Mohr, F. C., & Fewtrell, C. (1987) *J. Cell Biol. 104*, 783–792.
Narasimhan, V., Holowka, D., & Baird, B. (1990) *J. Biol. Chem. 264*, 1459–1464.
O'Sullivan, A. J., Cheek, T. R., Moreton, R. B., Berridge, M. J., & Burgoyne, R. D. (1989) *EMBO J. 8*, 401–411.
Peatfield, A. C., Piper, P. J., & Richardson, P. S. (1982) *Br. J. Pharmacol. 77*, 391–393.
Puustinen, T., Scheffer, M. M., & Samuelsson, B. (1988) *Biochim. Biophys. Acta 960*, 261–267.
Rouzer, C. A., & Kargman, S. (1988) *J. Biol. Chem. 263*, 10980–10988.
Rouzer, C. A., Ford-Hutchinson, A. W., Morton, H. E., & Gillard, J. W. (1990) *J. Biol. Chem. 265*, 1436–1442.
Riendeau, D., Falgueyret, J.-P., Nathaniel, D. J., Rokach, J., Ueda, N., & Yamamoto S. (1989) *Biochem. Pharmacol. 38*, 2313–2321.
Thastrup, O., Dawson, A. P., Scharff, O., Foder, B., Cullen, P. J., Drøbak, B. K., Bjerrum, P. J., Christensen, S. B., & Hanley, M. R. (1989) *Agents Actions 27*, 17–21.
Thastrup, O., Cullen, P. J., Drøbak, B. K., Hanley, M. R., & Dawson, A. P. (1990) *Proc. Natl. Acad. Sci. U.S.A. 87*, 2466–2468.
Tripp, C. S., Mahoney, M., & Needleman, P. (1985) *J. Biol. Chem. 260*, 5895–5898.
White, K. N., & Metzger, H. (1986) *J. Immunol. 141*, 942–947.
Wong, A., Hwang, S. M., Cook, M. N., Hogaboom, G. K., & Crooke, S. T. (1988) *Biochemistry 27*, 6763–6769.
Wong, A., Cook, M., Marshall, P., Sarau, H. M., Foley, J. J., & Hwang, S. M. (1991) *Biochemistry 30*, 9346–9354.
Yu, K.-T., Lyall, R., Jariwala, N., Ziberstein, A., & Haimovich, J. (1991) *J. Biol. Chem. 266*, 22564–22568.

Conserved Histidine Residues in Soybean Lipoxygenase: Functional Consequences of Their Replacement†

Janusz Steczko,‡ Greg P. Donoho,§,‖ James C. Clemens,§,⊥ Jack E. Dixon,§,⊥ and Bernard Axelrod*,§

Department of Biochemistry, Purdue University, West Lafayette, Indiana 47907-1153, and Institute of Catalysis and Surface Chemistry, Polish Academy of Sciences, 30-329 Krakow, Poland

Received December 13, 1991; Revised Manuscript Received January 24, 1992

ABSTRACT: Sequences of 13 lipoxygenases from various plant and mammalian species, thus far reported, display a motif of 38 amino acid residues which includes 5 conserved histidines and a 6th histidine about 160 residues downstream. These residues occur at positions 494, 499, 504, 522, 531, and 690 in soybean lipoxygenase isozyme L-1. Since the participation of iron in the lipoxygenase reaction has been established and existing evidence based on Mössbauer and EXAFS spectroscopy suggests that histidines may be involved in iron binding, the effect of the above residues has been examined in soybean lipoxygenase L-1. Six singly mutated lipoxygenases have been produced in which each of the His residues has been replaced with glutamine. Two additional mutants have been constructed wherein the codons for His-494 and His-504 have been replaced by serine codons. All of the mutant lipoxygenases, which were obtained by expression in *Escherichia coli*, have mobilities identical to that of the wild-type enzyme on denaturing gel electrophoresis and respond to lipoxygenase antibodies. The mutated proteins H499Q, H504Q, H504S, and H690Q are virtually inactive, while H522Q has about 1% of the wild-type activity. H494Q, H494S, and H531Q are about 37%, 8%, and 20% as active as the wild type, respectively. His-517 is conserved in the several lipoxygenase isozymes but not in the animal isozymes. The mutant H517Q has about 33% of the wild-type activity. The inactive mutants, H499Q, H504Q, H504S, and H690Q, become insoluble when heated for 3 min at 65 °C, as does H522Q. The other mutants and the wild-type are stable under these conditions. Although the essentiality of His-499, -504, and -690 is not proven, they are tentatively considered to be prime candidates for iron ligands. Judgment on the role of H-522 is more uncertain, since mutant H522Q has weak but detectable activity. The K_m values of the active mutants and the wild-type L-1, when determined against linoleic acid, differ only moderately, indicating that His replacements do not greatly influence the binding of the substrate.

