03-Crystallography of Biological Macromolecules

03.07 – Catalytic Mechanism of Hydrolysis

DS–03.07.01 MUTATIONAL ANALYSIS OF DNaseI–DNA–INTERACTIONS by E.Wolf, I.Brukner, V.Nwosu, S.Weston and D.Suck*, European Molecular Biology Laboratory – Structures Department, Meyerhofstrasse 1, 6900 Heidelberg, Germany

Desoxyribonuclease I (DNaseI) from bovine pancreas is an endonuclease which degrades double–stranded DNA in an unspecific manner but shows a strong sequence–dependence of its cutting rates. The solved structures of two DnaseI–DNA complexes show that DnaseI makes contacts to the minor groove and both sugar–phosphate backbones of its DNA substrate and that binding of DNaseI to DNA leads to a widening of the minor groove and a bending of the DNA towards the major groove. These X–ray results suggest that the cutting rates of DNaseI are strongly affected by the minor groove width and depth as well as bendability of the DNA.

A number of single mutants have been studied to probe the importance of certain residues for DNA binding and catalysis.

We are presently analysing DNaseI mutants which have been designed to from additional contacts to the major or minor groove of the DNA. These additional contacts are expected to increase the sequence–specificity of the enzyme.

We have constructed mutants which certain insertions at position 72 in the minor groove binding loop of DNaseI as well as at positions 138 and 173. Residues 138 and 173 are part of two loops located opposite the major groove in the DNaseI–DNA complexes. Mutants containing one, two or three inserts were expressed in E.coli and characterized according to their sequence–dependence.

Changes in sequence–dependence could be detected by digestion of radioactively labelled double–stranded DNA fragments, separation of the digestion products on denaturing polyacrylamid gels and evaluation of band intensities in terms of cleavage probabilities for different phosphodiester bonds.

Some of the analysed mutants exhibit a significantly increased sequence–specificity, which could be due to specific contacts between functional groups of the bases and the inserted amino acids.

DS–03.07.02 CRYSTALLOGRAPHIC STRUCTURES OF METALLOENZYMES FOR DNA REPAIR: THE [4Fe-4S] ENZYME ENDONUCLEASE III AND THE Mg ENZYME EXOMUCLEASE III by *Che-Fu Kuo[a], Duncan E. McRee[a], Cindy L. Fisher[a] Suzanne O'Handley[b], Richard P. Cunningham[b], and John A. Tainer[a]; [a] Department of Molecular Biology, The Scripps Research Institute, La Jolla, CA 92037; [b] Department of Biological Science, Center for Biochemistry and Biophysics, State University of New York at Albany, Albany, NY 12222

We are examining the structural basis for the activity of the DNA-repair metalloenzymes. We have crystallized and collected 2 Å resolution data on the DNA-repair enzyme exonuclease III (*1*). We have crystallized the DNA-repair [4Fe-4S] containing enzyme endonuclease III, in the presence of glycerol and solved its atomic structure (*2*). Structural characterization and analysis of both enzymes is in progress.

The crystal structure of the DNA-repair enzyme endonuclease III has been solved to 2.0Å resolution and refined to an R-factor of 0.185 (*3*). This [4Fe-4S] enzyme is elongated and bilobal with a deep cleft separating two similarly sized domains: a novel, sequence-continuous, six-helix domain (residues 22–132), and a Greek key four-helix domain formed by the N-terminal and three C-terminal helices (residues 1-21, 133-211), in addition to the [4Fe-4S] cluster. The cluster is bound entirely within the C-terminal loop with a ligation pattern (Cys-X_6-Cys-X_2-Cys-X_5-Cys) distinct from all other known [4Fe-4S] proteins (Fig. 1). Sequence conservation and the positive electrostatic potential of conserved regions identify a surface suitable for binding duplex B-DNA across the long axis of the enzyme, matching a 46-Å length of protected DNA. The primary role of the [4Fe-4S] cluster appears to involve positioning conserved basic residues for interaction with the DNA phosphate backbone. The crystallographically determined inhibitor binding region, which recognizes the damaged base thymine glycol, is a seven-residue β-hairpin (residues 113–119). Its location and side-chain orientation at the base of the inhibitor binding site implicate Lys^{120} in the β-elimination mechanism. Overall, the structure reveals a novel fold and a new biological function for iron-sulfur clusters, and provides a structural basis for studying recognition of damaged DNA and the *N*-glycosylase and apurinic/apyrimidinic-lyase mechanisms.

