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Effects of N-Acetylglucosamine and α-methylglucoside on bacteriophage T4 adsorption to *Escherichia coli* B23

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The role of lipopolysaccharide in the receptor function for bacteriophage T4 was studied by using *Escherichia coli* K-12 B23 strain. The receptor activity for T4 was monitored by inhibiting phage adsorption by saccharides that mimic sugars located at the receptor site on LPS. Coliphage T4 adsorbed to intact cells and acetone-ether treated cells, but was not adsorbed to heat-treated cells. The adsorption of phage T4 to acetone and ether-treated cells was inhibited by glucose derivatives like N-acetylglucosamine and α-methylglucoside but was not inhibited by maltose. The inhibition effect of the N-acetylglucosamine and α-methylglucoside is consistent with the observation that T4 adsorbs to the two glucoses on C-terminus of LPS's R-core in *E. coli* B23. However, wild type T4 is also capable of absorbing to *E. coli* B23 via OmpC, a major outer membrane protein, and phage adsorption via this protein might influence results obtained. With this confounding factor, a conclusion cannot be stated confidently until the adsorption is simplified by using OmpC mutants or OmpC$^-$ *E. coli*.

Carbohydrate binding proteins, lectins, are distributed widely in nature. These proteins, responsible for cell surface sugar recognition (1), occur as soluble or membrane associated molecules and can be found in plants, yeasts, mammals, birds, invertebrates, and bacteria (2). Examples include bacterial toxins, animal receptors, and plant toxins. In certain cases, bacterial surface lectins appear to function in the initiation of infection by mediating bacterial adherence to susceptible cells (3).

As resistances toward various antibiotics spread among the prokaryote kingdoms, demands for more effective prevention plans and treatments for pathogenic bacteria rise. Thus, protein-carbohydrate interactions between bacterial lectins and surface carbohydrate of host cells that aid in adherence of these prokaryotes to hosts has been actively investigated to determine whether this interaction may lead to new approaches for treatment or prevention of infectious diseases (3). Various literatures indicate that infection of bacterial cells via bacterial viruses such as coliphage T4 involves protein-sugar interaction that may resemble method of adherence by pathogenic bacteria to susceptible cells (4). Therefore, studying the mechanism of bacteriophage adsorption to their hosts offers a simpler and more economical alternative in investigation of lectin-sugar interaction between bacteria and eukaryotic cells.

Specificity of bacteriophage adsorption resides in the cell wall. The cell wall of gram-negative bacteria such as *Escherichia coli* is composed of two major components: the peptidoglycan layer and the outer membrane. The outer membrane is composed of lipopolysaccharide (LPS), proteins and phospholipids and it contains the receptor sites for bacteriophage (5). LPS, as the name implies, contain a lipid portion and a polysaccharide portion (5). It is localized mainly in the outer leaflet of the outer membrane bilayer in such a way that the hydrophilic polysaccharide portion extends outwards from the cell into the aqueous environment. It is known that core LPS in the outer membrane is the receptor site for various rough specific phages including T4, T3, and T7 (6). More specifically, the carbohydrates in the core of LPS form the bacteriophage receptor site.

The T4 bacteriophage adsorbs to its host bacterial initially by the tail fibres (7), based on efficiency of plating, phage-binding kinetics and phage-inactivation studies, the glucosyl-α-1,3-glucose disaccharide on rough LPS of *E. coli* B forms the best receptor for coliphage T4 (7). However, even though the receptor site is known, limited amount of studies has been done on the actual attachment mechanism. The focus of this project is to investigate the means of attachment of T4 to LPS of *E. coli* K-12 B23 in wild type strain. The results obtained may then be used as an attachment model system for cellular interaction and recognition between two organisms.

The host cell of choice for this experiment is *Escherichia coli* since is it is one of the better-characterized bacteria. Coliphage T4 is chosen as phage to be tested, as it is a commonly studied phage and is readily available. To determine that the disaccharides on R-core of LPS is indeed the receptor site, small carbohydrate molecules that are known to form the LPS core on *E. coli* are tested for their ability to inhibit phage T4 adsorption. These molecules include N-acetylglucosamine, α-methylglucoside, and maltose (4); all these sugars are derivatives of glucose except

maltose, which is made up by two glucose molecules linked by an ester linkage at carbon one of one molecule and carbon four on the other. As structures of these sugars mimic part of the disaccharides on LPS, their presence should create competition for the T4 binding site between LPS and the free sugar molecules, which would inhibit adsorption of the phage to the cell wall. A similar approach has been used in studying the antigenic determinants of complex polysaccharides, but it has not been applied to analysis of specific interactions between organisms (8).

MATERIALS AND METHODS

Bacterial and Phage Strains. *Escherichia coli* strains B23, SF8, HB101, DH5α, and InVα were used in this study (see Table 1) where B23 was used most often. All strains were provided Dr. W. Ramey of Department of Microbiology and Immunology of the University of British Columbia. All strains were maintained in Luria broth (see appendix A) except B23, which was stored in M9 supplemented with 0.2% glycerol (see appendix A). New stock of coliphage T4 was grown from the original T4 stock stored in this laboratory. It was kept in Hershey's broth at pH 7.4 (see appendix A) with approximately 0.5 ml chloroform added.

Table 1: Bacterial Strains and Their Characteristics

E. coli Strain	Type	Plasmid
B23	Wild-type	None
SF8	N/A	p309.1
HB101	N/A	pBR322.5
DH5α	N/A	pUC19
InVα	N/A	None

Cell Culture. Before each experiment, an overnight culture of *E. coli* was set up in either M9 supplemented with 0.2% glycerol or in Hershey's broth, pending requirements of the experiment. The culture was achieved by adding 10μl of *E. coli* stock to 9.99 ml of Hershey's broth or glycerol supplemented M9. Cultures were then incubated in 37 ^{0}C shaking water bath (power level 4) for 16-24 hours, or until saturation was reached.

