significantly affect the near-UV CD bands; further increase in methanol up to 5 M concentration made the near-UV

Table 2. Analysis of Secondary Structure of Lipoxygenase in the Presence of Monohydric Alcohols

sample	concn (M)	% α-helix	% β-sheet	% β-bend	% a periodic
control		29.0	12.0	14.0	45.0
methanol	1.25	32.0	19.0	23.0	26.0
	2.50	35.0	19.0	23.0	23.0
	3.75	35.0	20.0	23.0	22.0
	5.00	28.0	27.0	20.0	25.0
	6.25	32.0	16.0	20.0	32.0
ethanol	0.85	31.0	23.0	24.0	22.0
	1.70	29.0	26.0	22.0	23.0
	2.55	31.0	18.0	23.0	28.0
	3.40	32.0	22.0	22.0	24.0
n-propanol	0.67	31.0	16.0	18.0	35.0
	1.34	35.0	22.0	20.0	23.0
	2.01	37.0	24.0	17.0	23.0
	2.68	38.0	23.0	16.0	23.0
n-butanol	0.11	35.0	16.0	21.0	28.0
	0.22	34.0	19.0	21.0	26.0
	0.44	30.0	18.0	19.0	33.0
	0.55	33.0	21.0	21.0	25.0

CD bands disappear completely. Ethanol, n-propanol, and n-butanol also disrupted the tertiary structure with increasing concentration of alcohol as reflected in diminution of the CD bands. In disrupting the tertiary structure, methanol is less effective then ethanol followed by n-propanol and n-butanol.

The effect of monohydric alcohols on the secondary structure of lipoxygenase was quantitated by the far-UV CD measurements (Figure 2B). Methanol up to 5 M concentration enhanced the ordered structure with concomitant decrease in aperiodic structure. Only at very high concentration (6.25 M) did it increase the aperiodic structure. In promoting the ordered structure, for a given molarity of alcohol, n-butanol is more effective than n-propanol, ethanol, and methanol (Table 2).

In the case of alcohols where miscibility with water was high, measurements could be made up to 8.0 M. The change in mean residue ellipticity values at 216 nm as a function of added alcohol molarity is shown in Figure 3A. Such plots for different alcohol-induced helical formation indicated the cooperativeness of transition, and from the sigmoidal curves, it is apparent that the transition from the native to helical intermediates appeared to be complete at 8 M concentration of alcohol. The midpoint of transition occurred at 4.1, 3.3, and 2.2 M for methanol, ethanol, and n-propanol, respectively. Plateau regions where the ellipticity values did not change significantly were observed at low and high alcohol concentrations. These values corresponded to those of the native protein and helical intermediate, respectively, and these were different for different alcohols. From these data, f_N (the fraction of protein in the native state) was calculated as a function of alcohol concentration. A plot of f_N versus alcohol molarity is given in Figure 3B, and the equilibrium constants estimated for the transition from the native to helical intermediates were 0.24, 0.30, and 0.46 M^{-1}, respectively. The secondary structural changes induced by alcohols were reversible as checked by dilution and dialysis (data are shown in Figure 3A).

Difference Spectra Measurements. The difference spectra in the presence of various alcohols in the region 220–340 nm were recorded (data not shown). In the case of methanol (up to 5 M), ethanol (3.5 M), and n-propanol (2.68 M), positive difference spectra were obtained in the region 240–300 nm suggesting a more apolar environment around the aromatic chromophores.
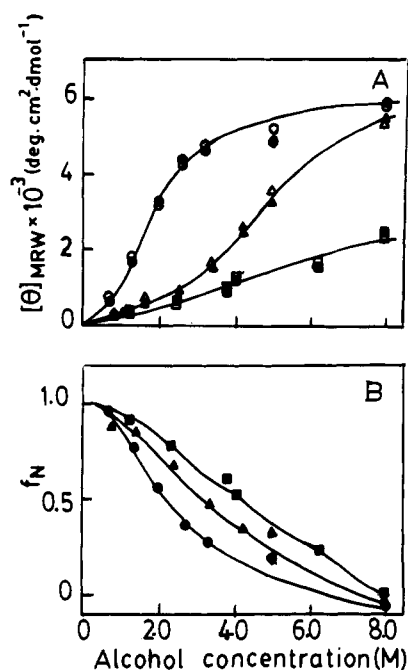
Figure 3. (A) Change in (Θ) MRW values at 216 nm as a function of added alcohol concentration. (B) f_N versus added alcohol concentration (■, methanol; ▲, ethanol; ●, n-propanol). Open symbols represent reversibility data.