Lipoxygenases catalyze the hydroperoxidation of lipids containing one or more *cis,cis*-pentadiene moieties. All lipoxygenases thus far examined contain one atom of non-heme, non-sulfur iron per molecule. It is well established that iron participates in the reaction. When we determined the sequence of soybean lipoxygenase isozymes L-1[1] (Shibata et al., 1987) and L-2 (Shibata et al., 1988), we noted a cluster of six histidine residues in a motif of 38 amino acid residues in both enzymes and in L-3 (Yenofsky et al., 1988). Because histidine frequently participates as an iron ligand in proteins, we suggested that the histidine-rich region was involved in the binding of this metal. Histidine is a common ligand of iron in a

† This work was supported by Grant PCM 8819688 from the National Science Foundation (to B.A.). This is Journal Paper 13255 from the Purdue University Agricultural Experiment Station.
* To whom correspondence should be addressed.
‡ Polish Academy of Sciences.
§ Purdue University.
‖ Present address: Department of Biochemistry, Stanford University, Stanford, CA 94304-5307.
⊥ Present address: Department of Biological Chemistry, University of Michigan, Ann Arbor MI 48109-0606.

[1] Abbreviations: L-1, L-2, and L-3, soybean lipoxygenase isozymes; PAGE, polyacrylamide gel electrophoresis.

0006-2960/92/0431-4053$03.00/0

		494	499	504	517	522	531	690	Δ
L-1	494	H Q L M S H W L N T H A A M E P F V I A T H R H L S V L H P I Y K L L T P H	531	... H(690)	159
5-LH	362	H Q T I T H L L R T H L V S E V F G I A M Y R Q L P A V H P I F K L L V A H	399	... H(550)	151
L-2	522	H Q L M S H W L N T H A A M E P F I I A T N R H L S A L H P I Y K L L T P H	569	... H(718)	149
L-3	513	H Q L V S H W L N T H A V I E P F I I A T N R H L S V V H P I Y K L L H P H	550	... H(709)	159
P-2	517	H Q L V S H W L N T H A V V E P F V I A T N R H L S C L H P I Y K L L L P H	554	... H(713)	159
P-3	519	H Q L M S H W L N T H A V I E P F V I A T N R Q L S V V H P I N K L L A P H	556	... H(726)	170
SC5	520	H Q L V S H W L N T H A V I E P F A I A T N R H L S V L H P I Y K L L Y P H	556	... H(726)	170
R	516	H Q L I S H W L N T H A V M E P F V I A T N R Q L S V A H P V H K L L L P H	553	... H(716)	163
5-LR	362	H Q T I T H L L R T H L V S E V F G I A M Y R Q L P A V H P L F K L L V A H	399	... H(711)	158
12-LH	355	H E I Q Y H L L N T H L V A E V I A V A T M R C L P S I H P V F K L I V P H	392	... H(550)	151
12-LP	356	H E L H S H L L R G H L M A E V I A V A T M R C L P S I H P I F K L L I P H	393	... H(541)	148
15-LH	355	H E L Q S H L L R G H L M A E V I V V A T M R C L P S I H P I F K L I I P H	392	... H(540)	148
15-LR	355	H E L N S H L L R G H L M A E V F T V A T M R C L P S I H P V F K L I V P H	392	... H(540)	148

FIGURE 1: Comparison of sequences of 38-residue motifs in lipoxygenases. Boldface letters refer to conserved sequences. Δ is number of residues between the carboxyl end of the motif and the sixth conserved His. L-1, soybean lipoxygenase L-1 (Shibata et al., 1987); 5-LH, human leukocyte 5-lipoxygenase (Dixon et al., 1988; Matsumoto et al., 1988); L-2, soybean lipoxygenase L-2 (Shibata et al., 1988); L-3, soybean lipoxygenase L-3 (Yenofsky et al., 1988); P-2, pea seed lipoxygenase, "L-3-like" (Ealing & Casey, 1988); P-3, pea seed lipoxygenase, "L-2-like" (Ealing & Casey, 1989); SC5, soybean cotyledon lipoxygenase (Shibata et al., 1991); R, rice seed isozyme (Shibata, personal communication); 5-LR, rat reticulocyte (Balcarek et al., 1988); 12-LH, human platelet 12-lipoxygenase (Funk, C. D. et al., 1990); 12-LP, porcine leukocyte 12-lipoxygenase (Yashimoto et al., 1990); 15-LH, human reticulocyte 15-lipoxygenase (Sigal et al., 1988); 15-LR, rabbit reticulocyte 15-lipoxygenase (Fleming et al., 1989).

