

Investigating the Induction and Purification of OGG1 Protein and Its Role in DNA-Protein Cross-Linking

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Background of DNA-Protein Cross-Linking

DNA-protein cross-links (DPC's) occur when a protein reacts with a DNA strand. DPC's can lead to cell death if it is not repaired due to its potential to interfere with cellular processes such as DNA replication and transcription because it causes a bulky distortion in the DNA double helix. Various chemical agents, such as 1,2,3,4-Diepoxybutane (DEB), are known to induce DPC's because of their alkylating functions. When uncontrollably created in cells, DPC's have a harmful effect, but they can have a therapeutic function if they are specifically induced in cells and controlled. Thus, with its potential lethality to cells, DPC's have been studied as a possible cancer treatment by trying to target its production in tumor cells and observing its biological activity. To better understand, DPC's, this project focused on the creation of DPC's using 8-oxoguanine glycosylase (OGG1) and the OG oligonucleotide duplex. Specific focus was placed on the induction and purification of the OGG1 protein and cross-linking it to OG.

Approach to Create and Observe DPC's

Insertion of OG Oligonucleotide in pDONR Plasmid

OG oligonucleotide + pDONR plasmid

Creation of DPC via Cross-Linking Reaction

OGG1 protein + pDONR plasmid with OG oligonucleotide

Transfect Plasmid with DPC into Mammalian Cells and Observe Effects of DPC

Observe Effects of DPC

OGG1 Protein

Encoded by the OGG1 gene, this protein is involved in base excision repair. The OGG1 protein contains a hexahistidine tag that allows for its selection using nickel agarose affinity chromatography (Ni-NTA).

OG Oligonucleotide Duplex

5' ——— CCGC **O** CGATC ——— 3'
3' ——— GGCGCGCTAG ——— 5'

This 158 base pair oligonucleotide duplex contains an 8-oxoguanine residue site for cross-linking. The oligonucleotide duplex was created by annealing and ligating six oligonucleotides and was then purified using a 6% polyacrylamide gel.

