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Authors

Larson, Steve
Day, John
Greenwood, Aaron
et al.

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Preliminary Investigation of Crystals of the Neutral Lipase from *Pseudomonas fluorescens*

Steve Larson[1], John Day[1], Aaron Greenwood[1], Joel Oliver[2], Donald Rubingh[2] and Alexander McPherson[1]†

[1]*Department of Biochemistry*
University of California at Riverside
Riverside, CA 92521, U.S.A.

[2]*Procter and Gamble Company*
Miami Valley Laboratories
Cincinnati, Ohio, U.S.A.

The neutral lipase from the bacteria *Pseudomonas fluorescens*, marketed under the trade name LpL-200S, has been crystallized in a form suitable for X-ray diffraction analysis from 35% *n*-propanol at pH 8·5. The crystals are monoclinic prisms and are of space group $C2$ with $a = 91\cdot00$ Å, $b = 47\cdot17$ Å, $c = 35\cdot21$ Å and $\beta = 121\cdot43°$. There is one molecule of the protein as the asymmetric unit of the crystals. The diffraction pattern extends to at least 1·6 Å resolution and the crystals are extremely robust in terms of X-ray exposure.

Keywords: lipase; X-ray diffraction; protein crystals

The neutral lipase from *Pseudomonas fluorescens* has a molecular mass of 33,515 daltons and a chain length of 317 residues. The enzyme is particularly interesting because it operates directly at the interface between water and lipid and, therefore, may incorporate the properties of both lipophilic and hydrophilic proteins in one molecule.

Recently, two other lipases have been solved by X-ray diffraction analysis and both are characterized by parallel β sheets with frequent helical con .ections (Brady *et al.*, 1990; Winkler *et al.*, 1990). The active sites in both cases included a constellation of amino acids, including a serine and histidine residue, strikingly similar to that found at the active sites of serine proteases such as subtilisin and trypsin (Kossiakof, 1987). It is, therefore, likely that the mechanism of lipase enzymes will be very similar to this other class of hydrolytic enzymes. It remains to be seen, however, if structural classes as diverse as subtilisin and trypsin exist among the various lipases.

Indeed, some significant variations in structural motif are almost certain among the lipases. For example, the lipase from *Pseudomonas fluorescens* can have but one possible disulfide bridge while there are three demonstrated in the lipase from

Mucor miehei. The two previously described lipases, in addition, have substantially different polypeptide chain lengths and show little or no amino acid sequence homology to that reported upon here.

The lipase from *Pseudomonas fluorescens* was obtained from Amano International Enzyme Co. of Japan and purified to homogeneity at the Procter and Gamble Co., Cincinnati, OH. Before attempting crystallization, a protein stock solution of 16 mg/ml was prepared from lyophilized material and dialyzed against distilled water for 24 hours. It was stored at $-20°C$ between crystallization trials.

A broad investigation of crystallization conditions using the procedures described by McPherson (1990) was undertaken using a variety of salts, polyethylene glycols, and a variety of other precipitants over a wide range of pH. In addition, the effects of metal ions and a spectrum of detergents, including β-octylglucoside (BOG: McPherson *et al.*, 1986), were examined as well. This matrix demonstrated that microcrystals of the lipase could be obtained from at least six different salts over nearly the entire pH range, and in some cases from polyethylene glycol as well. In no cases, however, were crystals large enough for even preliminary X-ray diffraction analysis to be obtained. In spite of intensive effort and hundreds of optimization trials, no improvement was obtained for well over a year. Eventually, it was discovered that larger crystals

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† Author to whom all correspondence should be addressed.

could be obtained from 30 to 38% (v/v) *n*-propanol in water at pH 8·4 to 8·6 at both 4 °C and 22 °C.

The crucial variable was, ultimately, identified as the pH, and for the growth of large crystals this must be within the very narrow range of 8·45 to 8·6. At lower pH values, no matter what the precipitating agent, only microcrystals form, and above that point no crystals appear. Isopropanol also yields crystals at this pH value identical in habit but of lesser quality and size than *n*-propanol. Currently, crystals of 0·8 mm × 0·3 mm × 0·2 mm are routinely grown.

