Toward the High Resolution Structures of DNA Repair Enzymes: Endonuclease IV and Manganese Superoxide Dismutase

Yue Guan,[1] David N. Silverman,[2] Richard P. Cunningham,[3] John A. Tainer[1]
[1]The Scripps Research Institute, Department of Molecular Biology MB-4,
10550 N. Torrey Pines Road, La Jolla, CA 92037 USA;
[2]University of Florida, Department of Pharmacology & Therapeutics,
1600 S W Archem Road, Gainesville, FL 32610-0267 USA;
[3]State University of New York at Albany, Department of Biological Sciences,
1400 Washington Avenue, Albany, NY 12222 USA.

Endonuclease IV (endoIV) is a damage-inducible apurinic/apyrimidinic (AP) endonuclease in the base-excision repair (BER) pathway (Cunningham *et al.*, 1986). Its activity is to catalyze the cleavage of an abasic site created by DNA glycosylases such as MutY after the removal of damaged bases. Exonuclease III and endoIV are responsible for most of the AP endonuclease activity in the cell. To address the structural basis for the DNA base-excision repair pathway in damaged base recognition and removal, and backbone cleavage we have been focusing on the determination of three-dimensional structures of endonuclease IV in its uncomplexed form and in complex with a mononucleotide and oligonucleotide substrates.

We are working on two different kinds of endoIV, namely *E. coli* endoIV and *Thermotoga M* endoIV. The *E. coli* endoIV crystallized in monoclinic crystal form with the space group P21. The unit cell dimensions are a=50 Å , b=60 Å , and c=51 Å , and β=110°. With a complete 1.4 Å resolution native data set collected we have been scanning extensively heavy atom derivatives. During the December 1997 ALS run, we have collected one potential gold derivative data set to 1.7 Å resolution with flash cooling cryogenic device. The crystal was well frozen. We recently found a new crystal form with the unit cell dimensions of a=49 Å, b=59 Å, and c=94 Å, and β =96°. The crystallization conditions make it easier for the heavy atom derivative scan.

MAD phasing with selenium-methionine (SeMet) has been proven to be a powerful tool in protein crystallography. We have obtained the isomorphous crystals for selenium-methionine endoIV which diffract poorly (~5 Å resolution). It's so critical for us to collect better than 3 Å data of SeMet endoIV to do MIR/MAD phasing. During the December run, we have, for the first time, collected a complete 2.6 Å resolution SeMet endoIV data set from a tiny crystal with the bright synchrotron X-ray beam at ALS. This data set greatly improved the MIR/MAD phasing of endoIV. We are in the progress of solving the structure of this key DNA repair enzyme.

The function of MnSOD is to protect mitochondria against oxidative damage. The 2.2 Å resolution crystal structure of human MnSOD reveals a homotetrameric assembly and the active site of hMnSOD ligates the manganese with three histidines (His 26, 74, 163), one aspartate (Asp 159), and a water molecule (hydroxyl anion) as the fifth ligand in a trigonal bipyramidal arrangement (Borgstahl *et al.*, 1992). Azide, a competitive inhibitor of MnSOD, is thought to bind to the active site Mn ion in a manner similar to the superoxide substrate. We have recently obtained co-crystals of azide-MnSOD and collected a 2.8 Å resolution data set from a crystal flash frozen at liquid nitrogen temperature using a Rigaku rotating anode generator and a MAR image plate detector. This resolution has been extended considerably when we collected a 2.0 Å resolution data set during the December 1997 ALS run. These data enable us to derive a more accurate model for azide-inhibited MnSOD and help to understand the function of this enzyme. Great efforts have

been made to functional mutants to further our understanding on the mechanism of MnSOD (Guan *et al.*, 1997).

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Principal investigator: John A. Tainer, Scripps Research Institute. Email: jat@scripps.edu.