

Bacteriophage adsorption efficiency and its effect on amplification

Zachary J. Storms · Eric Arsenault ·
Dominic Sauvageau · David G. Cooper

Received: 30 September 2009 / Accepted: 23 December 2009 / Published online: 12 January 2010
© Springer-Verlag 2010

Abstract Existing models for bacteriophage adsorption are modified with the addition of a new term, adsorption efficiency, and applied to a T4–*Escherichia coli* system. The adsorption efficiency is the fraction of phage that adsorbs irreversibly to the host. Adsorption kinetics were modeled using the adsorption rate constant (k) and the adsorption efficiency (ϵ). Experimental data demonstrated that the adsorption rate constant depends strongly on the condition of the host while the adsorption efficiency is a property of the bacteriophage population. The adsorption efficiency exhibited a marked dependence on the concentration of L-tryptophan. The system was used to study the effect of adsorption kinetics on bacteriophage amplification. Increasing adsorption efficiency had an effect similar to increasing the initial multiplicity of infection; the number of phages produced during amplification decreased. Optimizing the adsorption efficiency by manipulating the L-tryptophan concentration yielded a 14-fold increase in the number of phages produced.

Keywords Bacteriophage T4 · *Escherichia coli* · Amplification · Adsorption efficiency · Kinetics · L-Tryptophan

Introduction

Bacteriophage production on an industrial scale has been slow to develop since Felix d’Herelle first discovered what

he referred to as an “infravisible parasite of bacteria” [1]. However, with the ever increasing number of bacteria resistant to antibiotics [2–4] as well as opportunities in diagnostics [5] and recombinant protein production [6–9], there is a growing interest in bacteriophage applications. Realizing these will require efficient, large-scale bacteriophage production.

An important factor in production is the first step in the bacteriophage infective process: adsorption to the host cell. Studies on T-even and T-odd bacteriophages have revealed that a number of environmental factors, such as ion concentrations, organic cofactors, pH, and temperature, can have a significant impact on the adsorption of the virus to the host cell [10–13]. One of these cofactors is the amino acid L-tryptophan.

T. F. Anderson reported in 1945 that the presence of L-tryptophan greatly increased the plaque count when plating certain strains of bacteriophage T4 on a synthetic medium agar. Further experimentation indicated that L-tryptophan was interacting with the bacteriophage in a process called activation to enable adsorption to the host cell. Other organic cofactors were shown to have similar effects but not as pronounced as those of L-tryptophan [13]. The work of Anderson was corroborated and extended in a series of articles published by Stent and Wollman [14–17], who determined that the rate of activation of the phage virion by L-tryptophan was proportional to the fifth power of the tryptophan concentration at low concentrations (<3 mg/L) suggesting that five molecules of L-tryptophan are necessary for activation. The interaction between the virus and the L-tryptophan molecule is a reversible reaction, and dilution of the phage in tryptophan-free media was shown to lead to deactivation. Finally, decreasing temperature decreased the fraction of phages activated at a given L-tryptophan concentration. At high L-tryptophan concentrations (>3 mg/L),

Z. J. Storms · E. Arsenault · D. Sauvageau · D. G. Cooper (✉)
Department of Chemical Engineering, McGill University,
3610 University, Montreal, QC H3A 2B2, Canada
e-mail: david.cooper@mcgill.ca

the rate of activation is independent of L-tryptophan concentration.

The mechanism of bacteriophage T4 activation by L-tryptophan was elucidated and modeled by Kellenberger et al. in 1965 [18]. The authors proved that in order for phage T4 to adsorb to its host, it must be in an active state, a configuration with all six tail fibers away from the body of the virus. The virus uses the tail fibers to first reversibly bind to the host cell before irreversibly locking its tail to the cell surface. The deactivated state, or retracted state as it is commonly referred to, has the tail fibers retracted against the tail sheath. The authors suggested that there are cofactor-requiring strains of T4 which need six molecules of L-tryptophan per virus in order to enter that activated state, one for each tail fiber. Other studies indicate that the L-tryptophan molecule interacts with endogenous tryptophan residues located on the base plate or tail sheath, leading to the active tail fiber configuration [19, 20].

Due to the wealth of knowledge concerning cofactor-requiring strains of T4, the T4–*Escherichia coli* relationship can be easily manipulated to study how the adsorption capability of a bacteriophage affects the amplification of the bacteriophage population. This article first presents data on the adsorption kinetics of the phage–host system and then investigates how these relate to bacteriophage amplification.

Materials and methods

Host and bacteriophage

The host bacterium was *Escherichia coli* ATCC 11303. The growth medium was either Tryptic Soy Broth (TSB; Becton, Dickinson and Company, Sparks, MD), Nutrient Broth (NB; Becton, Dickinson and Company, Sparks, MD), or a minimal salt medium (MSM) consisting of 0.014 g/L Na₂EDTA, 0.01 g/L FeSO₄·7H₂O, 0.01 g/L CaCl₂·2H₂O, 0.2 g/L MgSO₄·7H₂O, 6.0 g/L Na₂HPO₄, 4.0 g/L KH₂PO₄, 4.0 g/L NH₄NO₃, 2.5 g/L glucose, and 0.1 g/L Bacto Yeast Extract (Becton, Dickinson and Company, Sparks, MD). MSM was supplemented with different concentrations of L-tryptophan when required (Sigma-Aldrich, St. Louis, MO). These media were also used as infection media.

When samples of host were required, these were grown overnight in shake flasks at 30 °C to a concentration of approximately 3×10^9 colony-forming units per mL (cfu/mL).

