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CIRCULAR DICHROISM OF LIPOXYGENASE-1 FROM SOYBEANS

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Summary

The circular dichroism spectra of the three forms of lipoxygenase-1 from soybeans show characteristic differences in the region between 300 and 600 nm. Native lipoxygenase-1 only shows a negative dichroic band around 330 nm. Yellow lipoxygenase-1, obtained by addition of an equimolar amount of 13-L-hydroperoxylinoleic acid to the native enzyme, shows a positive Cotton effect at 425 nm, while the negative band at 330 nm has increased in intensity. The blue enzyme, representing a complex of yellow enzyme with 13-L-hydroperoxylinoleic acid exhibits a negative dichroic band at 580 nm and positive bands at 410 and 391 nm.

The near-ultraviolet CD spectra of the three forms of lipoxygenase are very similar, showing several well resolved positive dichroic bands at 0°C.

Using the method of Chen et al. (Chen, Y.-H., Yang, J.T. and Martinez, H.M. (1972) Biochemistry 11, 4120—4131) the contents of α-helix, β- and unordered form of native lipoxygenase-1 were estimated to be 34, 27 and 39% respectively.

Introduction

Lipoxygenase-1 from soybeans (linoleate:oxygen oxidoreductase, EC 1.13.11.12) is a mononuclear non-heme iron dioxygenase, which catalyses the oxygenation of poly-unsaturated fatty acids, containing a 1,4-*cis*,*cis*-pentadiene system to yield the 1,3-conjugated hydroperoxy fatty acids [1].

Three forms of lipoxygenase-1 can be distinguished, the native (colourless) enzyme, the yellow enzyme, obtained upon reaction of native enzyme with an

equimolar amount of 13-L-hydroperoxylinoleic acid and the blue enzyme, which arises upon interaction of yellow lipoxygenase-1 with a molar excess of 13-L-hydroperoxylinoleic acid.

The iron in the various enzyme species shows different paramagnetic properties [2].

The ultraviolet-visible spectrum of the yellow enzyme shows an increase in absorbance around 330 nm as compared with the spectrum of the native enzyme [3]. De Groot et al. [4] found an additional absorption band for the blue lipoxygenase-1 with a maximum at 570 nm.

In view of the different absorption spectra of the various enzyme species it appeared worthwhile to investigate the optical properties of the chromophoric groups by means of CD spectroscopy. To see whether the interaction between lipoxygenase-1 and 13-L-hydroperoxylinoleic acid would give rise to significant conformational changes also the far and near ultraviolet regions of the CD spectra were considered.

For several non-heme iron proteins it has already been shown that Cotton effects in the visible region of the spectrum are characteristic for the coordination of iron to amino acid residues in the protein [5,6,7,8].

Materials and Methods

Lipoxygenase-1 was isolated from soybeans * according to Finazzi-Agrò et al. [9]; specific activity 235 μmol $O_2 \cdot min^{-1} \cdot mg^{-1}$. The iron content, as determined by flameless absorption spectrometry was 1.04 gatom per mol enzyme (molecular weight = 100 000). Enzyme concentrations were determined at pH 7.0 by measuring the absorbance at 280 nm, using $A_{280}^{0.1\%} = 1.6$. Linoleic acid (purity >99%) was purchased from Lipid Supplies (St. Andrews University, St. Andrews, Scotland). 13-L-Hydroperoxylinoleic acid was prepared by incubation of linoleic acid with soybean lipoxygenase-1 at pH 9.0 and 0°C according to Veldink et al. [10]. Absorption spectra were recorded on a Cary 118 C spectrophotometer, with thermostated cell holders at 0°C with 1-cm pathlength cells. Circular dichroism spectra were recorded on a Dichrograph II (Jouan) with automatic slit control, at 0°C under a constant nitrogen flush. The CD spectrometer was calibrated with epi-androsteron (Jouan) ($\Delta\epsilon_{304nm} = 3.310 \pm 0.007\ M^{-1} \cdot cm^{-1}$). Cells with either a 1-cm (for the near-UV and visible region) or 0.01-cm (for the far-UV region) light path were used. Instrument base lines were determined using 0.1 M sodium borate buffer (pH 9.0).

