Investigation of a chemical ligand that specifically targets 5fC in DNA

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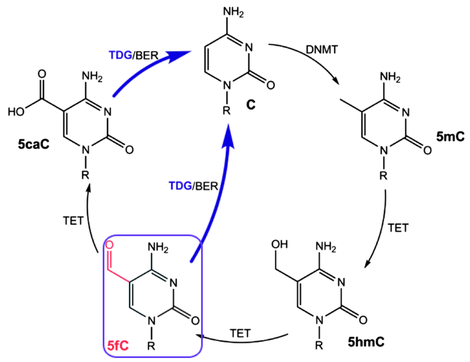
Abstract

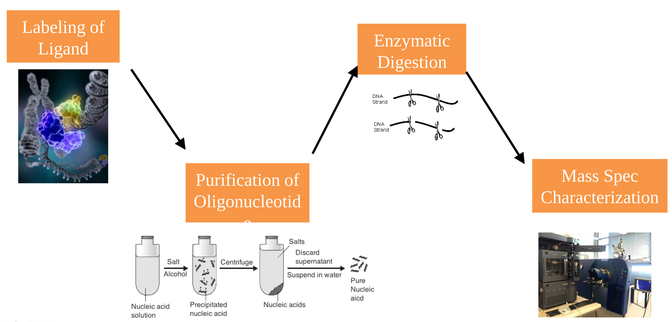
The fifth position on the Cytosine molecule in DNA has different modifications, each with its own different effects. One of the most intriguing and crucial modifications is the one called 5-formylcytosine (5fC). This occurs when a formyl group bonds with 5' position of Cytosine. This specific ligand has epigenetic implications on the cell, can be linked to various diseases, and has a variety of effects on the human body. Our research was directed in targeting and interacting with 5fC using a compound known as O-(4-Nitrobenzoyl)hydroxylamine, an aldehyde that is known to be one of the most reactive out of all compounds containing hydroxylamine. The experiment boiled down to four overarching steps: labeling, purification, digestion, and characterization. The implications for interaction with 5fC may be groundbreaking in the medical field.

Purpose and Background



The purpose of this study was to target 5-formylcytosine (5fC) sites in DNA so that interaction with it would be possible. This 5-formylcytosine modification in cytosine is linked to epigenetics, DNA methylation specifically. DNA methylation controls various cellular functions such as gene expression, X-Chromosome inactivation, maintenance of genomic stability, cellular identity maintenance and differentiation, and embryo development. Anomalous patterns of DNA methylation can cause cancer and other human diseases. Particularly, 5fC has a significant role in active DNA demethylation, whose process may provide novel fingerprints of cellular identity.

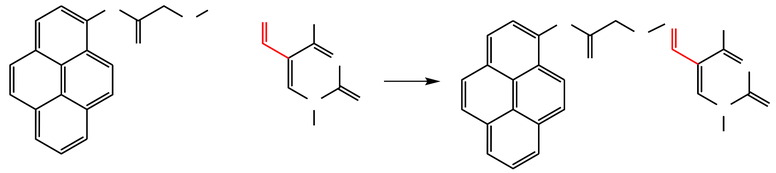
Methods and Approach



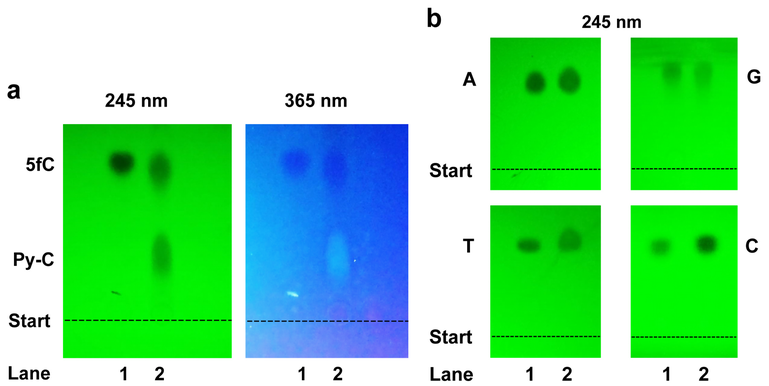
Images from Google Images

In order to label the ligand, a solution of 5fC (100 mM), O-(4-Nitrobenzoyl)hydroxylamine (1 mM), Sodium Phosphate (pH = 6.0; 20mM), and water was prepared with a final volume of 50 µl. For the purification of the oligonucleotide, 250 µl of ethanol was added to the solution in order to perform ethanol precipitation. We had to proceed with the digestion step next after precipitation. S1 Nuclease and Shrimp Alkaline Phosphatase were utilized for DNA digestion. Lastly, we had to characterize the 5fC using mass spectrometry.

