Crystallographic Data

	Native	NAG	NAG2	NAG3
Anomeric form		β	α	β
		Co-cryst.	Soaking	Co-cryst.
Space group	$P2_1$	C2	$P2_1$	C2
a (Å)	38.07	68.00	38.22	87.49
b (Å)	33.20	33.25	33.14	32.94
c (Å)	46.12	48.99	46.00	49.00
β (°)	110.06	98.54	109.78	119.14
Z	2	4	2	4
Resolution	1.30	1.77	1.55	1.77
R-value	0.181	0.173	0.175	0.177

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CRYSTALLIZATION AND PRELIMINARY X-RAY CRYSTALLOGRAPHIC ANALYSIS OF THE RHIZOPUS DELAMAR TRIACYLGLYCERIDE LIPASE. By Y. Wei*, U. Derewenda, M. Haas†, R. Joerger†, L. Swenson, R. Green and Z. S. Derewenda, MRC Group in Protein Structure and Function, Department of Biochemistry, University of Alberta, Edmonton, Alberta, Canada T6G 2H7, and †USDA, ARS, Eastern Regional Research Center, 600 East Mermaid Lane, Philadelphia, Pennsylvania 19118, USA.

An extracellular lipase from a fungus *R. delamar* had been a subject of numerous studies. Its cDNA derived sequence showed that it belongs to the closely homologous family of enzymes purified from various filamentous fungi. One of them, from *Rhizomucor miehei* was among the first lipases to be studied by X-ray crystallography (Brady *et al.*, 1990). Another one (from *Penicillium camembertii)* is reported elsewhere in these proceedings, while a lipase from *Humicola lanuginosa* had also been solved by our laboratory at 1.8Å resolution. In order to investigate the molecular basis of differences and similarities between the *Rhizopus delamar* lipase and other homologous enzymes we have undertaken systematic studies leading to the crystallization of the protein.

The R. delamar lipase crystallized in a wide variety of conditions yielding crystals with diverse morphology, albeit the quality of the crystals, judged by X-ray diffraction, was almost invariably poor. Among the many crystal forms grown, ammonium sulfate precipitation produced two different forms of tetragonal crystals up to 1 mm long. In an attempt to find more suitable crystallization conditions we resorted to the use of detergents. Two new monoclinic crystal forms were obtained. One in the presence of LDAO (N,N-Dimethyl-dodecylamine-N-oxide), space group $P2_1$, a=71.4Å, b=141.38Å, c=87.49Å, β=114.6° (4 molecules in the asymmetric unit) and another in the presence of C_{18}DAO, space group C2, a=92.6Å, b=128.8Å, c=78.3Å, β=135.8° (2 molecules in the asymmetric unit). The latter form diffracts to beyond 2.5Å resolution and preliminary studies of non-crystallographic symmetry indicate a clear two-fold axis relating both molecules. It is hoped that this crystal form will eventually yield an X-ray structure of the enzyme.

Ref: Brady *et al.* (1990) *Nature* 343: 767-770.

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THE CRYSTAL AND MOLECULAR STRUCTURE OF A NOVEL MONO- AND DIGLYCERIDE SPECIFIC FUNGAL LIPASE. By U. Derewenda*, S. Yamaguchi†, Y. Wei, L. Swenson, R. Green and Z. S. Derewenda, MRC Group in Protein Structure and Function, Department of Biochemistry, University of Alberta, Edmonton, Alberta, Canada T6G 2H7, and †Tsukuba Research Laboratories, Amano Pharmaceutical Co. Ltd., 22 Miyukigaoka, Tsukuba, Iberaki 305, Japan.

Diglycerides function as central intermediates in the synthesis of triglycerides and phospholipids, but they also function as second messengers formed when a phospholipase C is activated by an extracellular signal. The molecular basis of diglyceride recognition by proteins is therefore of considerable interest.

To date structures of only three lipases have been elucidated by X-ray crystallography. All three hydrolyze triacylglycerides. We have determined the molecular and crystal structure of a mono- and diglyceride specific enzyme from a filamentous fungus *Penicillium camembertii*. This protein is a single polypeptide chain made up of 279 residues and belongs to the same homologous family as the *Rhizomucor miehei* lipase (structure reported by Brady *et al.*, 1990) and the *Humicola lanuginosa* enzyme (refined recently at 1.8Å resolution in the authors' laboratory). The amino acid sequence shows 39% identity with the *Humicola* protein and 27% identity with *Rhizomucor*.

The *P. camembertii* lipase crystallizes in space group P1 (a=45.6Å, b=47.1Å, c=33.5Å, α=79.5°, β=112.1°, γ=70.3°) with one molecule in the asymmetric unit. The crystals grow as twins, and data were collected from such a crystal using the Xentronics/Siemens area detector. The diffraction pattern from a single crystal was successfully resolved and autoindexed. Data to 2.1Å resolution were reduced to give R_{merge} of 0.05. The structure was solved using a rotation function and the 1.8Å complete atomic model of the *Humicola* lipase. A starting model for refinement was manually constructed by substituting individual sidechains The first round of refinement involving rigid body refinement and simulated annealing reduced the R factor from 0.57 to 0.41. Least-squares refinement is now in its final stages with an R-factor of 0.17 and all but five amino acids and over 200 water molecules clearly resolved. In general terms the *P. camembertii* lipase is quite similar to the other members of this homologous family. However, significant differences occur close to the C-terminal end of the molecule, where the polypeptide is longer than any of the other lipases by ca. 10 amino acids. This region is close to the substrate binding site (as determined from the studies of the *R. miehei* complexes). It is hoped that specific amino acids responsible for specific substrate recognition can be identified in that region.

Ref: Brady *et al.* (1990) *Nature* 343: 767-770.