Bacteriophage T4 (ATCC 11303-B4) was used in all experiments. A stock solution of bacteriophage T4 suspended in TSB was prepared and stored at 4 °C, and the titer was verified periodically to ensure no loss in infectious

activity. The phage titer of this TSB stock solution was 3×10^{10} plaque-forming units per mL (pfu/mL) over the course of this study. A second stock solution of phages was prepared by infecting *E. coli* growing in MSM. This stock, referred to as the MSM stock, was also stored at 4 °C. It had a titer of 1×10^{11} pfu/mL.

Phage titer

Phage titers were determined using a modified version of the agar layering technique [21] with an *E. coli* ATCC 11303 lawn to determine the free phage titer. Four milliliters of TSB with 0.75% agar (w/v) was mixed with 10 µL of *E. coli* fully grown culture and then poured over a 1.5% (w/v) agar-TSB base. Phage samples were diluted in TSB and aliquots were dropped onto the soft agar lawn and incubated overnight at 37 °C for at least 12 h before the number of plaques was counted. Titers were determined in duplicate, and the average is reported.

Adsorption experiments

Adsorption experiments were carried out at either 24 or 37 °C. In all the cases, the phages were allowed to equilibrate in the infection medium in an incubator for 45 min before experiments were performed. Two types of adsorption experiments were performed: (1) standard infection where the host was grown and infected in the same medium and (2) host-cross infection where the host was grown in one medium and then infected in a different medium. Adsorption experiments were carried out in one of following three media: TSB, NB, or MSM.

Phage and host solutions were prepared separately in sterilized tubes. Phage solutions were prepared by diluting the phage stock in 11 mL of infection medium to the predetermined titer to be tested. A 1-mL sample of this solution was taken to determine the initial phage titer. The host solution was prepared by centrifuging 10 mL of an overnight host culture, removing the supernatant and resuspending the pellet in 10 mL of the desired infection medium at the desired temperature. Bacteria concentrations were diluted to approximately $\sim 10^8$ cfu/mL and infected at a multiplicity of infection (MOI) of 0.01 unless otherwise stated. Immediately following resuspension, the host and phage solutions were mixed together in a 30-mL syringe and incubated at the appropriate temperature. Samples of the free phages in the mixture were taken during the experiment by filtering 1 mL of the infection mixture through a 0.2-µm SFCA Corning syringe filter (Corning Inc, Corning, NY). A fresh syringe filter was used for each sample. Control experiments showed no statistically significant change of the free phage concentration when the medium was passed through the filter.

Bacteriophage amplification experiment

Bacteriophage amplification experiments were carried out in MSM with different concentrations of L-tryptophan (ranging from 0 to 50 mg/L) at 37 °C. A 0.4-mL aliquot of phage and a 0.4-mL aliquot of *E. coli* diluted in the desired growth medium were mixed in a microcentrifuge tube at the desired MOI (0.01–1.0) and host concentration (~10⁸ cfu/mL) to produce the infection mixture. After incubating for 5 min to allow for adsorption to the host cell, the infection mixture was transferred to a shake flask containing 100 mL of the desired growth medium in an incubator shaker at 37 °C.

Bacterial growth was monitored throughout the experiment by measuring the optical density of the culture at 600 nm. The infection process was determined to be over after population-wide lysis—determined by the OD₆₀₀ falling to a value below 50% of its peak value. Once the culture had been lysed, a sample was removed and passed through a 0.2-µm SFCA Corning syringe filter and assayed to determine the phage titer.

Model development

Phage adsorption was initially described with a simple first-order reaction mechanism (Eq. 1) [21], where *P* is the phage particle, *H* is the host cell, and *PH* is the phage–host complex.



In such a model, the rate of disappearance of phage can be described by Eq. 2, where *C_p*, *C_{h0}*, and *k* are the free phage concentration, the initial host concentration, and the rate constant, respectively.

$$\frac{dC_p}{dt} = -kC_{h0}C_p \tag{2}$$

Equation 2 can be solved for the concentration of free phages in solution as a function of time, yielding Eq. 3, where *C_{p0}* is the initial phage concentration.

$$C_p(t) = C_{p0}e^{-kC_{h0}t} \tag{3}$$

A number of modifications to this simple model have been proposed. The most widely accepted is a two-step adsorption process consisting of a reversible and irreversible reaction, proposed by Stent and Wollman [22], which has the advantage of not only being in close agreement with the physical phenomenon, but also providing an adequate fit for multiple phage/host systems [22–24]. However, in the case of activated bacteriophage T4, the virus particles have a very low probability of becoming detached once their tail fibers come in contact

with the cell surface [25]; hence the reaction scheme can be approximated by the model shown in Eqs. 1–3.

The situation is complicated for cofactor-requiring strains of T4 because the fraction of T4 particles that are in the active state depends on the concentration of the cofactor. Only phage particles in the activated state are able to irreversibly bind to a host cell. In order to quantify the adsorption capability of a specific virus population, a new term is introduced, adsorption efficiency (*ε*). Adsorption efficiency is defined as the fraction of a phage population that irreversibly binds to a host cell while those particles that remain free in solution are deemed the free phage fraction (*F*). Note that *F* = 1 – *ε*.

