Crystallization and Preliminary X-ray Investigation of Lipoxygenase 1 from Soybeans*

(Received for publication, March 12, 1990)

Janusz Steczko‡, Christine R. Muchmore§, Janet L. Smith¶, and Bernard Axelrod∥
From the Departments of ‡Biochemistry and §Biological Sciences, Purdue University, West Lafayette, Indiana 47907 and the ¶Institute of Catalysis and Surface Chemistry, Polish Academy of Sciences, Cracow, 30-239 Poland

Soybean lipoxygenase 1 has been crystallized by the vapor diffusion method in 8–10% polyethylene glycol (average M, 3400), 0.2 M sodium acetate buffer, pH 5.2–5.6, at a protein concentration of 6–12 mg/ml. Microseeding was employed to obtain growth of large single crystals. The crystals, which diffract to at least 2.2-A spacings, are monoclinic and of space group P21. Cell constants are: a = 95.4, b = 94.2, and c = 50.4 Å and β = 91.4°. The calculated value of Vm (3.41 Å³/ Da) is consistent with the probable presence of one molecule of lipoxygenase/crystallographic asymmetric unit (Z = 2).

Lipoxygenases constitute a class of non-heme, non-sulfur iron oxygenases that act upon lipids containing a cis,cis-pentadiene moiety. Dioxygen is incorporated into the primary reaction products, hydroperoxides. These enzymes are widely distributed among plants, animals, and microorganisms. The presence of lipoxygenases in mammalian tissue has attracted considerable interest since their natural substrate, arachidonic acid, is the precursor of a number of highly potent physiological effectors (1).

Soybeans contain a number of distinct isozymes. The three major isozymes that have been purified from seeds and have been studied are L-1, L-2, and L-3. The primary sequences of L-1 (2) and L-2 (3) have been reported from this laboratory, whereas that of L-3 has been reported by Yenofsky et al. (4). L-1, L-2, and L-3 have M, values of 94,038, 97,035, and 96,541, respectively. All contain a single iron atom/molecule. L-2/L-1 and L-2/L-3 are 81 and 74% identical in amino acid sequence, respectively. L-1 is activated by its own hydroperoxide product (5). Activation is accompanied by a shift in the optical spectrum to longer wavelength (6–9). L-2 (8, 10) and L-3 (8) behave in similar fashion. Activation results in the conversion of high-spin Fe⁺ to high-spin Fe³⁺ (11, 12). This change is reflected in the appearance of an EPR signal at g = 6 in L-1 (6, 7, 13), L-2 (8, 10), and L-3 (8).

The soybean isozymes share a homologous histidine-rich region, which we first suggested as a possible iron-binding site (3). Extended x-ray absorption fine structure (14, 15) and Mössbauer (16) studies are not inconsistent with histidine residues as ligands for the iron in L-1. However, it has been reported (17) that 2 of the histidine residues in the histidine-rich region in the 5-lipoxygenase are not reported for activity. The sequences of human reticulocyte 5-lipoxygenase (18, 19), human reticulocyte 15-lipoxygenase (20), and rabbit reticulocyte 15 lipoxygenase (21) have recently been determined. The histidine-rich region noted above occurs in the animal enzymes with reasonably good conservation. Another feature common to the soybean and mammalian lipoxygenases is the presence of a moderate but clearly conserved stretch of 12–13 amino acids which occurs between the histidine-rich region and the carboxyl terminus. Although the animal enzymes are about 150 amino acids shorter than L-1, all of them have greater than 60% similarity to L-1 in the carboxyl-terminal half of their polypeptide chains. The mammalian enzymes catalyze the same primary reaction as the soybean lipoxygenases, namely the hydroperoxidation of a cis,cis-1,4-pentadiene moiety in lipids; and they all contain one atom of iron/molecule of enzyme. It is therefore likely that they have a common tertiary structure, at least in part, and that L-1 may serve as a model for at least a portion of the structure of other lipoxygenases, including the highly important human enzymes (1).

In this paper, we describe the crystallization and the preliminary characterization of crystals of L-1, which diffract to 2.2-A spacings and appear to be suitable for structure determination by x-ray crystallography.

MATERIALS AND METHODS

Enzyme Preparation—L-1 was prepared from defatted soybean seeds as previously described (22), except that DE52 ion-exchange cellulose (Whatman) was used instead of DEAE-Sephadex. The final preparation had a specific activity of 210 units/mg of protein, which is the maximum activity of the pure enzyme. Concentrated protein solutions were prepared by centrifugation through a CentriCell 60 tube with a M, cutoff 30,000 filter (Polysciences, Inc.).