Fig. 1

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DS–03.07.03 CRYSTAL STRUCTURES AND CATALYTIC MECHANISM OF α-MOMORCHARIN. By Jingshan Ren*†, Yaoping Wang‡ Yicheng Dong‡ and David I. Stuart†. †: Laboratory of Molecular Biophysics, Oxford University, U.K. ‡: Institute of Biophysics, Academia Sinica, Beijing, China.

α-Momorcharin (αMMC) is a type I ribosome-inactivating protein (RIP) isolated from the Chinese herb Kuguazi, the seeds of *Momordica Charanti* of the Cucurbitaceae family. RIPs inactivate ribosomes by hydrolytically removing a specific adenine residue from a highly conserved, single-stranded loop of rRNA. The anticancer and antiviral activities of these proteins, especially inhibition of HIV replication, have attracted much attention. The native, two adenine-bound and formycin 5'-monophosphate (FMP) bound structures of αMMC (in space group R3) have been determined and refined at high resolution. These structures allow unambiguous definition of the catalytic residues of the enzyme and illuminate the N-glycoside bond hydrolysis pathway. The protein has also been crystallized in space group $P2_1$ and an adenine-bound structure of this crystal form solved. Of the above three adenine-bound structures, two were obtained by diffusing dAMP and 3'-dAMP into the crystals respectively. This demonstrates that the enzyme has dAMP and 3'-dAMP nucleosidase activity.

There is a deep cleft roughly acrosing the middle of the molecule. The active site is located in the middle of the cleft as a deep tetrahedral hydrophobic box, where the residues are either invariant or highly

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conserved across the RIP family. Two charged residues, Glu160 and Arg163, are on the left-hand side of the box, making a salt bridge with each other. The side chain of Tyr70, sitting at the front, undergoes significant conformational changes upon substrate binding. The right-hand side of the box consists of the main chains of Gly109, Asn110 and Tyr111. The side chain of Tyr111 projects across the cleft to form a H-bond with OE1 of Glu160.

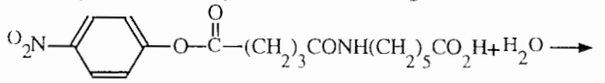
Unexpected features of the conformation of the bound FMP are (i) the ribose and base are in the anti conformation, rather than the syn conformation observed in the crystal structure of FMP, (ii) the furanose ring pucker is C1′-exo, different to that observed for the crystal structures of both FMP and AMP. This suggests that FMP may be bound in a strained high energy conformation. In the product bound structures, the adenine rings are in essentially the same position. However, this position is rotated by 15° (around an axis approximately along C6-N1) in comparison to the orientation in the FMP-bound structure, so that N9 moves 0.8Å toward the side chain of Tyr111. Two active site water molecules are conserved in all the five structures, both are H-bonded to FMP. OH2(247) forms a tight H-bond (2.67Å) to O3′; OH0(247) makes four H-bonds to OP2, O5′, O4′ and N8 of the FMP molecule. Thus OH0(247) could possibly be ionized by the negatively charged phosphate. The latter may therefore be the most likely candidate for nucleophilic attack on the C1′ atom of the ribose.

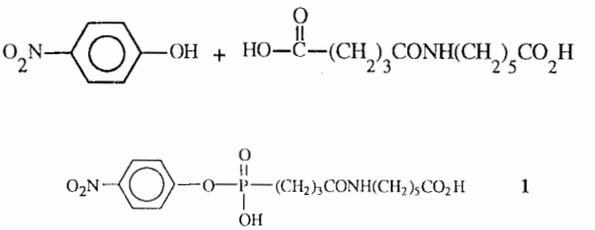
The hydrolysis of the N-glycoside bond by αMMC appears to proceed via a carboninum intermediate. The substrate adenosine is probably bound in a strained conformation (as observed in the bound FMP) thereby weakening the glycoside bond. Electrostatic and ring-ring interactions probably result in a force on the adenine ring which drives atom N9 of the substrate towards Tyr111. The strain is then secured by tight contacts, locking the ribose in position. Protonation at N3 by Arg163 or partial protonation at N3 and N1 by the H-bonds from Arg163 and Ile71 would then break the N-glycoside bond and produce the oxycarbonium ion on the ribose. Rotation of the freed adenine ring would then allow nucleophilic attack at C1' and protonation at N9 of the adenine.