The adsorption model presented in this article is a modification to the first-order rate equation (Eq. 2) that takes into account the adsorption efficiency of the virus. This is accomplished by replacing the phage titer (*C_p*) in Eq. 2 with the effective titer (*C_p* – *C_{p0}F*). The effective titer represents only those phages that successfully adhere to a host cell by accounting for the portion of the phage population that remains free in solution (*C_{p0}F*). Solving for *C_p* as a function of time and replacing *F* with (1 – *ε*) gives Eq. 4.

$$C_p(t) = C_{p0}\epsilon e^{-kC_{h0}t} + C_{p0}(1 - \epsilon) \tag{4}$$

Equation 4 differs from the traditional first-order model (Eq. 3) in two ways. First, the coefficient of the exponent is no longer the initial phage titer but rather the initial effective phage titer, *C_{p0}ε*. This gives a more realistic representation of the proportion of phages available for adsorption. Second, the expression on the right-hand side of the equation—*C_{p0}(1 – ε)*—is an indication of the “inefficient” phages, that is, those phages that fail to adsorb. This term is a constant for a given set of conditions. Note that with an efficiency of one, Eq. 4 reduces to the traditional first-order model (Eq. 3). Equation 4 can be manipulated to express the normalized free phage concentration as a function of time.

$$\frac{C_p(t)}{C_{p0}} = 1 - \epsilon(1 - e^{-kC_{h0}t}) \tag{5}$$

The efficiency for each medium is approximated by averaging the normalized free phage concentration once adsorption has ceased. The rate constant is determined from the kinetic data. This model is useful because it offers greater insight into the phage adsorption process—by incorporating the competing effects of kinetics and efficiency—than the approaches used in the literature.

Results

Typical results from some of the adsorption experiments are presented in Fig. 1. These are standard infections

of *E. coli* 11303 using the TSB phage stock in four different media: MSM, NB, TSB, or MSM supplemented with 1.0 g/L L-tryptophan. The MSM supplemented with 1 g/L L-tryptophan was used to observe adsorption kinetics in a medium that was unequivocally saturated with tryptophan. As each experiment had slightly different initial concentrations of phage, the data for each experiment were normalized relative to the initial phage concentration. Data from each of the four experiments exhibited the same trend: an initial rapid decay followed by a period of no further adsorption. Other experiments (data not shown) all demonstrated that once the final free phage fraction (F) was reached, the value remained unchanged for at least 60 min. The proposed adsorption model (Eq. 5) was fitted to the data for each medium and is shown as solid lines in Fig. 1.

Table 1 contains the values of the adsorption rate constant (k) and the adsorption efficiency (ϵ). Reported are averages from at least two experiments. The rate constants vary more than an order of magnitude among the four media, with MSM displaying the greatest rate constant and TSB exhibiting the smallest. It is important to note that there was no apparent correlation, positive or negative, between the rate of adsorption and the efficiency of adsorption. For example, phage T4 had the fastest rate of adsorption but the lowest efficiency in MSM. In TSB, the virus had the highest efficiency, but the slowest rate of

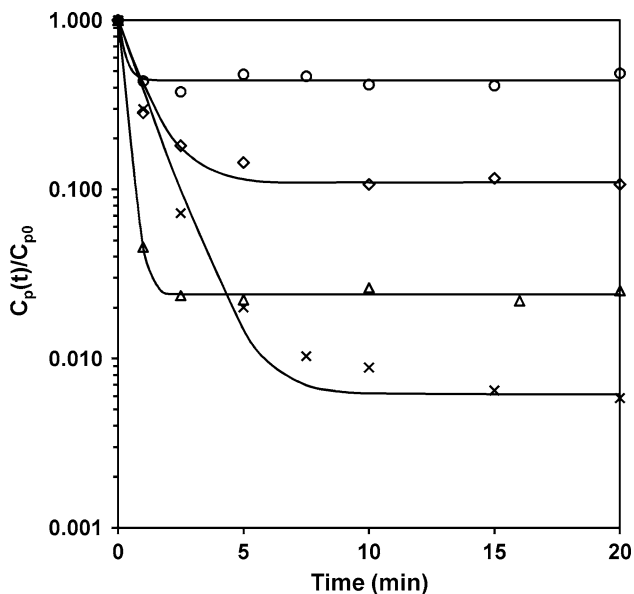


Fig. 1 Adsorption experiments of bacteriophage T4 to *E. coli* ATCC 11303 in four different infection media under standard infection conditions: (open circle) MSM, (open diamond) NB, (cross) TSB, and (open triangle) MSM + 1.0 g/L L-tryptophan. The phage concentration data are normalized with respect to the initial phage concentration. All experiments were carried out at 24 °C with a host concentration of 10^8 cfu/mL and an MOI ~ 0.01 with a phage stock prepared in TSB. The proposed adsorption model is also shown for each medium

adsorption. In MSM supplemented with L-tryptophan, the virus exhibited both a fast rate of adsorption and a high adsorption efficiency. Table 1 also shows that increasing the temperature from 24 to 37 °C during adsorption resulted in 8-fold and 2-fold increases in the rates of adsorption in MSM and TSB, respectively, but, statistically, led to no change in the adsorption efficiency.

Figure 2 shows the adsorption efficiency of phage T4, grown from two different phage stocks, in MSM as the infection medium, as a function of L-tryptophan concentration. The trend for the TSB phage stock displayed a small increase in adsorption efficiency at low L-tryptophan concentrations (1–7.5 mg/L). At higher concentrations (7.5–20 mg/L), the efficiency was a strong function of L-tryptophan concentration and increased to nearly 100%. Above 20 mg/L, the addition of L-tryptophan had no further effect. The efficiency of the MSM phage stock showed no dependence on the L-tryptophan concentration, remaining above 97% in MSM supplemented with 1 mg/L L-tryptophan and 75 mg/L L-tryptophan.