CD results are expressed as mean residual molar ellipticities (θ, degrees $cm^2 \cdot dmol^{-1}$) in the far-ultraviolet CD spectra and as molar circular dichroism ($\Delta\epsilon$, $M^{-1} \cdot cm^{-1}$) in the near-ultraviolet and visible CD spectra. From amino acid analyses a mean residue weight of 113 was calculated. Estimation of the secondary structure of native and yellow lipoxygenase was performed by conformation analysis according to Chen et al. [11]. The circular dichroism at any wavelength is expressed as follows, $X = f_H X_H + f_\beta X_\beta + f_R X_R$. The f values are the fractions of the helix (H), β- and unordered form (R). Reference data of the

* Registered Williams Soybeans, Lot T-735, obtained from Tabor Seed Division, 4248 West Main, Decatur, IL., U.S.A., December 1976.

three conformations, based on five globular proteins were used [12]. The far-ultraviolet CD spectra of native and yellow lipoxygenase were fitted to the reference spectra by means of a least-squares method.

Results and Discussion

The wavelength range from 300 to 700 nm

The absorption spectrum of a diluted solution of native lipoxygenase-1 (Fig. 1a) shows only the protein absorption, although at a high enzyme concentration shoulders are discernable at around 330 nm ($\epsilon_{330} = 1380\ M^{-1} \cdot cm^{-1}$) and 400 nm ($\epsilon_{400} = 446\ M^{-1} \cdot cm^{-1}$) (Fig. 1b), which probably stem from the coordination of iron to the polypeptide chain.

The CD spectrum of native lipoxygenase-1 (Fig. 2) shows a small negative band at around 330 nm, probably related to the shoulder at the same wavelength in the absorption spectrum.

CD spectra of the visible region were recorded not further than 600 nm because of limitations of the apparatus.

Upon addition of an equimolar amount of 13-L-hydroperoxylinoleic acid to a solution of native lipoxygenase-1, the colour of the solution turned into yellow. The absorption difference spectrum of the yellow and the native enzyme exhibits a shoulder at around 350 nm (Fig. 3a). During the conversion of the native enzyme, 13-L-hydroperoxylinoleic acid is completely converted. The reaction products contain about 20% 13-oxooctadecadienoic acid, which has an absorption maximum at 285 nm ($\epsilon_{285} = 22\,000\ M^{-1} \cdot cm^{-1}$) with a tail as far as 350 nm [13]. When the residual absorption in this region is taken into account, a molar absorption at 330 nm of $1800\ M^{-1} \cdot cm^{-1}$ was calculated.

The CD spectrum of the yellow species (Fig. 4) shows an increased negative Cotton effect at around 330 nm as compared to the native enzyme. When the organic reaction products are removed by gelfiltration, the negative CD band of the purified yellow enzyme has its maximum at 345 nm.

Negative CD bands around 330 nm have also been reported for other non-heme iron (III) enzymes such as protocatechuate 3,4-dioxygenase [7] and pyrocatechase [8].

In the CD spectrum of the yellow enzyme an additional positive dichroic band is present at 425 nm, which has apparently no distinct counterpart in the absorption spectrum. Since several non-heme iron proteins in the oxidized form show Cotton effects near 425 nm, e.g. xanthine oxidase at 432 nm [6], spinach ferredoxin at 426 nm [14], adrenodoxin at 435 nm [14] and rubredoxin at 400 and 440 nm [15] the positive CD band of the yellow enzyme probably reflects chelation of ferric iron by amino acid residues of the protein.

Addition of a molar excess of linoleic acid to the yellow Fe(III)-enzyme under anaerobic conditions leads to reduction of the enzyme to a colourless form having a decreased CD band at 330 nm and no bands in the visible region, just as described for the native enzyme. This observation gives additional evidence for the involvement of iron in the origin of the Cotton effects in the region between 300 and 600 nm.

The blue enzyme is formed upon addition of a molar excess of 13-L-hydroperoxylinoleic acid to the yellow enzyme. The absorption difference spectrum

