ELSEVIER

Affecting the activity of soybean lipoxygenase-1

Hanqing Wu

Department of Chemistry, University of Wisconsin-Milwaukee, Milwaukee, Wisconsin

The iron content in soybean lipoxygenase-1 is important for enzyme activity. If the iron is removed by a chelating agent, the activity of the enzyme will decrease. The active center includes the iron ligands and the surrounding environment, and any conformational change in the active center may affect the activity of the enzyme. It is shown that the activity of soybean lipoxygenase-1 is enhanced by chloride anion, phosphate, formate, borate, etc., especially at a lower concentration of substrate. It is also shown that one of four thiols in soybean lipoxygenase-1 is accessible to DTNB at 0.1% SDS without losing great activity, and that all four thiols are accessible to DTNB at 1% SDS and lose all activity. Two or three of the four thiols are accessible to mercuric cyanide without losing great activity. These results support the hypothesis that only one, or possibly two cysteines are responsible for the loss of activity. Two-substrate and two-product binding site models are proposed here and discussed in view of high-resolution X-ray crystal structure. © *1996 by Elsevier Science Inc.*

Keywords: Soybean lipoxygenase-1, fatty acid, nonheme, iron, enzyme, activity, active center, kinetics, substrate, product, activation, inactivation, inhibition, binding site, modification, thiol

INTRODUCTION

Lipoxygenase (EC 1.13.11.12) catalyzes dioxygenation of polyunsaturated fatty acids possessing a *cis,cis*-1,4-pentadiene unit to yield *cis,trans*-conjugated diene hydroperoxides. The enzyme is a monomer with a molecular mass of about 100,000 Da (839 amino acids) and contains 1 mol of iron per monomer.

In 1975, Groot et al.[1] proposed an acceptable reaction scheme for the activation of soybean lipoxygenase-1 on the basis of electron paramagnetic resonance (EPR) experiments. Under anaerobic conditions, and in the presence of both substrate (fatty acid) and product (hydroperoxide), it was proposed that a dissociation of the fatty acid free radical–enzyme complex takes place, leading to the free enzyme in its ferrous state. The latter enzyme species is oxidized to the active ferric state by hydroperoxide, with the formation of alkoxy free radicals and hydroxyl ions. The end products of the anaerobic reaction are derived from further reaction of fatty acid free radicals, alkoxy free radicals, and fatty acid. Kemal et al.[2] proposed a pathway for the conversion of the ferric enzyme to the inactive ferrous form by studying reductive inactivation of soybean lipoxygenase-1 by catechols. These authors mentioned that inhibition of lipoxygenase-1 by nordihydroguaiaretic acid (NDGA) is due to the reduction of E_{ox} and not the intermediate $E_{red}R$ or $E_{red}ROO$. Mansuy et al.[3] also studied the effect of phenidone and BW 755C on soybean lipoxygenase-catalyzed oxidation in the presence of hydroperoxide by examining EPR spectra and ultraviolet (UV) peaks at 234 nm. Their main conclusion is that 2 mol of hydroperoxide is required for the two-electron oxidation of 1 mol of phenidone into its dehydro derivative. Van der Zee et al.[4] studied the formation of free radical metabolites in the reaction between soybean lipoxygenase and its inhibitors by EPR studies. They proved that the formation of inhibitor radicals depends on the presence of the lipoxygenase and hydroperoxide. From the above-mentioned papers, the catalytic cycle of dioxygenation or oxidation, and the free radicals formed, are coupled with the changing lipoxygenase state, that is, from ferrous state to ferric state or from ferric state to ferrous state. The iron in the lipoxygenase plays an important role in the catalysis of the reaction from substrate to product and in the formation of free radicals.

Reactive compounds structurally related to substrates, or specific inhibitors of the enzyme, have also been used in many investigations of the active site. In favorable cases such reagents enter the active site of the enzyme and react specifically with nearby amino acid residues. If partial or complete loss of activity occurs, the involvement of the modified residue in the catalytic process of the enzyme can often be inferred. This phenomenon is called *self-inactivation* or simply *inactivation*. Many authors have contributed to the current state of understanding in this field.[5–9]

Work[10] shows that soybean 15-lipoxygenase inhibition is

Address reprint requests to: Hanqing Wu, Department of Chemistry, University of Wisconsin-Milwaukee, Milwaukee, Wisconsin 53201.

Paper submitted as part of the Electronic Conference of the Molecular Graphics and Modelling Society.

655 Avenue of the Americas, New York, NY 10010

0263-7855/97/$15.00
PII S0263-7855(97)00006-4

dependent on the lipid solubility and coordination structure of the chelators, and the inhibition is associated with the formation of chelator–iron complexes. Conditions for measuring pH-dependent lipoxygenase activity in reverse micelles have been the subject of further study.[11] This article shows that 1,10-phenanthroline chelator may bind to the iron in the enzyme at 63 ± 3°C to form an iron complex, and lead to a loss of enzyme activity. The effect on enzyme activity brought about by the addition of a high concentration of anion to the substrate solution, and by chemical modification of thiols in the enzyme, are also discussed, as are two-substrate and two-product binding site models.

