Kinetic and Structural Studies on the Interaction of Surfactants with Lipoxygenase L1 from Soybeans (*Glycine max*)†

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The interaction of various surfactants with lipoxygenase (LOX1) was followed by activity measurements, kinetics of oxidation of linoleic acid at pH 9.0 as reflected in K_m, V_{max}, and V/K ratio, and conformational changes in the enzyme. The conformational changes were probed by circular dichroism, fluorescence, and difference spectra measurements. The characteristic features of the enzyme activity in different surfactants are as follows: (i) at relatively low concentrations of surfactants there is enhancement of activity; (ii) at relatively higher concentrations of surfactants there is inhibition of the enzyme activity. The inhibition of the enzyme activity followed the order CTAB > Tween 20 > SDS > Brij 35 > DOC. The changes in the kinetic behavior of the LOX1 in different surfactant solutions can be explained in terms of altered V/K ratio. At increasing concentrations Brij 35, DOC, and SDS induced α-helix formation and CTAB and Tween 20 increased the β-structure of the enzyme. Difference spectra and fluorescence measurements indicate that the aromatic chromophores are perturbed to different extents in these surfactant solutions.

INTRODUCTION

Lipoxygenase L1 (linoleate:oxygen oxidoreductase EC 1.13.11.12) is the predominant isozyme from soybean, which catalyzes the oxidation of linoleic acid and other polyunsaturated fatty acids containing a *cis,cis*-1,4-pentadiene system at pH 9.0 (Axelrod et al., 1981). Lipoxygenases have been extensively characterized with respect to molecular characteristics, enzymology, and kinetics (Schewe et al., 1986; Vliegenthart and Veldink, 1982). Various nonionic surfactants such as Tween 20 have been used for solubilization of linoleic acid in the in vitro assay. Surfactants affect enzyme activity and structure in a complex way; for example, SDS at lower concentrations (<1 mM) protects against denaturation (Steinhardt and Reynolds, 1969) and induces ordered structure, but at higher concentrations it inactivates and denatures the enzyme. Many of the surfactants are known to stimulate enzyme activity.

The conformation of the enzyme in solution would be dependent on its primary structure and the surrounding environment provided by the solvent. The hydrophobic environment provided by surfactants may induce an ordered conformation in polypeptides and proteins that contain a sequence with α-helix or β-structure forming potential. The induced α-helix is stable in surfactant solutions below or above its critical micellar concentration (cmc). If the peptide chain has both potentials, the induced β-structure is usually converted back to unordered conformation or transformed to α-helix if the surfactant concentration is above its cmc (Wu and Yang, 1981). Surfactants have a unique role to play in micellar enzymology (Martinek et al., 1986). The ionic nature of the surfactant may also play a role in these interactions.

There are no systematic studies on the behavior of LOX1 from soybeans in various surfactant solutions in terms of either activity or structure. Most of the studies on denaturation of proteins by surfactants are aimed at establishing the stoichiometry and structure of protein–surfactant complexes. In this study, we report the effects of various surfactants of different ionic natures and hydrophobicities on the activity and structure of LOX1. We have measured the effect of surfactants on relative activity and kinetic parameters such as K_m, V_{max}, and V/K values, and structural changes were followed by difference spectroscopy, fluorescence, and circular dichroism measurements.

MATERIALS AND METHODS

Soybean lipoxygenase (LOX1) was purified according to the method of Axelrod et al. (1981) using the Braggs variety of soybean, after dehulling and defatting by hexane. The purified, pooled, and lyophilized enzyme had a specific activity of 100–110 units/mg of protein. The following chemicals were used: linoleic acid from Nucheck Prep Inc., Elysian, MN; sodium dodecyl sulfate (SDS; approximately 99%) and Brij 35 from Sigma Chemical Co., St. Louis, MO; Tween 20 from Aldrich Chemical Co. Inc., Milwaukee, WI; cetyltrimethylammonium bromide (CTAB) from E. Merck, Darmstadt, Germany; deoxycholate (DOC) from Hi-Media, Bombay, India; DEAE-Sephadex-A50 from Pharmacia Fine Chemicals, Uppsala, Sweden. All other chemicals were of analytical grade.