In the case of n-butanol, even at 0.33 M concentration negative difference spectra were obtained. The observed difference spectra could be due to both burying of chromophores and clustering of the alkyl moiety of the alcohol about the chromophore to modify its behavior.

Fluorescence Emission Spectra Measurements. Lipoxygenase at pH 9.0 had an excitation maximum at 285 nm and an emission maximum at 333 nm, implying that the fluorescence emission was due to tryptophan residues in a hydrophobic environment. The effect of various alcohols on the fluorescence emission spectra is shown in Figure 4. Because of the addition of alcohols, there was enhancement of fluorescence intensity, and at higher concentrations of alcohol, the fluorescence emission shifted toward red.

Thermal Stability. The thermal stability of lipoxygenase as affected by alcohols was measured by determining the midpoint of irreversible thermal inactivation. Lipoxygenase was inactivated irreversibly at 55 °C (Figure 5), the midpoint of transition being 44 °C. Addition of alcohols decreased the midpoint of transition (Figure 5). The thermal inactivation of lipoxygenase followed first-order kinetics, and the estimated rate constants as a function of temperature for lipoxygenase in the presence and absence of monohydric alcohols are given in Table 3. The rate constants increased with an increase in temperature. The Arrhenius plot for the thermal inactivation of lipoxygenase is shown in Figure 6. The plots were linear in the range of the temperature studied. The estimated activational parameters for thermal inactivation are given in Table 3. Activation free energy (ΔG^*), enthalpy (ΔH^*), and entropy (ΔS^*) were calculated at each temperature. Table 3 shows that ΔH^* and ΔS^* values were not significantly affected for control by temperature. Addition of alcohols slightly increased the ΔG^* values, but ΔH^* and ΔS^* values increased.

DISCUSSION

Monohydric alcohols inhibit lipoxygenase activity. With increase in carbon chain length, the hydrophobic-

Table 3. Activational Parameters for Thermal Inactivation of Lipoxygenase 1

sample	temp (K)	rate constant k_r ($\times 10^{-4}$ s^{-1})	ΔG^* (kcal)	ΔH^* (kcal)	ΔS^* (eu)
control	303	0.6	20.7	17.0	−12.0
	309	1.0	21.4	17.0	−14.0
	315	1.5	22.0	17.0	−16.0
	319	2.9	22.8	17.0	−18.0
methanol	303	0.26	20.2	33.6	44.0
	309	1.13	21.5	33.6	39.0
	315	2.00	22.2	33.6	36.0
	319	5.60	23.2	33.6	33.0
ethanol	303	0.42	20.5	29.0	28.0
	309	1.26	21.6	29.0	24.0
	315	2.13	22.3	29.0	21.0
	319	5.90	23.2	29.0	18.0
n-propanol	303	0.70	20.8	34.5	45.0
	309	1.96	21.8	34.5	41.0
	315	5.20	22.8	34.5	37.0
	319	14.60	23.5	34.5	35.0
n-butanol	303	1.1	21.1	35.6	48.0
	309	4.7	22.8	35.6	41.0
	315	8.3	23.1	35.6	40.0
	319	29.2	24.2	35.6	35.0

ity of the alcohol increases (Herskovits et al., 1970). Since the extent of inhibition is dependent on carbon chain length, hydrophobic interactions must be playing a dominant role. The average hydrophobicity index of lipoxygenase 1 calculated from amino acid composition based on the Tanford scale (Nozaki and Tanford, 1971) is −1139 cal.

In the case of kinetics of oxidation of linoleic acid, the K_m increased, the V_{max} decreased, and the V/K ratio decreased in the presence of alcohols which are indicative of mixed type inhibition. Similar observation has been made by Mitsuda et al. (1967) for the inhibition of lipoxygenase by lower chain length alcohols. The concept of a mixed type of inhibition is compatible with the observation of Slappendel et al. (1982). Alcohols bind in the vicinity of iron on the yellow form; the catalytic site is close to but distinctly different from the site with which the alcohol binds. With higher alcohols, from C_6 to C_{12} carbon chain length, the competitive type of inhibition is reported (Kuninori et al., 1992). However, with higher alcohols it is conceivable that there would be an optimal chain length to fit into the substrate binding site.

