

03-Crystallography of Biological Macromolecules

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RNase F1, a guanine-specific ribonuclease from *Fusarium moniliforme* 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 comparison 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 52 to 58 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'-*endo* pucker, which is different from that found in the complex with RNase T1. In the catalytic site of the RNase F1 complex with 2'GMP, one water molecule was 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.

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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 P2₁ with cell

dimensions of a=79.5Å, 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 asymmetric unit. Self rotation function revealed the presence of a non-crystallographic dyad axis located at $\Phi=42^\circ$, $\Psi=90^\circ$, suggesting two dimers per asymmetric unit. Two potential heavy atom derivatives have been identified so far, with K₂PtCl₄ and KAuCl₄.

PS-03.07.17 CRYSTAL STRUCTURE OF LYSOZYME FROM *STREPTOMYCES ERYTHRAEUS* AND CRYSTALLIZATION OF LYSOZYME FROM *STREPTOMYCES GLOBISPORUS*
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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 (P4₁2₁2 (P432₁2), a=b=63.09, c=121.44Å and P6₁22 (P6₅22), a=b=128.9, c=144.0Å). 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. By K. Harata, National Institute of Bioscience and Human-Technology, Tsukuba, Ibaraki 305, Japan

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.