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Mason Conen
mason.conen@umontana.edu

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The E. coli Protein YbgL: A Novel DNA Repair Enzyme?

Mason H. Conen, Brooke D. Martin, Kent D. Sugden, Savannah Whitfield

Background

Pentavalent chromium has been shown to create DNA damage by oxidizing the base guanine to two unusual hydantoin moieties: Sp and Gh. These lesions cause G→T transversion mutations and can be recognized by the BER enzyme Nei. Because of its structure and proximity to Nei in the genome, we hypothesize that YbgL, an uncharacterized E. coli protein, recognizes the Sp and Gh lesions.

YbgL is a member of the LamB/YcsF superfamily of proteins. This family is named because of its homology to LamA which hydrolyzes cyclic lactam molecules such as 2-pyrrolidone moieties (Katz et al. 1989). Due to the hydantoin chemical scaffold of the chromium-induced lesions Sp and Gh, we are investigating whether YbgL (and the LamB/YcsF superfamily to which it belongs) perform similar chemistry to the hydantoinase and cyclic amidohydrolase superfamily of enzymes. We wish to investigate whether YbgL is capable of hydrolyzing hydantoin-containing DNA lesions to Gh as E. coli cells lacking the YbgL gene cannot make Gh endogenously nor in response to chromium treatment (Savannah Whitfield~2016 in preparation). Specifically, we wish to investigate whether YbgL is required for the initial step in the following hydrolysis of Sp (Fig. 1).

Figure 1. (above) Mechanism of oxidation from Sp to Gh.

Figure 2. (right) Cr(III)-Salen crystals, the precursor for the Cr(V) carcinogen, were synthesized according to Coggon and McPhail (1970).

Methods

Oligo Oxidation

To test this hypothesis, we began by oxidizing 100 µM of a synthetic oligo (5’ ACCAGCGXCCGACCAGT, X is 8-oxo-G) with 20 µM Cr(V) at a pH of 8.05 in 100 mM K2HPO4 for 30 min. at room temperature.

HPLC Protocol

The oligonucleotide oxidation products were separated using a Dionex Nucleopac PA-100 4 x 250-mm anion exchange column using 10% acetonitrile with a gradient of 10-100% 1.5 M Ammonium Acetate in 10% AcCN pH 6.01 over 20 min. (Fig. 3).

Figure 3. Chromatogram of the control and Cr(V)-reacted synthetic oligo with peaks labelled based on Sugden et al. (2001).

Conclusions

Oligonucleotides contain Sp or Gh were synthesized and purified. Following analysis by mass spectrometry, these will be used to measure the binding of modified oligos to YbgL. Structural modeling indicated a structural similarity of YbgL to the active site of hydantoinase molecules as well as other manganese containing enzymes (Fig. 4).

Figure 4. Above is an overlay of one subunit from each of three LamB/YcsF proteins: YbgL (green; PDB: 1xw8) and two putative lactam utilization proteins from Pyrococcus horikoshii (yellow; PDB: 1v6t) and Thermos thermophiles (orange; PDB: 2fa). Note the histidine cluster of YbgL.

Future Directions

The next step in this project will be to demonstrate binding of YbgL to oxidized DNA lesions in vitro. To provide more insight into the structure and function of YbgL x-ray crystallography studies will be conducted to determine whether the tri-histidine cluster of YbgL requires a metal cofactor to stabilize and form and active protein.

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References

