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Crystallization and Preliminary X-ray Analysis of Soybean Lipoxygenase-1, a Non-heme Iron-containing Dioxygenase*

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Crystals of lipoxygenase-1 from soybeans have been grown by the method of vapor diffusion in the presence of sodium formate, ammonium acetate, and lithium chloride at pH 7.0. This enzyme contains a non-heme iron and is closely related to a human lipoxygenase found in leukocytes that participates in the biosynthesis of leukotrienes and lipoxins. The crystals are monoclinic space group C2 with cell dimensions of a = 183.8 Å, b = 123.2 Å, c = 94.3 Å and β = 102.9 °. They diffract beyond 2.7 Å, are stable for several days in the x-ray beam, and appear to be suitable for x-ray diffraction studies.

Lipoxygenases (EC 1.13.11.12) are widely distributed in both animals and plants and specifically catalyze the oxidation of polyunsaturated fatty acids containing a *cis,cis*-1,4-pentadiene system to yield *cis,trans*-1,3-conjugated hydroperoxy acids using molecular oxygen (Papatheofanis and Lands, 1985). Because of their abundance and relative ease in isolation, plant lipoxygenases have been characterized far more extensively than their animal counterparts. Lipoxygenase-1[1] is one of four distinct isozymes of lipoxygenase isolated from soybeans, the richest known source of this enzyme. The cDNA for this enzyme has been cloned revealing a single polypeptide sequence of 838 amino acid residues with a molecular weight of 94,038 (Shibata *et al.*, 1987). There is a cluster of 5 conserved histidines within a region of 40 amino acid residues that is highly conserved in the 9 lipoxygenases that have been sequenced (Funk *et al.*, 1989; Sigal *et al.*, 1988; Fleming *et al.*, 1989; Yoshimoto *et al.*, 1990). This same region has been proposed to be involved in iron binding (Shibata *et al.*, 1987). Lipoxygenase-1 has been observed to contain an essential non-heme iron that cannot be removed by any of the normal chelators unless a reducing agent is present (Pistorius and Axelrod, 1974). Both the ferrous and ferric states of iron are thought to be parts of the catalytic cycle (Cheesbrough and Axelrod, 1983).

Sequence homology between lipoxygenase-1 and the 5-lipoxygenase from human leukocytes has been noted previously (Dixon *et al.*, 1988; Funk *et al.*, 1989). Using the alignment algorithm of Smith and Waterman (1981), we observed a 27% sequence identity between lipoxygenase-1 and the 5-lipoxygenase from human leukocytes (Matsumoto *et al.*, 1988; Dixon *et al.*, 1988). Amino acid sequence homology is found predominantly in the C-terminal half of the proteins with 34% sequence identity observed in the last 312 residues. The human enzyme oxidizes arachidonic acid to 5-hydroperoxy-6,8,11,14-eicosatetraenoic acid and then further to leukotriene A_4, an unstable epoxide intermediate. Other enzymes transform LTA_4[2] into four more leukotrienes: LTB_4, LTC_4, LTD_4, and LTE_4. These four leukotrienes have multiple effects especially on the peripheral airways and vascular system including mediation of inflammation and immunity responses. The 5-lipoxygenase in conjunction with 15-lipoxygenase also participates in the biosynthesis of lipoxins, a class of trihydroxylated tetraene derivatives of arachidonic acid. The biological effects of these compounds are similar but quite distinct from those of leukotrienes (Samuelsson *et al.*, 1987).

Because 5-lipoxygenase products are the precursors to such powerful effectors, the elucidation of the structure and the mechanism of lipoxygenases are currently of great interest. We have therefore undertaken three-dimensional structural studies of soybean lipoxygenase-1 in the resting ferrous form and report below the preparation of crystals suitable for x-ray diffraction studies and their preliminary crystallographic analysis.

EXPERIMENTAL PROCEDURES

Purification and Crystallization—Lipoxygenase-1 from soybeans was prepared in 100–200-mg amounts in less than 48 h by a modification[3] of the Axelrod method (Axelrod *et al.*, 1981). A final high pressure liquid chromatography purification step (Ramadoss and Axelrod, 1982) employed a preparative anion exchange column (Dionex, ProPac PA1, 9 × 250 mm). Activities were 180 ± 10 μmol/min/mg using the assay of Axelrod modified to include 1–2 μM catalase (Kemal *et al.*, 1987). The protein was identified as the lipoxygenase-1 isozyme by comparing activity at pHs 9 and 6.8 (Christopher *et al.*, 1970) and by isoelectric focusing on agarose gels (FMC Isogel, pH range 3–7; the pI at 15 °C was found to be 5.8 in agreement with the reported pI of 5.6–5.7 (Christopher *et al.*, 1972)).