Crystallization—Crystals were grown at room temperature (21–23 °C) by vapor diffusion using either the hanging-drop method or the sitting-drop method (23) in depression plates. Typically, in the hanging-drop method, 1 ml of precipitant solution was placed in one well of a Linbro plastic tissue culture plate (Flow Laboratories, Inc.), and 5 μl of protein stock solution + 5 μl of well fluid were mixed on the siliconized cover glass. In the sitting-drop method, the depressions in a six-place depression plate were covered with a thin layer of silicone grease. Ten μl of precipitant solution was mixed with 10 μl of stock protein solution in each depression. The depression plate was sealed with duct tape in a container made of two plastic Petri dish tops, which held the reservoir solution. Enzyme solutions were passed through 0.22-μ Millipore filters prior to use. All solutions used in crystallization trials contained 0.50 mM sodium azide as an inhibitor of microbial growth.

X-ray Diffraction—for x-ray diffraction experiments, the crystals were mounted in thin-walled glass capillaries between plugs of precipitant solution. Precision photographs were recorded on a Sopper precession camera using CuKα radiation from an Elliot GX-20 ro
CRYSTALLIZATION OF SOYBEAN LIPOXYGENASE 1

Crystallization of Soybean Lipoxygenase 1

Protein stock solution concentration was 12 mg/ml. The best crystals were obtained with precipitant solutions of 0.2 M buffer, pH 4.9, in 14% PEG. One of these was crushed with a horsehair in a depression plate well containing 20 μl of crystallizing solution (10 μl of protein stock solution + 10 μl of precipitant solution). Ten μl of the crystal suspension was transferred to a second well containing 10 μl of crystallizing solution and mixed. In this manner, serial dilutions were continued for a total of 11 dilutions. In all cases, small uniform thick platelets were obtained. Suspensions of these crystals were used directly for seeding, without grinding, under the conditions noted above. Good single crystals, generally appearing as rods, were obtained in at least 75% of the sitting-drop experiments. Continued experimentation indicated that the following conditions were optimum: 0.2 M sodium acetate buffer, pH 5.2-5.6, 8-10% (w/v) PEG 3400, and 6-12 mg/ml protein concentration. The isoelectric point of L-1 is 5.68 (25). All concentrations refer to stock solutions.

To save time, when evaluating the suitability of the seed dilutions, a preliminary test was carried out under the optimum conditions shown, except that 14% PEG was employed. At this concentration, enough growth was seen after 2 or 3 h to permit selection of a dilution that would generate a small number of crystals. Based on the results of the preliminary tests, 1-3 μl of the selected seed dilution was added to 20 μl of crystallization droplets. Usually 2-3 days under the optimum conditions noted above was sufficient for producing crystals that were suitable for diffraction studies.

A typical crystal is shown in Fig. 1. The crystals often appeared six-sided in end view, but were not regular hexagons. They were mechanically robust and were not unusually sensitive to x-radiation. Diffraction extended to 2.2 Å spacings on a still photograph after 1 h of exposure. The symmetry and systematic absences observed in the diffraction pattern are consistent with monoclinic space group P2₁. The 0kl zone is shown in Fig. 2. Cell constants are: a = 95.4, b = 94.2, and c = 50.4 Å and β = 91.4°. From the volume of the unit cell and the molecular mass (94,038 Da) of L-1, one obtains a Vₐ value of 2.41 Å³/Da and a solvent content of ~48% assuming the presence of one molecule of enzyme/asymmetric unit (Z = 2). Vₐ is reasonable, based on the compiled data of Matthews (26) for globular proteins.²

² Since submission of this manuscript, a report (27) of the crystallization and preliminary x-ray characterization of another isozyme of soybean lipoxygenase has appeared. The authors suggest that their isozyme is lipoxygenase-2. The crystal form differs from what we report here, and the lattices appear unrelated.

REFERENCES

1. Samuelsson, B., Dahlén, S.-E., Lindgren, J. A., Rouzer, C. A., and Serhan, C. N. (1987) Science 237, 1171-1176
2. Shibata, D., Steczko, J., Dixon, J. E., Hermodson, M., Yazdanparast, R., and Axelrod, B. (1987) J. Biol. Chem. 262, 10080-10085
3. Shibata, D., Steczko, J., Dixon, J. E., Andrews, P. C., Hermodson, M., and Axelrod, B. (1988) J. Biol. Chem. 263, 6816-6821
4. Yenofsky, R., Fine, M., and Liu, C. (1988) Mol. Gen. Genet. 211, 215-222
5. Haining, J. L., and Axelrod, B. (1958) J. Biol. Chem. 232, 193-202
6. Pistorius, E. K., Axelrod, B., and Palmer, G. (1976) J. Biol. Chem. 251, 7144-7148

Since submission of this manuscript, a report (27) of the crystallization and preliminary x-ray characterization of another isozyme of soybean lipoxygenase has appeared. The authors suggest that their isozyme is lipoxygenase-2. The crystal form differs from what we report here, and the lattices appear unrelated.