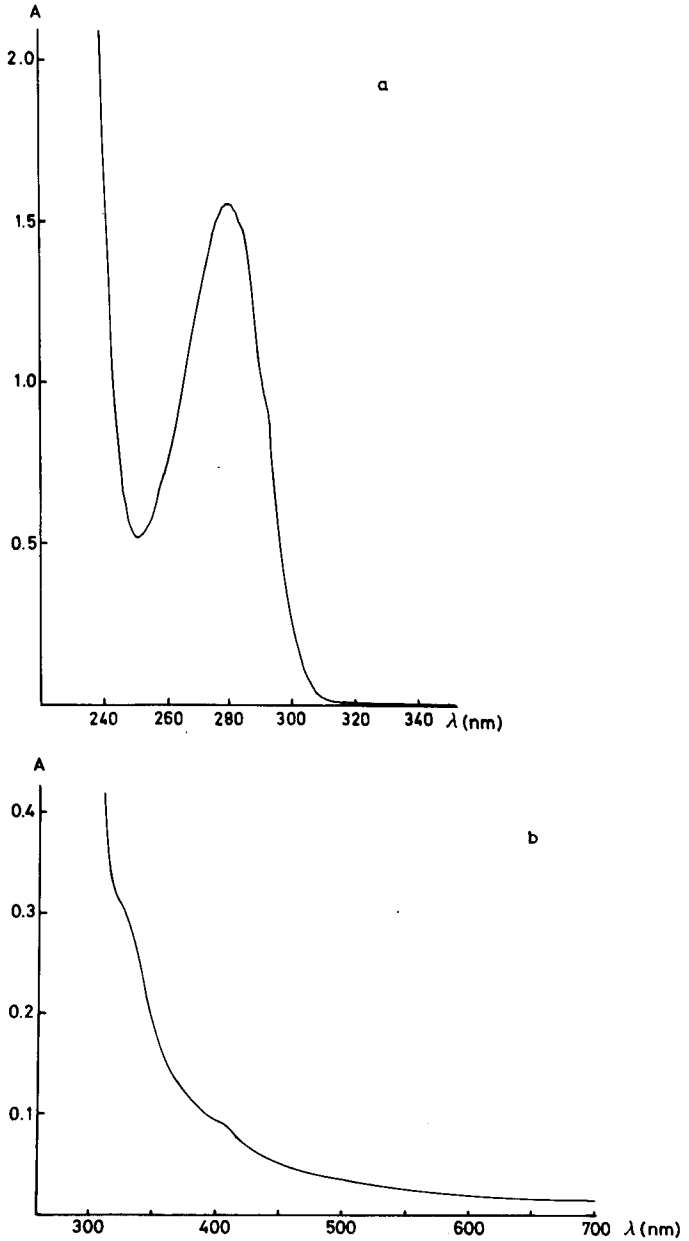


Fig. 1. (a) Ultraviolet absorption spectrum of native lipoxygenase-1 (enzyme: 9.7 μM, 0.1 M sodium borate, pH 9.0). (b) Absorption spectrum of native lipoxygenase-1 from 300 to 700 nm. (enzyme: 212 μM, 0.1 M sodium borate, pH 9.0).

of the blue and the native enzyme (Fig. 3b) shows a decreased intensity at 330 nm and new maxima at 360 nm ($\epsilon_{360} = 1960\ M^{-1} \cdot cm^{-1}$) and at 578 nm ($\epsilon_{578} = 1320\ M^{-1} \cdot cm^{-1}$).

Comparison of the CD spectra of the blue (Fig. 5a) and the yellow enzyme

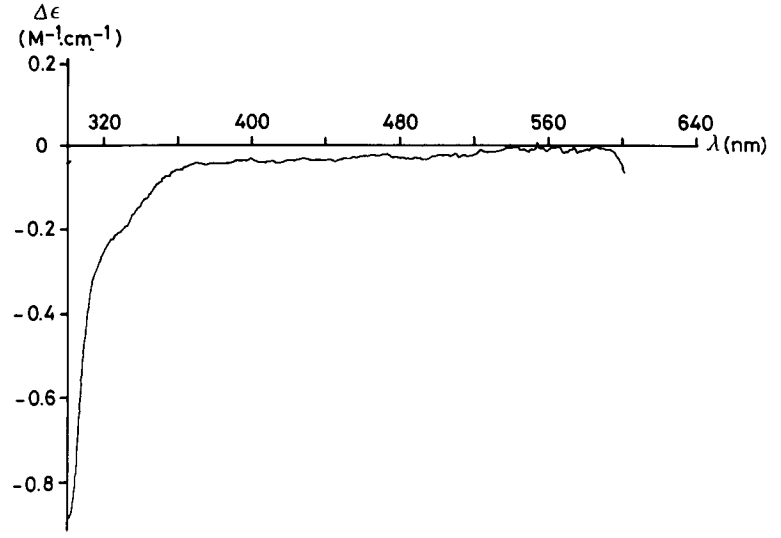


Fig. 2. CD spectrum from 300 to 600 nm of native lipoxygenase in 0.1 M sodium borate buffer, pH 9.0. Enzyme concentration, 649 μM.