EXPERIMENTS

Soybean lipoxygenase-1 is prepared according to Axelrod et al.,[12] and the protein is further purified by high-performance liquid chromatography (HPLC). Reagents used for chelating studies are the following: sodium phosphate buffer, 0.02 M, pH 7.0 and 7.5; 1,10-phenanthroline, 0.005 M in 0.02 M sodium phosphate buffer, pH 7.5; sodium borate buffer, 0.2 M, pH 9.2; sodium linoleate, 0.01 M. [To prepare, weigh out 100 mg of linoleic acid, add an equal weight of Tween 20, plus 5.7 ml of O_2-free water. Homogenize by drawing back and forth in a Pasteur pipette, avoiding air bubbles. Add sufficient 0.5 N NaOH to yield a clear solution [0.79 ml]. Make up to 35.7-ml total volume. Distribute the solution into 1-ml portions in small screw-cap vials, flush with N_2 before closing, and keep frozen until needed.] The substrate is prepared by taking 0.5 ml of 0.01 M sodium linoleate, adding it to 59.5 ml of 0.2 M sodium borate buffer, then adding 60 μl of catalase.

The samples prepared with and without 1,10-phenanthroline are as follows: The samples with 1,10-phenanthroline have 0.5 ml of protein (1.5 mg/ml), 0.5 ml of 0.005 M 1,10-phenanthroline, and 1 μl of catalase; the samples without 1,10-phenanthroline have 0.5 ml of protein, 0.5 ml of 0.02 M sodium phosphate, pH 7.5, and 1 μl of catalase. Place 50-μl aliquots of the above-described sample into 1-ml plastic tubes, and heat at 60, 62, 64, and 66°C in a hot water bath. The heating times are set at 0, 5, 10, 15, 20, 25, 30, 40, 45, and 50 min, respectively. When each sample is removed from the water bath, 0.95 ml of 0.02 M sodium phosphate buffer (pH 7.5) is added immediately. Twenty microliters of this solution are added to 1 ml of substrate and the change in OD_{234} are measured. Kinetic studies show that the specific activity of soybean lipoxygenase-1 is enzyme concentration dependent; in this article, a 3 nM concentration of enzyme is used as the final enzyme concentration under all conditions. The excess sulfhydryl regents are removed by passing the sample through a G-50 column during centrifuge. The number of sulfhydryl groups is determined by spectrophotometric titration with 5,5′-Dithiobis(2-nitrobenzoic acid (DTNB).

RESULTS

The iron in the enzyme plays an important role in activation. If the iron in the enzyme is removed by chelators, the activity of the enzyme will decrease. Other workers have found that inactivation by chelating agents such as *o*-phenanthroline occurs in the presence of reducing agents, which can be explained by a cooxidation mechanism. The author's work on soybean lipoxygenase-1 inhibited by 1,10-phenanthroline shows that the inactivation is temperature and pH dependent (pH ~9.0–10 gives optimized value). The change in local conformation near the active center in the enzyme occurs during inactivation, owing to the formation of chelator–iron complexes. Figure 1 shows that the relative activity decreases with increasing heating time at different temperatures (60, 62, 64, and 66°C) without 1,10-phenanthroline, and the higher the heating temperature, the faster decrease in the relative activity. Figure 2 supports this pattern of action. By comparing Figure 2 with Figure 1, it can be seen that for each heating temperature group, the absolute activity with 1,10-phenanthroline at $t = 0$ min does not change compared with the absolute activity without 1,10-phenanthroline at $t = 0$ min; when the heating time is longer, the difference between these two relative activities increases.

The decrease in relative activity at different heating temperatures without 1,10-phenanthroline may be caused by many unknown factors, but the difference in relative activity with and without 1,10-phenanthroline may be caused simply by the percentage of active protein chelated by 1,10-phenanthroline. From Figure 3, the chelating factor (the relative activity with 1,10-phenanthroline divided by the relative activity without 1,10-phenanthroline) can be calculated at different temperatures and different heating times.

More results show that, in the absence of any externally added product, substrate at high concentration binds to more than two binding sites and that the surfactants affect only the affinity but not the number of binding sites of substrate on the enzyme. The kinetic results indicate that the "apparent" affinity of the substrate for the enzyme is dependent on the concentration of the enzyme above 3 nM, but not below or at 3 nM. At high salt concentration, the "apparent" affinity is increased. Figure 4 shows that at low concentration of substrate and at high concentration of borate or formate, the specific activity ($\nu_0/[E_T]$) will be increased; the same result was obtained for phosphate (see Figure 5) and other salts.