The formation of a covalent intermediate is prohibited simply by the long distance from Glu160 to the carbon atom C1′ of the ribose (3.96Å , adjusting the side chain alone can not produce a sufficiently close approach to the C1′ atom).

DS-03.07.04 CRYSTAL STRUCTURE OF AN ABZYME HAVING AN ESTERASE ACTIVITY. By B. Golinelli[1], B. Gigant[1], T. Bizebard[1], J. Navaza[2], P. Saludjian[2], R. Zemel[3], Z. Eshhar[3], B.S. Green[3] and M. Knossow[1]* (1) Laboratoire de Biologie Structurale Bat 433 Université Pais Sud 91405 Orsay Cedex France (2) Laboratoire de Physique, Centre Pharmaceutique, 92296 Chatenay Malabry Cedex France (3) Department of Chemical Immunology, Weizmann Institute, Rehovot Israël.

Antibodies catalysing the following paranitrophenol ester hydrolysis have been obtained by immunising mice with the phosphonate transition state analog **1** conjugated to KLH ; Fab fragments of these were prepared and purified ; they were shown to have the same specific activity as the immunoglobulin.





Crystals of one of these Fab fragments have been obtained ; they belong to space group P1 with : a= 99.35 Å, b= 68.06 Å, c= 83.66 Å,α= 71.9°,β= 112.1°,γ= 119.6°, Z=4. Diffraction has been measured to 3 Å resolution, the R_{merge} value being 0.07 for a 93% complete data set . This abzyme has a kcat/kuncat value of 1600 and exhibits turnover. The crystal structure has been solved by molecular replacement and refined ; the R factor value is 0.21 , with a standard stereochemistry.

In the antibody combining site several tyrosine residues are oriented in such a way to enable them to make hydrogen bonds to the nitro and phophonate groups of **1**; they are therefore most likely to be involved in the transition state stabilisation ; their importance is confirmed by labelling experiments, which also indicate the presence of an argininine and an histidine. These crystallographic and biochemical results will be described and their implications for the mechanism of catalysis discussed.

DS-03.07.05 CONVERSION OF THE 3-D STRUCTURE OF ACETYLCHOLINESTERASE TO BUTYRYLCHOLINE- STERASE: MODELING AND MUTAGENESIS by M. Harel[a]*, I. Silman[b] & J. L.Sussman, [a]Dept. of Structural Biology [b]Dept. of Neurobiology Weizmann Institute of Science, Rehovot, Israel

In vertebrates, two enzymes efficiently catalyze acetylcholine (ACh) hydrolysis: acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). The principle role of AChE is the termination of impulse transmission at cholinergic synapses. BChE derives its name from the fact that it hydrolyses butyrylcholine (BCh) at rates similar to or faster than ACh, whereas AChE hydrolyses BCh over 100-fold more slowly. AChE and BChE are further distinguished by their differential susceptibility to various inhibitors. There exists a striking sequence homology between AChE and BChE with 53% identity and no deletions or additions. This marked structural similarity encouraged us to use the three-dimensional structure of AChE[1] to model BChE, in order to gain an understanding of how the structural differences between the two enzymes might account for the known differences in specificity between them.

The catalytic triad in AChE is located close to the bottom of area 20A deep marrow cavity, which we named the aromatic gorge, since about 40% of its surface area is lined with the rings of 14 aromatic amino acids. Six aromatic residues in the gorge of AChE are substituted by a non-aromatic residue in BChE. We suggested a plausible model for the docking of ACh, in an all-trans configuration, within the active site of AChE. This model was confirmed by structures of complexes of AChE with several competitive inhibitors[2]. When we model a bound BCh it is clear that the bulkier butyryl moiety of BCh cannot fit into the 'esteratic' locus. In the BChE model, however, the bottom of the gorge is enlarged by the substitution of two aromatic amino acids by smaller residues, i.e. F288L and F290V, permitting the butyryl group to fit into the larger 'esteratic' pocket. Following the predictions, it was possible to convert AChE to an efficient BCh-hydrolysing enzyme by site-directed mutagenesis of only two residues[3].