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 ($Cys\text{-}X_6\text{-}Cys\text{-}X_2\text{-}Cys\text{-}X_5\text{-}Cys$) 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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