Enzyme Assay. The enzyme was assayed according to the method of Axelrod et al. (1981) by monitoring the appearance of conjugated diene at 234 nm. One unit of enzyme activity is defined as the amount of enzyme required to form 1 μmol of the product/min at 30 °C under the assay conditions. The enzyme concentration was determined by measuring the absorbance of the solution at 280 nm and using a value of 0.7 mg of protein/mL per absorbance unit (Pistorius and Axelrod, 1974). For comparative purposes, all of the relative activity measurements and determination of Michaelis–Menten constant (K_m), and velocity maximum (V_{max}) were made with ethanol-solubilized substrate instead of Tween 20-solubilized linoleic acid as reported by Axelrod et al. (1981), in the presence and absence of surfactants.

Ethanol-Solubilized Substrate. Seventy milligrams of linoleic acid was dissolved in 6.25 mL of distilled ethanol and the final volume made up to 25 mL with 0.2 M sodium borate buffer (pH 9.0). The relative activity is expressed as percentage ratio of enzyme activity in the presence of surfactant solution to enzyme activity in the absence of surfactant at the end of 3 min. V_{max} and K_m were calculated using both the nonlinear regression

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program of Duggleby (1981) and least-squares analysis. For the determination of K_m, V_{max}, and V/K the substrate concentration was in the range 2×10^{-6}–2×10^{-5} M.

Absorption Spectroscopy. Difference spectra measurements were made at 25 °C using tandem cells of 10-mm path length with a Shimadzu double-beam spectrophotometer UV-160A in the wavelength region 240–340 nm. Difference spectra were obtained by placing the protein solution in the reference cell and protein solution containing surfactant in the sample cell. The molecular weight of the protein was assumed to be 94 038 (Shibata et al., 1987); the concentration of the protein was 1.14×10^{-6} M, and the concentration of surfactants was in the range 0.1–10 mM.

Fluorescence Measurements. Fluorescence measurements were made with a Shimadzu RF-5000 automatic recording spectrofluorometer with 5-nm bandwidth for both excitation and emission monochromators. The emission spectra of enzyme were recorded after excitation at 285 nm at 25 °C in the region 300–360 nm in the presence and absence of surfactants at pH 9.0, 0.2 M borate buffer. The protein solutions having absorbance of less than 0.1 at 280 nm were used to prevent inner filter effect. The temperature of the cell was maintained by circulating water from a constant-temperature water bath. Corrections were made for the fluorescence intensity of surfactants with appropriate blanks.

Circular Dichroism Spectroscopy. Circular dichroism measurements were made with a JASCO J20C automatic recording spectropolarimeter, calibrated with (+)-10-camphorsulfonic acid. Dry nitrogen was purged continuously into the instrument before and during experiments. Slits were programmed to yield 10-Å bandwidths at each wavelength. The measurements were made at 25 °C in 0.2 M borate buffer (pH 9.0) using a quartz cell of 1-mm path length. The mean residue ellipticity values were calculated using a value of 112 for mean residue weight.

The secondary structure of lipoxygenase in different surfactant solutions was analyzed by a computer program C.D. PROT (Menendez-Arias et al., 1988). In this method, the secondary structure of protein is calculated as a linear combination of reference spectra based on proteins of known tertiary structure. The methods are those of Chang et al. (1978), Bolotina et al. (1980), and Yang and Kubota (1985).

RESULTS

In the in vitro assay of LOX1 the natural substrate of lipoxygenase, linoleic acid, was solubilized with the surfactant Tween 20 (Axelrod et al., 1981). Since we are comparing the activity of LOX1 in the presence of various surfactants including nonionic surfactant Tween 20 for in vitro activity measurements, the substrate was solubilized with ethanol as mentioned under Materials and Methods. The kinetic plot of the data measured as increase in OD at 234 nm as a function of time in the presence of surfactants is shown in Figure 1. The relative activity of LOX1 as a function of log surfactant concentration is shown in Figure 2. The two nonionic surfactants Tween 20 and Brij 35 enhanced the enzyme activity up to 150% of the control at lower concentrations of surfactant. Both of them enhanced the enzyme activity in a concentration-dependent manner up to 1×10^{-5} M. Further increase in surfactant concentration resulted in a decrease of activity. The cationic surfactant CTAB at lower concentrations did not enhance the activity significantly but inhibited the LOX1 activity over a very narrow range of CTAB concentration. The anionic surfactant SDS enhanced the activity very slightly up to 1×10^{-5} M and then inhibited the enzyme activity over a fairly broad range of concentrations. The other anionic surfactant DOC enhanced the relative activity up to 150% over the concentration range 1×10^{-6}–1×10^{-3} M and then inhibited the enzyme activity.

