Creation of a DNA Substrate for the Investigation of DNA-Protein Crosslinks
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Background:
Bialkylating agents form DNA-protein crosslinks (DPCs) as a side reaction See Fig. 1. Bialkylating agents belong to a class of drugs used to treat cancer that function by forming DNA-DNA crosslinks and interfering with DNA function. To a lesser extent, they also form DNA-protein crosslinks (DPCs). DPCs are known to be harmful to the cell, but they have not been well-studied.

Mechlorethamine Chlorambucil

FIG. 1. Bialkylating agents

The aim of our research was to study these DPCs and better understand their mechanism of action. A better understanding of the function of DPCs could one day contribute to the development of novel and more efficacious drugs used to treat cancer.

Past attempts made by previous researchers to study DPCs have been hindered because techniques used to form DPCs also create many other types of cellular damage. There are no drugs that exclusively induce DPCs. In this situation, results become hard to interpret because nothing can be exclusively attributed to the presence of the DPC. To solve this problem, we created a method to specifically create a substrate molecule containing a defined DNA-protein crosslink.

Creation & Purification of DNA duplex
I. Phosphorylate 5' ends of oligonucleotides 2, 3, 5, and 6
II. Anneal complimentary strands
III. Ligate oligonucleotides
IV. Run DNA duplex on a 6% polyacrylamide denaturing gel
V. Cut out desired band
VI. Elute DNA from gel
VII. Reanneal strands
VIII. Run DNA duplex on a 6% polyacrylamide non-denaturing gel

Expression and Purification of hOgg1
I. Overexpress hOgg1 (Human 8-oxoguanine DNA glycosylase) in E. coli cells
II. Cells were lysed and the lysate collected
III. Hexahistidine tagged protein was purified using Ni²⁺ affinity chromatography

Crosslinking Reaction
I. Set up a reaction containing the DNA substrate containing the 8-oxoG, hOgg1, and NaCNBH₃ or NaBH₄. See Fig. 48

Future Assays with the DPC
In vitro Assay
I. Incubate DPC with nuclear extract and assay for repair

In vivo Assay
I. Ligate DNA duplex containing DPC attachment site into plasmid
II. Perform a crosslinking reaction
III. Introduce plasmid with DPC into mammalian cells
IV. Assay for repair

References
Loeber R., et. al. (2008). Cross-linking of the DNA repair protein O6-alkylguanine DNA alkyltransferase to DNA in the presence of 1,2,3,4-diethylbutane. Chemical Research in Toxicology. 5, 645-64.