Table I: Mutagenic Primers Used in the Preparation of His Substitution Mutants of Soybean Lipoxygenase L-1[a]

His position	primer	codon change	new residue
494	3′-CTTGCTACCAACAACTCATGA-5′	CAT → CAA	Gln
494	3′-GACTCTTGCTACAGTCAACTCATGAGC-5′	CAT → AGT	Ser
498	3′-TCATGAGCCAATGGTTAAATA-5′	CAT → CAA	Gln
504	3′-TAAATACTCAAGCGGCGATGG-5′	CAT → CAA	Gln
504	3′-TGGTTAAATACTAGTGCGGCGATGGAG-5′	CAT → AGT	Ser
517	3′-CACACCGACAACTTAGCGTGC-5′	CAT → CAA	Gln
522	3′-GCGTGCTTCAGCCAATTTACA-5′	CAC → CAG	Gln
531	3′-TGACTCCTCAGTATCGTAACA-5′	CAC → CAG	Gln
690	3′-CAGCTCTCCAAGCAGCCGT-5′	CAT → CAA	Gln

[a] Altered codons are underlined.

number of proteins, for example, transferrin (Chasteen, 1983), catechol oxidases (Que, 1983), hemerythrin (Wilkins & Harrington, 1983), uteroferrin (Antanaitis et al., 1985), the photosynthetic reaction center of *Rhodopseudomonas viridis* (Diesenhofer et al., 1985), and ribonucleotide reductase (Nordlund et al., 1990).

Examination of 10 additional sequences of lipoxygenase from higher plants and mammals as they became available in the literature revealed that five of the six His residues were conserved. An additional His residue, about 150–160 residues downstream from the histidine-rich region, was also present in all 13 sequences. The regions of interest are shown in Figure 1.[2]

Various spectroscopic studies support the view that spherically coordinate iron bearing 5 or 6 ligands is present in L-1. Thus EXAFS (extended X-ray absorption fine structure) studies indicate liganding to 4 ± 1 N (imidazole) atoms and 2 ± 1 O atoms (Navaratman et al., 1988; Feiters et al., 1990). Mössbauer spectroscopy is consistent with 6 ligands which may be O or N (Funk, M. O., Jr., et al., 1990; Dunham et al., 1990). Magnetic susceptibility experiments rule out the coordination of dioxygen to iron (Petersson et al., 1985). Magnetic circular dichroism studies indicate an octahedral 6-coordinate system having a small rhombic distortion (Whittaker & Solomon, 1988). Zhang et al. (1991) concluded from molecular circular dichroism measurements that at least two His residues are present as ligands. The possibility that H_2O provides an O ligand has been suggested (Nelson et al., 1990; Nelson, 1988). Iron is strongly bound in native L-1, resisting removal by all high-affinity Fe(II) chelators which do not contain divalent sulfur (Pistorius & Axelrod, 1974). Iron liganding in soybean L-1 is discussed in reviews of plant lipoxygenases by Gardner (1991) and Siedow (1991).

To determine which, if any, of the conserved His residues shown in Figure 1 might possibly be involved in the liganding of iron, we have created single mutants of L-1 in which they have replaced by Gln. Mutants in which His-494 and His-504 were replaced with Ser were also prepared for comparison with the corresponding mutants of human leukocytic 5-lipoxygenase

[2] In the course of verifying the sequence of the mutated genes which we constructed for these studies an omission of three nucleotides was detected in our previously published sequence of L-1 (Shibata et al., 1987). TG should be inserted after nucleotide 1520 and C inserted after nucleotide 1529. The amino acid residues 479–481 (Ala-Thr-Ser) become 479–482 (Trp-Leu-Leu-Ala), and the succeeding residue numbers are increased by 1. The revised M_r is 94 262, less the molecular weight of the amino-terminal blocking group which is absent from the *Escherichia coli*-expressed recombinant.

which have been studied earlier (Funk et al., 1989).

