Virology 485 (2015) 355–362

Contents lists available at ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/yviro

Review

Modeling tailed bacteriophage adsorption: Insight into mechanisms

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ARTICLE INFO

Article history:
Received 3 June 2015
Returned to author for revisions
5 August 2015
Accepted 8 August 2015

Keywords:
Adsorption kinetics
Sequential model
Adsorption efficiency
Phage amplification
Phage therapy

ABSTRACT

The process of a bacteriophage attaching to its host cell is a combination of physical diffusion, biochemical surface interactions, and reaction-induced conformational changes in receptor proteins. Local variations in the physico-chemical properties of the medium, the phage's mode of action, and the physiology of the host cell also all influence adsorption kinetics. These characteristics can affect a specific phage's binding capabilities and the susceptibility of the host cell to phage attack. Despite the complexity of this process, describing adsorption kinetics of a population of bacteriophages binding to a culture of cells has been accomplished with relatively simple equations governed by the laws of mass-action. Many permutations and modifications to the basic set of reactions have been suggested through the years. While no single solution emerges as a universal answer, this review provides the fundamentals of current phage adsorption modeling and will guide researchers in the selection of valid, appropriate models.

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Introduction

Bacteriophages have fallen in and out of favor among researchers since their discovery almost a century ago (d'Herelle, 1917; Twort, 1915). Euphoria over the existence of a natural prophylactic agent that could prevent and cure bacterial infections gave rise to snake-oil salesmen peddling bacteriophages as a solution to nearly everything from gallstones to herpes (a virus) (Harper et al., 2014). Inadequate understanding of phage biology led to many unsuccessful attempts at using phage therapy to treat bacterial infections in humans and animals (Pirnay et al., 2011; Sulakvelidze et al., 2001). By the late 1920s, the discovery of penicillin, an indiscriminate weapon against gram-positive pathogens, quenched whatever residual enthusiasm for phage therapy may have remained in the majority of the scientific world (Pirnay et al., 2012), save for countries of the Eastern Bloc.

Bacteriophages found new life in other circles though; in fact, much of our understanding of modern genetics is owed to studies involving bacteriophages (Ptashne, 2004). Even now, modern genetic engineering and synthetic biology techniques make heavy use of bacteriophage promoters, polymerases, and genes as tools to achieve recombinant or novel biological systems. Where would we be today without the temperature sensitive promoters of phage λ or the hyper-expression levels of the T7 promoter?

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http://dx.doi.org/10.1016/j.virol.2015.08.007
0042-6822/

Today, bacteriophages are used in situations far beyond what the original practitioners envisioned: vehicles of drug delivery (Dickerson et al., 2005; Lee et al., 2009; Tao et al., 2013a, 2013b), highly specific biological sensors (Guntupalli et al., 2012; Huang et al., 2008; Monk et al., 2010; Tawil et al., 2012) – particularly the luciferase-expressing reporter phages (Loessner et al., 1996; Schofield et al., 2009), viral-based electronics (Dang et al., 2011), and nanotechnology (Petrenko and Smith, 2011). But the original vision has also made a resurgence. Bacteriophages are being used as bio-control agents in agriculture and food processing applications (Adriaenssens et al., 2012; Fox, 2000; Fujiwara et al., 2011; Guenther et al., 2012; Jones et al., 2007; Loc Carrillo et al., 2005; Park and Nakai, 2003; Schnabel and Jones, 2001) and their use as anti-bacterial agents in the treatment of humans and animals is once again *au goût du jour* (Merabishvili et al., 2009; Pirnay et al., 2011, 2012; Rhoads et al., 2009; Verbeken et al., 2012; Wright et al., 2009).

While advances in modern imaging techniques such as Cryo-TEM have enabled visualization of the mechanism of bacteriophage infection at the nanometer scale (Hu et al., 2013), a comprehensive explanation of bacteriophage adsorption kinetics has not been reported in the literature, mostly due to the diversity of mechanisms exploited by different phages (Guerrero-Ferreira et al., 2011; Hu et al., 2013). In this review, we examine how researchers have dealt with modeling the often unintuitive nature of adsorption kinetics. We give some history around the progression of scientific understanding of bacteriophage adsorption and highlight the remarkably prescient hypotheses the early phage researchers used to explain the mechanics of phage adsorption. Next, we summarize the major types of adsorption models used to describe phage population dynamics in bacterial cultures. Finally, we comment on the approach to selecting an adsorption model most suitable for a specific virus–host system.