(Fig. 4) shows the disappearance of the negative Cotton effect at 330 nm and a shift of the positive CD band from 425 to 410 nm. The latter strong CD band overlaps a band centered at 391 nm. However, it cannot be excluded that upon the transition from yellow to blue enzyme new CD bands arise, super-

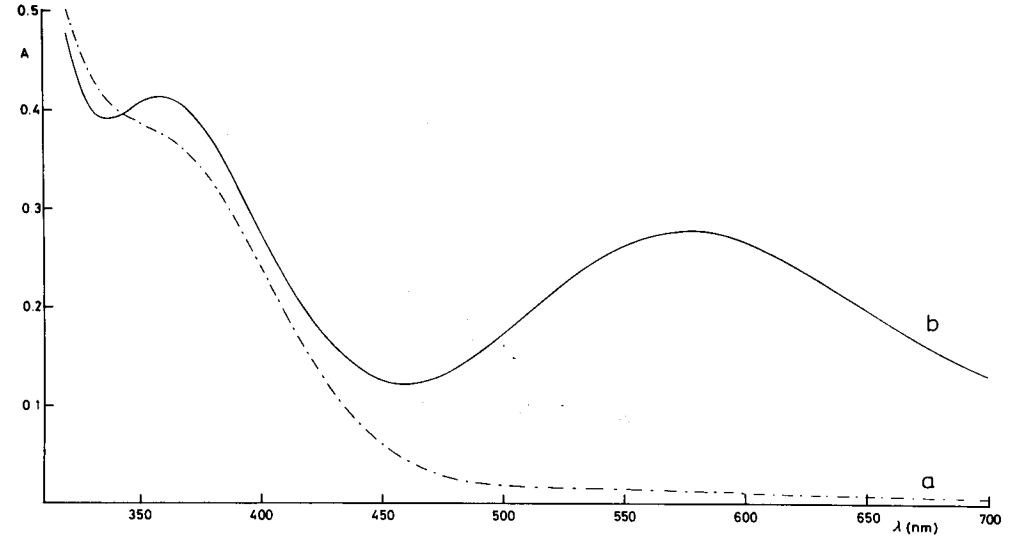


Fig. 3. Effect of 13-L-hydroperoxylinoleic acid on the absorption spectrum of lipoxygenase-1. (a) Absorption difference spectrum of yellow and native lipoxygenase in 0.1 M sodium borate buffer, pH 9.0. Enzyme concentrations, 212 μM. In order to obtain yellow enzyme, 2.9 μl 149 mM 13-L-hydroperoxylinoleic acid was added to 2 ml 212 μM native lipoxygenase-1 in the sample cuvet. (b) Absorption difference spectrum of blue and native enzyme in 0.1 M sodium borate buffer, pH 9.0. Enzyme concentrations, 212 μM. 5.8 μl 149 mM 13-L-hydroperoxylinoleic acid was added to the sample cuvet containing yellow enzyme.

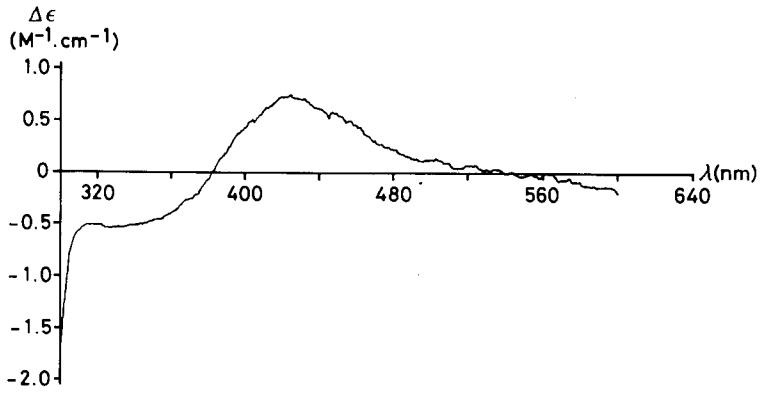


Fig. 4. CD spectrum of yellow lipoxygenase-1 in 0.1 M sodium borate buffer, pH 9.0. Enzyme concentration, 162 μM. The yellow enzyme was prepared by addition of 2.6 μl 63 mM 13-L-hydroperoxylinoleic acid to 1 ml 162 μM native lipoxygenase-1.

imposed on the CD band around 425 nm of the yellow enzyme due to an additional chromophore. In order to estimate the intrinsic CD bands of the blue chromophore, the CD spectra of the blue and the yellow enzyme were subtracted. This difference CD spectrum shows a maximum at 380 nm and a shoulder at 403 nm (Fig. 5b). The new negative Cotton effect at 580 nm has its counterpart in the absorption spectrum at 578 nm.

