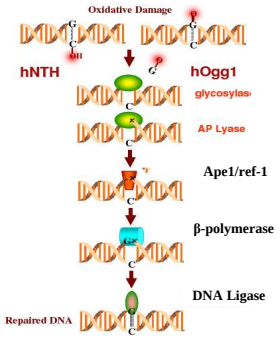
Structural Studies on the Mammalian Apurinic/Apyrimidinic Endonuclease (APE1) DNA repair enzyme

Sandrea M. Francis†,‡, Federico M. Ruiz†,‡, Jerónimo Bravo†,* and Carmen Fábrega†,*
† Signal Transduction Group, Structural Biology and Biocomputing Programme, CNIO, Melchor Fernández Almagro 3, E-28029 Madrid, Spain
‡ Equal contribution, *Corresponding authors: cfabrega@cnio.es, jbravo@cnio.es

Apurinic/apyrimidinic endonuclease (APE1) is a versatile protein having two unrelated and independent functions, one being DNA repair and the other transcriptional regulation of gene expression. For this last reason, it is implicated to interact with a variety of different proteins in several complexes. On the other hand, as part of the DNA base excision repair pathway, APE1 removes mutagenic and cytotoxic abasic sites often created by spontaneous alkylation or oxidative DNA damage. Since repair of damaged DNA is one of the major factors conferring resistance to chemo- or radio-therapeutic treatments in cancer, recent focus has been put in the development of inhibitors for this enzyme. Here we present the expression and purification of the APE1 protein in its soluble form. Crystallization studies have also been undertaken as a prerequisite for the structural study of protein-inhibitor or protein-protein complexes. Diffraction experiments have already been carried out at the ID-14 beamline of ESRF.

INTRODUCTION

Apurinic/Apyrimidinic Endonuclease 1 (APE1/Ref1)

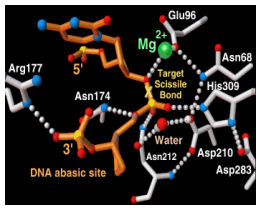


BASE EXCISION REPAIR PATHWAY [1]

- APE1: crucial function in the Base Excision Repair pathway which repairs DNA damage caused by alkylating agents/radiation
- Inhibition of this enzyme can overcome the resistance caused due to DNA repair in response to chemotherapeutic agents
- Binds to inhibitor molecules like Resveratrol, Soy isoflavones, Lucanthone, E3330 and other lead compounds developed
- Redox function: Transcriptional coactivator in the binding of several transcription factors to DNA like NFκB, Fos/Jun, Egr-1, HIF-1α, p53, CREB, PEBP-2, Pax-5 and -8, TTF-1 etc.

- Two independent domains for DNA repair and redox functions, also containing a nuclear localization signal at N-terminal
- Binds to minor groove of abasic DNA

MECHANISM OF DNA REPAIR [2]



- Hydrolyses phosphodiester backbone immediately 5´ to abasic site in a divalent metal ion involved catalysis

OBJECTIVES

- To obtain soluble APE1 protein in suitable amounts for crystallization studies
- To set up complex crystallization with inhibitor compounds and other interacting partners

CONCLUSIONS

- We obtained crystals of APE1-ΔN36 protein which were diffracted under cryo conditions at the ID-14 beamline of the ESRF synchrotron facility
- Diffraction data were collected and analyzed to a resolution of 1.8 - 2.0 Å

References:
1. Kremer T.M. et al., Respiratory Research 2004, 5-16
2. Mol C.D., et.al, Nature, 2000, 403, 451-456

Financial Entity: Fondo de Investigaciones Sanitarias (06/1250)

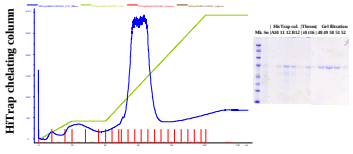
EXPERIMENTAL SECTION

Cloning, expression and purification of APE1 full length protein

The APE1 full length sequence (318 AA) was cloned into the pET28a vector, using NdeI/XhoI restriction enzymes. The recombinant plasmid was used to transform into BL21 and Rosetta *E. coli* strains for protein expression

- Grown in 1 L LB media containing Kanamycin 50 mg/ml at 37 ºC
- Induction at 30 ºC with 1mM IPTG

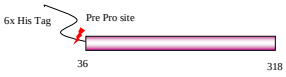
The purification protocol included 2 main steps: Immobilized-metal affinity chromatography (HiTrap) and Size exclusion (Sephadex 200 26/60)



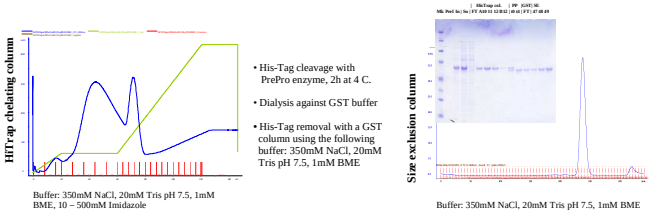
Cloning, expression and purification of APE1 ΔN36 protein

The APE1-ΔN36 construct was cloned into the modified pET28a vector with a PreScission Protease™ cleavage site, using NheI/XhoI restriction enzymes.The recombinant plasmid was used to transform BL21 and Rosetta *E. coli* strains for protein expression

- Grown in 1 L LB media containing Kanamycin 50 mg/ml at 37 ºC
- Induction at 30 ºC with 1mM IPTG

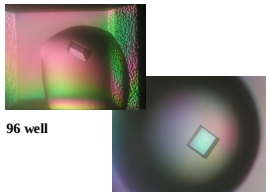


The purification protocol included 4 main steps: Ni affinity, His-tag cleavage, His-Tag removal by GST column and Size exclusion Chromatography



The protein crystallized at 30-40 mg/ml. The crystallization conditions of the APE1-ΔN36 construct were obtained at 20 ºC from Hampton Index screening conditions as given in the table :

Condition
0.1 M Bis-Tris pH 5.5, 25 % w/v PEG 3350
0.2 M Ammonium sulfate, 0.1 M Bis-Tris pH 5.5, 25 % w/v PEG 3350
0.2 M Sodium chloride, 0.1 M Bis-Tris pH 5.5, 25 % w/v PEG 3350



96 well

24 well