

Project Description

Title: Structure and Function: The Crystal Structure of Dpo4 with N-Acetyl-2-Aminofluorene

Abstract: Cancer is one of the leading causes of death in America. Cancer-causing agents, known as carcinogens, have the ability to attack DNA, creating DNA lesions that can potentially cause mutations. If these mutations are in critical genes that involve cell growth and maintenance, they can be passed on to daughter cells and lead to tumor formation and potentially cancer. How DNA lesions are handled by the cell has been a key question in understanding cancer. One way is through a group of specialized DNA polymerases that perform lesion bypass, termed TLS (translesion DNA synthesis).

Dpo4 is a TLS polymerase found in archaeal cells. While the structure of this DNA polymerase with undamaged DNA has been identified, little is known about the mechanism by which this polymerase is able to bypass DNA lesions. In order to further discover the mechanism of DNA lesion bypass, it is my goal to determine the structure of Dpo4 with the model liver carcinogen N-Acetyl-2-Aminofluorene (AAF).

Research Question: How does the structure of Dpo4 contribute to its ability to bypass the model liver carcinogen N-Acetyl-2-Aminofluorene?

Introduction:

Cancer accounts for 1 in 4 deaths in America. . In 2009 alone, the American Cancer Society predicted 1,479,350 new cancer cases in the United States, with 562,350 of these causing death. That is over 1,500 people per day dying of cancer. Environmental exposure from sunlight, radiation, and a variety of chemicals have all been identified as sources of cancer-causing agents, known as carcinogens. Carcinogens have the ability to attack DNA, creating DNA lesions that wreck havoc on the normal metabolic processes of the cell. When cells are unable to repair the damage, there is a high probability of mutation, producing cells with altered function. If these mutations are in critical genes that involve cell growth and maintenance, they can be passed on to daughter cells and lead to tumor formation and potentially cancer. How DNA lesions are handled by the cell has been a key question in understanding cancer.

One way cells handle DNA lesions is through a group of specialized DNA polymerases discovered in 1999 that perform lesion bypass, termed TLS (translesion DNA synthesis). Normal replicative DNA polymerases are blocked by lesions, and so the cell must recruit the TLS polymerases, also known as the “Y-family” polymerases, to continue DNA replication. The exact mechanism of how the Y-family polymerases are recruited, and how they are able to replicate past DNA lesions is not entirely known. However, structural information about the TLS polymerases themselves and the polymerases combined with undamaged DNA has revealed that they have a much more open and looser active site than the replicative polymerases. Therefore, while the replicative polymerases get caught and stopped by the DNA lesion, the open active site of the Y-family polymerases may allow them to accommodate DNA lesions. This means they are not caught by the lesions and can continue DNA replication past them. Each Y-family polymerase is thought to have its own signature of replication across different DNA lesions due to different amino acid sequences and other structural components.

Dpo4 is a TLS polymerase that is found in archaeal cells. While the structure of this DNA polymerase with undamaged DNA has been determined, the structure with certain DNA

lesions in the active site has yet to be discovered. Dpo4 is known to have a spacious active site that might be able to accommodate bulky DNA lesions. It is used as a model Y-family polymerase because of its similarity to the human TLS polymerase known as Polk.

N-Acetyl-2-Aminofluorene (AAF) was originally synthesized as a potential insecticide before it was discovered to cause mammalian cancer. It now serves as a model compound for other aromatic amines like 2-naphthylamine, which is a bladder carcinogen. Since aromatic amines are used in industrial processes to produce dyes, pharmaceuticals, antioxidants and pesticides, it is important to study aromatic amines such as AAF to determine how the cell handles the potential carcinogens.

As with other DNA lesions, when a replicative DNA polymerase encounters AAF, the lesion gets caught within the polymerase, causing it to be locked in an open, inactive formation. A TLS polymerase, such as Dpo4, needs to move in to bypass the lesion so DNA replication can continue. It is the structure of the Dpo4 polymerase, including its open active site, which allows it to bypass the AAF lesion, and that is why determining the structure of Dpo4 with AAF in its active site is so important.

Once the crystal structure of Dpo4 with AAF is determined, it can be used as a model for the study of the structures of other Y-family polymerases with other carcinogens. The study of this specialized group of DNA polymerases is important because it may help us determine why some people are more susceptible to cancer than others. For example, it was discovered that in humans, the lack of the TLS polymerase Polh can cause xeroderma pigmentosum, a genetic disorder that greatly enhances predisposition to sun-induced skin cancers.

The study of Y-family polymerases, like Dpo4, may lead to discoveries that allow us to prevent cancer. As one of the leading causes of death, cancer affects almost every person in the United States, whether directly or through family members and friends. After determining the structure of Dpo4 complexed with AAF, we will set the stage for the functional analysis of the DNA polymerase. This may include mutating the residues of Dpo4 to determine how they affect the function of the TLS polymerase.