Materials and Methods

Materials. Sources of chemicals, reagents, plasmids, and other reagents are as given previously (Steczko et al., 1991).

Site-Directed Mutagenesis. The construction of an expression plasmid containing L-1 cDNA, pT7/L-1, was previously described (Steczko et al., 1991). The gene coding for L-1, which was inserted in the plasmid, contained its natural start and stop codons. The mutagenic nucleotides employed in these studies are shown in Table I. The procedure of Eckstein (Sayers et al., 1988; Potter & Eckstein, 1984; Vesberg & Eckstein, 1982) was followed, using the Amersham Mutagenesis kit. Specific nucleotide changes were confirmed by DNA sequencing of the appropriate mutated region in the plasmid construct.

Expression. Cultures of *Escherichia coli* BL21(DE3) were transfected with pT7-7 plasmids carrying the various mutations. Cells were grown out from single colonies using freshly transfected cells. Medium was 2YT broth containing 50 μg/mL ampicillin. Growth at 37 °C with shaking was continued until absorbancy at 600 nm reached 0.6. Fresh medium augmented with 2.5% ethanol was inoculated from this culture in the proportion of 0.5 mL/L. It was previously shown that low concentrations of ethanol increased the expression of the wild-type recombinant by about 40%. When the absorbancy attained a value of 0.8–1.0, the culture was transferred to a 15 °C shaking incubator for 16 h. Although the L-1 gene in the plasmid was under the control of the *lac* promoter, we had previously found that expression of L-1 did not require induction with isopropyl β-thiogalactopyranoside. The cultures were allowed to grow until densities of 2.8–3.0 were obtained. A culture of wild-type cells were always included for purposes of comparison. Enzyme yields for the wild type corresponded to 7–9 units/mL of culture.

Enzyme Purification and Gel Electrophoresis. Enzyme purification was slightly modified from the previous procedure (Steczko et al., 1991) by including treatment with 0.5% poly(ethylenimine) at pH 7.5 to remove nucleic acid. This treatment was applied to the supernatant solution obtained after centrifuging the homogenate from the French press. Following removal of the poly(ethyleneimine)–nucleic acid precipitate by centrifugation, the solution was worked up as described previously. SDS–PAGE analyses were carried out as before. The expressed proteins were visualized by Western transfer blots carried out with 3–6 μL of centrifuged homogenate.

Enzyme Assay. Lipoxygenase was assayed by the spectrophotometric and the polarographic methods as described earlier (Steczko et al., 1991). The polarographic method which permitted the testing of large amounts of sample was useful for identifying null mutants.

Heat Stability. Two hundred microliters of the supernate from the homogenate was quickly introduced into 1.5-mL microfuge tubes, which had been preheated in a 65 °C water bath. The tube was held in the water bath for 3 min with gentle agitation, cooled in an ice bath, and then centrifuged to remove denatured protein. An aliquot of the supernatant solution (3–6 μL) was subjected to SDS–PAGE and Western blotting. When the blot was negative, the precipitate was resuspended in 200 μL of the electrophoresis sample buffer and analyzed as before.

Results

Effect of Replacement of His Residues on Expression of L-1. All preparations gave rise to Coomassie blue-staining bands in the M_r 94 000 region upon SDS–PAGE analysis (not shown). Readily detectable Western blots, corresponding to these bands, were obtained (Figure 2). In addition to the mutants formed by substituting the six conserved His residues (Figure 1), a seventh mutant, H517Q, not a member of this group, was also tested. It was included because we found His-517 of L-1 was conserved in all of the soybean lipoxygenase isozymes. We proceeded to construct it before the sequences of the animal-derived lipoxygenases were available in the literature and thus before it was known that it was not conserved in these species.

