Purification and Characterization of Linoleate 8-Dioxygenase from the Fungus Gaumannomyces graminis as a Novel Hemoprotein*

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The fungus Gaumannomyces graminis, which causes the major root disease of wheat known as "take-all," can metabolize linoleic acid to (8R)-hydroperoxylinoleic acid. The enzyme linoleate 8-dioxygenase abstracts hydrogen and introduces molecular oxygen in an antarafacial way at C-8. We have now purified the enzyme 1000-fold to a specific activity of 1.8 μmol/min/mg of protein. Acetone powder of mycelia of G. graminis was subjected to extraction and ammonium sulfate precipitation with solubilization. The 8-dioxygenase was purified by hydrophobic interaction chromatography, size-exclusion chromatography, anion-exchange chromatography, and immobilized metal ion affinity chromatography. The active enzyme appeared to consist of four subunits since the active enzyme had an apparent molecular mass of 520 kDa determined by gel filtration, while SDS-polyacrylamide gel electrophoresis showed a protein band of 520 kDa. The absorption maximum shifted to 429 nm after reduction with dithionite and to 421 nm after treatment of the reduced enzyme with carbon monoxide. BW4A4, a hydroxamic acid derivative, inhibited the enzyme by >90% at 10 μM. The pH optimum was 7.2-7.4, the isoelectric point was 5.2 by chromatofocusing, and the K_m values were 8 μM for linoleic acid and 30 μM for oxygen. We conclude that linoleate 8-dioxygenase appears to be a tetrameric hemoprotein distinct from other fatty-acid dioxygenases.

Dioxygenases oxygenate aromatic rings or aliphatic structures by inserting molecular oxygen (Webb, 1992). Almost all dioxygenases contain loosely bound ferrous or ferric iron, and only a few dioxygenases are hemoproteins.

Polysaturated fatty acid can be oxygenated by two major classes of dioxygenases, namely lipoxygenases, which contain non-heme iron, and PGH1 synthases, which contain heme. Lipoxygenases occur in mammals and plants and catalyze the first step in biosynthesis of biologically active products, e.g., leukotrienes from arachidonic acid in animals or jasmonic acid from linoleic acid in plants (Gardner, 1993; Siedow, 1991; Yamamoto, 1992). Many lipoxygenases have been purified, cloned, and sequenced, and their reaction mechanism is known in detail. Lipoxygenases abstract a bisallylic hydrogen of the 1Z,4Z-pentadienyl group of the polysaturated fatty acid and insert molecular oxygen in an antarafacial way at C-1 or C-5, with formation of a cis,trans-conjugated double bond. The three-dimensional structure of soybean lipoxygenase-1 has been determined, and non-heme iron has been unequivocally demonstrated at the active site (Boyington et al., 1993; Minor et al., 1993). PGH synthases catalyze the double dioxygenation of arachidonic acid to PGG2 (the cyclooxygenase activity) and the reduction of PGG2 to PGH2 (the peroxidase activity) (Smith and Marnett, 1991; Hla and Nelson, 1992). The first step in the biosynthesis of PGG2 is abstraction of the pro-S-hydrogen at C-13 of arachidonic acid and antarafacial insertion of oxygen at C-11 to generate the 11R-peroxy radical (Hamberg and Samuelsson, 1967; Smith and Marnett, 1991; Oliw et al., 1993b). The three-dimensional structure of PGH synthase-1 has been determined (Picot et al., 1994). The enzyme is dimeric and requires heme for both its cyclooxygenase and peroxidase activities.