Fig. 1. Typical lipoxygenase 1 crystals. Conditions were: 10% (w/v) polyethylene glycol, 12 mg/ml lipoxygenase, and 0.2 M sodium acetate buffer, pH 5.2. The length of the longest crystal is 1.75 mm.

Fig. 2. 15° precession photograph of 0kl zone. The distance from crystal to film was 75 mm, and exposure time was 6 h.
Crystallization of Soybean Lipoxigenase 1

7. Pistorius, E. K., and Axelrod, B. (1974) *J. Biol. Chem.* **249**, 3183–3186
8. Pistorius, E. K. (1974) Ph.D. thesis, Purdue University
9. Pistorius, E. K., Christopher, J. P., and Axelrod, B. (1976) *Proceedings of the 10th International Congress on Biochemistry, Hamburg*, p. 393
10. Feiters, M. C., Aasa, R., Malmström, B. G., Veldink, G. A., and Vliegenthart, J. F. G. (1986) *Biochim. Biophys. Acta* **873**, 182–189
11. Cheesbrough, T. M., and Axelrod, B. (1983) *Biochemistry* **22**, 3837–3840
12. Slappendel, S., Malmström, B. G., Petersson, L. E., Ehrenberg, A., Veldink, G. A., and Vliegenthart, J. F. G. (1982) *Biochem. Biophys. Res. Commun.* **108**, 673–677
13. de Groot, J. J. M. C., Veldink, G. A., Vliegenthart, J. F. G., Boldingh, J., Wever, R., and van Gelder, B. F. (1975) *Biochim. Biophys. Acta* **377**, 71–79
14. Vliegenthart, J. F. G., and Veldink, G. A. (1987) *J. Am. Oil Chem. Soc.* **64**, 642
15. Spek, A. L., Duijzenberg, A. J. M., and Feiters, M. C. (1983) *Acta Crystallogr. Sect. B Struct. Crystallogr. Cryst. Chem.* **39**, 1212–1214
16. Funk, M. O., Carroll, R. J., Thompson, J. F., Sans, R. H., and Dunham, W. R. (1987) *Biophys. J.* **51**, 14 (abstr.)
17. Funk, C. D., Gunne, H., Steiner, H., Izumi, T., and Samuelsson, R. (1988) *Proc. Natl. Acad. Sci. U. S. A.* **86**, 2592–2596
18. Dixon, R. A. F., Jones, R. E., Diehl, R. E., Bennet, C. D., Kargman, S., and Rouzer, C. A. (1988) *Proc. Natl. Acad. Sci. U. S. A.* **86**, 416–420
19. Funk, C. D., Hoshiko, S., Matsumoto, T., Radmork, O., and Samuelsson, B. (1989) *Proc. Natl. Acad. Sci. U. S. A.* **86**, 2587–2591
20. Sigal, E., Craik, C. S., Highland, E., Gronberger, D., Costello, L. L., Dixon, R. A. F., and Nadel, J. A. (1988) *Biochem. Biophys. Res. Commun.* **157**, 457–464
21. Fleming, J., Thiele, B. J., Chester, J., O'Prey, J., Janetzki, S., Aitken, A., Anton, I. A., Rapoport, S. M., and Harrison, P. R. (1999) *Gene (Amst.)* **279**, 181–188
22. Axelrod, B., Cheesbrough, T. M., and Laakso, S. (1981) *Methods Enzymol.* **71**, 441–451
23. McPherson, A. (1982) *Preparation and Analysis of Protein Crystals*, John Wiley & Sons, New York
24. Fitzgerald, P. M. D., and Madsen, N. B. (1986) *J. Crystal Growth* **76**, 600–606
25. Christopher, J. P. (1972) Ph.D. thesis, Purdue University
26. Matthews, B. W. (1968) *J. Mol. Biol.* **33**, 491–497
27. Stallings, W. C., Kroa, B. A., Carroll, R. T., Metzger, A. L., and Funk, M. O. (1990) *J. Mol. Biol.* **211**, 685–687
Crystallization and preliminary X-ray investigation of lipoxygenase 1 from soybeans.

J Steczko, C R Muchmore, J L Smith and B Axelrod

_J. Biol. Chem._ 1990, 265:11352-11354.

Access the most updated version of this article at [http://www.jbc.org/content/265/19/11352](http://www.jbc.org/content/265/19/11352)

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at [http://www.jbc.org/content/265/19/11352.full.html#ref-list-1](http://www.jbc.org/content/265/19/11352.full.html#ref-list-1)