Preparation of Phage Stock The method was adapted from Sambrook *et al*(9). An inoculum of 10^5 pfu of T4 mixed with 0.1 ml of saturated *E. coli* B23 culture was plated with standard plaque overlay technique and incubated for 24 hours at 37^{0}C to produce a confluent lysis of a bacterial lawn. After confluent lysis has occurred, overlay agar containing T4 and *E. coli* was scraped off into a sterile centrifuge tube via a sterile spatula. Five mL of Hershey's broth was then added to the plate to rinse off the remaining overlay agar and the rinse was then added to the centrifuge tube. One-tenth of a millilitre of chloroform was added to the agar suspension to kill any live bacteria that might be present in the culture. The suspension was incubated in shaking water bath (power level 4.5) at 37^{0}C for 15 minutes and then centrifuged at 4000g for 10 minutes at room temperature. The supernatant was recovered and 200μl of chloroform was added to it. The phage stock was titred with the plaque count in the standard overlay assay.

Preparation of Killed *E. coli* B23 was done by treatment with acetone-ether, heat, and chloroform. The acetone-ether treatment is adapted from method used by Watanabe (4). *E. coli* B23 cells in overnight culture grown in Hershey's broth were collected via centrifugation at 9000rpm for 15 minutes using Centra-4B centrifuge (International Equipment Company). The pellets were then washed twice with 5 ml of 0.85% saline and were collected via centrifugation at 9000rpm for 15 minutes after each wash. The cells were re-suspended in 1 ml 0.1 *M* tris (hydroxymethyl)-aminomethane (Tris) – HCl buffer at a pH of 7.4 and 10 volumes (10 ml) of cold acetone were added to the cell suspension. For 5 minutes, the mixture was vortexed at an interval of 1-minute vortexing and 30 seconds chilling on ice. The suspension was centrifuged at 9000rpm for 10 minutes and the acetone wash was repeated on the pellets, then twice with 10 volumes each of cold petroleum ether. Finally, cells were suspended in 5 ml of Hershey's broth as a stock of acetone-ether treated cells and were stored at 4^{0}C. These cells were used for some experiments instead of the intact live cells since propagation of coliphage and host cell might occur during the incubation in the case of live cells (4). Heat-treated cells were prepared by heating 20 ml of B23 culture at 0.4 $O.D_{660}$ for 15 minutes in an 100^{0}C water bath. Chloroform-treated cells were made by adding 10 ml of chloroform to 20 ml of B23 culture at 0.4 $O.D_{600}$ at a light absorbance of 660nm. The mixture was vortexed at high speed for 5 minutes to ensure that all bacterial cells were properly exposed, and then part of the cell lysate was removed for infection assay.

Efficiency of T4 Adsorption to Different Live *E. coli* Strains. Several *E. coli* strains were tested for their receptiveness to T4 binding. These strains include B23, HB101, SF8, DH5α, and InVα. Cells were grown in overnight cultures and then were sub cultured and grown until 0.4 $O.D_{660}$, then 15 μl of T4 stock at a concentration of 3 x 10^8 pfu/ml was added to the cultures to obtain a MOI of 1:1000. The cultures were incubated for 0-18 minutes at 37^{0}C in a shaking water bath (power level 4). Every 6 minutes, 0.5 ml of sample from each culture was taken and microfuged with an Eppendorf Microcentrifuge 5414C at 10,000g for 4 minutes. Supernatant was withdrawn and the concentration of unbound phage was assayed.

Inhibition of T4 Adsorption to Live *E. coli* B23 by Saccharides. Host cells from overnight culture were sub cultured in either M9 supplemented with different concentration of saccharides and 0.02% Tryptophan or Hershey's Broth and grown until 0.4 $O.D_{660,}$ then 15 μl of T4 stock (3 x 10^8 pfu/ml) was added to bacterial cultures to obtain a MOI of 1:1000. Different volumes of 10% N-acetylglucosamine or 30% maltose or 50% α-methylglucoside were added to cultures to attain a final concentration of 0.1 *M*, 0.03 *M*, and 0.5 *M*, respectively. These concentrations of saccharides were used as they were reported as the minimal concentration that would inhibit phage adsorption (4). The infected cultures were incubated for 0-18 minutes in shaking water bath (power level 4) at 37^{0}C and samples of culture were taken every 3 minutes. Each sample was microfuged with an Eppendorf Microcentrifuge 5414C at 10,000g for 4 minutes and supernatant was withdrawn for assaying of the number of unabsorbed phage.