Effect of Surfactants on V_m, K_m, and V/K Ratio. To obtain better insight into the mechanism of interaction

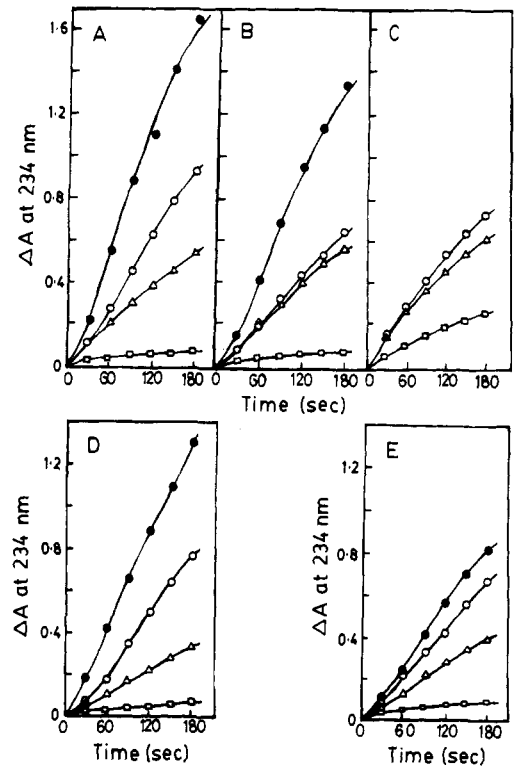

Figure 1. Time course of soybean lipoxygenase L1 activity in different surfactants at pH 9.0. The reaction mixture contained 0.2 M sodium borate buffer (pH 9.0) and 0.1 mM sodium linoleate. The concentration of LOX1 was 2.5×10^{-9} M. (A) Tween 20: ○, control; ●, 1×10^{-5} M; △, 1×10^{-3} M; □, 1×10^{-2} M. (B) Brij 35: ○, control; ●, 1×10^{-5} M; △, 1×10^{-3} M; □, 1×10^{-2} M. (C) CTAB: ○, control; △, 1×10^{-6} M; □, 5×10^{-5} M. (D) DOC: ○, control; ●, 1×10^{-3} M; △, 5×10^{-3} M; □, 1×10^{-2} M. (E) SDS: ○, control; ●, 1×10^{-5} M; △, 5×10^{-4} M; □, 1×10^{-2} M.

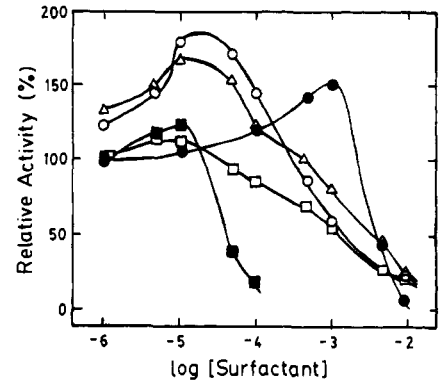

Figure 2. Effect of varying the concentration of different surfactants on the relative activity of the enzyme (○, Tween 20; △, Brij 35; ■, CTAB; ●, DOC; □, SDS).

of surfactants with LOX1, the effect of surfactants on the kinetic parameters K_m, V_{max}, and V/K was measured in the presence of different concentrations of surfactants. The double-reciprocal plots for different surfactants with varying concentrations are shown in Figures 4A, 5A, 6A, 7A, and 8A. The corresponding values of K_m, V_{max}, and V/K are shown in Table I. The common feature of both nonionic surfactants Tween 20 and Brij 35 was enhanced V/K values at 5×10^{-5} M and decreased V/K values at 5×10^{-4} M. The cationic surfactant CTAB at both concentrations used (5×10^{-6} and 1×10^{-5} M) decreased V/K values. The anionic surfactant DOC increased V/K values at lower concentrations and decreased V/K values at higher concentrations of surfactant. The other anionic surfactant, SDS, decreased both K_m and V_{max} values with increasing

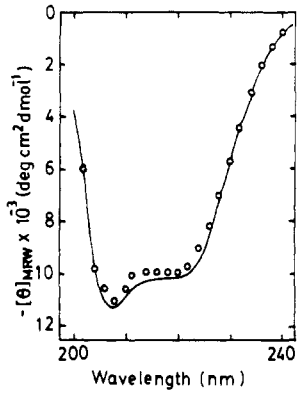

Figure 3. Secondary structure analysis of lipoxygenase L1. [—, experimental value; O, theoretical value according to the method of Bolotina et al. (1980)].