On the basis of the experimental results presented here, and on the results of others, Scheme 1 is proposed. There are two substrate-binding sites: one is the active binding site, which can bind substrate, with the subsequent forma-

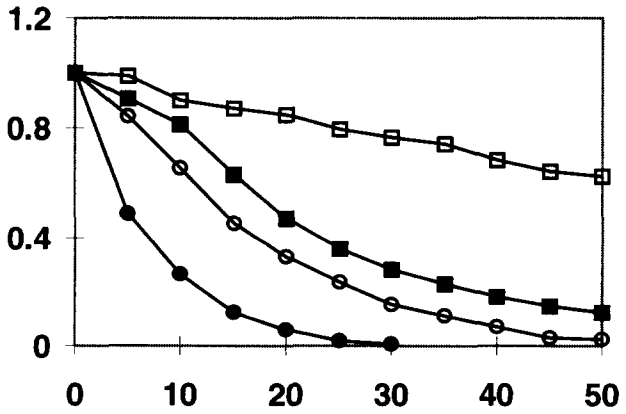


Figure 1. The relative activity vs heating time at different temperatures of 60°C (□), 62°C (■), 64°C (○), 66°C (●) without 1,10-phenanthroline.

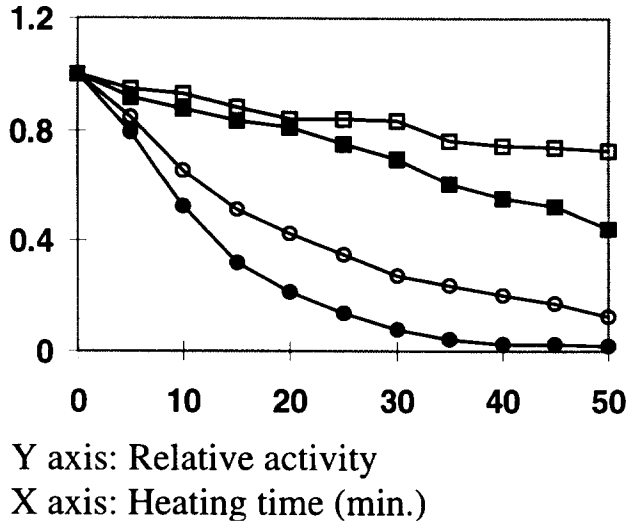


Figure 2. The relative activity vs heating time at different temperatures of 60°C (□), 62°C (■), 64°C (○), 66°C (●) with 1,10-phenanthroline.

tion of product catalyzed by the enzyme; the other is the inhibitory binding site, which can bind substrate, without the subsequent formation of product. The active binding site may be more hydrophobic than the inhibitory binding site. Salt anion ligands can be bound to the inhibitory binding site, and affect the affinity of the substrate for the enzyme.

The parameters have the following relationships: $K_{xs} > K_s >> K_{is}$. For the stock protein (old protein), the K_s (affinity of the substrate for the active binding site) may not change, but K_{is} may become small (higher affinity of the substrate for the inhibitory binding site). Figure 6 shows the comparison of the curves of the inverse of specific activity ($[E_T]/v_0$) versus the inverse of the concentration of substrate (1/[S]) for the newly prepared protein and stock protein.

In the presence of product, the specific activity was also measured at pH 9.2 and 10.0 (see Figures 7 and 8). The

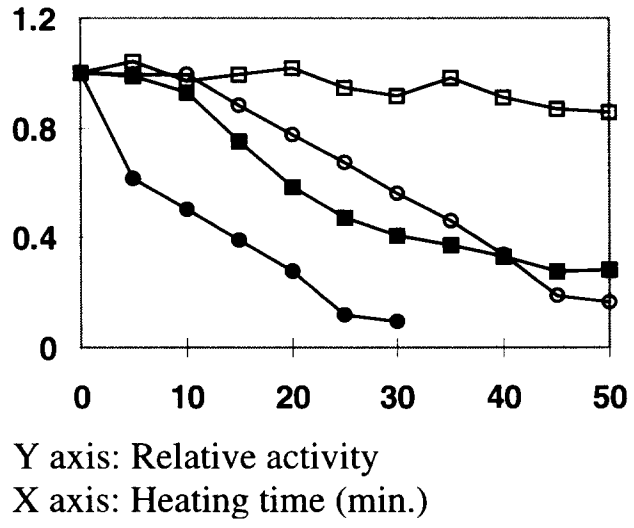


Figure 3. The chelating factor of 1,10-phenanthroline vs heating time at different temperatures of 60°C (□), 62°C (■), 64°C (○), 66°C (●).