The results point to a change in the symmetry of the iron-liganding upon binding of product 13-L-hydroperoxylinoleic acid (cf. Ref. 4).

The blue enzyme is rather unstable at room temperature. The colour slowly changes to yellow. The CD spectrum of this yellow enzyme is indistinguishable from that obtained after treatment of the native enzyme with an equimolar amount of 13-L-hydroperoxylinoleic acid.

The CD band at 425 nm of the yellow enzyme and the bands at 391 and 410 nm of the blue species do not coincide with maxima in the absorption spectra. However, it is entirely possible that those CD bands have their counterparts hidden under the tail of the absorption bands at 330 or 360 nm, respectively. Bossa et al. [16] suggested that, for a shift of dichroic bands with respect to the absorption maxima, in each absorption region more than one electronic transition might be involved contributing differently to absorption and rotation. There is no obvious reason to exclude this possibility for lipoxygenase.

For the electronic transitions in the yellow and blue enzyme the Kuhn dissymmetry factors g ($|\Delta\epsilon|/\epsilon$) [17] were calculated to be less than 10^{-2} (Table I), indicating that the observed transitions are electric dipole allowed [18]. It is interesting to note that the g values are of the same order of magnitude as those for the various CD bands of ferredoxin and adrenodoxin [14].

The near-ultraviolet region

The near-ultraviolet CD spectrum of native lipoxygenase-1 shows a number of remarkably well-resolved positive dichroic bands at 271, 279, 287, 293 and 300 nm, respectively (Fig. 6a). The bands at 279, 287, 293 and 300 nm probably stem from tryptophan or tyrosine residues in an asymmetric environment