I participated in a similar project to this last year which involved finding the crystal structure of Dpo4 with the free-radical 8-oxo-7,8-dihydroadenosine (8-oxo-A). This project introduced me to structural Biochemistry research and taught me many techniques that will assist with the AAF project. I was also able to obtain crystals of the 8-oxo-A complex, which is the most important part of this project. I have completed the 8-oxo-A project as far as I can for now. The next steps involve collaboration with the Aggarwal lab at Mount Sinai School of Medicine in New York, where the crystals will be x-ray diffracted using their synchrotron facility.

I have also participated in semester research in which I successfully added the AAF lesion to DNA. Now that I know the technique of crystallization and since I have DNA prepared, I will be able to crystallize AAF quickly and move on to study the structure in detail through various Biochemistry techniques. Determining how Dpo4 is able to replicate across AAF may provide us with important information on the process of mutagenesis and carcinogenesis.

Methodology:

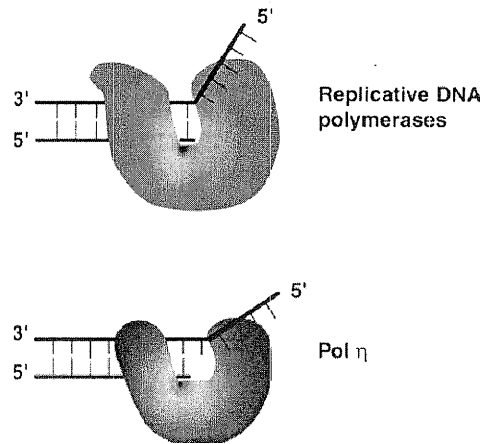
1) Production of purified Dpo4:

In order to determine the structure of Dpo4, we need large amounts of the DNA polymerase. This process has already been accomplished by other research students and by me. Dpo4 will be produced as needed through *E. coli* transformation and purified by column chromatography and FPLC.

- 2) Preparation of modified oligonucleotides:
Oligonucleotides are short strands of DNA. The AAF will be obtained commercially, and chemical reactions will be used to site-specifically modify the oligonucleotides. The resulting modified oligonucleotides will be purified by gel electrophoresis. This has already been accomplished as ATP semester research, and will continue as needed.
- 3) Crystallization of Dpo4 with AAF:
The crystallization of Dpo4 with AAF is necessary for determining the structure. The crystallization conditions of Dpo4 with undamaged DNA have been determined and will be used as the starting block for the crystallization with the AAF lesion. Presumably, tweaking the conditions determined for the undamaged DNA and Dpo4 crystal will result in the crystallization of the DNA with AAF and Dpo4. This will be accomplished by slight changes of precipitant, temperature and pH. However, this is a trial and error process which means many crystallization screenings may need to be preformed. These will be done on a small scale until the ideal conditions are determined, at which time larger scale crystallizations will be performed to obtain crystals suitable for diffraction studies.
- 4) Structure determination:
Once suitable crystals are obtained, the structure of Dpo4 complexed with AAF can be determined. Crystals of the Dpo4/AAF complex will be escorted or mailed to Brookhaven National Lab in New York. Diffraction data collection and data processing of the crystals will be measured at a high energy x-ray synchrotron source within Brookhaven National Lab. Structure determination and model building will be done with standard computer software.
- 5) Biochemical analysis:
Various methods of biochemical analysis will be use to study Dpo4 and its method of bypass over AAF. This includes studying if Dpo4 will incorporate the incorrect nucleotide across the lesion. This is generally studied using gel electrophoresis and radioactive tagging, however Bridgewater State College does not have the license or facilities for radioactive studies. Therefore, we will be using Invitrogen SYBR Gold dye to study nucleotide incorporation. I have already determined during semester research that this dye will work for our purposes.

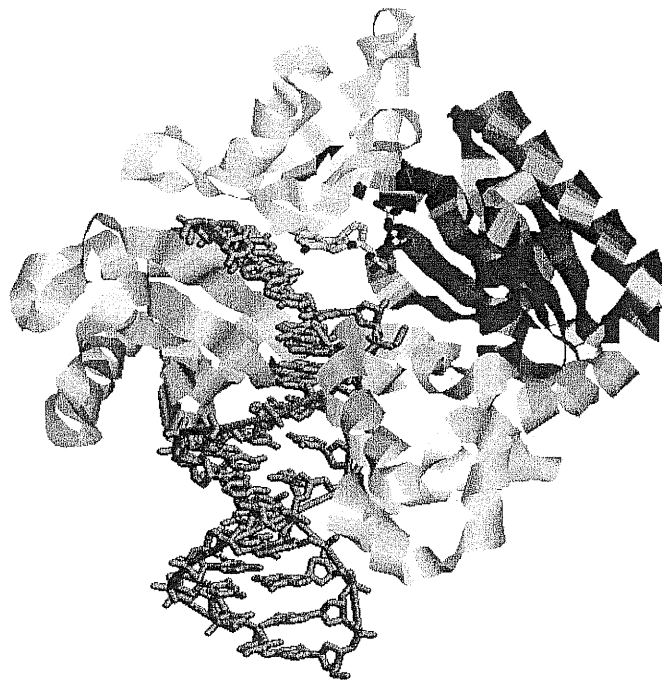
We will also be using other method of functional analysis such as protein residue mutagenesis to determine how the residues affect the function of Dpo4.