In order to verify whether the adsorption efficiency represented an equilibrium situation between the virus particles and the host, adsorption experiments were carried out under a variety of host concentrations and MOIs. Figure 3 shows the variation of efficiency with MOI at 24 °C for two different media, TSB and MSM. Statistical analyses showed no correlation between these parameters.

Several sets of host-cross infections, in which host cells were grown in one medium, collected by centrifugation and infected in a second medium, were carried out. The data for host-cross infections, in which the cells were grown in MSM and infected in either NB or TSB with the TSB phage stock, are presented in Fig. 4. Standard experiments where the cells are grown in TSB or NB and infected in the same medium are also shown. As seen in Fig. 4, for infections in both NB and TSB, the normalized free phage concentration approached F more quickly in the cross infection experiments than in the standard adsorption experiment; the adsorption rate constants increased by an order of magnitude for both cases (2.6×10^{-10} and 3.7×10^{-10} mL/s, respectively). However, for cross infections carried out in both TSB and NB, the adsorption efficiencies were approximately equal to the efficiencies of the respective infection media, changing by less than 7%. This small difference is amplified in Fig. 4 because of the logarithmic scale.

Figure 5 displays the final phage yield versus initial MOI for phage T4 amplification experiments carried out in MSM at various L-tryptophan concentrations. Each curve demonstrated the same trend. As the initial MOI was increased, the final phage titer decreased. As the L-tryptophan concentration increased from 1 to 15 mg/L, there was a 14-fold decrease in phage yield.

Table 1 Efficiency (ϵ) and rate constants (k) calculated for the TSB phage stock under standard conditions in the minimal salt medium (MSM), MSM with L-tryptophan, Tryptic Soy Broth (TSB), and Nutrient Broth (NB)

Medium	Temperature (°C)	L-Tryptophan concentration (mg/L)	ϵ^a (%)	k^a (mL/s)
MSM	24	Trace	56 ± 11	$3.7 \times 10^{-10} \pm 1.3 \times 10^{-10}$
MSM + 1 g/L Trypt	24	1,000	99 ± 0.9	$6.8 \times 10^{-10} \pm 3.5 \times 10^{-11}$
TSB	24	136	99 ± 1.0	$7.3 \times 10^{-11} \pm 2.5 \times 10^{-11}$
NB	24	36	87 ± 1.7	$3.4 \times 10^{-11} \pm 8.5 \times 10^{-12}$
MSM	37	Trace	50	3.0×10^{-9}
TSB	37	136	99	1.5×10^{-10}

^a Standard deviations are based on a minimum of two experiments

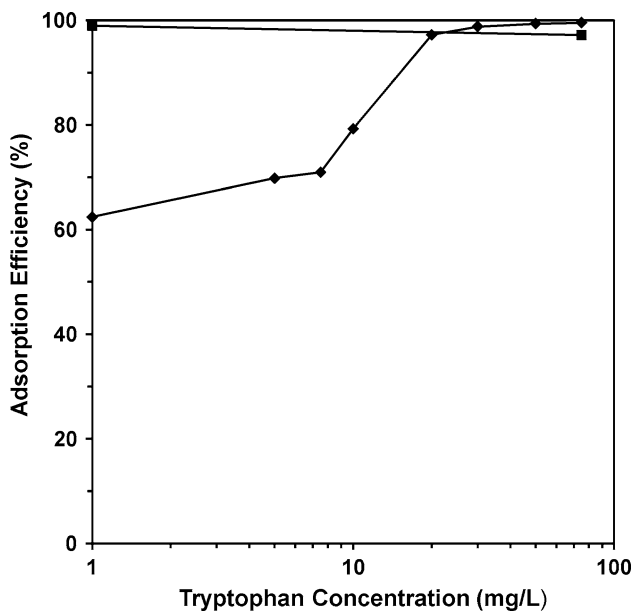


Fig. 2 The adsorption efficiency of phage T4 to *E. coli* in MSM versus L-tryptophan concentration under standard infection conditions. The adsorption efficiency was obtained by carrying out adsorption experiments in MSM at various L-tryptophan concentrations at 24 °C with a host concentration of 10^8 cfu/mL and an MOI ~ 0.01. Two different phage stocks were used: one stock produced and stored in MSM (filled square), the other produced and stored in TSB (filled diamond)

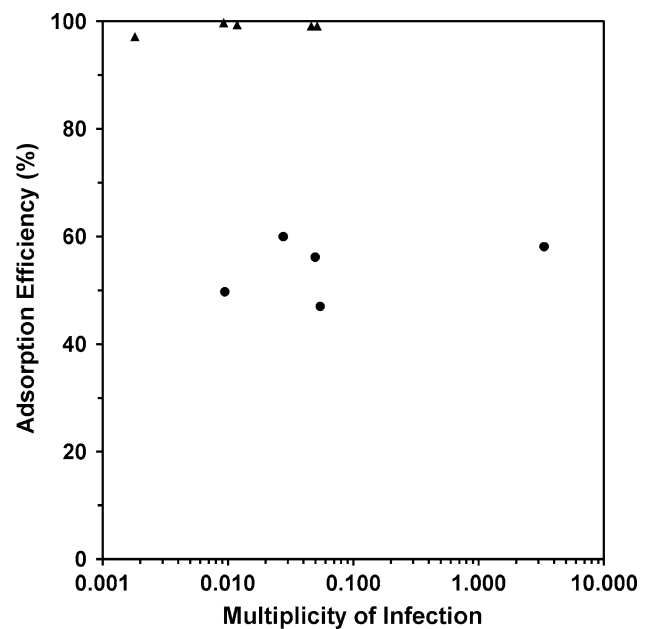


Fig. 3 Variation of the adsorption efficiency of phage T4 with initial multiplicity of infection in TSB (filled triangle) and MSM (filled circle) under standard infection conditions. All experiments were carried out at 24 °C with a host concentration of 10^8 cfu/mL with a phage stock prepared in TSB