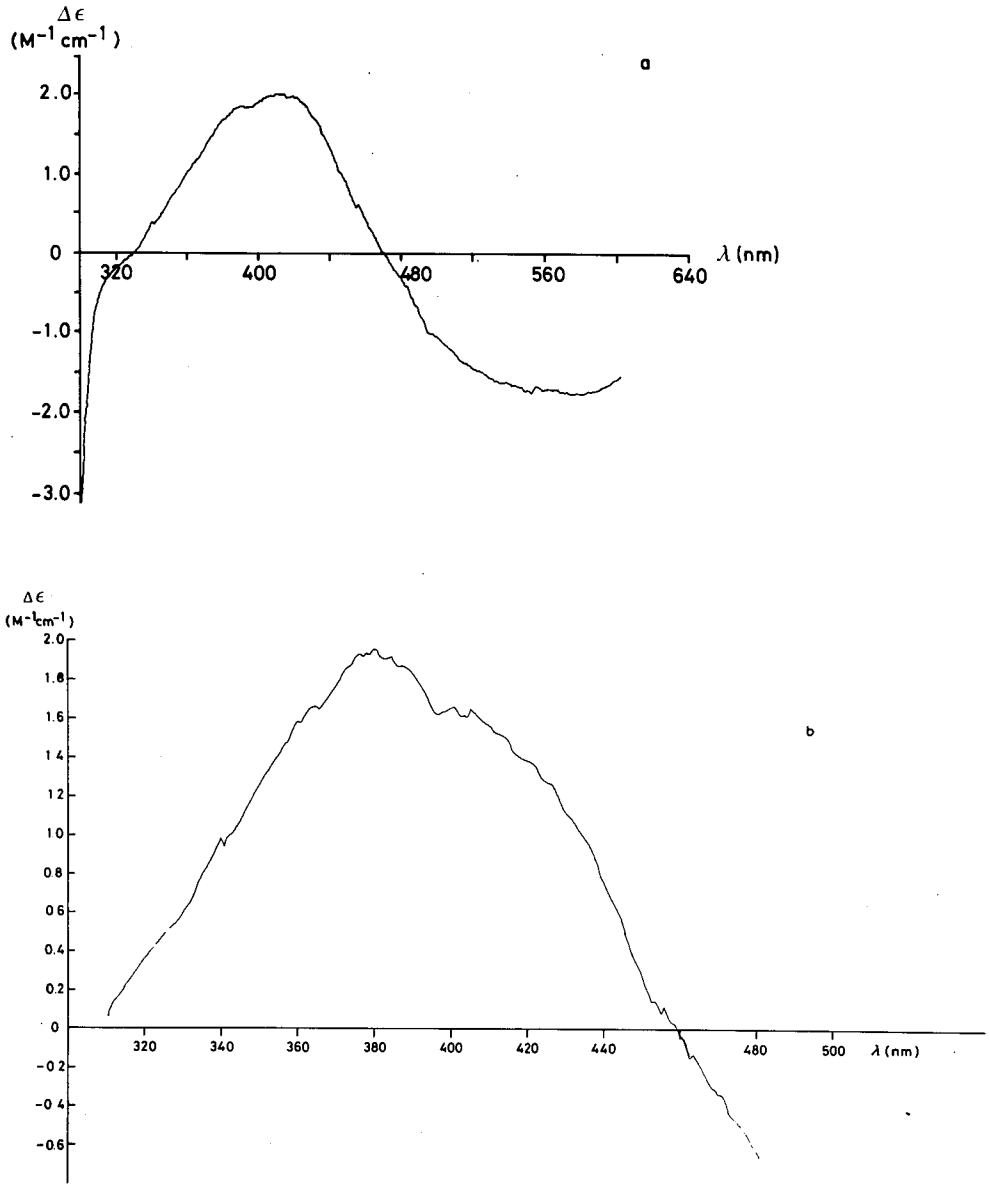


Fig. 5. (a) CD spectrum of blue lipoxygenase-1 in 0.1 M sodium borate buffer, pH 9.0. Enzyme concentration, 161 μM. 5.2 μl 63 mM 13-L-hydroperoxylinoleic acid was added to the reaction mixture described in the legend to Fig. 4. (b) Calculated difference CD spectrum of blue and yellow lipoxygenase-1.

[19]. The CD spectra of both the yellow (Fig. 6b) and the blue species (Fig. 6c) show only minor differences in the aromatic side chain region as compared with the spectrum of the native enzyme.