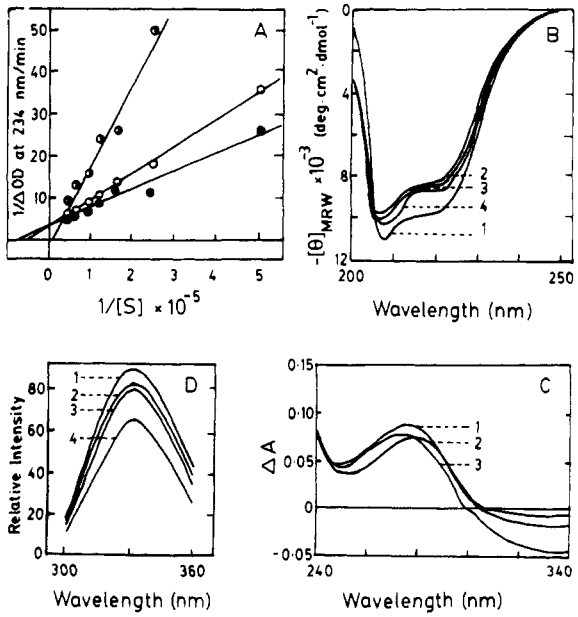

Figure 4. Effect of Tween 20 on enzyme kinetics and structure. (A) Plot of $1/v$ vs 1/[S]: O, control; ●, 5×10^{-5} M; ◑, 5×10^{-4} M. (B) Effect on CD spectrum: 1, control; 2, 1×10^{-4} M; 3, 1×10^{-3} M; 4, 1×10^{-2} M. (C) Difference spectra: 1, 1×10^{-4} M; 2, 1×10^{-3} M; 3, 1×10^{-2} M. (D) Fluorescence emission spectra: 1, control; 2, 1×10^{-4} M; 3, 1×10^{-3} M; 4, 1×10^{-2} M.

concentrations and decreased V/K values slightly at 5×10^{-4} M and increased V/K values at 5×10^{-3} M SDS.

Circular Dichroism Measurements in the Far-Ultraviolet Region. The far-ultraviolet CD spectrum of LOX1 in 0.2 M borate buffer (pH 9.0) is shown in Figure 3. The LOX1 spectrum is characterized by two minima, one at 220 nm with a mean residue ellipticity value of −9986 deg cm^2 dmol^{-1} and one at 208 nm with a mean residue ellipticity value of −11 204 deg cm^2 dmol^{-1}. Both minima are characteristic of α-helix. The secondary structure content of the enzyme as analyzed by different curve-fitting methods is presented in Table II. Of the three methods used, the method of Bolotina et al. (1980) gave a good fit to the experimentally observed curve with 29% α-helix, 12% β-structure, 14% β-bend, and 45% aperiodic structure (Figure 3). The far-ultraviolet CD spectra of LOX1 in the presence of different surfactants at varying concentrations are shown in Figures 4B, 5B, 6B, 7B, and 8B. Addition of surfactants at different concentrations to LOX1 affected the secondary structures in a complex way. The results are given in Table III. The nonionic surfactant Tween 20, at 10 mM concentration, increased the content of sum total of β-structure and