Fluorescence-quenching data by potassium iodide and acrylamide suggested the inaccessibility of chromophores in the native state. Addition of a small concentration of alcohols which inhibited the enzyme activity (~90%) did not affect the Stern−Volmer constant nor the fractional accessibility of fluorophores. Alcohols at these concentrations did not cause any detectable conformational changes in enzyme, which implied that the inhibition of lipoxygenase by monohydric alcohols could be linked to the binding of the alcohols at the substrate binding/catalytic site and is not due to structural changes in the enzyme.

To quantify the effect of high concentrations of various alcohols on the structure of the enzyme, the conformational changes were measured by circular dichroism in both near-UV and far-UV regions. In the near-UV region, the lipoxygenase exhibited peaks at 293, 288, 283, 274, and 266 nm. These bands could be due to tryptophan, tyrosine, and phenylalanine residues (Strickland, 1974). Assignment of the individual bands was difficult because of the overlapping nature of the tryptophan and tyrosine bands. However, the longer wavelength bands like 293 and 288 nm were possibly due to
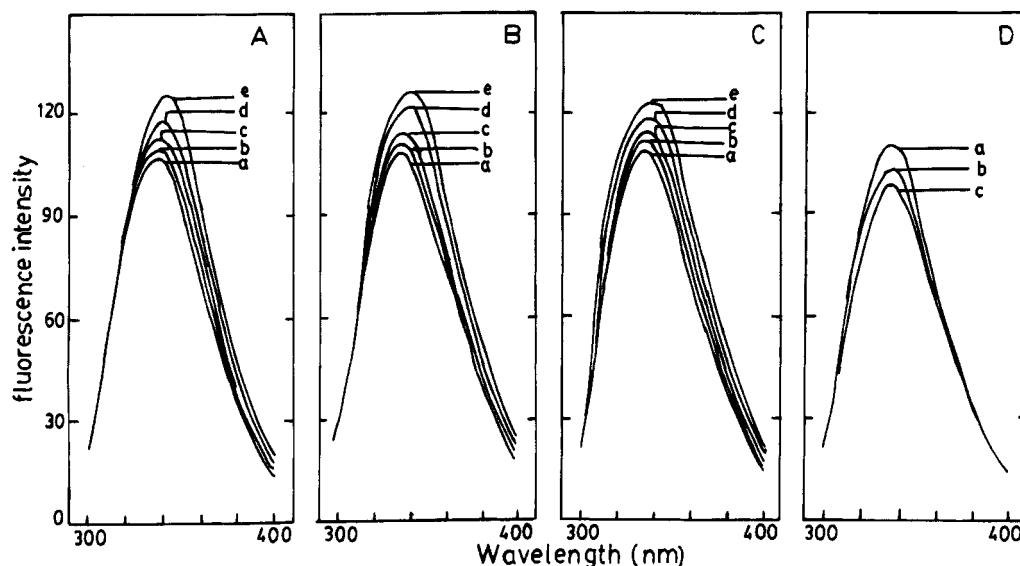
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Figure 4. Effect of monohydric alcohols on the fluorescence emission spectra of lipoxygenase in the presence and absence of alcohols. (A) Methanol: a, control; b, 1.25 M; c, 2.5 M; d, 3.75 M; e, 5.0 M. (B) Ethanol: a, control; b, 0.85 M; c, 1.7 M; d, 2.55 M; e, 3.4 M. (C) Propanol: a, control; b, 0.67 M; c, 1.34 M; d, 2.01 M; e, 2.68 M. (D) Butanol: a, control; b, 0.33 M; c, 0.66 M. The concentration of enzyme was 7.6×10^{-6} M.
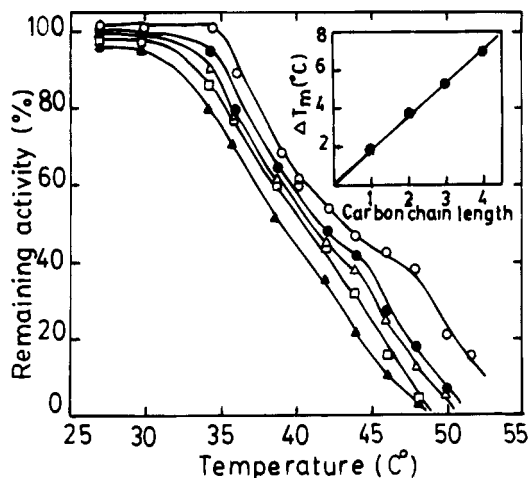

Figure 5. Effect of temperature on the irreversible thermal inactivation of lipoxygenase in the presence and absence of alcohols. Inset shows the change in T_m as a function of carbon chain length. (○) Control; (●) methanol (0.5 M); (△) ethanol (0.34 M); (□) n-propanol (0.27 M); (▲) n-butanol (0.22 M).