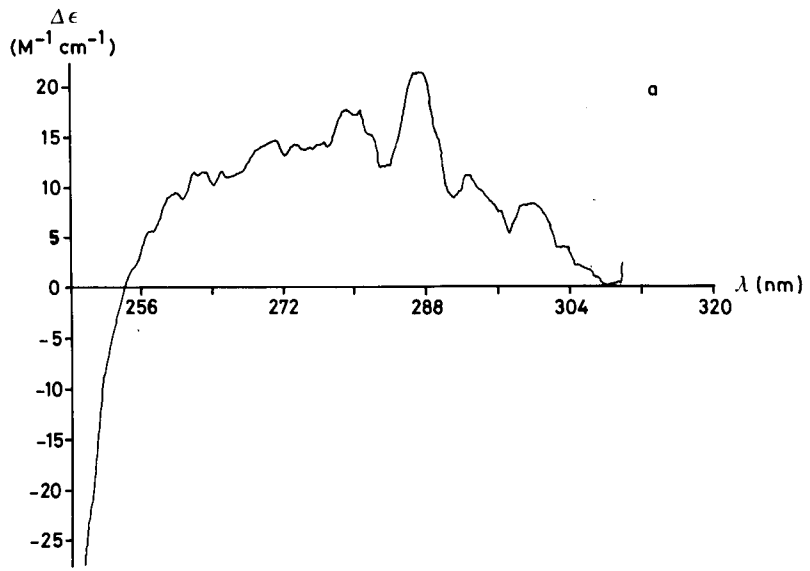
The far-ultraviolet region

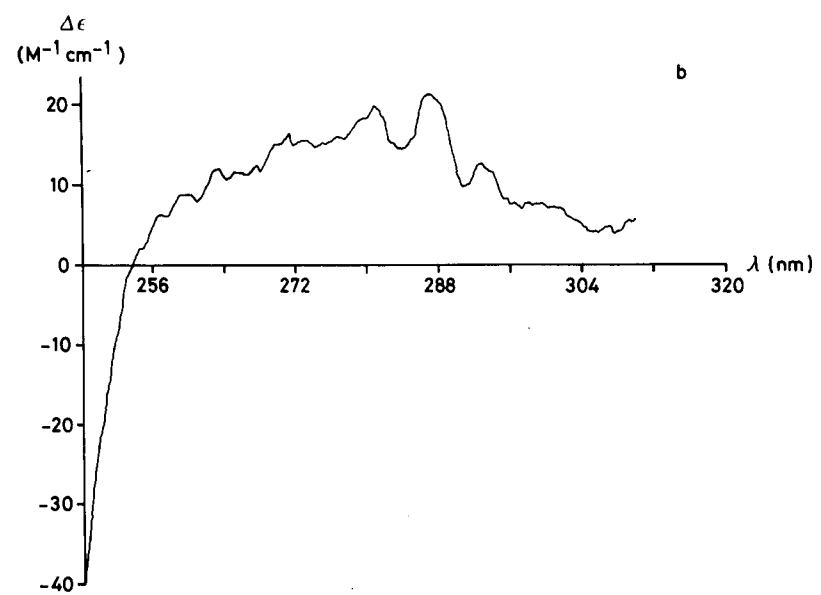
The strong negative dichroic bands in the far-ultraviolet CD spectrum of

TABLE I

SPECTROSCOPIC DATA AND KUHN DISSYMMETRY FACTORS FOR YELLOW AND BLUE LIPOXYGENASE-1

	λ (nm)	ϵ ($M^{-1} \cdot cm^{-1}$)	$\lvert\Delta\epsilon\rvert$ ($M^{-1} \cdot cm^{-1}$)	g
Yellow lipoxygenase	330	1800	0.52	$2.9 \cdot 10^{-4}$
	425	615	0.74	$1.2 \cdot 10^{-3}$
Blue lipoxygenase	391	1489	1.84	$1.2 \cdot 10^{-3}$
	410	1087	1.95	$1.8 \cdot 10^{-3}$
	580	1320	1.89	$1.4 \cdot 10^{-3}$





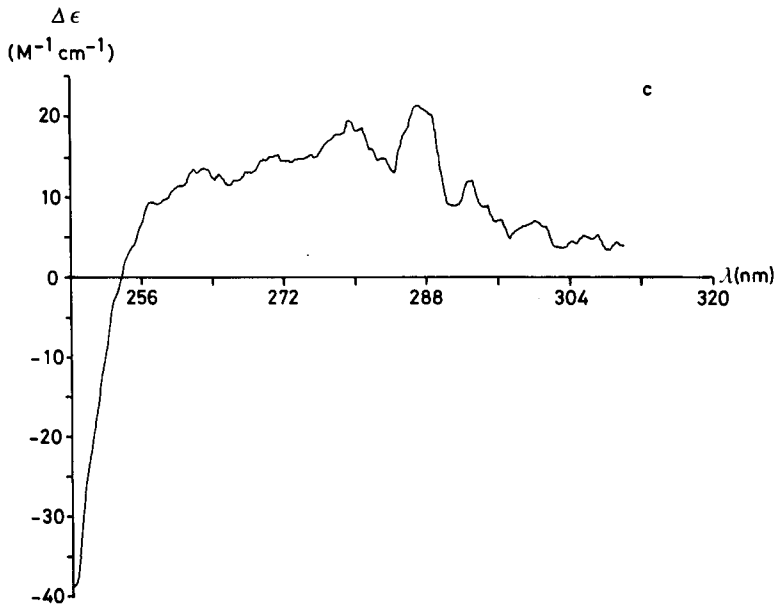


Fig. 6. Near-ultraviolet CD spectra of the three forms of lipoxygenase-1 in 0.1 M sodium borate buffer, pH 9.0. Enzyme concentrations, 5.4 μM. (a) Native lipoxygenase-1; (b) yellow lipoxygenase-1; (c) blue lipoxygenase-1.

native lipoxygenase-1 point to a highly ordered secondary structure of the enzyme (Fig. 7a). Conversion of native lipoxygenase-1 to the yellow form causes only small changes in the CD spectrum (Fig. 7b). These results indicate that there are no major conformational changes in the protein upon the reaction of native enzyme with an equimolar amount of 13-L-hydroperoxylinoleic acid.