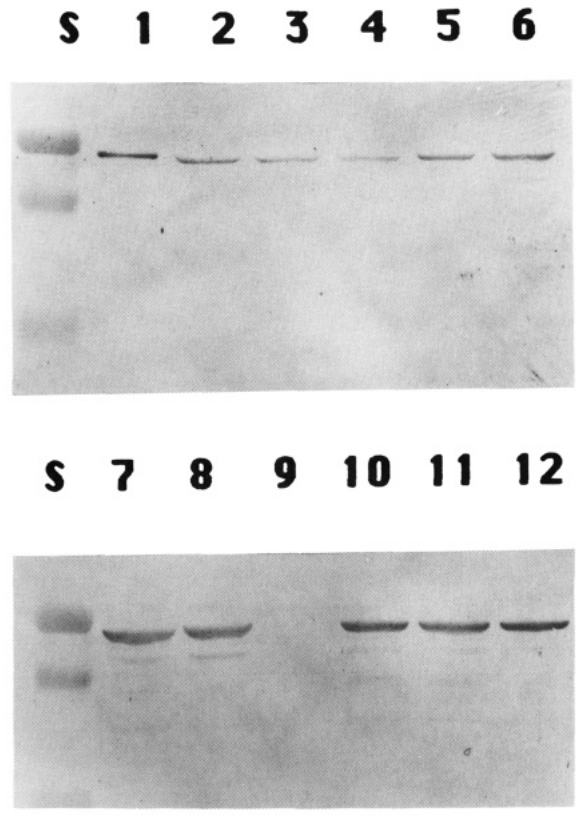

FIGURE 2: Western blots showing products formed by cells transfected with pT7/L-1 mutants. The sample identities are as follows: lanes S, molecular mass markers, in descending order, 106, 80, and 49.5 kDa; lane 1, pure L-1 expressed in *E. coli*; lanes 2–12, supernate of *E. coli* homogenates after treatment in French press. 2, H494Q; 3, H499Q; 4, H504Q; 5, H517Q; 6, H522Q; 7, H531Q; 8, H690Q; 9, control (host cells alone); 10, H494S; 11, H504S; 12, unmutated. Volume loaded per lane: lanes 1–6, 3 μL; lanes 7–12, 6 μL.

Although each of the mutant proteins was well expressed to approximately the same extent, enzyme activities ranged from 0% to 50% of the wild-type activity. Extracts were centrifuged and assayed for lipoxygenase activity as quickly as possible after preparation to minimize the possibility of inactivation, although no change in activity could be detected after storage at 4 °C for at least 24 h. The lipoxygenase activities of the mutants shown in Table II are normalized against the value obtained for the wild-type recombinant enzyme and are based on assays of the cell extract. Virtually all of the activity remained in the supernatant when the homogenate was centrifuged. The possibility that the results might reflect differences in the amount of lipoxygenase protein synthesized was considered. Differences may have existed, but they did not appear to be significant, judging from the intensity of Western blots. The ratios of the specific activities of the purified mutant enzymes to the activity of pure wild-type recombinant enzyme are qualitatively similar to the ratios when the corresponding crude supernates are compared (Table II). These results support the notion that the activities ob-

Table II: Lipoxygenase Activity and K_m of L-1 Mutants

mutant	relative activity[a] crude[b]	purified[c]	K_m (μM)[d]
wild type	100	100	11
H494Q	37 (49, 30, 31)	32	8
H494S	8 (7.7, 8.6, 8.3)	ND[e]	10
H499Q	<0.005	<0.005	ND
H504Q	<0.005	<0.005	ND
H517Q	33 (36, 30, 32)	46	12
H522Q	1 (1, 1, 0.75)	1.85	8
H531Q	20 (22, 13, 25)	30	17
H690Q	<0.005	<0.005	ND

[a] Averages from three preparations. [b] Activities refer to supernate from original homogenate. Value of recombinant wild type is taken as 100. Actual values were 7–9 lipoxygenase units/mL. [c] Values are relative specific activities of purified enzymes. Recombinant specific activity is taken as 100. Actual specific activity was 200 units/mL. [d] K_m was determined against linoleate. [e] ND, not determined.

served in the crude supernates of the active mutants probably reflect the presence of partially impaired protein species rather than diminished expression and survival of fully effective enzymes.

The relatively narrow range of K_m values found with the wild-type enzyme and the active mutants suggests that the His replacements have not influenced the substrate-binding region(s) of the enzyme.

The recombinant wild-type L-1 is relatively heat-stable (Steczko et al., 1991). When the mutant enzymes were subjected to 65 °C for 3 min (Figure 3), those that were devoid of activity, H499Q, H504Q, and H690Q, were found to be denatured while those that exhibited appreciable activity, H494Q, H517Q, and H531Q, were not. H522Q with an activity of only about 1% of the recombinant wild type was also denatured. The bands obtained on Western transfer blotting of the denatured proteins gave bands similar in position and intensity to those obtained with the unheated preparations, indicating that no significant proteolysis occurred during the heat treatment.