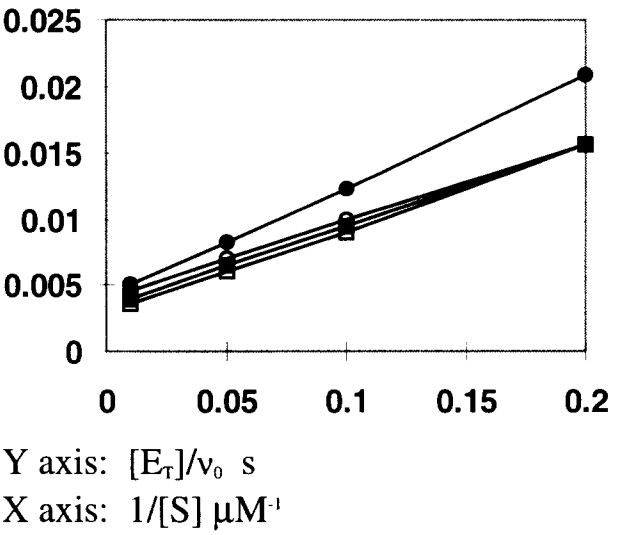


Figure 4. The concentration of borate or formate affect the specific activity ($v_0/[E_T]$) of the enzyme at different substrate concentrations. 0.0 M formate and 0.02 M borate (●); 0.5 M formate and 0.02 M borate (■); 0.0 M formate and 0.2 M borate (○); 0.5 M formate and 0.2 M borate (□).

product does inhibit the enzyme under both pH conditions. A fitting of the data measured at pH 10 suggests two binding sites for product (see Scheme 2).

According to the general scheme proposed in Figure 6 of Ref. 13, the steady state kinetic results in the presence of a normal concentration of substrate (5 to 50 μM) and a low concentration of product (2 to 20 μM) can be calculated by computer (the graphs and the program are not provided here): in the presence of a higher concentration of product or oxygen, a higher specific activity can be reached (activation); in the presence of a higher concentration of substrate, a less specific activity can be reached (inhibition). One paper[14] shows that under steady state conditions, at-

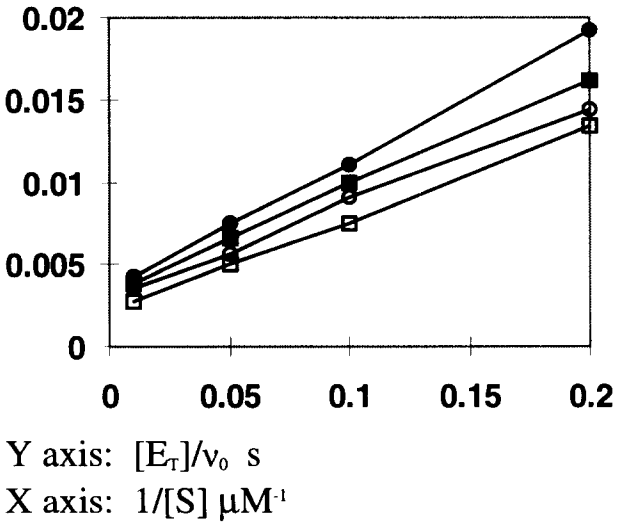
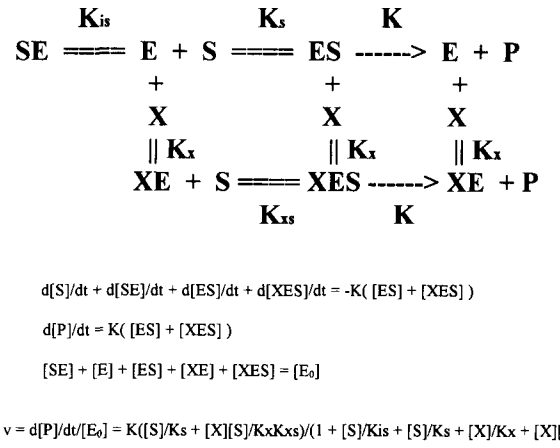


Figure 5. The concentration of phosphate affect the specific activity ($v_0/[E_T]$) of the enzyme at different substrate concentrations. 0.00 M Pi (●); 0.02 M Pi (■); 0.10 M Pi (○); 0.50 M Pi (□).



Scheme 1. Two substrate (S)-binding sites and salt anion ligand (X)-binding site.

mospheric oxygen enters the reaction pathway only after abstraction of hydrogen from substrate; these authors proposed that molecular oxygen is not productively bound by lipoxygenase but rather interacts directly with the substrate radical lipoxygenase to form the hydroperoxyl radical of linoleate. Unlike high-affinity nitric oxide (NO), which reacts with the ferrous iron in the protein to form an Fe–NO complex that gives an S = 3/2 with E/D ≈ 0.02 (D >> frequency) EPR signal (another paper reports that the D value is about 15 cm^{-1} for the ferrous mammalian lipoxygenase iron–NO complex[15]), the O_2 may not bind tightly to the lipoxygenase at all.