We have described a potential third class of fatty-acid dioxygenases. Two fungi, Gaumannomyces graminis and Laetisaria arvalis, contain a cytosolic oxygenase, linoleate 8-dioxygenase, which differs catalytically from lipoxygenases and PGH synthases (Brodowsky and Oliw, 1992, 1993; Brodowsky et al., 1992). This enzyme abstracts the pro-S-hydrogen at C-8 of linoleic acid and inserts molecular oxygen in an antarafacial way at C-8, leading to formation of 8HPDE (Hamberg et al., 1994). Minor products, possibly formed by 8-dioxygenase, were (11R)- and (10R)-hydroxyoctadeca-9Z,12Z-dienoic acids (Brodowsky et al., 1992). The enzyme oxygenated oleic, α-linolenic, and ricinoleic acids, while arachidonic, γ-linolenic, and stearic acids were not substrates.

It is noteworthy that the 8-dioxygenase abstracts a monoallylic hydrogen, which requires ~15% more energy (i.e. an additional 10 kcal/mol) than abstraction of a bisallylic hydrogen by lipoxygenases (Brodowsky, 1994; Porter et al., 1995). Lipoxygenases have never been found to abstract hydrogen and to insert molecular oxygen at the same carbon. PGH synthases can perform this reaction with at least one fatty acid, 5,8,11-Eicosatetraenoic acid, which cannot be converted to PGs, is oxidized to (13R)-hydroxy-5Z,8Z,11Z-eicosatrienoic acid by PGH synthase-1 and -2 in an "abortive" cyclooxygenase reaction (Elliott et al., 1986; Oliw et al., 1993b).

The mechanism of interaction between the enzyme, its substrate, and molecular oxygen at the active site of linoleate...
8-dioxygenase is uncharacterized, and only little is known about the biological function of the enzyme. Its product, 8-HPDE, can be further metabolized. Reduction yields the alcohol 8-HODE, 8-HODE was originally identified as a metabolite of L. arvalis (Bowers et al., 1986) and later as a factor, which induces premature sexual sporulation of Aspergillus nidulans (Champe and El-Zayat, 1989; Mazur et al., 1990). A microsomal hydroperoxide isomerase from G. graminis transforms 8-HPDE to a diol, 7,8-DiHODE, but L. arvalis lacks this enzyme (Brodowsky et al., 1992; Brodowsky and Oliw, 1993; Su et al., 1995). The biological functions of 8-HPDE, 8-HODE, and 7,8-DiHODE are unknown, but it is possible that these oxylinps could affect growth and reproduction. This may be worth investigation. The ascomycete G. graminis causes the wheat root disease "take-all", which causes substantial losses for farmers all over the world (Abelson, 1995).

The main objective of this study was to purify the 8-dioxygenase from G. graminis to homogeneity and to determine whether the enzyme contains heme or non-heme iron. We also wanted to determine some properties of the purified protein. Our results suggest that 8-dioxygenase may belong to a novel family of fatty-acid dioxygenases that are distinct from lipoxygenases and PGH synthases.

**EXPERIMENTAL PROCEDURES**

**Materials—** G. graminis var. graminis was obtained from Centraalbureau voor Schimmelcultures (Baarn, The Netherlands) and cultivated as described (Su et al., 1996). Mycelia were harvested by vacuum filtration and stored at –80 °C. [1-14C]Linoleic acid (55 Ci/mole) was from Amersham International (Amersham, Buckinghamshire, United Kingdom). Precasted TLC plates (0.25-mm Silica Gel 60A, 5

