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Modeling bacteriophage attachment using adsorption efficiency

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ABSTRACT

Typically, models of bacteriophage adsorption consider the process in two steps: reversible and irreversible attachment. In this study, a recently introduced one-step adsorption model, the adsorption efficiency model, is used to describe the adsorption of T-series bacteriophages to *Escherichia coli*. The adsorption efficiency model simplifies phage attachment to a single step: irreversible binding. The adsorption efficiency (ε) is used to account for unadsorbed phages. The model accurately describes T-series phage adsorption (T2, T4, T5, T6, and T7) under a variety of conditions. In addition, the model is compared to a commonly used two-step adsorption model, the sequential model. Experimental data support the assumptions of the adsorption efficiency model and suggest that the reversible first step of T-series phage adsorption is equivalent to irreversible attachment under the conditions tested. The adsorption efficiency model was not appropriate for a phage λ strain lacking side tail fibers. However, the model did agree with data previously published for a strain of phage λ possessing side tail fibers, as is the case of all T-series strains tested. This suggests that the adsorption efficiency model applies to phages containing side tail fibers

1. Introduction

Bacteriophages are the natural predator of bacteria. Consequently, they have received renewed attention in recent decades for potential applications as biological control agents in the agricultural and food packaging industries; in the control of water-borne pathogens; and in the treatment of bacterial diseases in both humans and animals [1–5]. In addition, their specificity makes them ideal candidates in diagnostic applications [6]. Finally, some researchers are examining their potential as vectors of recombinant protein production [7–10].

Because phage reproduction requires the introduction of its genome into the host cell, the adsorption of the virus particle to the host cell is a necessary step in almost all phage applications. Therefore, developing reliable models of virus–host cell interactions becomes essential for the successful application of bacteriophages. This work looks at the suitability of a recently developed adsorption model to predict the virus–host interactions of tailed phages, specifically, T-series and λ bacteriophages.

The main structural characteristics of the phages used in this study are summarized in Table 1. The phages represent three distinct families (myoviridae, siphoviridae, podoviridae) and exhibit significant differences in tail length and contractility. Also, there is a variety of tail fiber constructs represented. The tail fibers are the organelles used to identify the appropriate receptor protein on the surface of the cell during adsorption to the host. The T-even phages have six very large side tail fibers (140 nm) while the six fibers of phage T7 are quite small (23 nm) [11,12]. The siphoviridae studied here (T5, λ and Ur-λ) have one straight tail fiber connected to the distal end of the tail. Phages T5 and Ur-λ complement this end tail fiber with side tail fibers protruding outwards from near the base of the tail while λ has only the one end tail fiber [13–15]. The myoviridae (T2, T4, T6) and podoviridae (T7) do not have an end tail fiber.

The adsorption of a tailed phage particle to a host cell is a remarkable physiological phenomenon. Generally, upon contact with the host cell, the phage first reversibly attaches to the cell surface with its fibers, and then irreversibly binds to the appropriate receptor protein on the cellular membrane. This prompts conformational changes leading to the opening of the connector, allowing for the release of phage DNA from the capsid into the host cell [16].

A common modeling approach is to simplify phage adsorption into two steps: reversible and irreversible attachment. Numerous models have been published based on this mechanism [17–19]. Recently, we proposed an alternative interpretation of bacteriophage adsorption, known as the adsorption efficiency model, based on the interactions of bacteriophage T4 with *Escherichia coli* [20]. This model considers that the forward reaction in the reversible step is strongly favored, leading to an irreversible one-step approximation. In the present study, we examine the applicability of this model to other phage–host systems. We also compare and contrast

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Table 1
Characteristics of phage strains tested.

Phage	Structure and dimensions						
	Capsid	Tail		Side tail fibers		End tail fiber	References
	Size (nm)	Contractile	Length (nm)	Number	Length (nm)	Length (nm)	
T2	65 × 80	Yes	120	6	140	-	[11,46]
T4	65 × 80	Yes	120	6	140	–	[11,46]
T5	90 × 90	No	250	3	80[a]	50	[13]
T6	65 × 80	Yes	120	6	140	–	[11,46]
T7	60 × 60	No	23	6	32	–	[12,43,47]
λ	60 × 60	No	150	0	–	23	[14,48]
Ur-λ	Similar to λ	No	Similar to λ	6	80–85	Similar to λ	[15]

[a] Value estimated from Ref. [13].

the adsorption efficiency model with one of the most widely used two-step adsorption models, the sequential model.