Inhibition of T4 Adsorption to Heat Treated, Chloroform Treated, or Acetone-Ether Treated *E. coli* B23 by Saccharides. Half of a millilitre of heat, acetone-ether, or chloroform treated *E. coli* was added to Hershey's broth containing: 0.1 *M* N-acetylglucosamine or 0.03 *M* maltose or 0.5 *M* α-methylglucoside to make up a final volume of 10 ml. Optical density reading was taken for each suspension and amount of

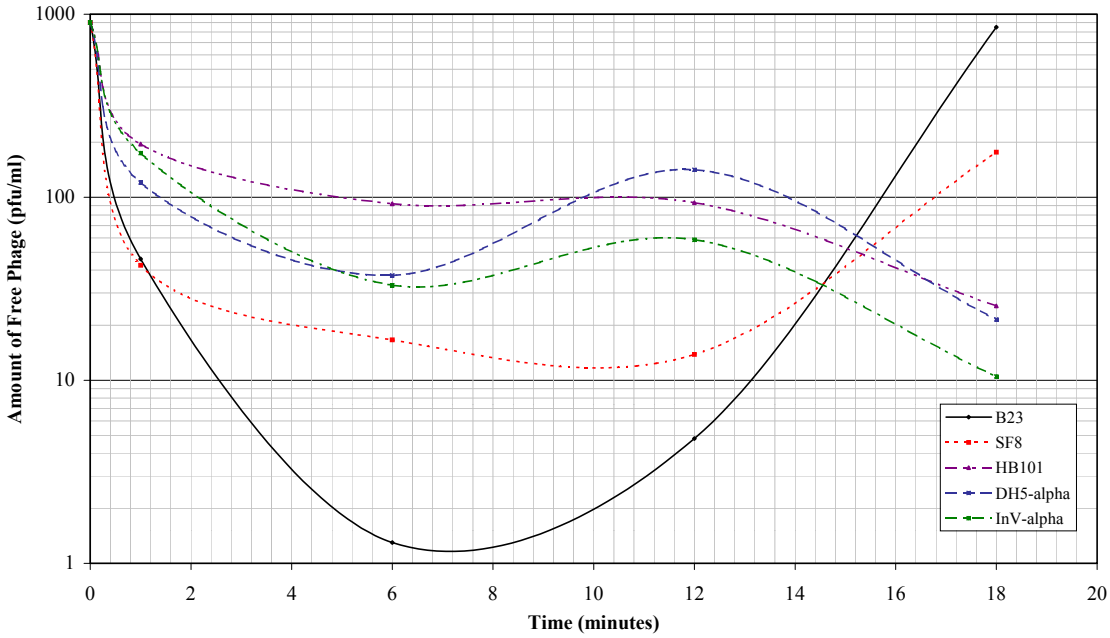
T4 stock added to each was calculated based on the optical density to obtain a MOI of 1:1000. After addition of phage, suspensions were incubated in shaking water bath (power level 4) at 37^{0}C for 0-30 minutes and samples of cultures were taken every 5 minutes. After centrifugating samples from different times at 10,000g for 4 minutes, supernatant was withdrawn and concentration of PFU was assayed.

Assay of Concentration of Unabsorbed Phage in Infected Cultures. The concentration of unattached T4 in an infected culture was assayed via overlay method. Small broth tube (13x100mm) containing 3 ml of molten soft overlay agar (see Appendix A) was placed into a 50^{0}C water bath. Supernatant of samples containing free T4 from adsorption assay at various time intervals was diluted to a concentration that, when plated, will give 30-300 plaques on a plate. 4 x 10^8 *E. coli* B23 (~0.1 ml of a saturated culture) was added to each tube as host cells, along with 0.1 ml or 0.2 ml of diluted phage samples, pending on the dilution. Tubes were mixed by phage style mixing, and then rapidly transferred to a plate containing bottom agar (see Appendix A). Plates were incubated for 16-24 hours at 37^{0}C and plaques were counted after incubation.

RESULTS

Efficiency of T4 Adsorption to Different Live *Escherichia coli* Strains. In order to find the strain of *E. coli* K-12 that T4 would attach to more efficiently, various strains of live *E. coli* were infected with phage and the binding efficiency was assayed. . As shown in Fig. 1, among all the strains tested, bacteriophage T4 bound to *E. coli* B23 strain with the greatest efficiency as it achieved a 30 fold drop in the concentration of free phage from the initial number of phage put in 6 minutes after time of infection, which was consistent with the reported value of 30-40 fold (10,11). Strain SF8 followed strain B23 in its binding efficiency to T4, as number of unattached phage in this culture was 20 fold lower 6 minutes after infection. Following SF8 were the two alpha strains, which were similar in their binding efficiency. The least efficient strain was HB101 as a drop of only 10 fold was seen after infection.

Fig. 1: Efficiency of T4 Adsorption to *Escherichia coli* B23, SF8, HB101, DH5-alpha and InV-alpha