Discussion

Mutants H494Q and H494S exhibited lipoxygenase activity, albeit at a reduced level. The mutants H504Q and H504S were virtually devoid of activity. These results must be contrasted to those of Funk et al. (1989), who reported that replacement of His with Ser in the corresponding positions in 5-lipoxygenase from human leukocytes did not "substantially alter" the activity of the expressed enzymes.[3]

While the present paper was being completed for publication, a most interesting evaluation of some of the conserved His residues of human leukocytic 5-lipoxygenase, by Nguyen et al. (1991), appeared. These workers produced single mutations at the 5 conserved histidines at positions 363, 368, 373, 391, and 400 using Ser. The corresponding assignments in L-1 are as follows: 494, 499, 504, 522, and 531 (Figure 1). Their study did not include the sixth conserved His corresponding to L-1 His-690. When they expressed their mutated genes using baculovirus as vector and *Spodoptera frugiperda* as host, they obtained substantial activity with H363S, H391S, and H400S relative to the wild-type recombinant but saw no activity with H368S and H373S. These results agree with our findings with the corresponding residues in L-1, His-499 and His-504, but are contrary to the conclusion of Funk et al. (1989) that His-373 in 5-lipoxygenase is not essential. Although we found that the corresponding mutants of L-1 (H494Q or H494S, H522Q, and H531Q) were substantially active, our results relative to the recombinant L-1 wild type were somewhat lower. The greatest difference was noted in comparing human 5-lipoxygenase mutant H391S, which had about two-thirds of the wild-type activity, with the corresponding mutant, L-1 H522Q, which was only 1% as active as its wild-type counterpart. It is interesting that the soybean L-1 mutant, H494Q, which had about one-third of the wild-type activity, was more than 4 times as active as H494S.

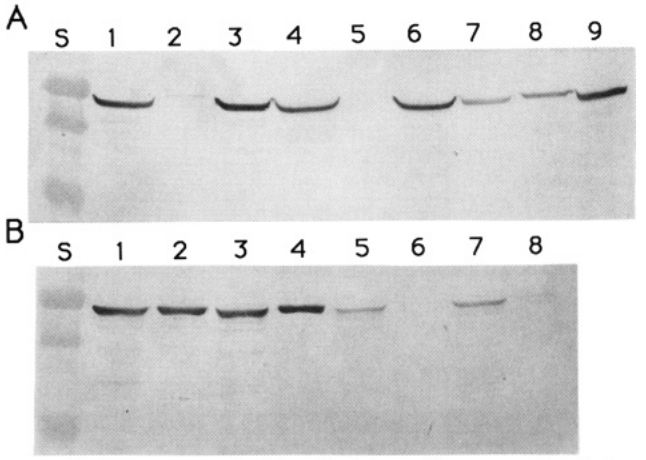

FIGURE 3: Western transfer blots of solutions of mutants of L-1 before and after heating at 65 °C for 3 min. (A) Lanes 1–3: (1) H499Q before heating; (2) after heating and centrifugation; (3) resuspended precipitate. Lanes 4–6: (4) H522Q before heating; (5) after heating and centrifugation; (6) resuspended precipitate. Lanes 7–9: (7) H517Q before heating; (8) after heating and centrifugation; (9) L-1 recombinant as marker. (B) Lanes 1 and 2: (1) Recombinant wild type before heating; (2) after heating and centrifugation. Lanes 3 and 4: (3) H494Q before heating; (4) after heating and centrifugation. Lanes 5 and 6: (5) H690Q before heating; (6) after heating and centrifugation. Lanes 7 and 8: (7) H504Q before heating; (8) after heating and centrifugation. Lanes S: Molecular mass markers, as in Fgiure 2.

Since substitutions in L-1 at His-499, His-504, and His-690 result in virtually complete loss of activity and substantial activity is retained with substitutions at His-494, His-517, and His-531, we suggest that the His residues in the first group may be involved in the binding of iron. This is not to say that the His residues in the second group could not be involved in iron binding, but it is possible that the carbonyls of Gln residues which have replaced them may act as binding sites, albeit less effective ones. It is also possible that the decrease in activity associated with substitutions of the second group of His residues may reflect alteration in protein structure not directly related to iron binding. In general, our findings are consistent with the conclusions reached by Nguyen et al. regarding the evaluation of the 5 conserved His residues even though the active mutants that are expressed in *S. frugiperda* retain a much higher proportion of the cognate wild-type activity than do our mutants. It should be noted however that 5-lipoxygenase appears to be an intrinsically less efficient enzyme than soybean L-1, the former having a specific activity of 24 units/mg (Percival, 1991) as compared to 200 units/mg for the latter (Axelrod et al., 1981).