Discussion

Validity of proposed model

The traditional first-order model for the adsorption of phages to their host (Eq. 3) is sufficient to describe many phage–host systems during the first few minutes of adsorption [21]. However, it implies that, provided with enough time, every member of a phage population will adsorb to a host. In fact, while 10 min after the onset of adsorption experiments, no further adsorption was observed in all experiments performed in this study, a fraction of the phage population, ranging from less than 1% to around 50% depending on experimental conditions

(see Fig. 2), always remained free in solution. Therefore, the traditional first-order model did not provide an adequate fit for the data obtained in these experiments.

An improvement to the traditional first-order model is the two-step mechanism proposed by Stent and Wollman. Adsorption described by this model is generally characterized by two exponential decay terms: a fast decay followed by a relatively slow decay. However, this model still has the problem that the phage population diminishes to zero, a phenomenon that can sometimes be extrapolated from the literature data, but is not supported by any of the literature surveyed. Certainly, the systems studied here did not behave in the manner predicted by this two-step mechanism.

The new model proposed here (Eq. 4) is a hybrid between these two earlier phage adsorption models.

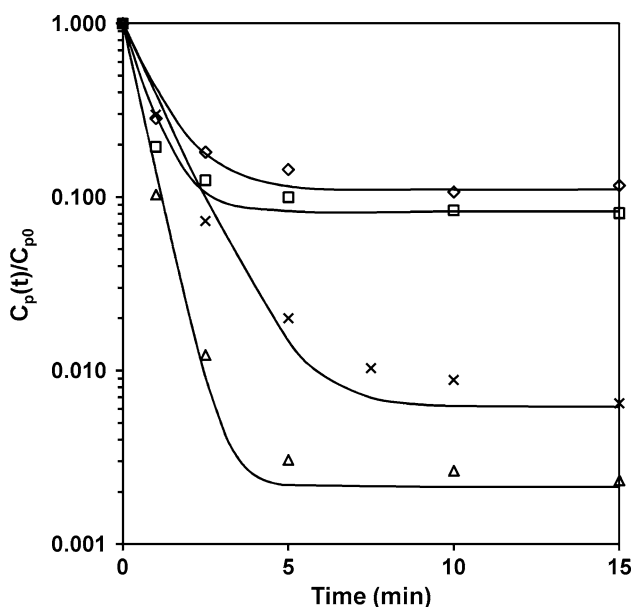


Fig. 4 Host-cross infection adsorption experiments. *E. coli* was grown in MSM and then infected in either NB (*open square*) or TSB (*open triangle*). Standard infections, where the host is grown and infected in the same medium—NB (*open diamond*) and TSB (*cross*)—are shown for comparison. All experiments were carried out at 24 °C with a host concentration of 10^8 cfu/mL and an MOI ~ 0.01 with a phage stock prepared in TSB. The proposed adsorption model is shown for each experiment

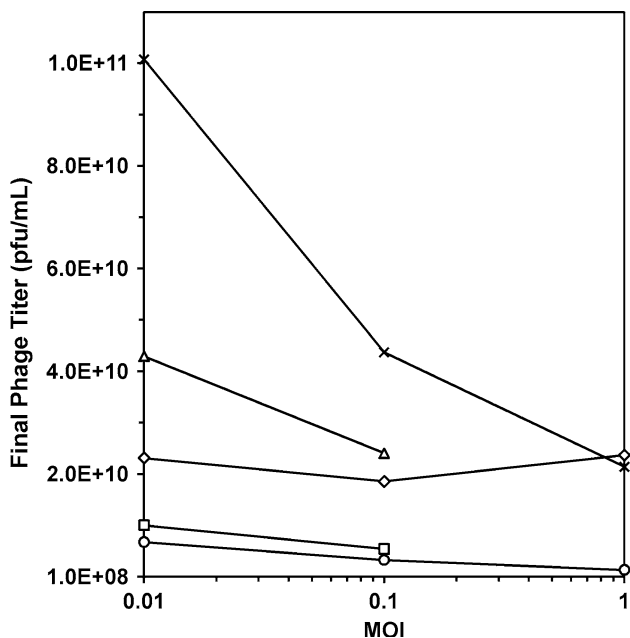


Fig. 5 Final phage titer versus initial MOI obtained from amplification experiments. Host cells were infected with a phage stock prepared in TSB and the experiments were carried out in MSM supplemented with L-tryptophan at the following concentrations: 1 mg/L (*cross*), 3 mg/L (*open triangle*), 5 mg/L (*open diamond*), 7.5 mg/L (*open square*), and 15 mg/L (*open circle*). All experiments were carried out in 100 mL shake flasks in an incubator shaker at 37 °C agitated at 200 rpm

It makes use of a first-order relationship while recognizing the two-step reality of phage adsorption. This model has the advantages of being simple, accurate, and robust. It has two modeling parameters: the adsorption rate constant (k) and the adsorption efficiency (ε). The former is an indication of how quickly adsorption takes place, the latter reveals what fraction of the phage population will undergo adsorption. The utility of this new model is that it augments the simpler kinetic model with a quantifiable property of the bacteriophage population, the adsorption efficiency.