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**Enzyme Assays—** All incubations were performed on ice for 5–10 min in a volume of 100 μl unless stated otherwise. An aliquot (0.2–10 μl, adjusted to 10 μl with buffer) from the chromatography fractions was mixed with 85 μl of 10 mM TEA, 5 mM EDTA, 1 mM GSH and incubated with 1 nmol of [1-14C]linoleic acid (10 μM), added in 5 μl of 30% ethanol in the same buffer. The amount of enzyme was usually adjusted so that the conversion of the substrate was <25%; the conversion was linear with the amount of added protein and with time. The effect of 0.01% CHAPS was assayed in duplicate or triplicate after 10 min of preincubation. After termination with ethanol and extractive isolation on Sep-Pak C18 reagents for SDS-PAGE were from Bio-Rad. Bovine albumin, bovine hemin (type I), CHAPS, and GSH were from Sigma. Tween 20 was from U. S. Biochemical Corp. Sulfob Enzyme Assays— All incubations were performed on ice for 5–10 min in a volume of 100 μl unless stated otherwise. An aliquot (0.2–10 μl, adjusted to 10 μl with buffer) from the chromatography fractions was mixed with 85 μl of 10 mM TEA, 5 mM EDTA, 1 mM GSH and incubated with 1 nmol of [1-14C]linoleic acid (10 μM), added in 5 μl of 30% ethanol in the same buffer. The amount of enzyme was usually adjusted so that the conversion of the substrate was <25%; the conversion was linear with the amount of added protein and with time. The effect of 0.01% CHAPS was assayed in duplicate or triplicate after 10 min of preincubation. After termination with ethanol and extractive isolation on Sep-Pak C18 reagents for SDS-PAGE were from Bio-Rad. Bovine albumin, bovine hemin (type I), CHAPS, and GSH were from Sigma. Tween 20 was from U. S. Biochemical Corp. Sulfob...
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### RESULTS

#### Purification of Linoleate 8-Dioxygenase

An outline of the purification of 8-dioxygenase and the results are summarized in Table I. A modification of this scheme gave similar results (see procedure B in Table I). The enzyme was purified >1000-fold up to a specific activity of 1.8 μmol/min/mg. The purified enzyme was associated with a pale yellow color. SDS-PAGE showed a major protein band of 130 kDa and only traces of other proteins on silver staining.

Although the 8-dioxygenase was mainly found in the cytosolic fraction, it appeared to share membrane protein properties and tend to form aggregates during chromatography. It was therefore necessary to use detergents. Several detergents were tested in order to solubilize the enzyme from the ammonium sulfate precipitate without inhibiting the enzyme. CHAPS (0.5 mM), sodium deoxycholate (0.5 mM), and Tween 20 (0.15%) were most effective. Tween 20 was compatible with the hydrophobic interaction chromatography. CHAPS improved the separation by gel filtration. Another problem was enzyme instability, particularly at the early stages of purification. It was essential to have EDTA (1–5 mM) and GSH (1 mM) present and to include sorbitol (0.75 M) during extraction.

Three media for hydrophobic interaction chromatography were evaluated: octyl-, phenyl-, and butyl-Sepharose. Chromatography on butyl-Sepharose 4FF resulted in a 10-fold purification with good recovery (Fig. 1A and Table I). The subsequent gel filtration (Sephacryl S-300 HR) was essential to remove large aggregates of proteins (Fig. 1B). Anion-exchange chromatography on Q-Sepharose FF purified the enzyme 5-fold (Fig. 1C).

Most of the remaining contaminating proteins were removed by HPLC on Mono Q HR 5/5 (Fig. 2A), followed by gel filtration on Biosepharose SEC-S3000 (Fig. 2B). However, our enzyme assay showed that hydroperoxide isomerase could be present even after HPLC. The linoleate 8-dioxygenase could be separated from the hydroperoxide isomerase by IMAC with Zn²⁺ as the ligand (Fig. 2C) and eluted with 40 mM imidazole or with 25 mM EDTA. We confirmed that 40 mM imidazole, 25 mM EDTA did not inhibit the hydroperoxide isomerase.

