RNase F1, a guanine-specific ribonuclease from *Fusarium monitiforme* was crystallized in two different forms, in the absence of an inhibitor and in the presence of 2'GMP. Both crystal structures were solved by molecular replacement method. The crystal structure of RNase F1 free form was refined to a final R-factor 18.7% at 1.3Å resolution. The crystal structure of the complex was refined to a final R-factor 16.8% at 2Å resolution. The two crystal structures of RNase F1 free form and the complex with 2'GMP are very similar to each other (r.m.s.d. 0.43Å for all C^{α} atoms). The main differences between the two structures are associated with binding of 2'GMP in the substrate binding site. A structural comparision between RNase F1 and RNase T1 shows a substantial similarity between all C^{α} atoms (r.m.s.d. 1.4Å). The loop from residues 32 to 38 was strikingly different between these two enzymes. The side chain of a catalytically active residue, His92, is shifted away from the catalytic site in RNase F1 by 1.3Å and 0.85Å with respect to the corresponding positions in RNase T1 free form and in the RNase T1 complex with 2'GMP, respectively. In the RNase F1 complex, the guanine base of 2'GMP has a *syn* conformation about the glycosyl bond, and the furanose ring assumes a 3'-*exo* pucker, which is different from that found in the complex with RNase T1. In the catlytic site of the RNase F1 complex with 2'GMP, one water molewas observed, which bridges the phosphate oxygen atoms of 2'GMP and the side-chains of the catalytically important residues, His92 and Arg77, through hydrogen bonds. A water molecule occupying the same position was found in the RNase F1 free form. This water molecule may play an important role during the catalytic reaction.

D.G. Vassylyev, K. Katayanagi, K. Ishikawa, M. Tsujimoto-Hirano, M. Danno, A. Pahler, O. Matsumoto, M. Matsushima, H. Yoshida and K. Morikawa (1993). *J. Mol. Biol.* 230, in press.

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CRYSTALLIZATION AND PRELIMINARY STUDY OF ENDOGLUCANASE (EGIII) FROM *Trichoderma reesei*

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The endoglucanase EGIII is one component of the enzyme mixture, called "cellulases" which contains multiple cellobiohydrolases (exoglucanases) and endoglucanases. All these cellulases act synergistically to degrade both crystallin and amorphous cellulose. EGIII from fungus *Trichoderma reesei*, has been crystallized. It is a low molecular weight (Mr=25,000 dalton) unglycosylated enzyme with pI=7.4. The EGIII gene has been cloned and the amino acid sequence has been deduced from genomic DNA. The crystals diffract at least to 2.8Å resolution. The crystals are monoclinic, space group $P2_1$ with cell dimensions of a=75.5Å, b=96.6Å, c=67.4Å, β=107.2°. The solvent content is 49% with V_m=2.4 if there are four molecules per assymetric unit. Self rotation function revealed the presence of a non-crystallographic dyad axis located at Φ=42°, Ψ=90°, suggesting two dimers per asymmetric unit. Two potential heavy atom derivatives have been identified so far, with K_2PtCl_4 and $KAuCl_4$.

PS–03.07.17 CRYSTAL STRUCTURE OF LYSOZYME FROM *STREPTOMYCES ERYTHRAEUS* AND CRYSTALLIZATION OF LYSOZYME FROM *STREPTOMYCES GLOBISPORUS*
By S. Harada*, C. Uematsu, Y. Kai, and N. Kasai Department of Applied Chemistry, Faculty of Engineering, Osaka University, Suita, Osaka 565, Japan.

Lysozyme is the enzyme which causes lysis of cell walls of bacteria by hydrolyzing the β-(1,4)-glycosidic bonds of the polysaccharide backbone of the peptidoglycan. On the basis of the homology of amino acid sequence, lysozyme is classified into four distinct types: (1) chicken, (2) phage, (3) goose and (4) bacteria. The crystal structure of a bacterial lysozyme produced by *Streptomyces erythraeus* (SEL) has been determined by X-ray diffraction analysis using the isomorphous replacement method. SEL consists of 202 amino acid residues and its amino acid sequence is totally different from other lysozymes whose crystal structures are known. The three-dimensional model of SEL shows that there are eight β-strands, six α-helices in the molecule. Six β-strands forms a parallel β- sheet. The parallel β-sheet, in which adjacent strands are connected by helices, is a barrel-like shape. Thus the folding pattern of SEL is topologically different from other lysozymes. A deep cleft which is identified as the active site exists on the C-terminal ends of the parallel β-sheet. The refinement of the structure is in progress. *Streptomyces globisporus* produces two kinds of lysozymes (M-1 and M-2 lysozymes) and secretes them in the cultural broth. The molecular weights are about 20,000 and 11,000 for M-1 and M-2 lysozymes, respectively. Their amino acid sequences are not known. M-1 lysozyme has been crystallized in two crystal forms ($P4_12_12$ ($P4_32_12$), $a=b=63.09$, $c=121.44$Å and $P6_122$ ($P6_522$), $a=b=128.9$, $c=144.0$Å). Since the amino acid sequence of M-1 lysozyme is supposed to be similar to that of SEL, the structure of M-1 lysozyme is intended to be determined by the molecular replacement method using the refined structure of SEL.

PS–03.07.18 CRYSTAL STRUCTURE OF TURKEY LYSOZYME COMPLEXES WITH OLIGOSACCHARIDES.
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Crystal structure of monoclinic turkey lysozyme and its complexes with N-acetylglucosamine(NAG) di-N-acetylchitobiose(NAG2), and tri-N-acetylchitotriose(NAG3) has been determined by the X-ray analysis. The NAG3 molecule occupies A,B, and C subsites and is bound in the manner that assumed in the catalytic reaction. The NAG molecule is found near the B subsite with the orientation different from that found in the NAG3 complex. One sugar residue of NAG2 with the α-anomeric form is bound near the D subsite while the other residue protrudes outside from the active site cleft. Therefore, the mode of binding differs according to not only the number of NAG residues but also the anomeric form of the terminal residue.

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.