The adsorption paradox

One of the early topics of debate surrounding bacteriophage adsorption was how to explain the paradoxical notion that nearly every collision between a virus particle and host cell leads to irreversible attachment.

Studies on phage adsorption have revealed that in the early minutes of adsorption the interactions between the phage particle (P) and bacterium (B) can often be described by the simplified reaction

$$B + P \rightarrow I \tag{1}$$

where I is the irreversibly adsorbed phage–bacterium complex. Many studies have demonstrated that this mechanism obeys a first order observed rate of reaction where the concentration of the host as an available binding entity remains constant (Delbruck, 1940; Krueger, 1931; Schlesinger, 1932). In this case, the virus concentration decreases exponentially and the rate of adsorption can be described by the rate function

$$r_{ads} = kBP \tag{2}$$

where k is the adsorption rate constant, B is the bacterial concentration and P is the free phage concentration or phage titer. Note that if B is assumed to be constant in Eq. (2), the reaction rate reduces from a 2nd order reaction rate (two variables: B and P) to a pseudo 1st order reaction rate (where P is the only variable, k and B remain constant). Experiments on the adsorption of phage to living and heat killed *Staphylococcus aureus* in excess bacterial concentrations led Krueger to propose the following pseudo 1st order model to describe the decrease of free phage concentration over time (Krueger, 1931):

$$\frac{dP}{dt} = -kBP \tag{3}$$

As long as the ratio of phage to bacteria was low enough to assume an unchanged available bacterial surface area during adsorption, Krueger concluded that B could be assumed constant. Schlesinger (1932) and, later, Delbruck (1940) applied the coagulation theory of von Smoluchowski (1917) to phage adsorption, treating the bacterium and phage particle as two molecules interacting in space. According to this theory, if all collisions between phage and bacteria lead to irreversible attachment, the maximum value of k is given by

$$k = 4\pi r D \tag{4}$$

where r is the radius of a sphere "equivalent" to the bacterium (r in this context is not to be confused with the adsorption rate of Eq. (3)) and D is the diffusion coefficient of the phage particle. The maximum rate constant predicted by this theory is on the same order of magnitude as k-values determined experimentally (Delbruck, 1940; Schwartz, 1976), implying that nearly every collision between phage and bacteria leads to irreversible adsorption. How this is possible when the binding sites of both phage and bacteria constitute only a small fraction of their respective surface areas has long been a topic of debate.

The mechanism of phage attachment

Delbruck (1940) postulated that greater than predicted rate constants under optimal growth conditions of the host could be due to larger cell sizes and increased cell motility. However, high adsorption rates were still recorded for experiments completed on heat-killed bacteria (Krueger, 1931) or on stationary phase cultures (Gallet et al., 2012; Storms et al., 2010, 2012). Furthermore, calculations have shown that the influence of cell motility on adsorption rate is insignificant (Berg and Purcell, 1977). A more comprehensive explanation for phage adsorption that focused on the individual interactions between the virus particle and the cell surface was offered by Anderson (1949). Observing that (1) interactions between phage and bacterium are highly specific, (2) nearly every collision leads to irreversible attachment in undisturbed media, and (3) almost no collisions lead to irreversible attachment in violently agitated media, Anderson hypothesized that small protruding elements located on the virus particle are the first point of contact between phage and cell. These small elements would have higher rates of Brownian motions relative to the larger bacterial surface and therefore result in many collisions while the phage particle diffuses over the cell. If one of these collisions results in the proper orientation of the element with the receptor, it would lead to a "steric fitting of the elements and the formation of a weak bond between virus and host" (Anderson, 1949). This bond would be weak enough that intense agitation could break it, but strong enough to keep the virus–host complex together until irreversible attachment in undisturbed media.

In a comprehensive study of phage λ, Schwartz (1976) demonstrated that Anderson's proposed mechanism provides an adequate description of the mechanics of phage attachment. Adsorption rate is proportional to not only collision frequency, but also the probability that the appropriate interactions between virus and host occur within the average collision time. Using Einstein's equation of Brownian movement, Schwartz estimated that the λ particle will spend on average 5×10^{-3} s close enough to the cell receptor during each collision, but that only 1.6×10^{-3} s of the collision time will see the phage tail oriented in the right position for meaningful interactions. Then, applying the classical kinetic theory of gases to the ligand–membrane interactions on the cell surface and making some simplifying assumptions, Schwartz derived an equation describing the probability that a phage will react with a receptor during the effective collision. For maltose-grown *Escherichia coli* cells with a λ phage receptor density of ~630 mol μm^{-2},