| Purification step | Total protein | Specific activity | Recovery | Purification |
|------------------|---------------|------------------|----------|--------------|
| Procedure A      |               |                  |          |              |
| Extract of 5 g of acetone powder | 687 | 0.94 | 100 | 1 |
| Ammonium sulfate cut | 186 | 2.91 | 90 | 3 |
| Butyl-Sepharose 4FF | 13.9 | 34.0 | 78 | 36 |
| Sephacryl S-300 HR | 3.75 | 92.2 | 57 | 98 |
| Q-Sepharose FF | 0.52 | 485 | 42 | 512 |
| Mono Q HR 5/5 | 0.10 | 743 | 12 | 787 |
| Biosepharose SEC-S3000^a | 1080 | 1150 | 90 | 1150 |
| Procedure B      |               |                  |          |              |
| Extract of 25 g of acetone powder | 3280 | 1.56 | 100 | 1 |
| Ammonium sulfate cut | 1340 | 2.64 | 69 | 2 |
| Butyl-Sepharose 4FF | 95.6 | 29.2 | 55 | 19 |
| Chelating Sepharose FF | 23.0 | 72.0 | 32 | 46 |
| Sephacryl S-300 HR | 10.7 | 134 | 28 | 86 |
| Mono Q HR 5/5 | 0.66 | 703 | 9 | 451 |
| Biosepharose SEC-S3000^a | 1860 | 1190 | 100 | 1190 |

^a Purification of aliquots from Mono Q HR 5/5 on this gel filtration column for HPLC resulted in a loss of ~30% of the applied enzyme activity but the specific activity increased. Enzyme activity was measured on ice.

#### Prosthetic Groups

The spectrum of the purified enzyme is shown in Fig. 3A. The native protein showed absorption peaks at 280 and 408 nm (γ, Soret) and weaker and broader absorption maxima at 504–535 nm (β), 565 nm (α), and 631 nm. The ratio of A_{408}/A_{320} was 0.54 ± 0.03 nm (S.D.) in six different enzyme preparations. Incubation of 0.3 mM linoleic acid with 0.56 μM purified enzyme appeared to shift the Soret absorption only slightly to 407 nm, and the A_{408}/A_{320} ratio appeared to decrease from 0.53 to 0.44. Treatment with sodium dithionite shifted the Soret band to 429 nm (Fig. 3B). After saturating the reduced enzyme with CO, there were two Soret bands at 421 nm and a transient shoulder at 440 nm, but no distinct peak at 450 nm (Fig. 3B).

The pyridine ferrohemochromogen of the purified enzyme showed absorption maxima at 556.5 nm (α) and 525 nm (β) as shown in Fig. 3C. These data were almost identical to the hemin standard and are indicative of a hemoprotein of the cytochrome group b (Webb, 1992). An enzyme preparation, which showed virtually no other proteins on SDS-PAGE than the 130-kDa protein, had a specific activity of 0.7 μmol/min/mg and contained 0.49 mg of protein/ml, corresponding to ~0.72 μM enzyme. The sample contained 2.0 μM heme as judged from absorption of the pyridine ferrohemochromogen at 557 nm. These figures indicated 2.8 mol of heme/mol of enzyme (520 kDa). Heme might be partly lost from the enzyme during purification. We conclude that the enzyme contains at least 2 mol of heme/mol of enzyme.

#### Molecular Mass and Oligomeric Structure

SDS-PAGE from the different stages of enzyme purification is shown in Fig. 4A. After Mono Q HR 5/5 column chromatography, the band at 130 kDa was intense on SDS-PAGE, while only traces of other proteins were found. Gel filtration (Biosepharose SEC-S3000) yielded enzyme activity associated with a protein peak around 520 kDa (Fig. 2B). Collecting small fractions over this peak showed that enzyme activity and the intensity of the 130-kDa protein band on SDS-PAGE appeared to vary in parallel, as discussed below (Fig. 2B). The size of the active enzyme on gel filtration SDS-PAGE was thus estimated to be 4-fold larger than on SDS-PAGE, ~520 and 130 kDa, respectively. SDS-PAGE with or without treatment of the sample with a strong reducing agent (β-mercaptoethanol) did not affect the results, indicating that the 130-kDa subunits were not covalently bound to each other by disulfide bridges.