A review of the protein sequence shows that there are four cysteines; previous results indicate that all of them are buried in the hydrophobic environment. One of the two conserved cysteines, Cys-492, is located in a region containing a cluster of six histidines and two tyrosines. This region has been proposed to be the iron-binding region. This cysteine is also the candidate for the substrate- and product-binding site. The present author is thus interested in determining the free SH groups under different conditions (partial denaturation, subsequent to addition of sulfhydryl reagents such as Hg^{2+}, etc.). Figure 9 shows that one of four thiols in soybean lipoxygenase-1 is accessible to DTNB at 0.1% sodium dodecylsulfate (SDS) without losing much activity, whereas all four thiols are accessible to DTNB at 1% SDS and lose all activity. After removal of excess sulfhydryl reagents, the number of sulfhydryl groups was determined by spectrophotometric titration with DTNB in the presence of 1% SDS at pH 8.0. Results from titration of the enzyme with mercury compounds show that one, possibly two sulfhydryl groups are responsible for the loss of activity (see Figures 10 and 11). The SH groups seem not to be involved in the tight iron binding. The result shows that the number of bound SH groups for mercury cyanide is consistent with the heavy atom number in the heavy atom derivative crystals.[16]

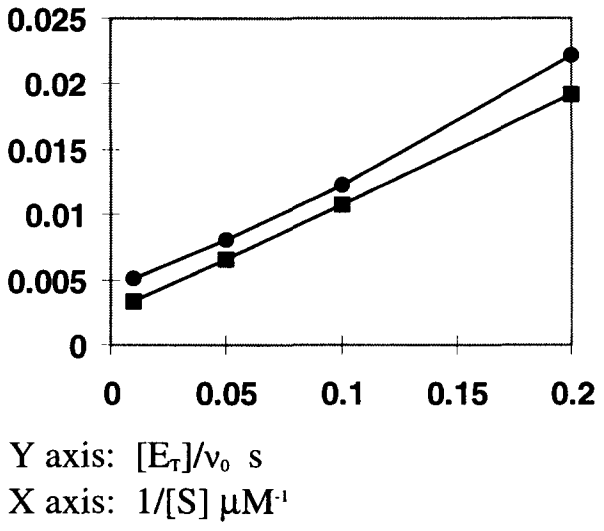


Figure 6. Comparison of the specific activities ($v_0/[E_T]$) of newly prepared protein (■) and stock protein (●) at different substrate concentrations.

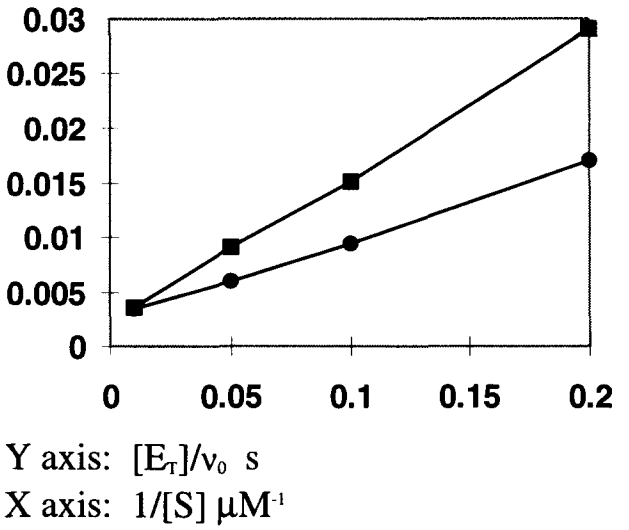


Figure 7. Product inhibition at 0.2 M borate pH 9.2 ●: at 0.0 μM [P]; ■: at 40.0 μM [P].

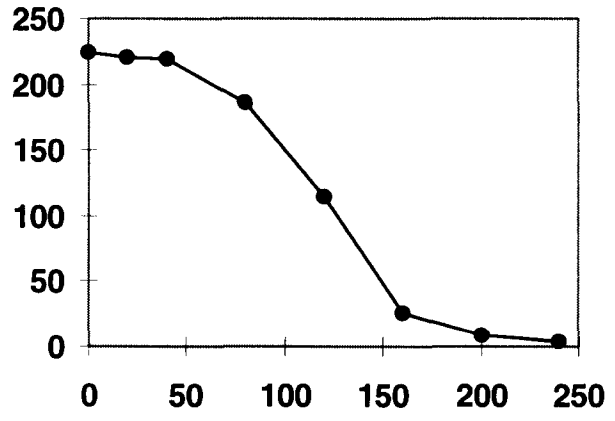


Figure 8. Specific activity ($v_0/[E_T]$) vs product ([P]) at 0.2 M borate pH 10 and [S] = 100 μM.

$$E + S \overset{K_s}{\underset{}{\rightleftharpoons}} ES \xrightarrow{K} E + P \overset{K_{p1}}{\rightleftharpoons} EP$$

$$\begin{array}{ccc} & + & + \\ & P & P \\ & \| K_{p2} & \| K_{p2} \\ PE + P & \overset{K_{p1}}{\rightleftharpoons} & PXP \end{array}$$

$$d[S]/dt + d[ES]/dt = -K\,[ES]$$

$$d[P]/dt + d[Ep]/dt + d[PE]/dt + d[PEP]dt = K[ES]$$

$$[E] + [ES] + [EP] + [PE] + [PEP] = [E_0]$$

$$v = d[P]/dt/[E_0] = K([S]/K_s)/(1 + [S]/K_s + [P]/K_{p1} + [P]/K_{p2} + [P]^2/K_{p1}K_{p2})$$

Scheme 2. The substrate turnover to the product and two product (P)-binding sites.