2. Materials and methods

2.1. Host and bacteriophage

The host bacterium used for the T-series phages was *E. coli* ATCC 11303. The following media were used for growth of the host and for adsorption experiments: Tryptic Soy Broth (TSB; Becton, Dickinson and Company, Sparks, MD), Nutrient Broth (NB; Becton, Dickinson and Company, Sparks, MD) supplemented with 0.5% NaCl (w/v), and a minimal salt medium (MSM; previously described in Storms et al. [20]) supplemented with 2 g/L glucose and 0.1 g/L Bacto Yeast Extract (Becton, Dickinson and Company, Sparks, MD).

The host bacterium for phage λ was *E. coli* MG1655, obtained from the *E. coli* Genetic Stock Center (CGSC #6300) of Yale University (New Haven, CT). It was cultured in MSM using either glucose or maltose as a carbon source at a concentration of 2 g/L (w/v).

The following four bacteriophage strains were used in this study: bacteriophage T2 (ATCC 11303-B2), bacteriophage T5 (ATCC 11303-B5), bacteriophage T6 (ATCC 11303-B6), bacteriophage T7 (ATCC 11303-B38) and bacteriophage λ_{cI857} isolated from a lysogenized strain of MG1655(λ_{cI857}) generously provided by SAIC-Frederick, NCI-Frederick (Frederick, MD). Bacteriophage strains T2, T5, T6 and T7 were amplified using *E. coli* ATCC 11303 in nutrient broth with 0.5% NaCl (w/v), as per ATCC guidelines. Amplified samples were filtered using a 0.2-μm SFCA Corning syringe-filter (Corning Inc., Corning, NY) and the filtrate was stored at 4 °C. The titer of each phage stock was verified periodically to ensure no loss in infectious activity. The data for bacteriophage T4 was from a previous study [20].

Adsorption experiments were performed using samples of host cultures grown overnight, in the medium to be tested, in shake flasks at 200 rpm and 37 °C to a concentration of approximately 1×10^9 colony-forming units per mL (cfu mL^{-1}).

2.2. Adsorption experiments and phage and infective sites titers

Adsorption experiments were carried out in one of the three media described in Section 2.1 (TSB, NB, or MSM) according to the method described in Storms et al. [20]. All adsorption experiments were carried out in the growth medium of the host. The host solution (~10^8 cfu mL^1) was added to the phage solution (~10^7 pfu mL^{-1}) in a 30-mL syringe at 24 °C to a total volume of 11 mL and a resulting multiplicity of infection (MOI) of 0.1. Periodically, 1-mL samples were filtered out of the syringe through a syringe-filter (0.2-μm SFCA syringe - filter-Corning Inc.) and assayed for free phage titer measurements. A fresh syringe-filter was used for each sample.

In some experiments, in addition to measuring the free phage concentration (C_P), the total number of infective centers (C_{IC}) in the phage–host mixture was assayed. This was done by removing a sample from the adsorption broth, diluting 1000 fold to stop further adsorption, and then plating on an agar plate using the modified layering technique described previously [20]. In this context, infective centers include all irreversibly adsorbed phages (infected cells) and free phages. From these measured values, it is possible to determine the concentration of irreversibly attached phages (C_I) using the equation $C_I = C_{IC} - C_P$. Moreover, in order to determine the concentration of reversibly attached phages (C_R), samples from the adsorption broth were diluted in tubes containing 2% chloroform (v/v), which lyses the cells and releases reversibly attached phage particles [21]. These samples were assayed with the agar layering technique, yielding the total concentration of free and reversibly attached phages (C_{PR}). By comparing these samples with free phage samples (C_P), the concentration of reversibly attached phages could be determined using the equation $C_R = C_{PR} - C_P$.

3. Theory

Two kinetic models of phage adsorption are summarized below. The first is the sequential model, a variation of the two-step mechanism proposed by Stent and Wollman as an improvement over the earlier, simple first order model [19]. This model remains widely used today [21–23]. The second is the adsorption efficiency model, describing the adsorption kinetics of bacteriophage T4 taking into account the incapacity of a portion of the phage population to efficiently bind to the host [20]. Note that in each of the models, the adsorption process has been simplified and that the adsorption rate constants k_i depend on many diffusive and physico-chemical properties of the medium, phage and host bacterium. For a more detailed treatment, including the stochastic diffusion of the initial stages of phage adsorption, see the work of Adam and Delbruck [24] or Berg and Purcell [25].

3.1. Sequential model

The sequential model describes phage adsorption as a two-step process consisting of a reversible reaction followed by an irreversible one, where P is the free phage particle, H is the host cell, R is the reversible phage–host complex, and I is the irreversible phage–host complex.

$$P + H \underset{k_2}{\overset{k_1}{\longleftrightarrow}} R \xrightarrow{k_3} I \quad (1)$$

Researchers applying this model have been able to show the reversibility of the first reaction and that the irreversible step is indeed necessary for a successful infection [18,26,27]. The concentration of each species over time can be calculated by