Figure 1



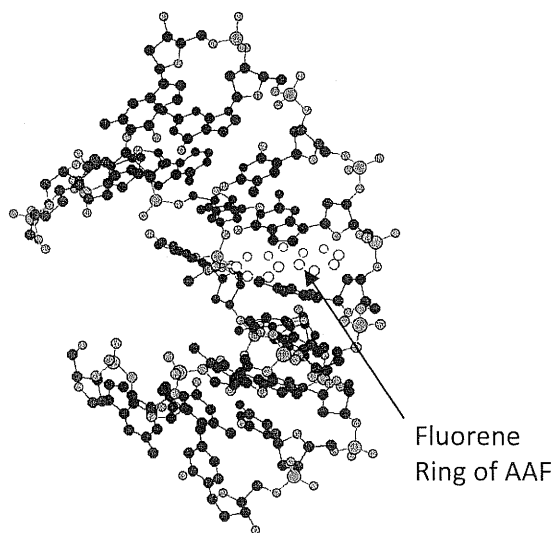
Pol η illustrates the open active site of TLS polymerases compared to replicative polymerases.

Figure 2



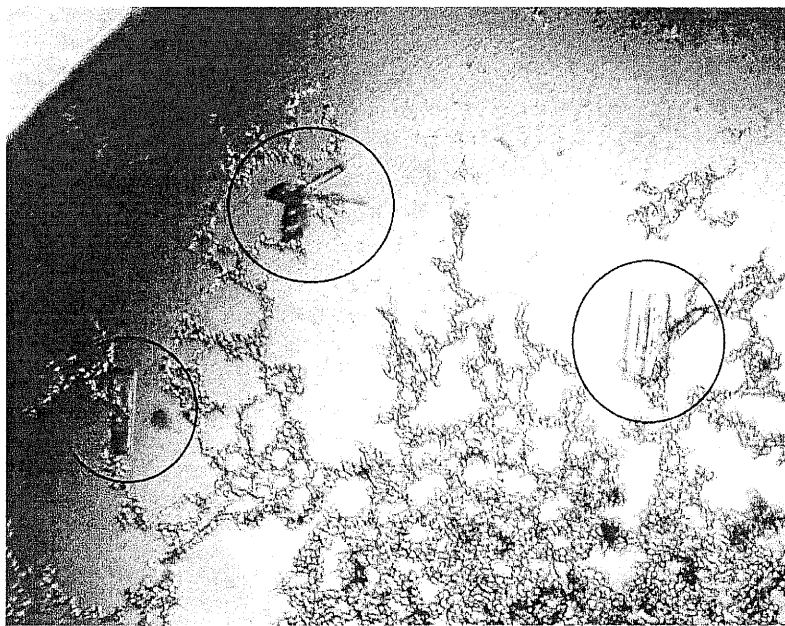
Undamaged Dpo4 shown with DNA and an incoming nucleotide.

Figure 3



AAF (yellow) attacks DNA,
causing conformation changes that
lead to potentially lethal
frameshifts in genes.

Figure 4



Crystals of Dpo4 with the free
radical lesion, 8-oxo-A. Obtained
during research in summer 2009.

Preliminary Bibliography

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Proposal Logistics

- This research will be conducted at Bridgewater State College in the Conant Science Building.
- The resources needed to complete this research will be provided by or commercially obtained by Doctor Samer Lone (research mentor) and the BSC chemistry department.
- Doctor Lone will be supervising the research on a daily basis.

Project timeline

Weeks 1-2: Production of purified Dpo4, preparation of modified oligonucleotides

Though the above steps have already been done throughout semester research, I will use the first two weeks of my project to ensure I have the necessary amount of protein and DNA to properly crystallize the DNA/protein complex.

Weeks 3-6: Crystallization of Dpo4 with AAF

It may take a few weeks to work out the crystallization conditions of Dpo4 with AAF. Generally it takes three days for crystals to solidify after a crystal tray has been set up.

Weeks 3-10: Biochemical Analysis

To analyze different biochemical properties of Dpo4, we will be using gel electrophoresis with SYBR Gold. Though we have determined that this dye works, we still have yet to develop a concrete method. While I am waiting for crystals to develop, I will work on developing this method. I will then use it to study Dpo4's efficiency of nucleotide incorporation.

Weeks 6-10: Structure determination

Once I have obtained good crystals of the protein complex, we will escort or mail them to the synchrotron facility in New York to be x-ray diffracted. We will then use the data to build a 3D computer model of the protein to determine how its structure relates to its function.

(Please note: Many steps of the project can be performed at the same time.)