Antiviral Capabilities of a DABCO-hydrocarbon molecules

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Microbiology

Research Plan

A) Rationale

Viruses constantly pose harm onto humans' lives and the need to eradicate them is vital. A virus is a microscopic parasite. Viruses are capable of infecting almost all living organisms such as plants, animals, or bacteria. They are infectious particles that use the resources of the living host cells they target and take them for their own propagation. Although only 219 species are known to be able to infect humans, there are over 320,000 different types of viral species identified today (Woolhouse, 2012). However, despite the antimicrobial techniques that have been created today to aid in killing bacteria, no antiviral methods have been perfected to inhibit viruses yet. Several researchers have begun to study possible antiviral techniques that could be manufactured. One group of researchers has identified a protein that can eliminate viruses and aid the body in naturally targeting the virus based on genetic patterns (Mandal, 2019). However, as viruses maintain their harmful capabilities in society today, the need to create more effective antiviral techniques is a growing issue.

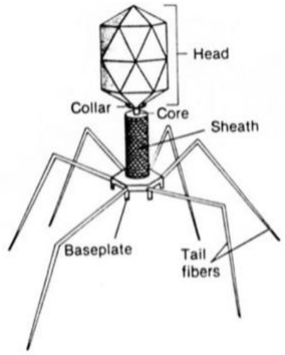


Figure 1: Structure of bacteriophage. Protein capsid (head) and negative tail fibers are shown.

A method to circumvent the infectious activity of viruses is the usage of a DABCO-hydrocarbon chain attached to a surface (cloth, paper, etc.). Past studies have shown that it is an effective method to reduce the infectious activity of viruses, specifically the T4 bacteriophage. Bacteriophage are one of the more common viruses, found very readily in the biosphere in freshwater and oceans, feces, sewage, soil, and many other environments (White, 2019). Despite their widespread abundance, these viruses are characterized by their high specificity to bacterial species along with their morphology and genome. For example, T4 bacteriophage is a specific virus in the family *Myoviridae* and is best known to kill the bacteria *E. coli*. Results have indicated that specific DABCO molecules **(Figure 2a)** attached to a cloth made of 100% cotton will be approximately 92% effective in reducing the number of T4 viral plaques in *E. coli* (Stirling, 2015). The DABCO (1,4- diazabicyclo [2. 2. 2] octane) molecule has shown several antimicrobial characteristics when attached to a hydrocarbon chain. The DABCO molecule is a nitrogen-based molecule that is positively charged and can be covalently bound to other carbohydrates or even proteins (Engel, 2008). The extra bond in the middle of the molecule serves as an extra coordination bond that attaches the two nitrogen molecules in a three dimensional formation, allowing the nitrogen

Figure 2: (A) Structure of diDABCO molecule with C6 hydrocarbon linker chain attached to cotton cloth. Molecule has +3 charge due to attachment to cloth that creates 4 bonds at nitrogen. (B) diDABCO-C6 molecule shown without cloth attachment (powder form). Without the cloth, the molecule now has a charge of +2.

Figure A:

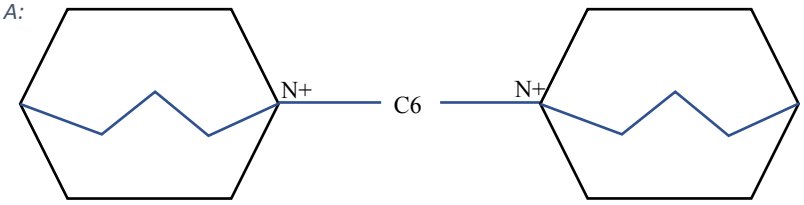
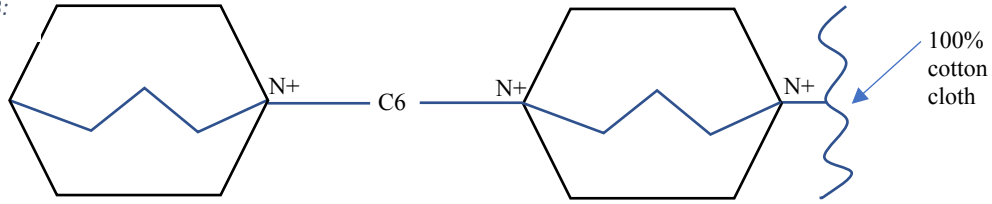


