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CRYSTALLIZATION NOTE

Crystallization and Preliminary X-Ray Analysis of a Lipase from *Staphylococcus hyicus*

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Single crystals of a lipase from *Staphylococcus hyicus* have been obtained using a combination of 18 to 24% dimethylsulfoxide and 10% isopropanol as a precipitant. The crystals grow at 4°C in 2–3 months. They belong to the orthorhombic space group $P2_12_12_1$ with a = 73.31 Å, b = 77.96 Å, and c = 169.81 Å, with two protein molecules per asymmetrical unit. The crystals diffract to at least 2.8 Å resolution and are suitable for an X-ray structure analysis.

Lipases (E.C. 3.1.1.3) are found in diverse organisms, including animals, plants, fungi, and bacteria (Jaeger *et al.,* 1994). They catalyse the hydrolysis of triacylglycerols into free fatty acids and glycerol. Because of their wide variety in substrate and reaction specificity lipases have found industrial application in the resolution of racemic mixtures, the synthesis of esters and in transesterification reactions (for a review see Santaniello *et al.* (1993)), and as an additive in laundry detergents. Lipolytic enzymes are often activated by lipid–water interfaces. To understand this activation, knowledge of their three-dimensional structures is essential. Since 1990 a number of lipase structures have been published, among which are those from human pancreatic lipase (Winkler *et al.,* 1990), *Rhizomucor miehei* (Brady *et al.,* 1990), *Geotrichum candidum* (Schrag *et al.,* 1991), and *Candida rugosa* (Grochulski *et al.,* 1993). Only one bacterial lipase structure is known so far, that from *Pseudomonas glumae*/*Chromobacterium viscosum* (Noble *et al.,* 1993; Lang *et al.,* 1994). All these lipases were found to have a catalytic triad consisting of Ser, His, and Asp (or Glu), as in the serine proteases. Their active site is buried beneath a helical segment, or "lid." This lid opens up, uncovering the active site, when a substrate or an inhibitor molecule is bound as witnessed from the crystal structure of *R. miehei* lipase complexed with an inhibitor (Brzozowski *et al.,* 1991) and from the structure of the human pancreatic lipase–procolipase complex bound to a nonsubstrate interface (van Tilbeurgh *et al.,* 1993). As a result a large hydrophobic patch becomes exposed to solvent and the active site becomes accessible. However, not only the presence of lipids can induce this conformational change but any hydrophobic interface may accomplish this. This was observed in the X-ray structures of the two homologous lipases from *G. candidum* and *C. rugosa.* The former was crystallized with polyethyleneglycol as a precipitant and showed a closed structure (active site buried), but the one from *C. rugosa* was crystallized in the presence of 2-methyl,2,4-pentanediol and is in an open conformation with the active site accessible. Therefore, it has been postulated that the presence of a hydrophobic environment will induce this conformational change, making the active site accessible for the substrate and thus may account for the so-called interfacial activation of lipases.

Besides *P. glumae* several other bacteria secrete lipases, such as the gram-positive *Staphylococcus hyicus.* The *S. hyicus* lipase is particularly interesting since it has phospholipase activity as well as

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lipase activity. It can remove the acyl chains of both the primary and secondary positions of a variety of natural and synthetic neutral lipids and phospholipids. In contrast to phospholipase A_2, which is stereospecific, the *S. hyicus* lipase hydrolyses both L and D phospholipids. It has a preference for dioctanoyl lecithin. Its pH optimum is around pH 9. A second interesting feature of this enzyme is that it shows interfacial activation only in the presence of lipid aggregates, but not with phospholipids (van Oort *et al.,* 1989). The *S. hyicus* lipase shows no homology to other lipases apart from the characteristic G–X–S–X–G sequence motif around the catalytic serine residue which is conserved in lipases (the so-called "lipase box") (Jäger *et al.,* 1992; Leuveling Tjeenk *et al.,* 1994). The enzyme has been cloned, sequenced, and expressed to high level in *S. carnosus* (Götz *et al.,* 1985) and *Escherichia coli* (Leuveling Tjeenk, 1994). The nucleotide sequence codes for a preprotein of 641 amino acid residues with a molecular weight of 71.4 kDa, much higher than that of other known lipases. Upon secretion into the medium the molecular weight is reduced to 46 kDa by tryptic cleavage with a concomitant 3-fold increase of the specific activity. Divalent cations such as Ca^{2+} and Sr^{2+} enhance the enzyme's activity another 40-fold, approximately (van Oort *et al.,* 1989).