The 130-kDa protein band was also present in the large
protein peak without enzyme activity, which eluted after the enzyme. Analysis of the Soret band at 408 nm during gel filtration gave important information. As shown in Fig. 4C, two major peaks of protein with absorption at 408 nm were separated by gel filtration (Biosep SEC-S3000). The first eluting peak (peak I) contained active enzyme, while the second peak (peak II) was inactive. SDS-PAGE and spectroscopic analysis of both peaks gave the same results, a protein band at 130 kDa and a Soret band at 408 nm, which shifted to 429 and 421 nm, as discussed above. When the material in peak I was concentrated, frozen, thawed, and reanalyzed by gel filtration under identical conditions, peaks I and II were present again (Fig. 4D). It appears as if peak I contains the active enzyme as a tetramer of \(520\) kDa, which is followed by peak II-containing subunits in oligomers of lower orders. The tetramer did not appear to be completely dissociated by 4M urea. The enzyme could be incubated in 4M urea for 1 h, followed by desalting (PD-10), with only partial loss of enzyme activity. Gel filtration in 4M urea showed the same elution pattern, with two major protein peaks with enzyme activity only in the first (after desalting).

Purified 8-dioxygenase thus showed a major band at 130 kDa and a faint diffuse protein band at \(-100\) kDa as judged by
silver staining (Fig. 4A). The intensity of the latter band varied in different batches, and it proved to be difficult to remove completely. The intensity of this band appeared to increase with storage of the partly purified enzyme since SDS-PAGE of several batches of 8-dioxygenase, which were purified almost without interruption, showed the highest purity.

Several observations indicated that the 130-kDa band, but not the 100-kDa band, was associated with the 8-dioxygenase activity. First, purification only moderately enriched the 100-kDa protein or proteins (Fig. 4A). Second, we occasionally obtained fractions during chromatography in which these two bands appeared to be of almost equal intensity, but these fractions did not have a very high specific enzyme activity. The 130-kDa protein must be cloned and expressed to obtain certainty, but it was reassuring to find that the amino acid sequences of four oligopeptides (with 7, 8, 11, and 14 sequenced amino acid residues), which were obtained by enzymatic digestion of the 130-kDa band, did not match any previously sequenced proteins.

3 C. Su, U. Hellman, and E. H. Oliw, unpublished observation.
Properties of Linoleate 8-Dioxygenase

pH Optimum—Enzyme activity was assayed at pH 6.4, 6.8, 7.2, 7.4, 7.6, and 8.0. The highest activity of the purified enzyme was noted at pH 7.2 and 7.4 (31% conversion of substrate at both pH values).

Isoelectric Point—Enzyme activity eluted as one peak on chromatofocusing (Mono P HR 5/5) at pH 5.2 (25°C). SDS-PAGE showed that this fraction contained a protein band at 130 kDa.

Stability—The enzyme was quite unstable at the early steps of purification, but active fractions from anion-exchange chromatography could be kept for 1 week on ice with only a small loss of enzyme activity. We routinely stored the enzyme at −80°C, but repeated freezing and thawing, particularly of dilute enzyme solutions, appeared to inactivate the enzyme, as discussed above (Fig. 4D).

Kinetic Constants—The enzyme was purified to a specific activity of 1.8 μmol/min/mg at 0°C (Table I). The turnover number on ice with 10 μM linoleic acid as a substrate was calculated to be 15/s. The K_{m} and V_{max} values for oxygen were calculated from incubations of enzyme (after Mono Q HR 5/5 purification; see Table I) with 0.2 mM linoleic acid at 25°C. Initial oxygen consumption was measured with an oxygen electrode. The reaction was started by adding the enzyme. The oxygen concentration was varied from 0.247 to 0.016 mM (five points in duplicate, r = 0.99). The V_{max} and K_{m} values were estimated to be −2.2 μmol/min/mg of protein and 30 μM, respectively. The K_{m} and V_{max} values for linoleic acid were determined in the same way, but with oxygen-saturated buffer using 7–200 μM linoleic acid (five points in duplicate, r = 0.99), and were found to be 8 μM and 4 μmol/min/mg of protein, respectively.

