Modification of Plasmid DNA by [Ir(9S3)Cl₃], [Rh(9S3)Cl₃], and [Rh(9S2O)Cl₃]

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Cis-platin, the benchmark chemotherapy drug, works mainly by crosslinking DNA by binding to Guanine. This act then activates apoptosis. However, there are several issues with cis-platin. For example, because it is delivered intravenously it damages healthy cells along with cancerous cells.
Because of this inherent issue, research is constantly going towards finding better chemotherapy agents that could also specifically target cells. One way to do such, would be to use compounds that are similar to cis-platin, but that are photosensitive to only certain wavelengths of light. In this way, even if a person received the compound intravenously, a physician could expose only the cancerous area to light of the correct wavelength, which would cause the chemical reaction to occur. Such a process would be ideal for skin and other such cancers.
Our laboratory is chiefly interested in the properties of \([\text{Ir}(9S3)\text{Cl}_3]\), \([\text{Rh}(9S2O)\text{Cl}_3]\), and \([\text{Rh}(9S3)\text{Cl}_3]\). These molecules are similar to cis-platin, in that they all have the chlorine leaving groups. However, recent literature suggests, they are different in that they show a tendency towards being photoactivated. To test this hypothesis, plasmid DNA was exposed to each of the following compounds in both time dependent and concentration dependent trials under two different wavelengths of light.
The Bacterial Plasmid pBR322

- 4361 base pairs in length
- Contains replicon region
- Unique restriction sites
- Really expensive ($5,612,000 per g)

http://parts.mit.edu/igem07/index.php/ETHZ/pbr322
Procedure

1. Dilution
   Concentrations of the different molecules were made specifically for the experiments.

2. Incubation
   Plasmid, TE buffer, and the molecule of interest were combined and then exposed to either 253.7 nm or 350.0 nm UV light for a specified amount of time.

3. Electrophoresis
   Our results are gathered qualitatively through electrophoresis, in which a researcher can determine if the molecule of interest is causing cleavage of the pBR322. Circular DNA will be visible further downfield, while nicked DNA will be upfield. Thus, if a molecule is causing cleavage some amount of nicked DNA should be apparent.
What is DNA Electrophoresis?

- Useful in the separation, identification, or purification of DNA.
- Agarose gel was used (can separate fragments from 100 b to 20 kb)
- DNA is a negatively charged molecule, so when current is supplied to the system, the fragments move towards the positive pole.
- TBE Buffer has salts to conduct current and controls the pH of solution
- Loading Dye – weighs down sample and acts as a marker
- Ethidium Bromide – intercalating dye that allows DNA to be detected by fluorescing when illuminated with ultraviolet light

For the concentration dependent trials, pBR322 was exposed to 10 uM, 50 uM, and 100 uM concentrations of one of the molecules. There was also a negative control (which was kept in complete darkness) and control A, which contained only plasmid and was exposed to the same light as the other samples. These samples were exposed 350.0 nm and 253.7 nm for 10 minutes.

For time dependent trials, pBR322 was exposed to 10 uM concentration of each molecule for 1 minute, 5 minute, and 30 minutes. The negative control was kept in darkness for a full 30 minutes. A control with just plasmid was run under both 350.0 nm and 253.7 nm for the same time lengths to serve as a standard of comparison.

“mm” on results represent molecular marker
Time Dependent Control

- 10 kb
- 6 kb
- 3 kb

350.0 nm

253.7 nm
Nuclease Activity of [Ir(9S3)Cl₃]

Time Dependent
350 nm light

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<th>CN</th>
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Concentration Dependent
350 nm light

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<th>CN</th>
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Time Dependent
254 nm light

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Concentration Dependent
254 nm light

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<th>100μM</th>
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Nuclease Activity of [Rh(9S2O)Cl₃]

Time Dependent
350 nm light

Concentration
Dependent
350 nm light

Time Dependent
254 nm light

Concentration
Dependent
254 nm light
Nuclease Activity of [Rh(9S3)Cl₃]

Time Dependent
350 nm light

Concentration Dependent
350 nm light

Time Dependent
254 nm light

Concentration Dependent
254 nm light
None of the complexes at our conditions seem to be active at 350 nm UV light

[Ir(9S3)Cl₃] is unreactive at 254 nm light for our conditions

[Rh(9S2O)Cl₃] shows nuclease activity in 254 nm light for concentrations greater than 50 uM

[Rh(9S3)Cl₃] shows nuclease activity in 254 nm light for concentrations greater than 10 uM
Future Direction

- Fluorescence Binding Assay with Calf Thymus DNA/Poly AT/Poly GC
- Nuclease Assay with Calf Thymus DNA
- E. coli cell cytoxicity
- Intracellular delivery
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