Significance of the adsorption rate constant (k) and the adsorption efficiency (ε)

Both the adsorption rate constant (k) and the adsorption efficiency (ε) were shown to be affected, often significantly, by different physico-chemical conditions of the media as well as by physiological conditions of the host. Furthermore, these two parameters were shown to be mutually independent by their different responses to these changes. In fact, k is largely dependent on the state of the host whereas ε is more a property of the state of the virus.

One of the earliest observed methods of increasing the rate of adsorption was by increasing the temperature of the infection medium [12]. This phenomenon was observed in the values of adsorption rate constants in Table 1. Note that other characteristics of the media such as pH, ion concentration, and viscosity [10–13] were not investigated in this study. Aside from the nutrient makeup, many properties of TSB, NB, and MSM are relatively similar (pH, viscosity, etc.).

In the past decade, studies have shown that the nutrient makeup of the medium in which the host is grown can have an effect on the rate of adsorption of the virus to the host [24, 26]. Hadas et al. found that the adsorption rate of bacteriophage T4 was linearly proportional to total host cell surface area when growing cells in a minimal medium supplemented with various carbon sources and nutrients. Depending on the carbon source, the total cell surface area and the adsorption rate varied over a factor of four [26]. Moldovan et al. found slightly faster adsorption rates could be obtained in a phage λ -Ymel system when the host cells were grown in a minimal medium in the presence of maltose rather than glucose [24]. It is known that the expression level of LamB, the surface receptor protein required for adsorption of phage λ , varies depending on the carbon source [24]. From these observations, it is evident that the cell size of the host and the density of receptor proteins on the surface of the host are dependent on the nutrient conditions during growth. These variations are, therefore, possible explanations for the order of magnitude differences in adsorption rate constants among the data tabulated in Table 1. However, these observations alone

do not rule out the possibility that the virus itself is accountable for the variation in rate of adsorption from one medium to the next.

Other experiments supported the importance of the growth conditions of the host on the rate of adsorption of the virus. The results in Fig. 1 and Table 1 indicated that under standard experimental conditions, that is, growing and infecting the host in the same medium, the fastest adsorption was observed in MSM. The slowest adsorption was observed in TSB. However, as shown in Fig. 4, when the host was grown in MSM and then infected in TSB, the rate of adsorption of the virus approaches the rates of adsorption attained in infections in MSM under standard conditions. This same trend was observed when growing the host in MSM but infecting with phage T4 in NB (Fig. 4). Since the experimental conditions of the virus remain unchanged while those of the host are manipulated in the host-cross infections, the changes in rates of adsorption observed must be caused by different conditions in the host cell. The most plausible explanation is that the morphological and physiological state of the host in MSM leads to the fastest rate of adsorption of the virus. These trends were also seen, although to a lesser extent, in the reverse experiments. When the host was grown in TSB and infected in MSM, the rate of adsorption was closer to that of standard adsorption experiments carried out in TSB.

The second influential factor in Eq. 4, the adsorption efficiency (ϵ), was introduced to account for the fact that the phage population is divided between those that readily adsorb to a host and those that fail to adsorb to a host even after an extended time period. As can be seen in Fig. 1 and Table 1, this efficiency is unique for each medium. Unlike the rate constant, the efficiency is independent of temperature between 24 and 37 °C—as reported in Table 1. In addition, the efficiency was found to be independent of the MOI (Fig. 3). Regardless of the ratio of virus to host, the fraction of phages that binds irreversibly to the host cells remains the same for a given medium in which the adsorption takes place. This suggests that the adsorption efficiency of the virus population is not merely an equilibrium between the host and bacteriophage and the complex they form, but in fact a property of the virus population. Finally, from Fig. 4, it is clear that the growth conditions of the host have minimal impact on the adsorption efficiency of the virus. In both TSB and NB, there is little change in the adsorption efficiency compared to the infection medium at standard conditions.

In fact, the only experimental condition that brought about significant changes in the adsorption efficiency was the L-tryptophan concentration of the infection medium. From Table 1, it can be seen that the increase in efficiency from MSM to NB to TSB parallels a similar increase in L-tryptophan concentration in the media. While it cannot be

said with absolute certainty that L-tryptophan is the only factor influencing the adsorption efficiency in these three media, Fig. 2 shows how important it is. Here the adsorption efficiency of phage T4 in MSM is increased from roughly 60% to nearly 100% simply by the addition of L-tryptophan to the infection medium.

However, the cofactor requirement of bacteriophage T4 appears to depend on the history of the virus particle. T. F. Anderson reported evidence in 1948 that the L-tryptophan requirement of phage T4 was an inheritable trait [27]. Experimentation in this study with the MSM stock suggests a slightly different conclusion. As shown in Fig. 2, phage samples from the MSM stock had an efficiency of over 97% in MSM with and without supplementation of L-tryptophan. Unlike phages produced in TSB, the offspring produced in MSM exhibited no L-tryptophan requirement for adsorption. Note that both the MSM and TSB phage stocks were amplified using the same ATCC strain. In addition, the L-tryptophan dependency was evident when phages produced in TSB were amplified in MSM and then reamplified in TSB. These observations suggest that the L-tryptophan requirement of phage T4 is a phenotypic quality that can be triggered or inhibited depending on the environment of the bacteriophage particle and not the result of a mutation in the phage DNA.