Crystallization experiments were performed by the hanging-drop vapor diffusion method (Blundell and Johnson, 1976). Crystals were grown reproducibly using a reservoir solution containing 4.6 M sodium formate, 1.0 M ammonium acetate, 600 mM lithium chloride, and 10 mM MES, pH 7.0. A drop containing 5 μl of 17 mg/ml protein in 10 mM MES, pH 7.0, mixed with 5 μl of reservoir solution was equilibrated with 1 ml of reservoir solution.

X-ray Diffraction Studies—Characterization of the crystals was carried out on an Enraf Nonius precession camera with nickel-filtered CuKα radiation generated from a Siemens Kristalloflex 710 x-ray generator operated at 40 kV and 35 mA. Intensity data for the native crystals were collected on a Nicolet area detector mounted on a Huber 4-circle goniostat using a Rigaku Rotoflex RU-200B rotating anode equipped with a graphite monochromator as the x-ray source. The

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[1] Using the substrate arachidonic acid as a standard, lipoxygenase-1 is also known as a 15-lipoxygenase, inserting a dioxygen at C_{15} (Kühn *et al.*, 1986).

[2] The abbreviations used are: LTA_4, leukotriene A_4; LTB_4, leukotriene B_4; LTC_4, leukotriene C_4; LTD_4, leukotriene D_4; LTE_4, leukotriene E_4; MES, 2-(*N*-morpholino)ethanesulfonic acid.

[3] B. J. Gaffney and D. V. Mavrophilipos, manuscript in preparation.

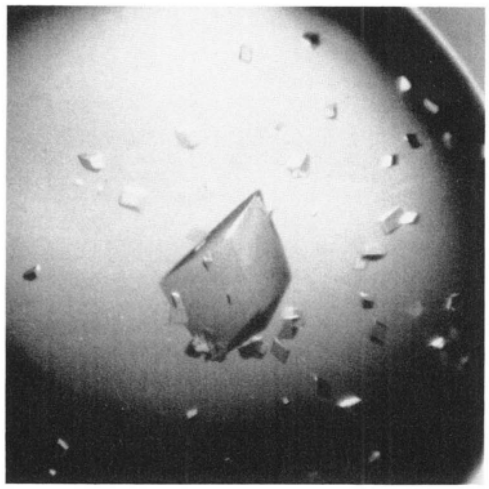

FIG. 1. **Crystals of soybean lipoxygenase-1.** The largest dimension is 0.5 mm.

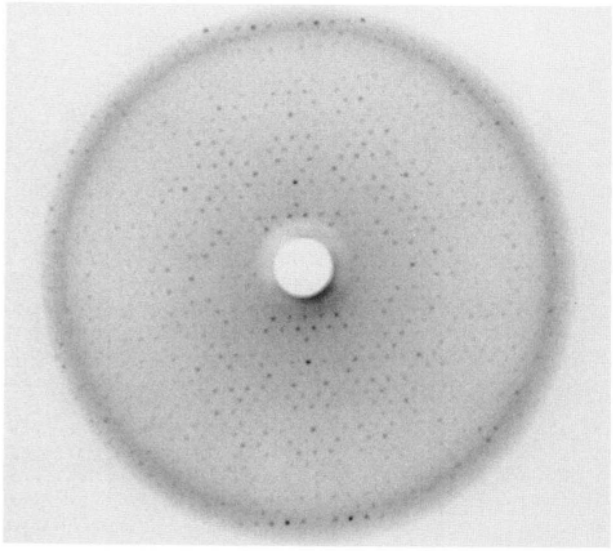

FIG. 2. **Precession photograph of the *hk*0 zone with $\mu = 8°$.** The horizontal axis is parallel to a* and the vertical axis is parallel to b*. Systematic absences of reflections are seen for which $h + k$ is odd.

data were processed and reduced using the XENGEN computer package (Howard *et al.*, 1987).

RESULTS AND DISCUSSION

Using solutions with approximately 15 mg/ml protein and starting at different pHs over 1000 crystallization conditions were screened using 19 different salts, 19 different organic compounds, and combinations thereof. Most conditions resulted in fine granular precipitation, but sodium formate (4.5–5.0 M) gave small needles within a week. Exploration of other additions showed that large chunky crystals were obtained when ammonium acetate and lithium chloride were included with the sodium formate in the concentrations described under "Experimental Procedures." Within 6 weeks crystals grew at room temperature to dimensions of approximately 0.2 × 0.4 × 0.5 mm (Fig. 1).