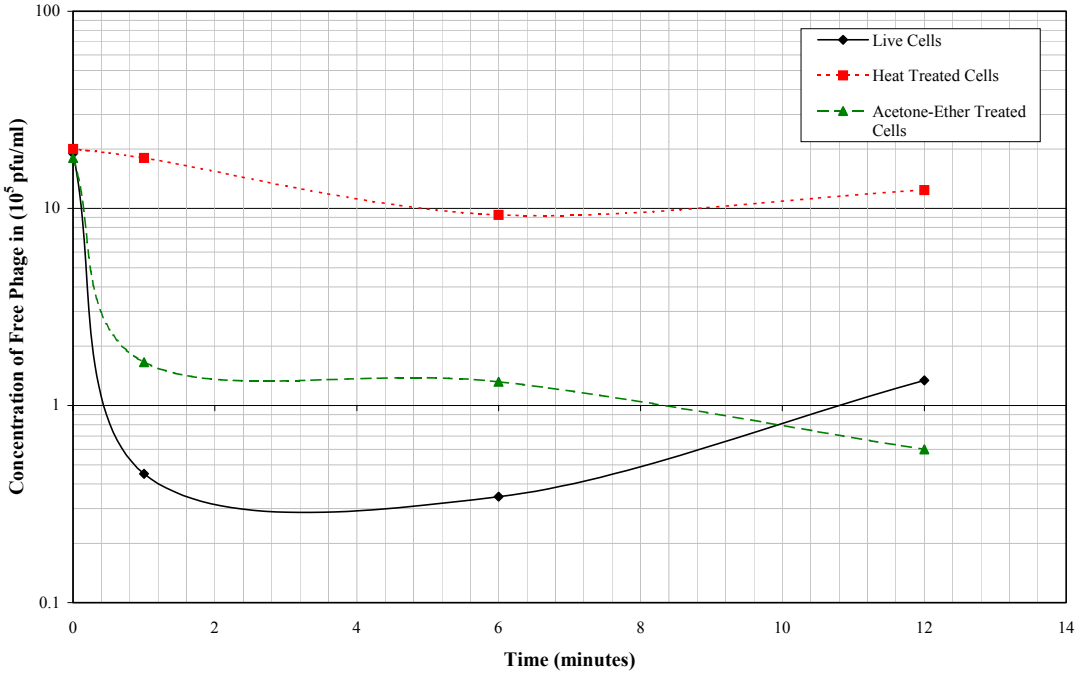


From Fig. 1, it is shown that concentration of PFU for both the B23 strain and SF8 strain started to increase 12 minutes after initial infection. This observation suggests that the phage was growing and was approaching the end of the latency period, which is the time that is required for host cells to produce enough phage to lyse the cell (10). Lysis of host cells would allow phage to escape and would result in the high concentration of PFU.

As live cells would allow phage to propagate and mask adsorption effects at later times of the assay, usage of dead host cells would enable observation of phage attachment to host cells and effects of carbohydrate molecules on inhibition of phage adsorption for a longer time span since bacteriophage growth is eliminated.

T4 Adsorption to Heat-treated, Chloroform-treated, and Acetone-Ether Treated *Escherichia coli* Cells in Hershey's Broth. When phage T4 suspension was incubated with the heat-treated, the acetone-ether treated, or untreated cells at 37^0C, the phage strongly adsorbed to intact, live *E. coli* cells as shown by the 20 fold drop in concentration of PFU by 3 minutes after infection. The phage was less adsorbed to acetone-ether treated cells, and did not attach to heat treated *E. coli* as there was a drop of less than 10 fold in concentration of unbound phage from initial concentration (Fig 2). One unexpected result is that the concentration of free phage in culture appeared to stabilize 2 minutes after infection, as if the rates of phage attachment and detachment to bacterial cells were in equilibrium.

Fig. 2: T4 Adsorption to Heat or Chemically Treated *Escherichia coli* B23 in Hershey's Broth



Inhibition of T4 Adsorption to Live *E. coli* B23 by Saccharides in Supplemented M9 Minimal Media or Hershey's Broth. The role of LPS of *E. coli* B23 in the receptor function for phage T4 infection was studied via infecting live bacterial cultures with addition of different sugars. The infection was conducted in supplemented M9 media initially; however, phage binding in this media was rather ineffective as only a 10 fold drop was seen by 10 minutes after infection (Fig. 3). Due to the low binding efficiency, which was suspected to be caused by lack of nutrition/cofactor in the minimal media, rich medium Hershey's broth replaced M9 and results obtained from infection in the new medium showed a 20 fold drop, which was still lower than the expected 40 fold drop but better than outcomes attained using supplemented M9 as the medium.

A drop in number of PFU was observed for all three cultures in the first 8 minutes after infection due to phage attachment to bacterial cells. However, as shown in Fig. 3 and 4, the number started to increase slowly 10 minutes after infection and this was probably caused by cell lysis of some bacterial cells, which allowed assembled phage in bacterial cells to escape.

In both supplemented M9 and Hershey's broth, the two sugars tested did not show inhibitory effects on T4 adsorption as the numbers of unabsorbed phage in cultures supplemented with N-acetylglucosamine or maltose were similar to the control at almost any given point of time when the infected cultures were assayed (Fig. 3, 4). The background noise for this experiment was quite high, which resulted in the bumps shown in both Fig. 3 and Fig. 4.

Fig. 3: Inhibitory Effects of N-acetylglucosamine or Maltose on T4 Adsorption to *Escherichia coli* B23 in M9 Minimal Media Supplemented with Saccharides