Figure B:



molecules to have four bonds and carry a positive charge. The negatively charged tails of the T4 bacteriophage are attracted to the positively charged DABCO molecule, creating a bond that would ultimately make the virus inactive or less infectious. Additionally, the DABCO's structure allows it to be combined with different carbon chains that vary in length or end moiety, changing the function of the DABCO molecule. For example, diDABCO molecules have been synthesized, creating two DABCO rings on each end of a hydrocarbon chain; this would enhance the overall charge of the molecule, making it more positive (see Figure 2a; one DABCO molecule has two positive charges, two DABCO's have 3 charges).

Since the DABCO molecule forms 4 bonds around each nitrogen, each nitrogen carries a positive charge. Therefore, when the DABCO molecule is attached to cloth, it creates an additional charge on the DABCO-hydrocarbon molecule as compared to the same molecule not bound to cloth. From previous studies, it has been proposed that the virus's negatively charged tail fibers are attracted to the positive

charges of the DABCO molecule and binds, when binding occurs, the tails fibers of the virus, which attach to bacterial host cells, would no longer be effective as sensors, thus decreasing viral activity.

A) Research Question

How does exposure to a DABCO-hydrocarbon molecule affect the infectivity of T4 bacteriophage?

Hypotheses

(1) If an electrostatic relationship exists between the DABCO molecule and T4 viral particles, then incubating diDABCO-C6 cloth with T4 bacteriophage would result in a decrease in viral plaques.

(2) If shorter hydrocarbon chains permit more attachments to viral tails by dissolved DABCO molecules, then diDABCO-C3 will have the greatest percent reduction of viral plaques when compared to longer DABCO chains.

B) Procedures

Virus Titer & Plaque Assays

In order to determine the ideal viral concentration for the *E. coli* strain used, I will conduct a virus titer. Before beginning the titer, nine petri dishes filled with tryptic soy agar (TSA) will be removed from 4ºC and allowed to come to room temperature. 200 µl of virus will be serially diluted to 10^{-6} in SM+G.

100 µl of each viral dilution will be incubated with 100 µl of *E.coli*. at 1 x 10^5 CFU. SM+G (virus stabiliting media) only and *E.coli* only tubes will also be made to serve as control groups. Once the *E.coli* and virus dilutions are mixed together, all tubes will be incubated at 37ºC for 20 minutes.

During this time, soft agar will be melted in a microwave. Then, 4 mL of soft agar will be added to seven 15 mL snap cap tubes and placed in the water bath at approximately 55ºC to prevent solidification.

100 µl of each phage-bacteria mixture will be added to individual snap cap tubes with soft agar. Then the mixtures will be poured onto 10 cm agar containing petri dishes. The solutions will be swirled around the plates to spread out the mixture and then left on the benchtop to solidify.

Once solidified, the plates will be placed in a 37°C incubator overnight and the results (number of viral plaques present) will be recorded the following day. The correct virus concentration that will be used

in the experiment will be chosen by observing which plate has a countable number of plaque forming units (PFU).

Cloth Testing

A plaque assay will be conducted to observe which cloths appeared to be most efficient in changing the activity of the virus. The virus will be serially diluted to the appropriate countable concentration. Then, several microfuge tubes will be prepared for each cloth (with a different DABCO-hydrocarbon) to be tested and 900 µl of SM+G will be added to every tube. 100 µl of virus will then be added to each new tube and pipetted up and down to mix. 2.25 cm^2 squares will be placed in each microfuge tube, which will be rocked overnight at 4º C.

After incubation, 100 µl of the liquid will be added to a new microfuge tube and combined with 100 µl of *E. coli*. The tubes will be incubated for 20 minutes at 37°C, plated with soft agar and incubated overnight at 37ºC.

Using sterilized forceps, the cloths will be removed from the original microfuge tubes and laid across a 10 cm petri dish containing TSA. A soft agar/*E. coli* mixture will then be poured directly over the cloth and swirled to evenly spread it out. The plates will be incubated overnight at 37º C and examined for plaques the next day.