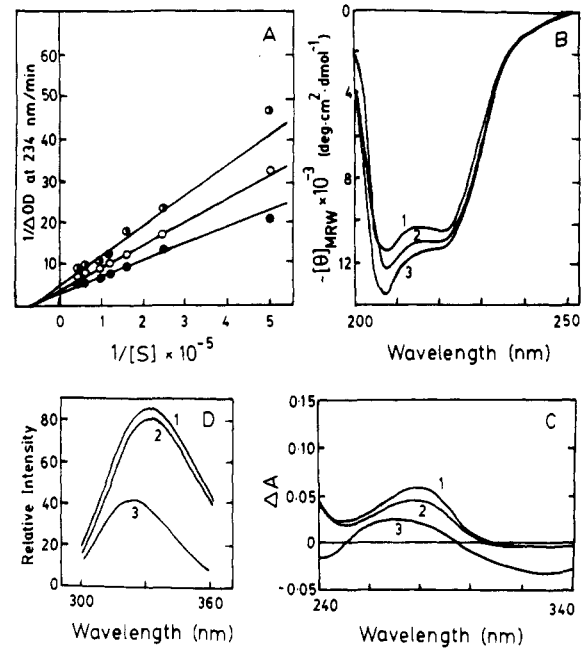

Figure 5. Effect of Brij 35 on enzyme kinetics and structure. (A) Plot of $1/v$ vs 1/[S]: O, control; ●, 5×10^{-5} M; ◑, 5×10^{-4} M. (B) Effect on CD spectrum: 1, control; 2, 1×10^{-3} M; 3, 1×10^{-2} M. (C) Difference spectra: 1, 1×10^{-4} M; 2, 1×10^{-3} M; 3, 1×10^{-2} M. (D) Fluorescence emission spectra: 1, control; 2, 1×10^{-3} M; 3, 1×10^{-2} M.

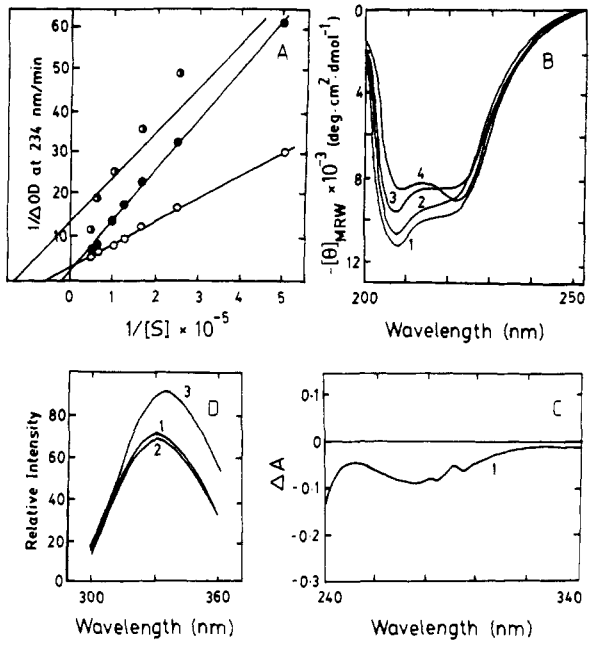

Figure 6. Effect of CTAB on enzyme kinetics and structure. (A) Plot of $1/v$ vs 1/[S]: O, control; ●, 5×10^{-6} M; ◑, 1×10^{-5} M. (B) Effect on CD spectrum: 1, control; 2, 1×10^{-5} M; 3, 5×10^{-5} M; 4, 1×10^{-4} M. (C) Difference spectra: 1, 1×10^{-1} M. (D) Fluorescence emission spectra: 1, control; 2, 1×10^{-6} M; 3, 1×10^{-2} M.

decreased aperiodic structure. In contrast, the other nonionic surfactant Brij 35 at both 1 and 10 mM concentrations increased the ellipticity values at all wavelengths. There was an increase in α-helix content at both 1 and 10 mM concentrations, and at 10 mM there was an increase in aperiodic structure with a concomitant decrease in β-content. The addition of cationic surfactant CTAB resulted in a decrease of the α-helix content and an increase in β-content of the LOX1 (Figure 6B). The anionic surfactant DOC had no effect at 0.5 mM concen-