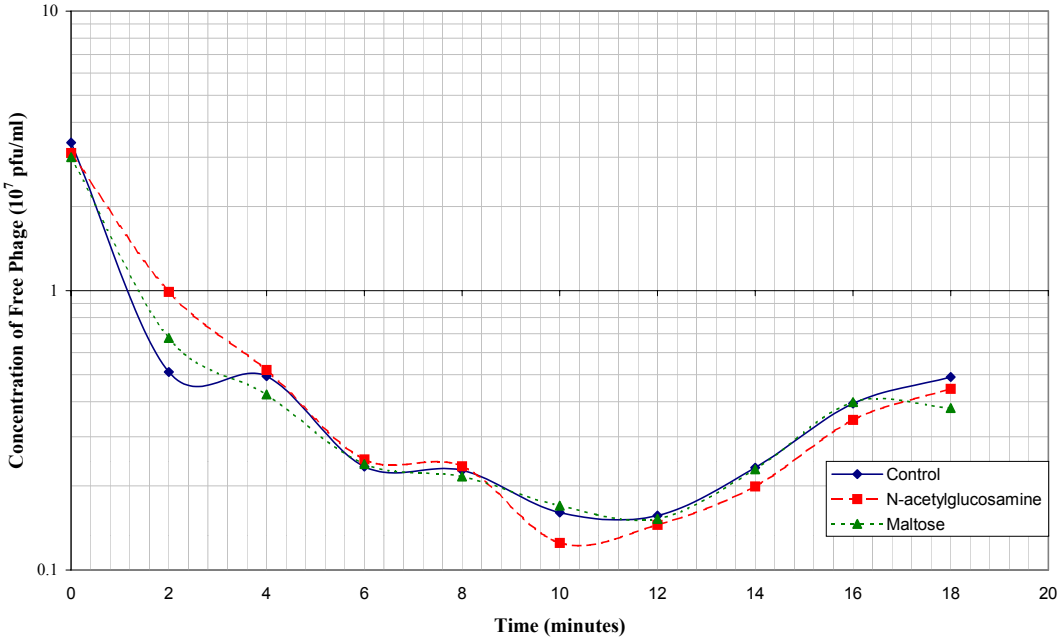
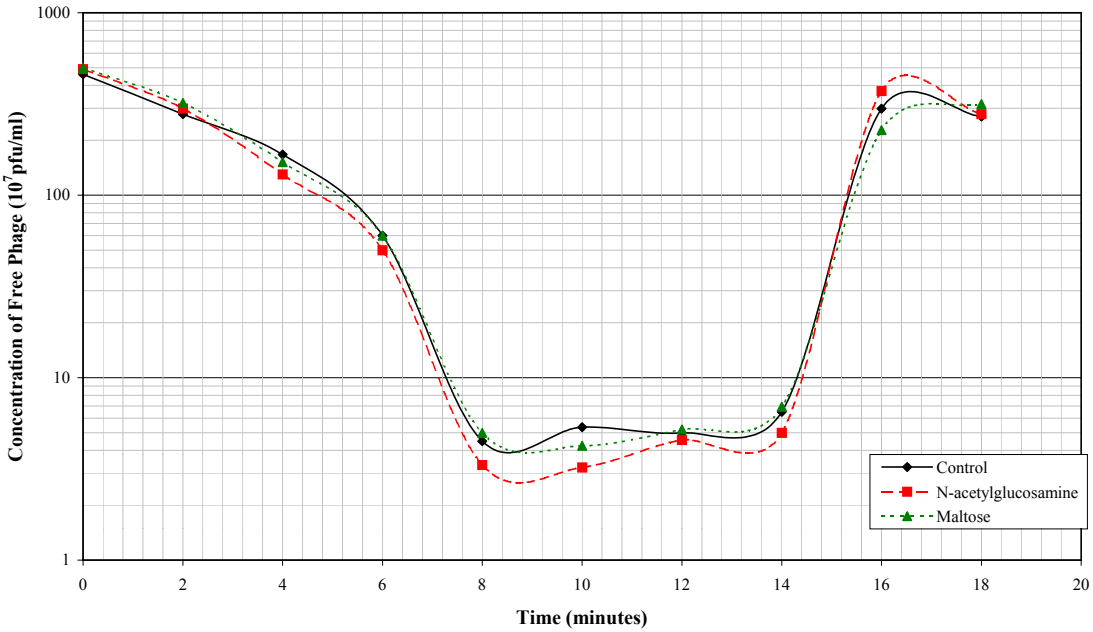
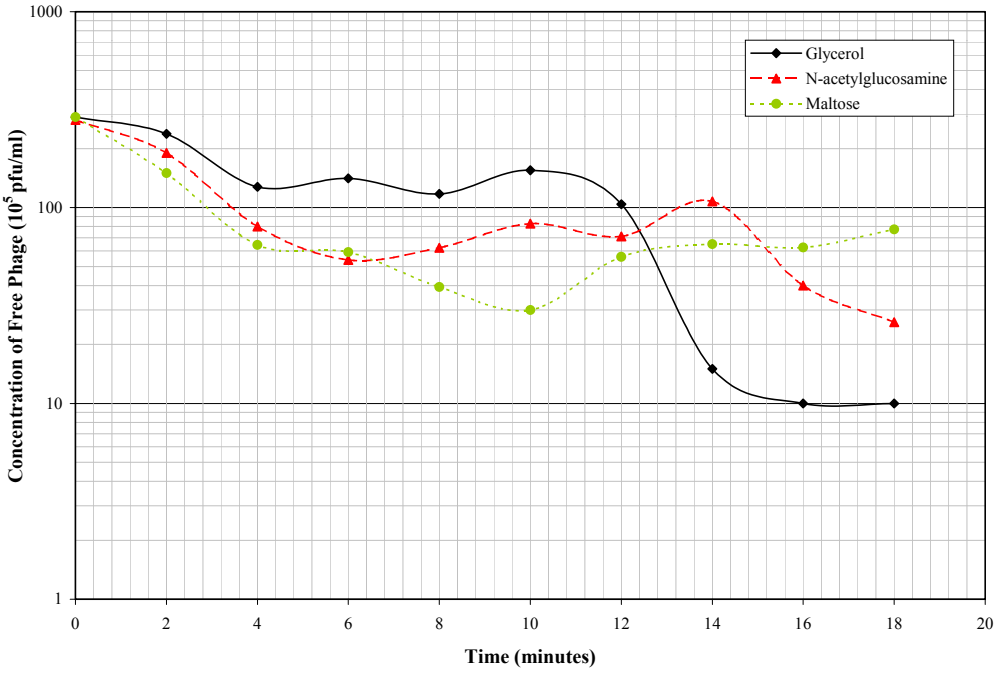


Fig. 4: Inhibitory of T4 Adsorption by Maltose or N-acetylglucosamine to Live *Escherichia coli* B23 in Hershey's Broth



Inhibition of T4 Adsorption to Chloroform Treated *Escherichia coli* B23 by Saccharides. To avoid phage propagation, chemically treated *E. coli* B23 was used instead of live cells. Chloroformed killed cells were first use since it was easier to prepare. From Fig. 5, it is shown that, surprisingly, phage adsorbed to bacterial cells in cultures supplemented with maltose or N-acetylglucosamine better for the first 6 minutes in the experiment. This appears to contradict the expectation that phage adsorption should be inhibited by the presence of carbohydrate. However, as shown on the graph, phage started to attach to *E. coli* cells in the control system approximately 10 minutes after infection and finally achieved a drop of 12 fold whereas the rate of phage adsorption in the maltose system stabilized after 6 minutes from the time of infection. The PFU concentration in the N-acetylglucosamine system continued to decrease 6 minutes after infection but at a slower rate than the first 6 minutes and finally reaching a drop of 10 fold.