Reaction between 5fC and the hydrazine group

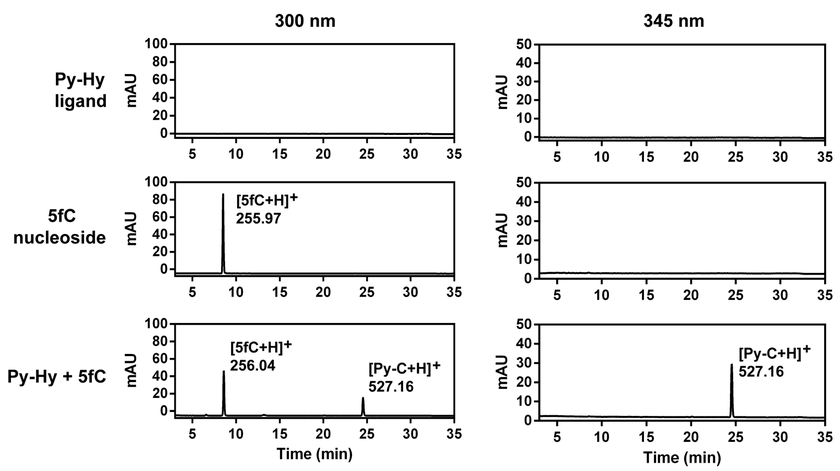


Thin Layer Chromatography



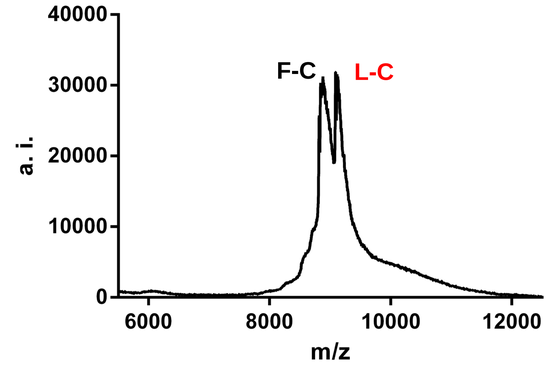
TLC analysis of selectivity of Py-Hy for different nucleosides. Lane 1: nucleoside only; Lane 2: reaction mixture of Py-Hy with different nucleosides.
(a) Images of 5-formylcytosine after reaction with Py-Hy. (b) TLC analysis of reaction of A, T, G and C with Py-Hy probe

HPLC-MS Analysis



After reaction between Py-Hy and 5fC nucleoside, the product emerged as a single new peak at 24.5 min retention time, which can be detected under both 300 nm and 345 nm wavelengths. Mass spec confirmed this new product was consistent with the hydrazone product.

MALDI-TOF Analysis



Strand No.	Sequence (5′-3′)	Mass (calc.)	Mass (found)
F-C DNA	CTACCGATAAGCAGA**X**GACCCTCTCCATG (**X** = 5fC)	8819.8	8819.1 [M-H]
L-C DNA	CTACCGATAAGCAGA**X**GACCCTCTCCATG (**X** = Py-C)	9090.9	9089.6 [M-H]
Difference before and after ligand-adduct formation		271.1	270.5

Discussion and Conclusions

Here, we investigated the reactivity and selectivity of this small ligand with 5-formylcytosine in both the level of nucleoside and the context of oligonucleotide. Our studies indicate that this ligand might be a useful chemical tool for further *in vivo* studies on 5fC related biological processes. Our strategy of combining a 5fC targeting group with a functional group may be used to inhibit DNA demethylation process and to further interrupt gene expression. Future studies will focus on the utilization of rationally designed ligands to manipulate the 5fC related functions in the genome.

References

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