S. hyicus lipase was purified from *E. coli* essentially as described by van Oort (1989) and modified by Leuveling Tjeenk (1994). A rapid initial screening of crystallization conditions was conducted using the sparse matrix methods described by Jancarik and Kim (1991) and by McPherson (1992). Based on the results of this screening (crystalline material at conditions where 35% (v/v) DMSO was present in the reservoir, but not in the drop), a more systematic search was done, varying the DMSO concentration in small steps and the temperature. Reproducible small plate-like crystals were obtained at 4°C in the presence of 30% (v/v) DMSO. Analysis of the crystals by SDS electrophoresis showed that they are composed of intact 46 kDa *S. hyicus* lipase. After optimization, the best crystallization conditions used the sitting drop vapor diffusion method. Drops, containing only protein at a concentration of 8.7 mg/ml in a low-salt buffer (13 m*M* NaCl in 1.6 m*M* Na succinate buffer, pH 6.5), were equilibrated against reservoir solutions with 18 to 24% (v/v) DMSO as precipitant. Crystals start to grow after a few weeks and need several months to reach their final shape (both plate- and rod-like) and size (up to $0.4 \times 0.5 \times 0.2$ mm^3). When DMSO was added to the protein solution at the start of the experiments no crystals were obtained. During crystallization, a considerable decrease in drop volume occurs, which eventually results in drying out of the crystals. To prevent this we started with large drops (15 μl or more) and added small amounts of a synthetic mother liquor (20% DMSO, 50 m*M* NaCl, and 10 m*M* Na succinate buffer, pH 6.5) in the course of the experiments. We determined by additional crystallization experiments that the growing of crystals is not caused merely by the increase in protein concentration during crystallization due to the reduction of drop volume, but that DMSO is necessary for the crystallization of *S. hyicus* lipase. Analysis by [^{1}H]NMR and gas chromatography showed that the drops contained DMSO after a few weeks. To further increase the quality of the crystals, several additives were tested. The best results were obtained with 10% isopropanol in both the drop and reservoir, and in the synthetic mother solution. The crystals are sensitive to temperature changes and are often very fragile. They diffract to 2.8 Å resolution at 4°C with X-rays from a synchrotron source (DESY-EMBL, Hamburg). The reflections could be indexed on an orthorhombic lattice ($P2_12_12_1$) with unit cell parameters $a = 73.31$ Å, $b = 77.96$ Å, and $c = 169.81$ Å. Using a molecular mass of 46 kDa, and assuming the presence of 8 monomers per unit cell (1 dimer per asymmetrical unit), a V_M value of 2.64 Å^3/Da was calculated, corresponding to a solvent content of 54%. These values are within the commonly observed range (Matthews, 1968).

To analyze the noncrystallographic symmetry in the asymmetrical unit, an X-ray data set was collected from one native crystal at the X31 beamline (EMBL) of the DESY Synchrotron in Hamburg, with a MAR image plate detector. The wavelength of the X-rays used was 1.0 Å. Reflection intensities were obtained with the program MOSFLM and scaled and merged with the ROTAVATA, AGROVATA, and TRUNCATE programs of the CCP4 package (Collaborative Computational Project No. 4, 1994). The final merged native data set, obtained from 140 059 measurements, consists of 22 931 unique reflections and was 98.8% complete in the resolution range 1000–2.87 Å. The R-merge (on intensities) was 8.8% in this range.

A self-rotation function (Crowther, 1972) calculated in the resolution range from 3.5 to 8 Å showed only one peak (42% of the height of the origin peak). The next highest peak had a height of 21% of the origin peak. The peak is consistent with a noncrystallographic twofold axis lying in the a–b plane and making an angle of about 37° with the a axis. A self-Patterson function calculated in the resolution range from 3.5 to 8 Å did not show any peak (except for the origin peak). This result is in agreement with two lipase molecules in the asymmetrical unit as was derived from the crystal density calculation.

The three-dimensional structure of *S. hyicus* li-

pase is being determined by the isomorphous replacement method. The search for heavy-atom derivatives is currently in progress.

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