Cross-Linking of OG and OGG1

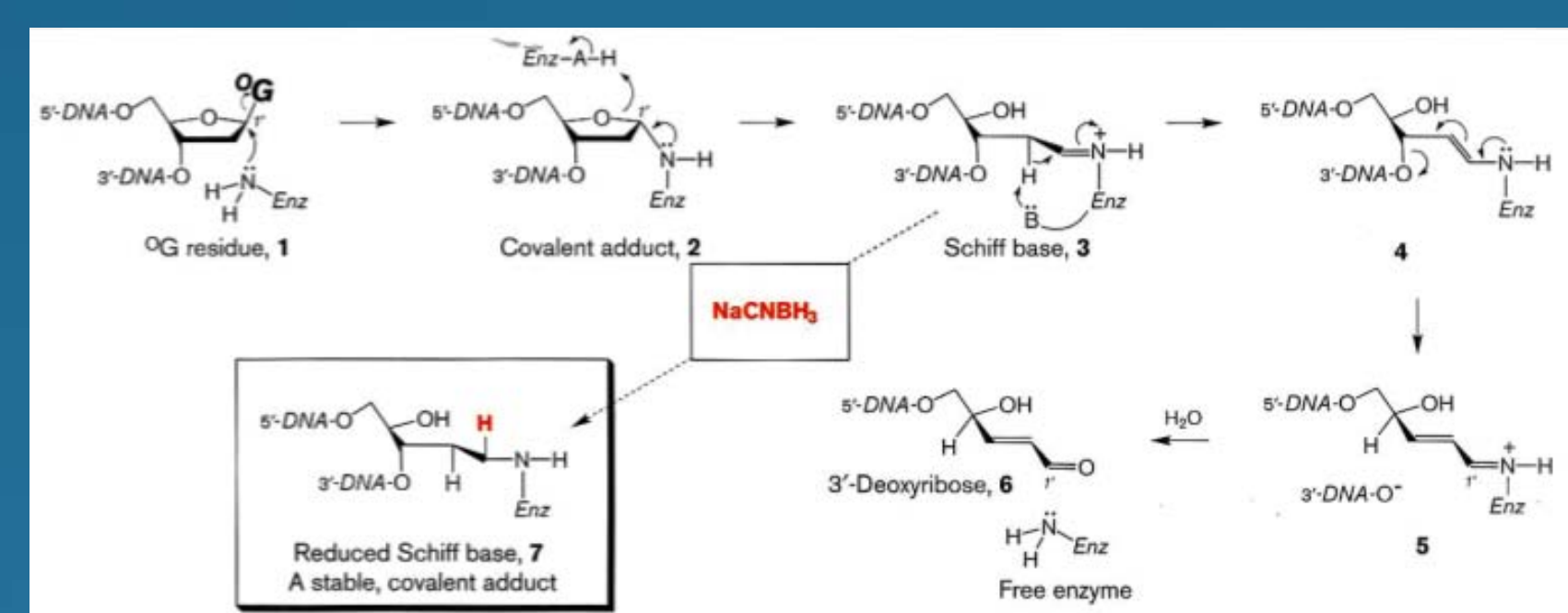


Image from Nash et al. (1997)

The above image depicts the mechanism of the reaction between OGG1 and the OG site of DNA in which a DPC is formed using a borohydride compound.

Induction and Purification of OGG1

Overnight Culture

Overnight at 37°C in 5µl Kanamycin/1 ml LB media

Induction

-At 37°C until optical density reached 0.4
-Added 1 mM IPTG and grew overnight at 16°C

Clear Lysate

-Lysed pellet with lysis buffer and sonication
-Recovered supernatant

Ni-NTA Purification

-Incubated with Ni-NTA Beads
-Wash Buffer x 3 and Elution Buffer x 3
-Recovered supernatant (purified OGG1 protein)

Optimizing OGG1 Recovery

To maximize the recovery of OGG1 protein, different dialysis and freezing methods were used:

1. Immediate Dialysis and -80°F Freezing

- Immediately dialyzed into 250 mM NaCl, 10% glycerol, 10 mM Tris-HCl overnight
- Stored at -80°F
- Result: Low protein yield because the low freezing temperature affected protein stability

2. Delayed Dialysis and -20°F Freezing

- Stored in 50% glycerol at -20°F
- Dialyzed into 50 mM NaCl, 20 mM Tris-HCl, and 1 mM EDTA overnight the day before using protein for cross-linking reaction
- Result: High protein yield after freezing, but protein precipitated out during dialysis

3. Shorter Dialysis Time and -20°F Freezing

- Stored in 50% glycerol at -20°F
- Dialyzed into 50 mM NaCl, 20 mM Tris-HCl, and 1 mM EDTA for 3 hours before using protein for cross-linking reaction
- Result: High protein yield after freezing and dialysis with no precipitate

OGG1 Recovery



Lane	Sample
1	Clear Lysate
2	Flow Through
3	Marker
4	1 st Wash
5	2 nd Wash
6	3 rd Wash
7	Undialyzed Elution
8	Dialyzed Elution

By immediately dialyzing and storing the recovered OGG1 protein at -80°F, there was a significant loss of protein as shown by comparing the highlighted bands in lanes 7 and 8.

Cross-Linking with NaBH₄ and NABH₃CN



Lane	Sample
1	OGG1
2	OGG1+ NaBH ₄
3	OGG1 +NABH ₃ CN
4	4 µg BSA
5	Marker
6	8 µg BSA
7	OGG1 +OG
8	OGG1 + NaBH ₄ + OG
9	OGG1 + NABH ₃ CN + OG

Lane 1 indicated a high yield of recovered OGG1 protein using a shorter dialysis time and -20°F freezing. Visualization was done using Coomassie Blue stain to detect a protein shift due to an increase in molecular weight if OGG1 successfully cross-linked to OG. The cross-linking reaction was successful using NaBH₄ and NABH₃CN as indicated by the upwards band shift in the highlighted bands in lanes 8 and 9. However cross-linking occurred at a higher rate using NaBH₄ with approximately 1/3 of the OG successfully cross-linking with OGG1.

Next Steps

Expression and purification of OGG1 were achieved at satisfactory efficacy and purity levels by using a shorter dialysis time and -20°F storage temperature because those conditions allowed for better maintenance of protein stability. Furthermore, the cross-linking of OGG1 to OG was achieved at satisfactory levels using NaBH₄.

The next steps are to insert OG into the pDONR and cross-linking OGG1 to OG using the aforementioned procedure. The plasmid will then be transfected into mammalian cells to observe the effects of DPC's.

References

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