DISCUSSION

According to the work of the present author, 1,10-phenanthroline chelator at a temperature ranging between 60 and 66°C binds to the iron in the enzyme to form its iron complexes, then destroys or changes the conformation of the active center, leading to loss of enzyme activity.

The enhanced activity of soybean lipoxygenase-1 brought about by most anions can be explained as follows: The anion occupies the inhibitory binding site for substrate, making the enzyme turn over from substrate to product; at the same time, the concentration of substrate relative to the active binding site is enhanced. This is particularly significant at low concentrations of substrate. At a high concentration of substrate, almost no activity is enhanced by the anion. Two-substrate or two-product binding site models are possibly confirmed from the high-resolution X-ray crystal structure.[17] Boyington et al.[16] identified two major cavities in the C domain. Cavity I is a conical invagination that leads from an ~10-Å-wide opening at the surface of the protein. Cavity II is a 40-Å-long, narrow, and winding cavity that extends in two directions from a bend near the iron atom. The more remote end of the cavity was defined by the side chains of residues Met-341 and Leu-480, approximately 30 Å from the iron atom; the closer end was not well defined. Minor et al.[17] state that cavity I is not as hydrophobic as Boyington et al.[16] mentioned; at least 22 water molecules are found in cavity I and within 20 Å of the iron atom. Cysteine-357 is one of four cysteines in the protein, which surrounds cavity I, and it may be accessible to DTNB at 0.1% SDS. Minor et al.[17] further define cavity II as two cavities, II_a and II_b, with II_a close to the iron and serving as the probable binding cavity for the fatty acid substrate; displacement of water from cavity II_b may be difficult, however, because the space is relatively confined. Sterically unhindered adjustments of the side chains of Thr-259 and Leu-541 appear to open a channel wide enough (>6 Å) for

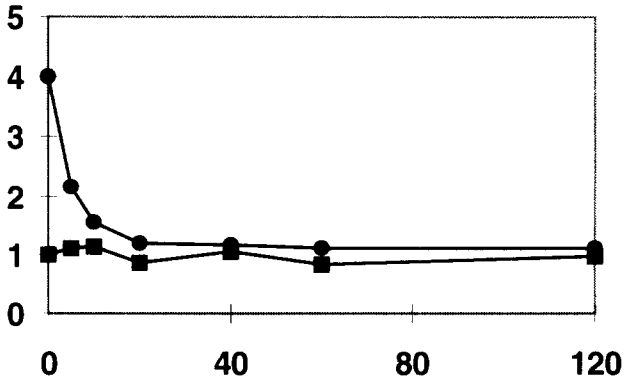


Figure 10. Free SH # (●) and relative activity (■) vs incubation time.

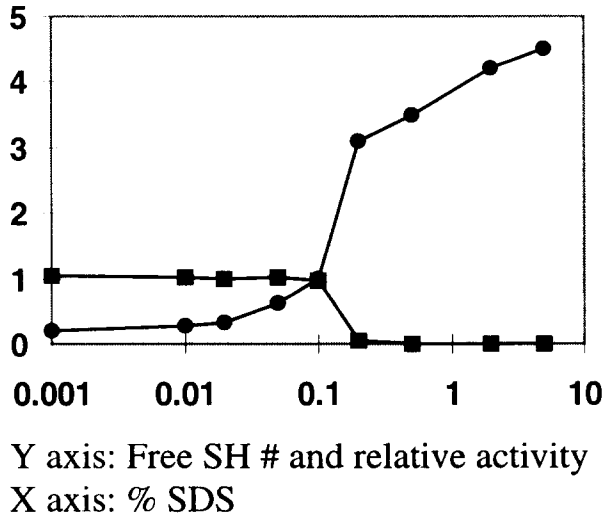


Figure 9. Free SH # (●) and relative activity (■) vs % SDS.

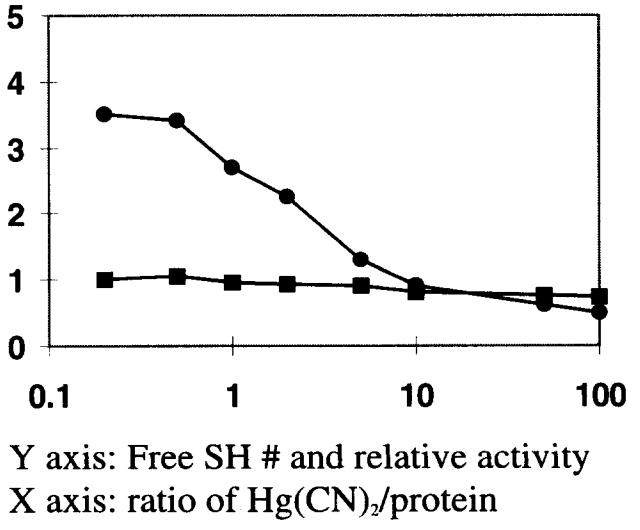
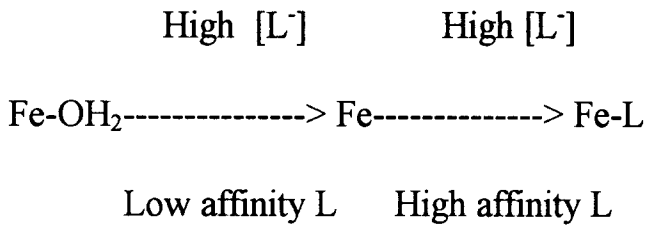


Figure 11. Free SH # (●) and relative activity (■) vs ratio of $Hg(CN)_2$/protein.