Fig. 5: Inhibitory Effects of Maltose or N-acetylglucosamine on T4 Absorption to Chloroform Treated *Escherichia coli* B23 in Hershey's Broth



A trendline analysis was done on the data as background noise in this experiment was relatively high, which might have attributed to the unexpected results. Trend lines are calculated via calculating the least square fit through the points with the following equation:

$$y = c \ln x + b \qquad \textbf{(1)}$$

where c and b are constant and ln is natural logarithm function. Results obtained from the analysis showed that, overall, phage in the control system attached to bacterial cells better when compared to the other two sugar systems (Fig. 6). This observation is the expected result and corresponded to observations reported in the literatures, but is less pronounced then the reported decrease.

Inhibition of T4 Adsorption to Acetone-Ether Treated *E. coli* B23 by Saccharides. Data obtained from adsorption assay using chloroform killed *E. coli* showed that T4 attached to the cells poorly as the maximum drop of number of PFU among the three systems was 12 fold, which is considerably lower than the expected 40 fold. Therefore, acetone-ether treated cells were used. This treatment, other than having the function of killing bacterial cells, also extracts organic solvent soluble molecules such as lipids and hydrophobic membrane proteins on cell surface and thus, T4 should more readily adsorb to bacterial cells as the proposed receptor sites, R-core of LPS, were more exposed.

Fig. 6: Inhibitory Effects of Maltose, N-acetylglucosamine, or Alpha-methylglucoside on T4 Adsorption to Acetone-Ether Treated *Escherichia coli* B23 in Hershey's Broth

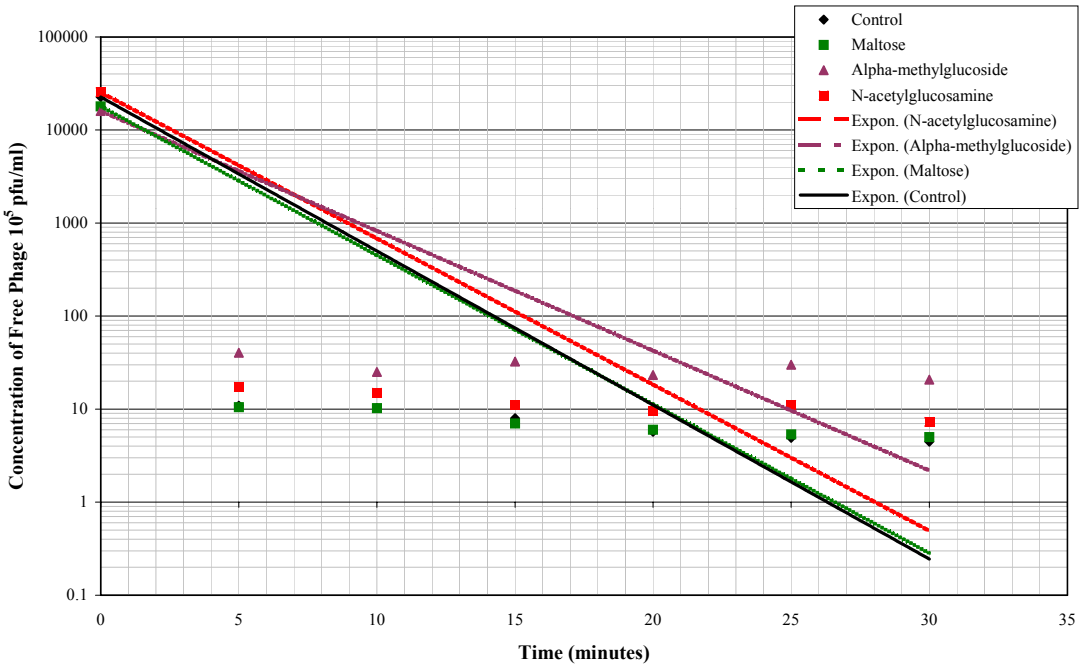
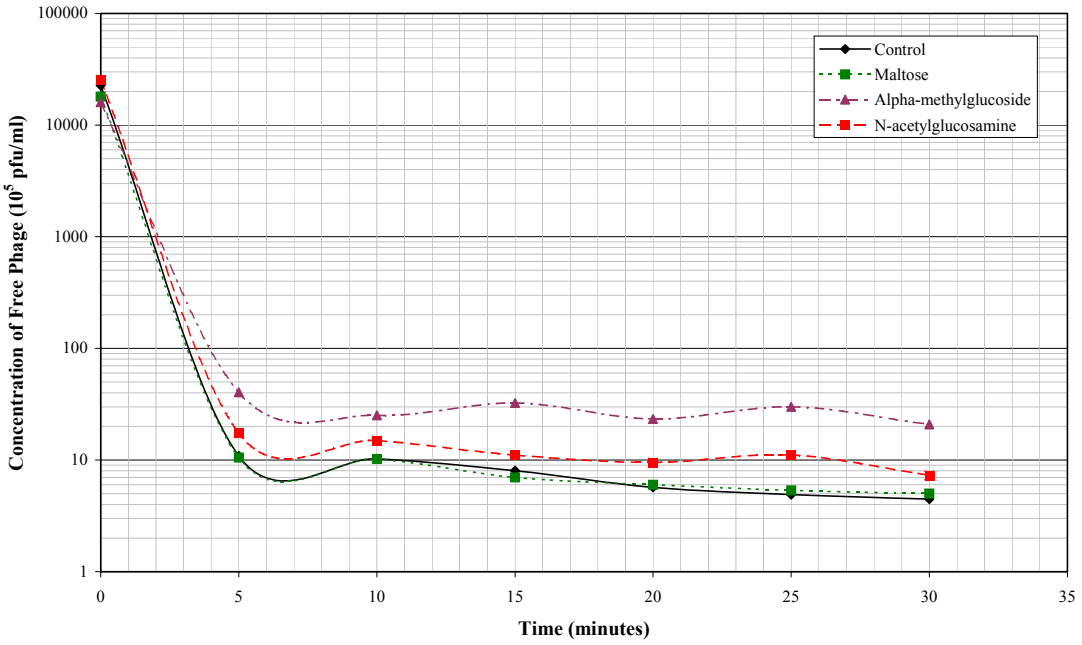