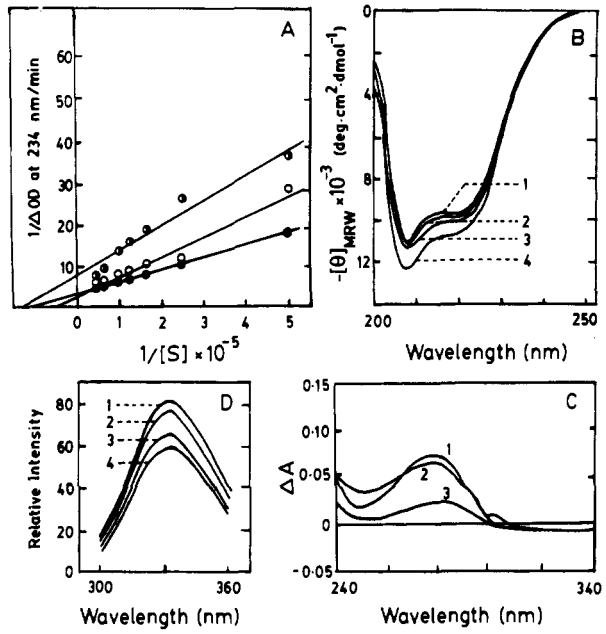

Figure 7. Effect of DOC on enzyme kinetics and structure. (A) Plot of $1/v$ vs $1/[S]$: ○, control; ●, 1×10^{-3} M; ◑, 4×10^{-3} M. (B) Effect on CD spectrum: 1, control; 2, 5×10^{-4} M; 3, 5×10^{-3} M; 4, 1×10^{-2} M. (C) Difference spectra: 1, 1×10^{-4} M; 2, 1×10^{-3} M; 3, 5×10^{-3} M; 4, 1×10^{-2} M. (D) Fluorescence emission spectra: 1, control; 2, 1×10^{-4} M; 3, 1×10^{-3} M; 4, 1×10^{-2} M.

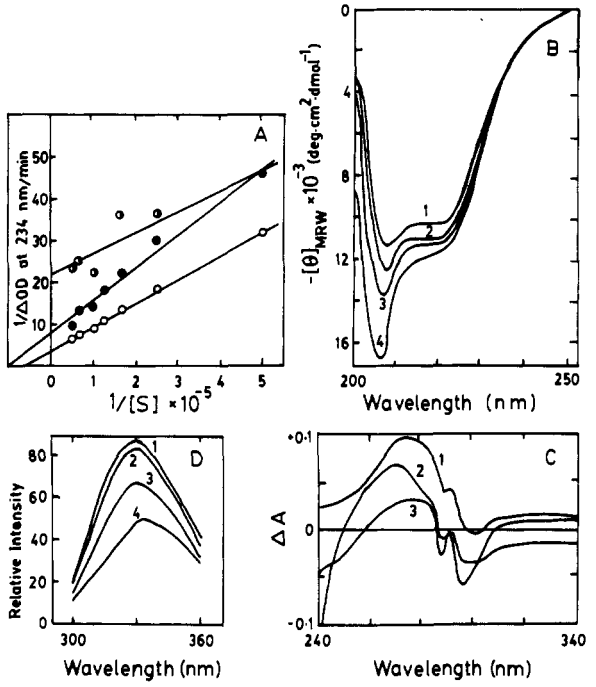

Figure 8. Effect of SDS on enzyme kinetics and structure. (A) Plot of $1/v$ vs $1/[S]$: ○, control; ●, 5×10^{-4} M; ◑, 5×10^{-3} M. (B) Effect on CD spectrum: 1, control; 2, 1×10^{-4} M; 3, 1×10^{-3} M; 4, 1×10^{-2} M. (C) Difference spectra: 1, 1×10^{-4} M; 2, 1×10^{-3} M; 3, 1×10^{-2} M. (D) Fluorescence emission spectra: 1, control; 2, 1×10^{-4} M; 3, 1×10^{-3} M; 4, 1×10^{-2} M.

tration and at 10 mM concentration increased the ellipticity values at all wavelengths. There was an increase in α-helix without a significant change in β-structure and a decrease in aperiodic structure. In the case of SDS, with increasing concentrations there was an increase in α-helix content and aperiodic structure (Figure 8B and Table III).

Difference Spectra Measurements. The effect of various surfactants on the conformation of enzyme was also followed by difference spectra in the region 340–240 nm, where the aromatic amino acids contribute to the spectra. In LOX1, there are 37 residues of tyrosine, 13 tryptophan residues, and 30 phenylalanine residues (Shibata et al., 1987). Nonionic surfactants Tween 20 and Brij 35 in the concentration range 0.1–10 mM perturbed the aromatic amino acids (Figures 4C and 5C), showing a positive difference spectra below 300 nm indicative of blue shift in the spectra. The chromophores could be in a hydrophobic environment in these surfactant solutions. In the case of cationic surfactant CTAB, measurements could not be made at lower concentrations due to turbidity. The difference spectra at 0.1 M CTAB had negative bands indicating a red shift of aromatic chromophores (Figure 6C). The anionic detergents SDS and DOC had differing effects on LOX1 as evidenced from difference spectra (Figures 7C and 8C). In the case of DOC, at all concentrations there was a positive difference spectrum, whose magnitude decreased with increasing concentrations, suggesting that the chromophores experience a less hydrophobic environment at higher concentrations due to the unfolding of protein. In the case of SDS there was a negative difference spectrum, suggesting that the chromophores experience a more polar environment due to the unfolding of the molecule.