Inhibition by BW A4C—The hydroxamic acid derivative BW A4C at 10 μM inhibited 8-dioxygenase by >90%, while 1 μM caused −50% inhibition.

CO, KCN, and H_{2}O_{2}—The enzyme was not inhibited by 1 mM KCN. The enzyme was mixed with 4 volumes of CO- or N_{2}-saturated buffer and then incubated with substrate. CO did not inhibit the enzyme. Finally, H_{2}O_{2} (up to 9 mM) did not support enzyme activity under anaerobic conditions.

DISCUSSION

We have purified linoleate 8-dioxygenase >1000-fold to one major protein band on SDS-PAGE (Fig. 4A and Table I). Linoleate 8-dioxygenase appeared to consist of four noncovalently bound subunits of 130 kDa. The enzyme contained heme. This finding was unexpected. Only a few dioxygenases contain heme (Webb, 1992). Linoleate 8-dioxygenase might be related to other heme-containing oxygenases, e.g., PGH synthases, tryptophan 2,3-dioxygenase, or cytochrome P450.

Linoleate 8-dioxygenase was inhibited by micromolar concentrations of BW A4C, which is a hydroxamic acid derivative and a potent lipoxygenase inhibitor (McMillan and Walker, 1992). Hydroxamic acid derivatives can chelate iron, but they may also have redox properties (Nelson et al., 1991). Some other lipoxygenase inhibitors and reducing agents can inhibit linoleate 8-dioxygenase (Brodowsky et al., 1994; Su et al., 1995). In spite of pharmacological similarities, linoleate 8-dioxygenase and lipoxygenases are clearly different. Lipoxygenases do not contain heme and do not catalyze hydrogen abstraction and oxygen insertion at the same carbon. Lipoxigenases and linoleate 8-dioxygenase differ in substrate requirements. The 8-dioxygenase can metabolize oleic acid (Brodowsky et al., 1992), while lipoxygenases require fatty acids with methylene-interrupted double bonds (Gardner, 1991; Siedow, 1991; Yamamoto, 1992). We conclude that lipoxygenases and 8-dioxo-
genase belong to different families of enzymes.

Linoleate 8-dioxygenase could be related to cytochrome P450, but the reaction mechanisms of 8-dioxygenase and P450 have little in common. This work shows that purified linoleate 8-dioxygenase does not appear to require any cofactors or other enzymes for full activity. P450 can hydroxylate fatty acids in the presence of NADPH and cytochrome P450 reductase. In addition, P450 cannot form hydroperoxy fatty acids enzymatically (Capdevila et al., 1981; Oliw et al., 1993a; Oliw, 1994). Cysteine is the proximal heme iron ligand of P450, and heme-thiolate proteins show a characteristic absorption at 450 nm after reduction and treatment with CO (Nelson et al., 1993; Oliw, 1994). It cannot be excluded that 8-dioxygenase also might be a heme-thiolate protein, but it seems unlikely. We were unable to obtain the characteristic spectrum with a distinct peak at 450 nm.

It seems relevant to compare linoleate 8-lipoxygenase with heme-containing dioxygenases. Tryptophan 2,3-dioxygenase has been well investigated (Schutz and Feigelson, 1972; Leeds et al., 1993), and its reaction mechanism is interesting. Tryptophan 2,3-dioxygenase degrades the indole ring of tryptophan and forms N-formylkynurenine. The initial reaction involves hydrogen abstraction from the indole nitrogen (N-1), migration of the C-2–C-3 double bond to N-1–C-2, insertion of molecular oxygen, and formation of a peroxyl radical at C-3 (Leeds et al., 1993). Tryptophan 2,3-dioxygenase from mammalian liver is a tetrameric hemoprotein with four identical 48-kDa subunits (Schutz and Feigelson, 1972; Masezono et al., 1990). The purified enzyme contains 2 mol of heme, and no other cofactor is associated with the enzyme. The ligand of heme iron is a nitrogen atom of a histidine residue. Exogenous heme stimulates the activity, which suggests a stoichiometry of one heme/subunit (Schutz and Feigelson, 1972; Leeds et al., 1993). The ferrous form of the enzyme is active, and the function of heme is likely to bind and activate molecular oxygen rather than the substrate (Leeds et al., 1993).