One way to interpret the tryptophan dependency of bacteriophage T4 is to consider it as a conditional lethal phenomenon. Conditional lethal is a term applied to virus mutants that are only able to replicate under permissive conditions. For example, some temperature-sensitive mutants of bacteriophage T4D can only propagate at low temperatures and are unable to grow at higher temperature [28]. However, as discussed here, the tryptophan dependency of bacteriophage T4 is not a genetic property, but rather a phenotypic characteristic of the virus. In this context, tryptophan dependency can be thought of as a conditional lethal phenotype with a permissivity dictated by the concentration of L-tryptophan.

Other studies in the literature suggest that L-tryptophan increases the rate of adsorption of phage T4 to its host [16]. This phenomenon was observed in this study, although only a small increase was observed. As shown in Table 1, the rate constant of the TSB stock increased approximately 1.8 fold when L-tryptophan was added to the MSM during adsorption experiments. This change is relatively minor compared to the order of magnitude increase in the rate constant observed between MSM and TSB or NB. L-Tryptophan had a much more pronounced effect on the adsorption efficiency of the TSB stock, as is evident in Fig. 2.

In summary, the growth medium of the host and the infection temperature have a significant impact on the adsorption rate constant while the phenotype of the phage

in response to its environment—including the L-tryptophan concentration of the infection medium—has the most pronounced effect on the adsorption efficiency. The data from this study suggest that the adsorption rate constant can be strongly influenced by the conditions of the host while the adsorption efficiency is mainly dependent on the conditions of the bacteriophage. Studies with other phage–host systems have suggested a similar conclusion [24, 26].

The new term introduced in this model (ε) could have a more general application. It is reasonable to assume that the concept of adsorption efficiency could be applied to other phage–host systems and possibly other virus–host systems.

Adsorption efficiency (ε) and the efficiency of plating

One historical context in which the adsorption efficiency could be applied is the efficiency of plating (EOP). Early phage researchers observed that the plaque count of the same phage preparation varied according to the environmental conditions of the assay. In order to address this phenomenon, Ellis and Delbruck introduced the concept of EOP [29]. The EOP can be defined as the plaque count determined under stated conditions relative to the plaque count under standard conditions [21]. While the EOP depends on many factors—virus diffusion through the agar, adsorption to the host cell, successful phage replication, lysis of the host cell, etc.—the adsorption efficiency of the bacteriophage is quite possibly the most significant mechanism explaining many of the previously reported changes in the EOP. Anderson observed a 10,000-fold increase in the plaque count of bacteriophage T4 when plating on nutrient agar compared to plating the same strain on a defined medium lacking amino acids [13]. This observation is consistent with the presence of tryptophan in the nutrient agar, which can lead to an adsorption efficiency of over 99% as reported in this article (see Table 1). The EOP can also change when assaying the same phage preparation with different susceptible bacteria [21] and by varying the salt concentrations of the agar [10]. The adsorption efficiency is almost certain to change with different susceptible hosts and is likely influenced by the ionic environment of the bacteriophage and its host. Therefore, the adsorption efficiency is a plausible mechanism behind the large variation in EOP reported in the literature. Additional experiments would have to be done to confirm this hypothesis.

Phage amplification

The data bring out an important property of L-tryptophan. While it can have a significant impact on the adsorption efficiency, it has only a minimal effect on the rate of

adsorption. In addition, growth experiments with *E. coli* indicated that L-tryptophan does not have an effect on the metabolism of the host cell (data not shown). Therefore, the addition of L-tryptophan should not affect the physiological parameters linked to adsorption. Consequently, amplifying bacteriophages in MSM supplemented with various L-tryptophan concentrations will only affect the adsorption efficiency of the virus while keeping other amplification parameters constant. It should be noted that the amplification experiments were carried out at 37 °C while the majority of the kinetic data was collected at 24 °C. As reported in Table 1, the warmer temperature increased the adsorption rate constant but caused no observable change in the adsorption efficiency.

The results shown in Fig. 5 shed an interesting light on the adsorption efficiency of the bacteriophage. Increasing the adsorption efficiency decreases the overall number of phages produced for given initial conditions of infection. As the L-tryptophan concentration increases from 1 to 15 mg/L, the adsorption efficiency increases from around 60% to over 95% (Fig. 2). This, in turn, corresponds to a roughly 14-fold decrease in the final bacteriophage titer obtained from amplification experiments (Fig. 5). This negative correlation between adsorption efficiency and final phage titer is due to the fact that a high efficiency of adsorption implies that a large portion of the host cells will be infected and die early in the amplification process. The entire bacterial culture will lyse before it has a chance to propagate to any significant degree. Consequently, more phages are produced at a low adsorption efficiency because it takes significantly longer for the bacteriophage population to lyse the host culture. This allows for the host culture to grow to a higher cell density and, therefore, produce more virus particles in the process. In this regard, increasing the efficiency is similar to increasing the initial MOI of the production broth.

It seems that the most dramatic changes in phage yield observed in Fig. 5 are seen at very low L-tryptophan concentrations (1–5 mg/L) where the efficiency ranges from 60 to 70% according to Fig. 2. As the L-tryptophan concentration is increased, the phage titer curves tend toward a limit. This happens because, as the L-tryptophan concentration increases, the efficiency becomes very high and the phage production period becomes very short, and consequently, the phage yield is low. As such, the differences in yield are much less pronounced. There seems to be a threshold of approximately 15 mg/L L-tryptophan, after which further increases in L-tryptophan concentration do not result in further decreases in phage yield. According to Fig. 2, this corresponds to an adsorption efficiency of roughly 95%. Therefore, it is reasonable to conclude that above efficiencies of 95%, no further effect on phage yield is observed during production.