Table I. Summary of K_m, V_{max}, and V/K Values in the Presence of Surfactants

	$K_m \times 10^{-5}$ mol	V_{max}, OD min^{-1}	$V/K \times 10^{-5}$ min^{-1} mol^{-1}
control	1.95	0.35	0.18
Tween-20			
5×10^{-5} M	1.77	0.39	0.22
5×10^{-4} M	11.80	0.60	0.05
Brij 35			
5×10^{-5} M	1.54	0.39	0.25
5×10^{-4} M	1.90	0.27	0.14
DOC			
1×10^{-3} M	0.88	0.30	0.34
4×10^{-3} M	0.76	0.13	0.17
SDS			
5×10^{-4} M	1.08	0.14	0.13
5×10^{-3} M	0.23	0.05	0.22
CTAB			
5×10^{-6} M	5.9	0.48	0.08
1×10^{-5} M	0.7	0.07	0.10

Fluorescence Measurements. LOX1 at pH 9.0 had an excitation maximum at 285 nm and an emission maximum at 333 nm, suggesting that fluorescence emission was due to tryptophan residues in a hydrophobic environment. With the addition of Tween 20 up to 1 mM concentration, there was no shift in spectral maximum and a slight quenching of fluorescence intensity. At 10 mM Tween 20 the spectrum was slightly red shifted accompanied by a decrease in fluorescence intensity (Figure 4D). In contrast, in Brij 35 solutions up to 1 mM there was no significant change in the intensity or in the emission maxima. At 10 mM Brij 35 the spectrum was blue shifted accompanied by a decrease in fluorescence intensity (Figure 5D). In the case of CTAB, at very low concentration (1 μM), where there was no turbidity, there was not much change in fluorescence intensity and wavelength of maximum; at 10 mM there was enhancement of fluorescence intensity, but the wavelength of emission maxima was shifted toward longer wavelength (Figure 6D). In the case of DOC at 0.1 mM concentration there was a slight blue shift and decreased intensity. At higher concentrations fluorescence intensity decreased without significant change in the wavelength of emission maximum

Table II. Analysis of Secondary Structure of Lipoxygenase (LOX1) by Different Methods

S1	method	$-(\theta)$ MRW $\times 10^{-3}$ at 208 nm		$-(\theta)$ MRW $\times 10^{-3}$ at 222 nm		% secondary structure			
		expt	theor	expt	theor	α-helix	β-sheet	β-bend	aperiodic structure
1	Bolotina et al. (1980)	11.2	11.1	9.8	9.7	29	12	14	45
2	Chang et al. (1978)	11.2	9.3	9.8	10.4	24	40	2	34
3	Yang and Kubota (1985)	11.2	10.5	9.8	9.9	12	43		45

Table III. Secondary Structure Content of Lipoxygenase (LOX1) As Affected by Surfactants

S1	surfactant	concn, M	$-(\theta)$ MRW $\times 10^{-3}$		% secondary structure			
			208 nm	222 nm	α-helix	β-sheet	β-bend	aperiodic structure
1	control		11.2	9.8	29	12	14	45
2	Tween-20	1×10^{-4}	9.8	8.5	24	13	14	49
		1×10^{-3}	9.7	8.9	25	12	14	49
		1×10^{-2}	10.3	8.5	29	17	19	35
3	Brij 35	1×10^{-3}	12.3	11.1	32	14	15	39
		1×10^{-2}	13.1	11.0	31	8	11	50
4	CTAB	1×10^{-5}	10.7	9.0	29	13	16	42
		5×10^{-5}	9.7	8.7	28	13	18	41
		1×10^{-4}	8.4	9.1	27	18	18	37
5	DOC	5×10^{-4}	11.2	9.6	28	12	15	45
		5×10^{-3}	11.3	9.8	31	15	16	38
		1×10^{-2}	12.4	10.5	33	10	15	43
6	SDS	1×10^{-4}	11.2	9.8	30	13	16	41
		1×10^{-3}	12.9	10.4	31	6	12	52
		1×10^{-2}	14.7	10.3	35	-1	14	52

(Figure 7D). In the case of SDS with increasing concentrations there was a decrease in intensity and emission maximum shifted toward red (Figure 8D).