Fig. 7: Inhibitory Effects of Maltose, N-acetylglucosamine, or Alpha-methylglucoside on T4 Adsorption to Acetone-Ether Treated *Escherichia coli* B23 in Hershey's Broth

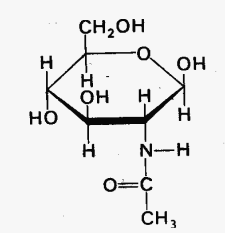


Phage adsorption to acetone-ether treated bacterial cells appeared to be more efficient as the concentration of free phage dropped by approximately 35 fold by 5 minutes after infection. This degree of drop corresponded to the reported literature value (11) and thus, acetone-ether treated cells were used in the latter part of the project.

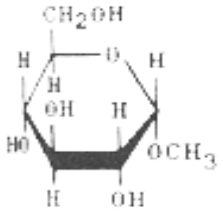
As shown in Fig. 7, adsorption of phage T4 to acetone-ether treated cells was inhibited by N-acetylglucosamine and α-methylglucoside but not by maltose. Trendline analysis of the results also showed inhibition of phage adsorption via the two sugars (Fig. 8). However, one unusual observation was that the rate of decrease in free phage number due to phage attachment decreased and eventually stabilized 5 minutes after infection whereas one would normally expect the initial rate would remain the same for minimum of 15 minutes, especially with a low MOI (1:1000). This phenomenon may be explained by establishment of steady state between the rate of phage attachment and rate of phage dissociation.

DISCUSSION

It is known that T4 receptor site on *E. coli* B is located at the glucose residue at distal end of LPS whereas in *E. coli* K-12, there is a also a similar glucose region but is masked by additional glucose and galactose molecules (12). Studies with LPS mutants of the K-12 strain showed that none of the T4-resistant mutants contained glucose in the LPS while T4-sensitive K-12 all contained glucose (6). This difference suggests that, for *E. coli* K-12, the core glucose linked to heptose is indispensable for wild type phage T4 receptor activity. In the acetone-ether treated cell system, both α-methylglucoside and N-acetylglucosamine showed inhibition effects on T4 adsorption, which is consistent with the reports T4 recognized the two glucose molecules located at the K-12's LPS core sequence: glucose → glucose → glucose (1,6-galactose) → heptose…(12). The figure below shows the structure of the two sugars tested for inhibition effect:



N-Acetylglucosamine

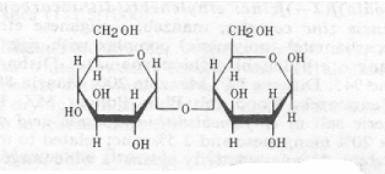


α-methylglucoside

From these structures, it is obvious that both sugars bear resemblance to glucose molecules. The α-methylglucoside is essentially the same as glucose except that the hydroxyl group on carbon 2 is substituted by a methyl group. The N-acetylglucosamine also resembles the structure of glucose except that the hydroxyl group on carbon 2 is replaced by an amine group that has an acetyl group attached to it. Studies have shown that a protein called gp37 on T4 tail recognizes structure of glucose on core of LPS and adsorb to it (3,13). As the N-acetylglucosamine and α-methylglucoside have structures that are closely related to glucose, gp37 might have recognized the sugars as glucose on LPS and formed attachment to them. Results from the assay showed high PFU concentration in cultures supplemented with the N-acetylglucosamine or α-methylglucoside, consistent with the hypothesis that the two sugars inhibited phage adsorption.

When inhibition effects of N-acetylglucosamine and α-methylglucoside are compared, it is obvious that α-methylglucoside inhibited phage adsorption more as the number of free phage in α-methylglucoside system was approximately 7 fold higher than the control while N-acetylglucosamine had a difference of 3 fold. The difference could be caused by several reasons: (i) the concentration of N-acetylglucosamine used was lower than the concentration of α-methylglucoside used (0.1 *M* and 0.5 *M*) and since less N-acetylglucosamine was present, the probability of phage attaching to it decreased while the probability of attachment to bacterial cells increased. Thus, the inhibition effect was not as obvious. (ii) Although N-acetylglucosamine resembles glucose, structurally it deviates from glucose more than α-methylglucoside as it has the additional amine and acetyl group. This difference might have hindered phage binding and resulted in a milder inhibition effect.