There is a specific reason to compare the reaction mechanism of linoleate 8-dioxygenase with that of PGH synthases. With 5,8,11-eicosatrienoic acid as a substrate, PGH synthase-1 and -2 can perform an abortive cyclooxygenase reaction by abstracting hydrogen and inserting molecular oxygen at C-13 (Elliott et al., 1986; Oliw et al., 1993b). This reaction appears to be similar to that of linoleate 8-dioxygenase. PGH synthase-1 contains two essential elements for its cyclooxygenase activity, heme and Tyr^{385}. This tyrosine residue is located at the end of the substrate channel within 10 Å of the heme iron (Picot et al., 1994). Tyrosyl radicals can be detected by electron resonance spectroscopy during enzyme catalysis, but how heme and Tyr interact is not known (Tsai et al., 1994). Whether the active site of linoleate 8-dioxygenase contains heme and tyrosine in close relation and whether tyrosyl radicals are formed during catalysis are unknown. Linoleate 8-dioxygenase can now be purified in milligram amounts, and it should therefore be possible to address this question.

Linoleate 8-dioxygenase is a tetramer hemoprotein with no other cofactors, and it contains at least 2 mol of heme. The low heme number indicates that it could be composed of two types of subunits of equal size. There are at least two possible oxygenation mechanisms for the enzyme, oxygen activation or substrate activation, as illustrated by tryptophan 2,3-dioxygenase and PGH synthase, respectively. In both models, the pro-S-hydrogen at C-8 is abstracted, a carbon-centered radical is formed at C-8, and molecular oxygen is inserted with inversion of configuration at C-8 (Hamberg et al., 1994). The (8R)-peroxy radical is then reduced to 8-HPODE. Additional work is needed to elucidate the reaction mechanism and the precise
interaction between substrate and enzyme, oxygen, and heme.

In summary, we have purified linoleate 8-dioxygenase from the ascomycete G. graminis, which is a devastating pathogen of wheat all over the world. The biological function of this enzyme is unknown, but it may affect sexual sporulation and fungal growth. Our results suggest that linoleate 8-dioxygenase is a hemoprotein that is distinct from previously described fatty-acid dioxygenases. The reaction mechanism of the enzyme may have properties in common with tryptophan 2,3-dioxygenase or PGH synthases.