Figure 6. Arrhenius plot of thermal inactivation of first-order rate constants of lipoxygenase (●, control; ▲, methanol; ■, ethanol; ▲, n-propanol; □, n-butanol).

tryptophan; the 283 and 274 nm bands could be due to tyrosine residues, while the 260 nm band could be possibly due to phenylalanine. Similar observation has been made for the near-UV CD spectra of lipoxygenase (Spaapen et al., 1979). All the alcohols disrupted the tertiary structure, at higher concentration, the effectiveness being dependent on the carbon chain length of the alcohol used.

Alcohols are known to increase the helical content of proteins. The transition induced by alcohols has been interpreted in terms of binding of alcohol to nonpolar groups on the protein molecule (Arakawa and Goddette, 1985). In case of lipoxygenase, results indicate an increase in both α-helical and β-structure with concomitant decrease in aperiodic structure (Table 2). The difference CD spectra point to a formation of β-structure (Figure 2B, inset). The estimated equilibrium constants are in the millimolar range and are comparable to other systems (Arakawa and Goddette, 1985). The free energy changes calculated for the transition are small but positive in nature (0.1—0.3 kcal/mol). At higher concentration of monohydric alcohols, helical structure
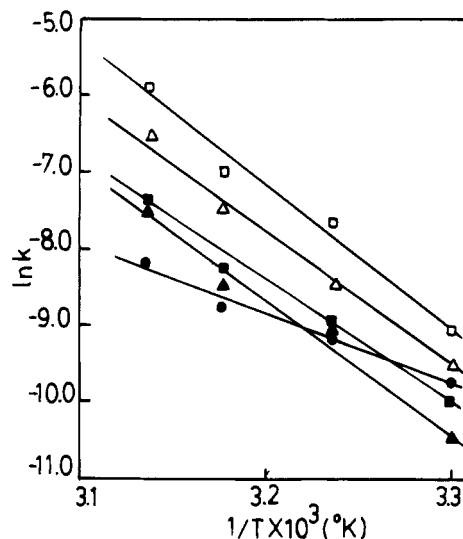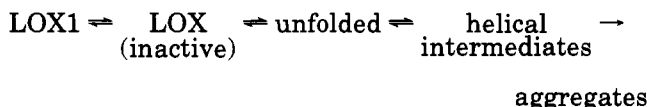
enhanced, whereas the tertiary structure was disrupted, suggesting possibly the tertiary structure of lipoxygenase was stabilized by the hydrogen-bonding networks between the side chains of the helices.

Monohydric alcohols decreased the thermal stability of lipoxygenase as reflected in the decrease in T_m and the increase in first-order rate constants of thermal inactivation. The linear relationship between carbon chain length and decrease in T_m suggests the predominance of hydrophobic interactions in the stability of the native molecule which is supported by increased activational enthalpy (ΔH^*) and entropy (ΔS^*) values derived from the Arrhenius plot in the presence of alcohols. Similar destabilization of other enzymes like lysozyme and ribonuclease by monohydric alcohols has been reported (Gerlsma and Stuur, 1974, 1976). Since the concentrations of alcohols used in thermal stability measurements are very low, they will have no effect on the dielectric constant of the media, and hence alcohols are very unlikely to affect the ionic interactions and van der Waals forces.

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In conclusion, it may be stated that lower alcohols inhibit the enzyme activity by mixed type inhibition. Based on the results obtained from the present studies, the following minimal scheme is proposed to account for the interaction.

$$LOX1 \rightleftharpoons LOX \rightleftharpoons unfolded \rightleftharpoons helical \rightarrow$$
$$(inactive) \qquad\qquad intermediates$$
$$aggregates$$

This mechanism can be explained in terms of the theory proposed by Arakawa and Goddette (1985). The first step involves the binding of one or two molecules of alcohols resulting in inactive lipoxygenase where there are no detectable structural changes. The second step accompanied by changes in tertiary structure can be explained by binding more molecules of alcohols to the intermediate than to the native state. The third step results in changes mainly in the secondary structure by less hydration sites for the helical state. The equilibrium constant obtained for the transition for the helical intermediates lends credence the theory proposed by Arakawa and Goddette (1985).

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