DISCUSSION

The relative activity vs logarithm of surfactant concentration profiles showed two distinct phases. In the initial phase activity was enhanced as a function of added surfactant concentration. In the second phase, there was a decrease in the enzyme activity over a narrow range of surfactant concentrations. The midpoint of the inhibition phase for CTAB was 4.8×10^{-5} M, for Brij 35 3.3×10^{-3} M, for Tween 20 1.6×10^{-3} M, for SDS 3×10^{-3} M, and for DOC 5.2×10^{-3} M. The inhibition of enzyme followed the order CTAB > Tween 20 > SDS > Brij 35 > DOC. The effect of surfactants on the activity profile can be explained in terms of V/K values. The increase in enzyme activity at lower concentrations of surfactants was reflected in higher V/K values, which was an index of affinity of substrate for the enzyme. Similarly, at higher concentrations there was a decrease in the V/K value indicating a lower affinity of the substrate to enzyme.

The K_m value obtained in the absence of surfactant for the ethanol-solubilized substrate was higher than that for Tween 20-solubilized substrate (Axelrod et al., 1981), suggesting the enzyme had a higher affinity for Tween 20-solubilized substrate. At the concentration of ethanol used in these measurements the conformation of the enzyme was not affected, as indicated by CD measurements (data not shown). It has been reported earlier (Ben-Aziz et al., 1970) that Tween 20 was a competitive inhibitor of LOX1. In contrast with the findings of Ben-Aziz et al. (1970), we found that the effect of Tween 20 was complex because of its dual effect on both V_{max} and K_m (Table I). In the in vitro assay of LOX1, according to the Axelrod et al. (1981) method, the final concentration of Tween 20 used in the assay medium is 2.28×10^{-5} M. From Figure 2 it is apparent that at this concentration of Tween 20 the LOX1 activity was enhanced (150% of the control).

The kinetic data were analyzed by Hill plot. The Hill coefficient, which was slightly less than one, for lipoxygenase did not change appreciably in the presence of various surfactants, suggesting that the number of binding sites for the substrate on the enzyme did not change in the presence of surfactants. Thus, the effect of surfactants was to affect only the affinity and not the number of binding sites of substrate on the enzyme.

The secondary structure of LOX1 was characterized by a high content of α-helix. From the primary amino acid sequence data of LOX1, Shibata et al. (1987) have predicted the presence of 29% α-helix in the molecule. The estimated secondary structure of LOX1 from the CD data (Figure 3) using the method of Bolotina et al. (1980) agreed well with this. Spaapen et al. (1979), using the method of Chen et al. (1972), have reported that LOX1 has 34% α-helix, 27% β-structure, and 39% aperiodic structure.

The two nonionic surfactants Tween 20 and Brij 35, which had similar effects on activity, differ in their effect on conformation of the enzyme. The two anionic surfactants, DOC and SDS, also differed in their effect on enzyme structure; SDS increased both α-helix and aperiodic structure at higher concentrations, whereas DOC increased α-helix with a concomitant decrease in aperiodic structure. Of the five surfactants, only cationic CTAB and anionic SDS were strong denaturants of the enzyme.

Both difference spectra and fluorescence emission spectra in the presence of various surfactants suggested that the aromatic chromophores were perturbed to different extents in these solutions. The reported fluorescence emission spectrum for LOX1 was in agreement with earlier observations (Finazzi-Agro et al., 1973). In the case of Tween 20, Brij 35, and DOC, at concentrations of surfactants where there was enhancement of enzyme activity there was a blue shift in the spectra. At concentrations of surfactants such as 1×10^{-2} M where there was no activity, there was a red shift of the chromophores due to unfolding of the protein molecule. In conclusion, the effect of surfactants on LOX1 is complex in terms of their effect on both activity and structure. The nonionic surfactants Tween 20 and Brij 35 at lower concentrations enhance the activity, possibly due to stabilization of protein

structure; the kinetics is complicated due to the effect of surfactants on protein structure and their resemblance to substrate. There is no correlation between the ionic nature of the surfactant and its effect on structure.

ABBREVIATIONS USED

LOX1, lipoxygenase L1; cmc, critical micellar concentration; K_m, Michaelis constant; V_{max}, velocity maximum; V/K, ratio of V_{max} to K_m; CTAB, cetyltrimethylammonium bromide; DOC, deoxycholic acid, sodium salt; SDS, sodium dodecyl sulfate.

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