Wash Test

I will conduct a wash test to observe if washing the DABCO cloths prior to plating will strip the virus off of them. The wash test will be conducted in the same way as the cloth testing procedure. However, for each type of cloth, two cloths will be placed in microfuge tubes. After rocking them overnight at 4°C, the cloths will be removed from the tubes and placed on sterile aluminum foil. Two small petri dishes will be filled with 6 ml of SM+G for the blank cloths and the diDABCO-C6 cloths. Cloths will be washed with SM+G once or three times. For example, in the blank cloths, one cloth will be quickly rinsed and then left to dry on sterile aluminum foil. The second blank cloth, identical to the first, will then be quickly rinsed in the SM+G, then placed on a rocker in a dish containing a new 6 mL of SM+G. After 5 minutes, the cloth will then be quickly rinsed again in a dish containing new 6 mL of SM+G and then left to dry on sterile aluminum foil. This process will be repeated for the diDABCO-C6 cloth. After all four cloths were left to dry for 5 minutes, they will be plated with soft agar mixed with 100 µl of *E. coli* again, similar to the cloth test procedure stated previously.

Detergents

SDS and TX-100 will be made at 5% dilutions using SM+G. 2 grams of SDS will be dissolved in 10 mL of SM+G in order to make the detergent at 20%. TX-100 will be in liquid form and therefore will be mixed in 8 mL of SM+G. Three microfuge tubes will then be filled with 675 ul of SM+G and 225 ul of 20% SDS to dilute the detergent to a 5% concentration. The same process will be used to dilute the 20% TX-100. This will result in a total of six microfuge tubes that would be allocated to the blank cloth, no cloth, and diDABCO-C6 cloth. The cloths will be cut into 2.25 cm^2 squares and placed into the microfuge tubes to be rocked overnight at 4^oC. The following day, the cloths will be plated in the same manner as the cloth testing procedure above using soft agar/E.coli mixture along with the appropriate T4 phage dilution. 100 ul of the liquid from each microfuge tube will also be extracted and plated as well. The plates will then be incubated overnight and results will be observed and recorded the following day.

Risk and Safety

During experimentation, gloves, goggles, and a lab coat are required when handling *E.coli* and T4 bacteriophage.

Data Analysis

Aa ANOVA test will be conducted to analyze the statistical significance of the data.

1. **Human Participants Research**

Not applicable.

2. **Vertebrate Animal Research**

Not applicable.

3. **Potentially Hazardous Biological Agents Research**

The experiment was a BSL 1. It was conducted on an open workbench within the laboratory. Although E.coli and bacteriophage are not extremely harmful to humans, safety precautions were still maximized to avoid contamination. Therefore, access to the lab was restricted and all materials were placed in cabinets. Additionally, all waste materials were then disposed with the usage of an autoclave, under my mentors supervision.

4. **Hazardous Chemicals, Activities, & Devices**

After all microorganisms and viral particles were used, they were then safely disposed using an accessible autoclave within the laboratory.

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Post Research Summary

Powder Mixes and Test

Following the results of the cloth tests, I began to question how effective the DABCO-hydrocarbon molecules would be without cloth attachment. Therefore, I used the starting materials of the DABCO molecules and chose 18 different powders to test. Each powder differed in characteristics including chemical structure, solubility, consistency, size, etc. 0.1 grams of each powder were weighed using an electronic scale and weigh boat. Each powder was then added to a microfuge tube containing 900 µl of SM+G, incubated and rocked overnight at 4°C. The liquid was then plated using soft agar the following day and combined with 100 ul of *E. coli*.

Data Analysis

A Welch's ANOVA test was conducted due to the unequal variances of the data. The Welch test was conducted to indicate if the data was statistically significant if the p-value less than 0.05. Following the Welch test, the Games-Howell post hoc test was used to determine which relationships were statistically significant in the DABCO-hydrocarbon cloths as well as the free floating (powder) molecules. The Welch's ANOVA test was also used to observe the effects of the detergents SDS and TX-100 on the number of viral plaques present.