A proposal that His-647 and His-657 serve as iron ligands in L-1 has been offered by Navaratam et al. (1988) on the

[3] While the present paper was under review, workers in the laboratory where the report by Funk et al. (1989) originated revised and extended the study of human 5-lipoxygenase (Zhang et al., 1992). It is no longer claimed that mutants carrying replacements of His-362 and -372 (which correspond to His-494 and -504 in soybean L-1) are not substantially altered. Their results are essentially similar to what we report here for soybean L-1 for all 6 conserved histidines.

basis of the proximity of these residues to a highly hydrophobic Trp-rich region as well as to acidic residues which presumably would supply oxygen ligands from the carboxyl group. This proposition appears to be untenable since His-647 is absent from the corresponding positions in all of the other lipoxygenases. Moreover, while His-657 is conserved in the plant lipoxygenases, it does not appear in the equivalent position in any of the animal lipoxygenases.

As noted, the inactive L-1 mutants, with substitutions at His-494, His-504, and His-690, lack the heat stability shown by wild-type L-1 whereas the mutants which show reasonable retention of activity, H494Q or H494S, H517Q, and H531Q, are heat-stable. In this connection, it has often been observed that removal of a metal from a metalloprotein results in its destabilization, e.g., Donovan and Ross (1975). Mutant H522Q, which has about 1% of the wild-type activity, appears as an exception, since it lacks the heat stability of the other active mutants but exhibits measurable activity. Perhaps the binding of the iron is compromised by the substitution, making it more dissociable. Work is underway to obtain sufficient amounts of purified mutants for the determination of iron content and affinity and for the characterization of the EPR spectra of the Fe(II) and Fe(III) forms of the enzyme. The ultimate identification of the His residues as well as other amino acid residues participating in the coordination of the iron must await solution of the X-ray structure of the enzyme.

References

Antanaitis, B. C., Peisach, J., Mims, W. B., & Aisen, P. (1985) *J. Biol. Chem. 260*, 4572–4574.

Axelrod, B., Cheesbrough, T. M., & Laakso, S. (1981) *Methods Enzymol. 71*, 441–451.

Balcarek, J. M., Thiesen, T. W., Cook, M. N., Varrichio, A., Hwang, S.-M., Strohsacker, M. W., & Crooke, S. T. (1988) *J. Biol. Chem. 263*, 13937–13941.

Chasteen, D. (1983) *Adv. Inorg. Biochem. 5*, 201–233.

Diesenhofer, J., Epp, O., Miki, K., Humbler, R., & Michel, H. (1985) *Nature 318*, 618–624.

Dixon, R. A. F., Jones, R. E., Diehl, R. E., Bennett, C. D., Kargman, S., & Rouzer, C. A. (1988) *Proc. Natl. Acad. Sci. U.S.A. 85*, 416–420.

Donovan, J. W., & Ross, K. D. (1975) *J. Biol. Chem. 250*, 6026–6031.

Dunham, W. R., Carroll, R. T., Thompson, J. F., Sands, R. H., & Funk, M. O., Jr. (1990) *Eur. J. Biochem. 190*, 611–617.

Ealing, P. M., & Casey, R. (1988) *Biochem. J. 253*, 915–918.

Ealing, P. M., & Casey, R. (1989) *Biochem. J. 264*, 929–932.

Feiters, M. C., Aasa, R., Malmström, B. G., Slappendel, S., Veldink, G. A., & Vliegenthart, J. F. G. (1985) *Biochim. Biophys. Acta 831*, 302–305.

Feiters, M. C., Boelens, H., Veldink, G. A., Vliegenthart, J. F. G., Navaratnam, S., Allen, J. C., Nolting, H.-F., & Hermes, C. (1990) *Recl. Trav. Chim. Pays-Bas 109*, 133–146.

Fleming, J., Thiele, B. J., Chester, J., O'Prey, J., Janetzki, S., Aitken, A., Anton, L. A., Rapoport, S. M., & Harrison, P. R. (1989) *Gene 79*, 181–188.