Precession photography revealed the space group to be C2 with cell dimensions $a = 183.8$ Å, $b = 125.2$ Å, $c = 94.3$ Å, and $\beta = 102.9°$ (Fig. 2). Crystals diffract beyond 2.7-Å resolution and remain stable in the x-ray beam for several days. Using two lipoxygenase molecules in the asymmetric unit, a value of 2.81 Å^3/Da is obtained for V_m (56% solvent), well within the range observed in protein crystals (Matthews, 1968). Since the crystals are suitable for x-ray diffraction analysis, a native data set was collected to 2.7-Å resolution using a Nicolet area detector and was processed using the computer package XENGEN. Intensities obtained from three different orientations of the same crystal gave an unweighted R-factor for multiple observations of 5.9%. Of the 49,796 reflections collected, 14,916 were recorded once, 21,928 twice, and the rest more than two times. Presently a search for heavy atom derivatives is under way.

The three-dimensional structure of lipoxygenase-1 is of interest for several reasons. Structural studies of the active site geometry will bring to light the manner in which the non-heme iron is coordinated and its possible role in the enzymatic mechanism. Studies of the enzyme with and without bound substrate analogues and products will provide considerable insight into enzyme-substrate interactions and the mechanism of binding and catalysis. The fatty acid binding site may reveal the factors that control the positional and stereochemical specificity of lipoxygenases. Analysis of the coordination of the non-heme iron will help understanding the manner in which oxygen binds to the enzyme. These insights will be very useful in helping one to elucidate the mechanism of 5-lipoxygenase, an enzyme of great physiological interest. Since lipoxygenase-1 and 5-lipoxygenase show no significant sequence homology with any other proteins in the National Biomedical Research Foundation Protein Database (Matsumoto *et al.*, 1988; Shibata *et al.*, 1987) it will be interesting to look at the overall fold of lipoxygenase and compare it with the folds of fatty acid binding proteins and lipases.

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REFERENCES

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

Blundell, T., and Johnson, L. N. (1976) *Protein Crystallography*, pp. 75–77, Academic Press, New York

Cheesbrough, T. M., and Axelrod, B. (1983) *Biochemistry* **22,** 3837–3840

Christopher, J., Pistorius, E. K., and Axelrod, B. (1970) *Biochim. Biophys. Acta* **198,** 12–19

Christopher, J., Pistorius, E. K., and Axelrod, B. (1972) *Biochim. Biophys. Acta* **284,** 54–62

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

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

Funk, C. D., Hoshiko, S., Matsumoto, T., Rådmark, O., and Samuelsson, B. (1989) *Proc. Natl. Acad. Sci. U. S. A.* **86,** 2587–2591

Howard, A. J., Gilliland, G. L., Finzel, B. C., Poulus, T. L., Ohlendorf, D. H., and Salemme, F. R. (1987) *J. Appl. Crystallogr.* **20,** 383–387

Kemal, C., Louis-Flamberg, P., Krupinski-Olsen, R., and Shorter, A. L. (1987) *Biochemistry* **26,** 7064–7072

Kühn, H., Schewe, T., and Rapoport, S. M. (1986) *Adv. Enzymol. Relat. Areas Mol. Biol.* **58,** 273–311

Matsumoto, T., Funk, C. D., Rådmark, O., Höög, J.-O., Jörnvall, H., and Samuelsson, B. (1988) *Proc. Natl. Acad. Sci. U. S. A.* **85,** 26–30; Correction (1988) *Proc. Natl. Acad. Sci. U. S. A.* **85,** 3406

Matthews, B. W. (1968) *J. Mol. Biol.* **33,** 491–497

Papatheofanis, F. J., and Lands, W. E. M. (1985) in *Biochemistry of Arachidonic Acid Metabolism* (Lands, W. E. M., ed) pp. 9–39, Martinus Nijhoff, Boston

Pistorius, E. K., and Axelrod, B. (1974) *J. Biol. Chem.* **249,** 3183–3186
Ramadoss, C. S., and Axelrod, B. (1982) *Anal. Biochem.* **127,** 25–31
Samuelsson, B., Dahlén, S.-E., Lindgren, J. Å., Rouzer, C. A., and Serhan, C. N. (1987) *Science* **237,** 1171–1176
Shibata, D., Steczko, J., Dixon, J. E., Hermodson, M., Yazdanparast, R., and Axelrod, B. (1987) *J. Biol. Chem.* **262,** 10080–10085
Sigal, E., Craik, C. S., Highland, E., Grunberger, D., Costello, L. L., Dixon, R. A. F., and Nadel, J. A. (1988) *Biochem. Biophys. Res. Commun.* **157,** 457–464
Smith, T. F., and Waterman, M. S. (1981) *Adv. Appl. Math.* **2,** 482–489
Yoshimoto, T., Suzuki, H., Yamamoto, S., Takai, T., Yokoyama, C., and Tanabe, T. (1990) *Proc. Natl. Acad. Sci. U. S. A.* **87,** 2142–2146