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REFERENCES

Abelson, P. H. (1995) Science 269, 1027
Bowers, W. S., Hoch, H. C., Evans, P. H., and Katayama, M. (1986) Science 232, 105–106
Boyington, J. C., Gaffney, B. J., and Amzel, L. M. (1993) Science 260, 1482–1486
Bradford, M. M. (1976) Anal. Biochem. 72, 248–254
Brodowsky, I. D. (1994) Acta Univ. Ups. 123, 1–46
Brodowsky, I. D., and Oliw, E. H. (1992) Biochim. Biophys. Acta. Acta 1124, 59–65
Brodowsky, I. D., and Oliw, E. H. (1993) Biochim. Biophys. Acta 1168, 68–72
Brodowsky, I. D., Hamberg, M., and Oliw, E. H. (1992) J. Biol. Chem. 267, 14738–14745
Brodowsky, I. D., Hamberg, M., and Oliw, E. H. (1994) Eur. J. Pharmacol. 254, 43–47
Capdevila, J., Chacos, N., Werringloer, J., Prough, R. A., and Estabrook, R. W. (1981) Proc. Natl. Acad. Sci. U.S.A. 9, 5362-5366
Champ, S. P., and El-Zayat, A. A. E. (1989) J. Bacteriol. 171, 3982–3988
Elliott, W. J., Morrison, A. H., Sprecher, H., and Needelman, P. (1986) J. Biol. Chem. 261, 6719–6724
Gardner, H. W. (1991) Biochim. Biophys. Acta 1084, 221–239
Hamberg, M., and Samuelsson, B. (1967) J. Biol. Chem. 242, 5336–5343
Hamberg, M., Zhang, L.-Y., Brodowsky, I. D., and Oliw, E. H. (1994) Arch. Biochim. Biophys. 309, 77–80
Hla, T., and Neillson, K. (1992) Proc. Natl. Acad. Sci. U.S.A. 89, 7384–7388
Laemmli, U. K. (1970) Nature 227, 680–685
Leeds, J. M., Brown, P. J., McGeer, M. G., Brown, F. K., and Wiseman, J. S. (1993) J. Biol. Chem. 268, 17781–17786
Maezono, K., Tashiro, K., and Nakamura, T. (1990) Biochim. Biophys. Res. Commun. 170, 176–181
Mazur, P., Meyers, H. V., Nakanishi, K., El-Zayat, A. A. E., and Champe, S. P. (1995) Tetrathraon Lett. 31, 3837–3840
McMillan, R. M., and Walker, E. R. H. (1992) Trends Pharmacol. Sci. 13, 323–330
Minor, W., Steczko, J., Bolin, J. T., Otwiinoski, Z., and Axelrod, B. (1993) Biochemistry 32, 6320–6323
Nelson, M. J., Batt, D. G., Thompson, J. S., and Wright, S. W. (1991) J. Biol. Chem. 266, 8225–8229
Nelson, D. R., Kamataki, T., Wexman, D. J., Guengerich, F. P., Estabrook, R. W., Feyerlein, R., Gonzales, F. J., Coon, M. J., Gunsales, I. C., Goto, O., Okuda, K., and Nebert, D. W. (1993) DNA Cell Biol. 12, 1–51
Ohsawa, K., and Ebata, N. (1983) Anal. Biochem. 135, 409–415
Oliw, E. H. (1994) Prog. Lipid Res. 33, 329–354
Oliw, E. H., Brodowsky, I. D., Hörnsten, L., and Hamberg, M. (1993a) Arch. Biochem. Biophys. 300, 434–439
Oliw, E. H., Hörnsten, L., Sprecher, H., and Hamberg, M. (1993b) Arch. Biochem. Biophys. 305, 288–297
Paul, K. G., Theorell, H., and Akesson, A. (1953) Acta Chem. Scand. 7, 1284–1287
Picot, D., Loli, P. J., and Garavito, R. M. (1994) Nature 367, 243–249
Porter, N. A., Caldwell, S. E., and Mills, K. A. (1995) Lipids 30, 277–290
Schutz, G., and Feigelson, P. (1972) J. Biol. Chem. 247, 5327–5332
Siedow, J. N. (1991) Ann. Rev. Plant Physiol. Plant Mol. Biol. 42, 145–188
Smith, W. L., and Marnett, L. J. (1991) Biochim. Biophys. Acta 1083, 1–17
Su, C., Brodowsky, I. D., and Oliw, E. H. (1995) Lipids 30, 43–50
Tsai, A., Hsi, L. C., Kulmacz, R. J., Palmer, G., and Smith, W. L. (1994) J. Biol. Chem. 269, 5085–5091
Tundro, P., and Johansson, K.-E. (1984) J. Biochim. Biophys. Methods 9, 171–179
Webb, E. C. (1992) Enzyme Nomenclature, Academic Press, New York
Yamamoto, S. (1992) Biochim. Biophys. Acta 1128, 117–131