Scheme 3. The change in coordination sites of the iron in the presence of low- and high-affinity ligand.

entry of a fatty acid. Two additional points where a channel might open to connect cavity II_a with the external surface are suggested by Minor et al.[17] The active binding site mentioned in this article is probable cavity II_a, which is close to the iron atom. Any cavities or points that are suitable for substrate binding but that do not allow substrate to enter near the iron are candidates for the inhibitory binding site mentioned in this article. The probable inhibitory binding site is cavity II_b, because rearrangement of the side chains of Arg-707 and Val-354 would be required to allow the fatty acid to pass from cavity II_b into II_a. Anionic ligand (X in Scheme 1) possibly binds to cavity II_b, because of the existence of positively charged amino acid Arg-707 in cavity II_b; its binding near the Arg-707 side chain will either block substrate access to the inhibitory binding site (cavity II_b), thus enhancing the enzyme "apparent" activity, or reduce the free volume in the active binding site (cavity II_a), thus leading to inhibition. Two product-binding sites are easily acceptable because product can bind to the cavities or points to which substrate may bind.

The author prefers the X-ray crystal structure determined by Minor et al.,[17] but does not deny the X-ray crystal structure determined by Boyington et al.,[16] because the crystals were prepared under different conditions by the two groups. Boyington et al.[16] prepared the crystal under high salt conditions, including formate, which affects enzyme activity (see Figure 4) and the EPR signal of active lipoxygenase-1.[18] From this paper, it is known that at high anion concentration, the conformation of the active center in the protein may differ from that at a low concentration of anion; the water molecule bound to the iron in the enzyme may be split out to form the two unoccupied coordination sites of the iron.[16,17,19] Another paper also shows that the enzyme undergoes a rapid change in fluorescence (changes in the conformation of the active center) when lipoxygenase is mixed with an equimolar concentration of 13(*S*)-hydroperoxy-9,11-(*E,Z*)-octadecadienoic acid (13-HPOD).[20] Low-affinity ligands (like Cl^-) may not bind to the ferrous iron in the enzyme even at high concentration, but high-affinity ligands (like NO) do bind to the ferrous iron in the enzyme[21]; thus Scheme 3 is proposed.

The author believes that the enzyme–ligand complexes are possibly formed as the immediate (the ligand may not be necessary as anion).[18,19] Some of the complexes are in the right conformation to catalyze the reaction from substrate to the product; some of them are not, and inhibit the reaction instead. By exterior ligand replacement, modified enzymes (like the enzyme–NO complex) can be designed for further studies.

The experimental results derived by the author and the discussion above are consistent not only with recent work,[22,23] but also support general concepts: Enzyme is affected by the concentrations of those substrate(s), activators, and inhibitors specific to the enzyme, and is affected nonspecifically by compounds such as salts and buffers, pH, ionic strength, temperature, etc., that might be present. The nature of the buffer can be very important here, since many buffers (including the salts) can upset enzyme behavior by (directly or indirectly) acting as inhibitors.

During the inactivation of lipoxygenase by suicide substrate, the active center is also changed or modified: free-radical intermediates are formed, and some amino acids near the active center are modified. These amino acids are possibly methionine or cysteine.[24–27] Cysteine-492 and Tyr-493 are possible from S–π bond; modification of Cys-492 possibly perturbs the conformation near the active site, both sterically and electronically.

CONCLUSION

1. Chelators can bind to the iron in the enzyme to form their iron complexes, then destroy or change the conformation of the active center, leading to loss of enzyme activity.
2. There are possibly two binding sites for both substrate and product: one of the substrate-binding sites is for catalytic reaction, the other is only for inhibition. At a high concentration of product, its inhibition occurs.
3. Two to three of the four cysteines can be accessible and can be modified by heavy metal ions. In the presence of 1% SDS, all four cysteines can be modified by DTNB and the activity is lost.