For preliminary X-ray characterization, crystals were sealed in quartz capillaries along with a small amount of mother liquor and photographs of the reciprocal lattice recorded using a Buerger precession camera. The X-rays were CuK_α and were produced by an Enraf–Nonius generator fitted with a fine focus tube operated at 45 kV and 32 mA.

Three-dimensional X-ray diffraction data were collected on a San Diego Systems Multiwire Area Detector (Hamlin *et al.*, 1981) using monochromated CuK_α radiation produced by a Rigaku RU-200 rotating anode source operated at 45 kV and 150 mA.

X-ray precession photographs of the lipase crystals showed that the reciprocal lattice contained a single mirror plane and that the reflections could be indexed on a monoclinic lattice corresponding to real unit cell dimensions of $a = 92·0$ Å, $b = 47·17$ Å, $c = 85·21$ Å   (1 Å = 0·1 nm)   and $\beta = 121·5°$. Examination of reciprocal space demonstrated further that reflections of the class $h + k \neq 2n$ were systematically absent. The monoclinic space group is, therefore, $C2$.

The volume of the unit cell is $V = 2·95 \times 10^5$ Å^3 and this is large enough to reasonably accommodate only four molecules of the lipase. The volume to mass ratio is $V_m = 2·20$ Å^3/dalton which is near the center of the range found for most protein molecules (Matthews, 1968). The asymmetric unit of the crystals must, therefore, be composed of a single lipase molecule.

The crystals diffract extremely well, and X-ray data have now been collected to about 1·8 Å resolution. The diffraction data do, however, extend well beyond that limit. Although an entire data set can be obtained from a single crystal, we have recorded those from four separate crystals to insure a high degree of redundancy and have merged them with a conventional residual of 0·08 to produce a native data set having 20,000 independent reflections with an intensity to sigma ratio greater than 3. The crystals appear to be quite resistant to X-ray exposure and many days of data collection result in little noticeable decay in the diffraction pattern.

The crystals of the lipase we describe here appear to be eminently suitable for a full three-dimensional structure analysis. Our attempts to utilize the structures of other lipases that have been determined in a molecular replacement search, have not yielded any indication that the *Pseudomonas fluorescens* lipase is similar in structure. Thus, we are proceeding with a full structure analysis based on conventional multiple isomorphous replacement.

References

Brady, L., Brzozowski, A. M., Derewenda, Z. S., Dodson, E., Dodson, G., Tolley, S., Turkenberg, J. P., Christiansen, L., Huge-Jensen, B., Norskov, L., Thim, L. & Menge, U. (1990). A serine protease triad forms the catalytic center of a triacylglycerol lipase. *Nature (London)*, **343**, 767–770.

Hamlin, R., Cork, C., Howard, A., Nielsen, C., Vernon, W., Matthews, D. & Xuong, Ng. H. (1981). Characteristics of a flat multiwire area detector for protein crystallography. *J. Appl. Crystallogr.* **14**, 85.

Kossiakoff, A. A. (1987). Catalytic properties of trypsin. In *Biological Macro-molecules and Assemblies. Active Sites of Enzymes* (Jurnak, F. & McPherson, A., eds), vol. 3, pp. 320–412, John Wiley and Sons, New York.

Matthews, B. W. (1968). Solvent content of protein crystals. *J. Mol. Biol.* **33**, 491–497.

McPherson, A. (1990). Current approaches to macromolecular crystallization. *Eur. J. Biochem.* **189**, 1–23.

McPherson, A., Koszelak, S., Axelrod, H., Day, J., McGrath, M., Williams, R. & Cascio, D. (1986). An experiment regarding crystallization of soluble proteins in the presence of β-octyl glucoside. *J. Biol. Chem.* **261**, 1969–1975.

Winkler, F. K., D'Arcy, A. & Hunziker, W. (1990). Structure of human pancreatic lipase. *Nature (London)*, **343**, 771–774.

Edited by A. Klug