Conclusion

The adsorption efficiency appears to be a property of the phage population that is a function of the environmental conditions of infection and the phenotype of the phage stock, while the adsorption rate constant is a property that can be significantly influenced by the conditions of the host. An adsorption model that takes into account the adsorption rate constant and adsorption efficiency provided an accurate fit for adsorption data under numerous experimental conditions. The number of phages produced during amplification was found to be negatively correlated with the adsorption efficiency of the virus, decreasing as the efficiency increased. This study demonstrates the importance of considering the adsorption efficiency in any attempt to optimize the production of bacteriophage particles in an industrial process.

Acknowledgments The authors would like to thank the National Science and Engineering Research Council of Canada, the Eugenie Ulmer-Lamothe Fund of McGill University, and the Richard H. Tomlinson Doctoral Fellowship of McGill University for providing financial support for this project.

References

- d'Herelle F (1926) The bacteriophage and its behavior. The Williams & Wilkins Company, Baltimore
- Glynn MK et al (1998) Emergence of multidrug-resistant *Salmonella enterica* serotype typhimurium DT104 infections in the United States. *N Engl J Med* 338:1333–1338
- Hofmann J et al (1995) The prevalence of drug-resistant *Streptococcus pneumoniae* in Atlanta. *N Engl J Med* 333:481–486
- Panlilio AL et al (1992) Methicillin-resistant *Staphylococcus aureus* in U.S. hospitals, 1975–1991. *Infect Control Hosp Epidemiol* 13:582–586
- Thiel K (2004) Old dogma, new tricks—21st century phage therapy. *Nat Biotechnol* 22:31–36
- Padukone N, Perettie SW, Ollis DF (1992) Characterization of the mutant lytic state in lambda expression systems. *Biotechnol Bioeng* 39:369–377
- Padukone N, Perettie SW, Ollis DF (1990) Lambda vectors for stable cloned gene expression. *Biotechnol Prog* 6:277–282
- Lin CS et al (1998) Characterization of bacteriophage lambda Q⁻ mutant for stable and efficient production of recombinant protein in *Escherichia coli* system. *Biotechnol Bioeng* 57: 529–535
- Oh JS et al (2007) Construction of various bacteriophage lambda mutants for stable and efficient production of recombinant protein in *Escherichia coli*. *Process Biochem* 42:486–490
- Hershey AD, Kalmanson GM, Bronfenbrenner J (1944) Coordinate effects of electrolyte and antibody on the infectivity of bacteriophage. *J Immunol* 48:221–239
- Tolmarch LJ, Puck TT (1952) The mechanism of virus attachment to host cells: III. *J Am Chem Soc* 74:5551–5553
- Puck TT, Garen A, Cline J (1951) The mechanism of virus attachment to host cells: I. The role of ions in the primary reaction. *J Exp Med* 93:65–88
- Anderson TF (1945) The role of tryptophane in the adsorption of two bacterial viruses on their host, *E. coli*. *J Cellular Comp Physiol* 25:17–26
- Stent GS, Wollman EL (1950) Studies on activation of T4 bacteriophage by cofactor II: the mechanism of activation. *Biochim Biophys Acta* 6:307–316
- Stent GS, Wollman EL (1951) Studies on activation of T4 bacteriophage by cofactor III: conditions affecting the activation process. *Biochim Biophys Acta* 6:374–383
- Wollman EL, Stent GS (1950) Studies on activation of T4 bacteriophage by cofactor I: the degree of activity. *Biochim Biophys Acta* 6:292–306
- Wollman EL, Stent GS (1952) Studies on activation of T4 bacteriophage by cofactor IV: nascent activity. *Biochim Biophys Acta* 9:538–550
- Kellenberger E et al (1965) Functions and properties related to the tail fibers of bacteriophage T4. *Virology* 26:419–440
- Brenner S et al (1962) On the interaction of adsorption cofactors with bacteriophages T2 and T4. *Virology* 17:30–39
- Gamow RI, Kozloff LM (1968) Chemically induced cofactor requirement for bacteriophage T4D. *J Virol* 2:480–487
- Adams MH (1959) Bacteriophages. Interscience Publishers, New York
- Stent GS, Wollman EL (1952) On the two-step nature of bacteriophage adsorption. *Biochim Biophys Acta* 8:260–269
- Gamow RI (1969) Thermodynamic treatment of bacteriophage T4B adsorption kinetics. *J Virol* 4:113–115
- Moldovan R, Chapman-McQuiston E, Wu XL (2007) On kinetics of phage adsorption. *Biophys J* 93:303–315
- Goldberg E, Grinius L, Letellier L (1994) In: Karam JD (ed) Molecular biology of bacteriophage T4. American Society for Microbiology, Washington
- Hadas H et al (1997) Bacteriophage T4 development depends on the physiology of its host *Escherichia coli*. *Microbiology* 143:179–185
- Anderson TF (1948) The inheritance of requirements for adsorption cofactors in the bacterial virus-T4. *J Bacteriol* 55:651–658
- Edgar RS, Lielausis I (1964) Temperature-sensitive mutants of bacteriophage T4D: their isolation and genetic characterization. *Genetics* 49:649–662
- Ellis EL, Delbruck M (1939) The growth of bacteriophage. *J Gen Physiol* 22:365–384