From these CD spectra the secondary structure of the native and the yellow species were calculated with the method of Chen et al. [11] (Table II), using the mean residue ellipticities of the three reference conformations as derived from the following globular proteins: spermwhale myoglobin, egg-white lysozyme, dogfish lactate dehydrogenase, papain and bovine pancreas ribonuclease [12].

Since it is assumed that the rotatory contributions are from peptide chromophores only, the parameters for the conformation should be considered as first approximation [11,20].

TABLE II

CALCULATED SECONDARY STRUCTURE PARAMETERS FOR NATIVE AND YELLOW LIPOXYGENASE

	α-Helix (± S.E.) (%)	β-Form (± S.E.) (%)	Random coil (± S.E.) (%)
Native lipoxygenase	34.1 ± 0.2	26.9 ± 0.9	39.0 ± 0.9
Yellow lipoxygenase	31.5 ± 0.2	24.9 ± 0.9	43.6 ± 0.9

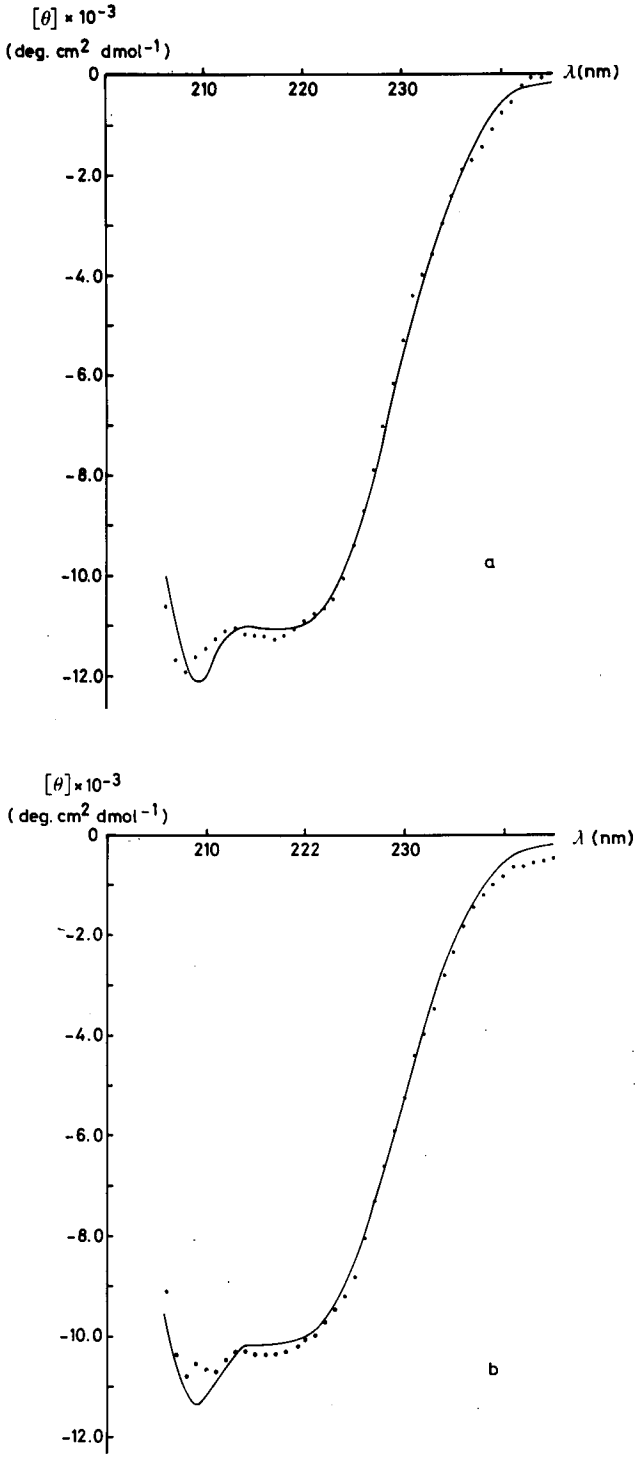


Fig. 7. Far-ultraviolet CD spectra of native lipoxygenase-1 (a) and of yellow lipoxygenase-1 (b) in 0.1 M sodium borate buffer, pH 9.0. Enzyme concentrations, 8.1 μM, 0.01 cm cell. (———), calculated spectra with the parameters from Table II; (· · · · · ·) experimental spectra.

Acknowledgements

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