Funk, C. D., Gunne, H., Steiner, H., Izumi, T., & Samuelsson, B. (1989) *Proc. Natl. Acad. Sci. U.S.A. 86*, 2592–2596.

Funk, C. D., Furci, L., & Fitzgerald, G. A. (1990) *Proc. Natl. Acad. Sci. U.S.A. 87*, 5638–5642.

Funk, M. O., Jr., Carroll, R. T., Thompson, J. F., Sands, R. H., & Dunham, W. R. (1990) *J. Am. Chem. Soc. 112*, 5375–5376.

Gardner, H. W. (1991) *Biochim. Biophys. Acta 1084*, 221–239.

Matsumoto, T., Funk, C. D., Rådmark, O., Höög, J.-O, Jörnvall, H., & Samuelsson, B. (1988) *Proc. Natl. Acad. Sci. U.S.A. 85*, 26–30.

Navaratnam, S., Feiters, M. C., Al-Hakim, M., Allen, J. C., Veldink, G. A., & Vliegenthart, J. F. G. (1988) *Biochim. Biophys. Acta 956*, 70–76.

Nelson, M. J. (1988) *J. Am. Chem. Soc. 110*, 2985–2986.

Nelson, M. J., Seitz, S. P., & Cowling, R. A. (1990) *Biochemistry 29*, 6897–6903.

Nguyen, T., Falgueyret, J.-P., Abramowitz, M., & Riendeau, D. (1991) *J. Biol. Chem. 266*, 22057–22062.

Nordlund, P., Sjöberg, B.-M., & Eklund, H. (1990) *Nature 345*, 593–598.

Percival, M. D. (1991) *J. Biol. Chem. 266*, 10058–10061.

Petersson, L., Slappendel, S., & Vliegenthart, J. F. G. (1985) *Biochim. Biophys. Acta 828*, 81–85.

Pistorius, K. E., & Axelrod, B. (1974) *J. Biol. Chem. 249*, 3183–3186.

Potter, B. V. L., & Eckstein, F. (1984) *J. Biol. Chem. 259*, 14243–14248.

Que, L., Jr. (1983) *Adv. Inorg. Biochem. 5*, 167–199.

Sayers, J. R., Schmidt, W., & Eckstein, F. (1988) *Nucleic Acids Res. 16*, 791–802.

Shibata, D., Steczko, J., Dixon, J. E., Hermodson, M., Yazdanparast, R., & Axelrod, B., (1987) *J. Biol. Chem. 262*, 10080–10085.

Shibata, D., Steczko, J., Dixon, J. E., Andrews, P. C., Hermodson, M., & Axelrod, B. (1988) *J. Biol. Chem. 263*, 6816–6821.

Shibata, D., Kato, T., & Tanaka, K. (1991) *Plant Mol. Biol. 16*, 353–359.

Siedow, J. N. (1991) *Annu. Rev. Plant Physiol. Plant Mol. Biol. 42*, 145–188.

Sigal, E., Craik, C. S., Highland, E., Grunberger, D., Costello, L. L., Dixon, R. A. F., & Nadel, J. A. (1988) *Biochem. Biophys. Res. Commun. 157*, 457–469.

Steczko, J., Donoho, G. P., Dixon, J. E., Sugimoto, T., & Axelrod, B. (1991) *Protein Expression Purif. 2*, 221–227.

Vesberg, H.-P., & Eckstein, F. (1982) *J. Biol. Chem. 257*, 6595–6599.

Whittaker, J. W., & Solomon, E. I. (1988) *J. Am. Chem. Soc. 110*, 5329–5339.

Wilkins, P. C., & Harrington, P. C. (1983) *Adv. Inorg. Biochem. 5*, 52–85.

Yenofsky, R. L., Fine, M., & Liu, C. (1988) *Mol. Gen. Genet. 211*, 215–222.

Yoshimoto, T., Suzuki, H., Yamamoto, S., Takai, T., Yokoyama, C., & Tanabe, T. (1990) *Proc. Natl. Acad. Sci. U.S.A. 87*, 2142–2146.

Zhang, Y., Gebhard, M. S., & Solomon, E. I. (1991) *J. Am. Chem. Soc. 113*, 5162–5175.

Zhang, Y. Y., Rådmark, O., & Samuelsson, B. (1992) *Proc. Natl. Acad. Sci. U.S.A. 89*, 485–489.