Maltose was also tested for its inhibition effect and from the results obtained, it appeared that this particular sugar did not have an obvious effect in inhibition of phage adsorption, which contradicted the findings by Watanabe (4) and the hypothesis. However, T4D strain was used in Watanabe's experiment while the wild type T4 strain was used in this project. As the phage tail proteins vary slightly with different strains of T4, it is highly probable that the wild type T4 was incapable of binding to maltose. In addition, the concentration of maltose used was 0.03 *M*, which was a value that was reported to have inhibitory effect on phage adsorption (4) yet the results obtained appeared to suggest otherwise. The concentration of maltose used was significantly lower than the concentration used for the other two saccharides (0.1 *M* and 0.5*M*). Lower concentration implies decreased probability of phage attachment to maltose as less sugar molecules lead to decreased chance of collision between phage and maltose in the proper orientation for binding. Alternatively, the size of maltose and the orientation of the glycosidic bond are quite different from N-acetylglucosamine and α-methylglucoside, which may have caused steric hindrance in phage attachment and thus, did not inhibit phage adsorption as effectively as the other two sugars.



Maltose

In order for T4 to infect a bacterial cell, it must undergo two binding stages: reversible and irreversible binding (11). Reversible binding is the initial binding between phage tail and LPS, and irreversible adsorption is the successful interaction between baseplate of T4 and bacterial cell. Since reversible binding is a relatively weak interaction (11), dissociation will occur if the baseplate of phage does not interact with the host cell. In the acetone-ether treated cells system, phage adsorption appeared to stop 5 minutes after infection since results showed no significantly decrease in concentration of PFU after the 5 minutes time span (Fig. 7). This suggested that either all receptor sites on the *E. coli* were bound by phage or the rate of adsorption and dissociation to bacterial cells reached equilibrium. The first explanation is less likely to be the cause as a MOI of 1:1000 (1 phage to 1000 host cells) was used in the system, thus there should be an excess of receptor sites. As phage adsorption to saccharides or LPS is a reversible process (11) and the two components would readily dissociates when lacking baseplate interaction, the second explanation is more probable. However, no conclusion can be without determining kinetics of phage adsorption.

The composition of LPS of bacteria belonging in the same strain is similar; therefore, phage T4 should attach to bacteria in the same strain with equal efficiency. However, the adsorption efficiency assay suggested otherwise since T4 adsorbed to the B23 strain most efficiently even though all the strains of bacteria tested were all *E. coli* K-12. This observation indicates that, although the composition of LPS of different types of bacteria in the same bacterial strain may be alike, differences still exist between them and these differences could be caused by factors such as the presence of plasmid or the type of environment that the cells were cultured. Phage adsorption to bacterial cells may very well be affected by these minor differences, which forms a part of the background noises in these experiments. Phage binding efficiency might also be affected by the strain of T4 tested. The strain of T4 used in these experiments has always been cultured with *E. coli* B23 as its host cells, which, over time, would have put T4 that could bind B23 efficiently in favour and select against those that could not. The selection pressure would eventually result in phage stock that contains T4 that would preferentially bind B23 over the other strains of *E. coli*, and caused the difference in adsorption efficiency.

Studies have shown that wild type T4 has one other receptor site on *E. coli* K-12, which is the transmembrane protein OmpC (8,13,14). Results obtained from binding assay with heat-treated cells showed that phage adsorption was inhibited when heat-treated cells were used. This observation is consistent with the reports that OmpC is also another phage receptor as heating would denature a protein (OmpC) and cause the observed inhibitory effects as the phage-binding site on the protein was likely destroyed. This additional receptor is a confounding factor in these experiments if the tested T4 adsorbed to host cells via OmpC instead of LPS, even though wild type T4 preferentially uses LPS as the receptor (13). T4 adsorption through OmpC would mask the phage adsorption-inhibiting activity of the saccharides, and result in data showing that saccharides tested lacked inhibiting ability.

These results obtained showed that phage adsorption was inhibited by heating the cells or by incubating the cells with N-acetylglucosamine or α-methylglucoside. The carbohydrate inhibitors suggest that interaction between T4 tails and its receptor sites (LPS) on bacterial cells could represent lectin-carbohydrates interaction to a certain degree

as both systems involves recognition between protein and carbohydrates. However, due to influence of OmpC in phage adsorption, the conclusion cannot be stated confidently until the effect of OmpC is excluded. Exclusion of OmpC can be done by conducting the assay using OmpC mutant or using $OmpC^-$ *E. coli*. The inhibitory effects on phage adsorption by carbohydrates should also be investigated further via varying the concentration of the saccharides tested. The role or protein on the interaction can be studied by using proteases or chemicals that specifically inactivates OmpC only (1).

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Appendix A – Media Composition

Hershey's Broth

Nutrient broth	8g
Peptone	5g
Sodium chloride	5g
Glucose	1g
Water to	1000 ml

pH adjusted to 7.4 with 1 *M* NaOH

Phage Top Agar

Tryptone	13g
Sodium chloride	8g
Sodium citrate	2g
Glucose	3g
Agar	7.5g
Water to	1000 ml

pH adjusted to 7.2

Phage Bottom Agar

Tryptone	13g
Sodium chloride	8g
Sodium citrate	2g
Glucose	1.3g
Agar	15g
Water to	1000 ml

pH adjusted to 7.2

M9 Minimal Medium

NaCl	0.5g
Na_2HPO_4	7.0g
KH_2PO_4	3.0g
NH_4Cl	1.0g
$MgSO_4 \bullet 7H_2O$	0.2g
Water to	1000ml