REFERENCES

1 de Groot, J.J.M.C., et al. Demonstration by EPR spectroscopy of the functional role of iron in soybean lipoxygenase-1. *Biochim. Biophys. Acta* 1975, **377,** 71–79

2 Kemal, C., et al. Reductive inactivation of soybean lipoxygenase-1 by catechols: A possible mechanism for regulation of lipoxygenase activity. *Biochemistry* 1987, **26,** 7064–7072

3 Mansuy, D., et al. Soybean lipoxygenase catalyzed oxidation by linoleic acid hydroperoxide: Different reducing substrates and dehydrogenation of phenidone and BW 755C. *Biochem. Biophys. Res. Commun.* 1988, **151,** 339–346

4 Van der Zee, J., et al. Superoxide and peroxyl radical generation from the reduction of polyunsaturated fatty acid hydroperoxides by soybean lipoxygenase. *Arch. Biochem. Biophys.* 1991, **290,** 153–159

5 Sok, D.E., et al. The possible role of 9(*S*)-hydroperoxy-octadecatrienoic acid as a suicide substrate of soybean lipoxygenase. *Biochem. Biophys. Res. Commun.* 1989, **162,** 1357–1362

6 Gardner, H.W. Soybean lipoxygenase-1 enzymatically forms both (9*S*)- and (13*S*)-hydroperoxides from linoleic acid by a pH-dependent mechanism. *Biochim. Biophys. Acta* 1989, **1001,** 274–281

7 Youseong-Gu, G.D., et al. Irreversible inhibition of soybean lipoxygenase-1 by hydroperoxide acids as substrate. *Arch. Biochem. Biophys.* 1991, **288,** 270–275
8 Sloane, D.L. A primary determinant for lipoxygenase positional specificity. *Nature (London)* 1991, **354,** 149–152
9 Schilstra, M.J. Mechanism of lipoxygenase inactivation by the linoleic acid analogue octadeca-9,12-diynoic acid. *Biochemistry* 1996, **35**(11), 3396–3401
10 Abeysinghe, R.D., et al. The environment of the lipoxygenase iron binding site explored with novel hydroxypyridinone iron chelators. *J. Biol. Chem.* 1996, **271,** 7965–7972
11 Rodakiewicz-Nowak, J., et al. The effect of linoleic acid on pH inside sodium bis(2-ethylhexyl)sulfosuecinate reverse micelles in isooctane and on the enzyme activity of soybean lipoxygenase. *Eur. J. Biochem.* 1996, **238,** 549–553
12 Axelrod, B., Cheesbrough, T.M., and Laakso, S. Lipoxygenase from soybeans. *Methods Enzymol.* 1981, **71,** 441–451
13 Nelson, M.J. and Seitz, S.P. The structure and function of lipoxygenase. *Curr. Opin. Struct. Biol.* 1994, **4,** 878–884
14 Glickman, M.H. and Klinman, J.P. Lipoxygenase reaction mechanism: Demonstration that hydrogen abstraction from substrate precedes dioxygenase binding during catalytic turnovers. *Biochemistry* 1996, **35**(39), 12882–12892
15 Wiesner, R., et al. Nitric oxide oxidises a ferrous mammalian lipoxygenase to a pre-activated ferric species. *FEBS Lett.* 1996, **389,** 220–232
16 Boyington, J.C., et al. The three-dimensional structure of an arachidonic acid 15-lipoxygenase. *Science* 1993, **260,** 1482–1486
17 Minor, W., et al. Crystal structure of soybean lipoxygenase L-1 at 1.4 Å resolution. *Biochemistry* 1996, **35,** 10687–10701
18 Gaffney, B.J., et al. Access of ligands to the ferric center in lipoxygenase-1. *Biophys. J.* 1993, **64,** 773–783
19 Scarrow, R.C., et al. X-Ray spectroscopy of the iron site in soybean lipoxygenase-1: Changes in coordination upon oxidation or addition of methanol. *Biochemistry* 1994, **33,** 15023–15035
20 Jones, G.D., et al. Role of lipid hydroperoxides in the activation of 15-lipoxygenase. *Biochemistry* 1996, **35**(22), 7197–7203
21 Nelson, M.J. The nitric oxide complex of ferrous soybean lipoxygenase-1. *J. Biol. Chem.* 1987, **262,** 12137–12142
22 Srinivasulu, S., et al. The detection of kinetic intermediates during the unfolding of lipoxygenase-1 by urea or guanidine hydrochloride. *Biochim. Biophys. Acta* 1996, **1294**(2), 115–120
23 Matsui, K., et al. Effect of modification of arginine residues on the activity of soybean lipoxygenase-1. *Z. Naturforsch.* 1995, **50c,** 37–44
24 Zakut, R., et al. Evidence for an essential methionine residue in lipoxygenase. *FEBS Lett.* 1976, **71,** 107–110
25 Tatsunami, S., et al. Kinetics of suicide substrates, steady-state treatments and computer-aided extract solutions. *Biochim. Biophys. Acta* 1981, **662,** 226–235
26 Rapoport, S., et al. Methionine sulfoxide formation: The cause of self-inactivation of reticulocyte lipoxygenase. *Eur. J. Biochem.* 1984, **139,** 573–576
27 Kühn, H., et al. The mechanism of inactivation of lipoxygenase by acetylenic fatty acids. *Eur. J. Biochem.* 1984, **139,** 577–589