VIRUS–CELL INTERACTION: PREDICTION OF THE TIME COURSE OF OBSERVABLE EFFECTS FROM VIRUS INTERACTION AT CELL INJECTION SITES, AND MECHANISMS LEADING TO ATTACHMENT

M. WONG, M. E. BAYER and S. LITWIN
The Institute for Cancer Research, The Fox Chase Cancer Center, Philadelphia, PA 19111, USA

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1. Introduction

Macromolecular collision and receptor recognition play a key role in biology. In this letter we consider the kinetics of events occurring during virus adsorption and infection, within the framework of a 3-state description [1]. The time course of the fraction of cells with one or more viruses attached to injection sites is predicted. This time course is related to that of observable effects arising from virus interactions at these sites. Such effects could serve to distinguish between possible mechanisms leading to viral attachment. We consider two such mechanisms here.

The results of a detailed analysis of the first stage of the adsorption event sequence is presented. A boundary value problem is solved containing one free parameter that is interpreted as a measure of the speed of the virus particle in the vicinity of the cell. The two attachment mechanisms lead to adsorption rates for which the dependence on this free parameter is markedly different. Comparison with experimental data is made for adsorption of the virus ϵ_{15} to the host cell *Salmonella anatum*.

2. 3-State description

We consider the phage to be in one of three states at any particular instant: in solution, on the cell surface, and over a DNA-injection site. Let $n_1(t)$, $n_2(t)$, $n_3(t)$ be the phage concentration at time t in each of these states, respectively. In order to give the time evolution of these quantities, we consider two specific mechanisms leading to eventual attachment (i.e., phage binding to an injection site). Each mechanism provides a way for the phage to arrive at a site after first colliding with the cell surface (see fig.1). In the first, the phage is assumed to diffuse 2-dimensionally on the cell surface (surface-diffusion mechanism); in the second, the phage undergoes 3-dimensional diffusion in the vicinity of the cell surface while making multiple collisions with it (encounter mechanism). Approximating the processes by first order kinetics, the concentrations $n_i(t)$ (i = 1,2,3) are then given by:

$$n_1(t) = n_0\, e^{-k_1 t} \tag{1}$$

$$n_2(t) = n_0 \frac{k_1}{k_1 - k_2} (e^{-k_2 t} - e^{-k_1 t}) \tag{2}$$

$$n_3(t) = n_0\, (1 - e^{-k_1 t}) - n_0 \frac{k_1}{k_1 - k_2} (e^{-k_2 t} - e^{-k_1 t}) \tag{3}$$

in the surface-diffusion mechanism,
and

$$n_1(t) = n_0\, e^{-k_1 t} \tag{4}$$

$$n_2(t) = 0 \tag{5}$$

$$n_3(t) = n_0\, (1 - e^{-k_1 t}) \tag{6}$$

in the encounter mechanism.
It is assumed in this approximation that the surface area available to the phage remains constant with time. In eq. (1)–(6) n_0 is the initial phage concentra-

Fig.1. Schematic of two possible mechanisms leading to phage attachment and eventual cell infection. (A) The phage, after colliding with the cell, stays on the cell surface and diffuses 2-dimensionally on it, eventually arriving at an injection site (surface-diffusion mechanism). (B) After first collision, the phage undergoes 3-dimensional diffusion in the vicinity of the cell surface while making multiple collisions with it. The phage can subsequently escape into solution or bind to an injection site as a result of a direct hit (encounter mechanism).

tion, and k_1 and k_2 are constants determined by the volume and surface diffusion, respectively. In terms of specific rate constants k_v and k_s, $k_1 = k_v N$ and $k_2 = k_s N_s$ where N is the cell concentration and N_s is the areal density of injection sites on the surface. The quantity $n_1(t)$ gives the depletion of phage from solution and can be obtained from traditional phage adsorption measurements as has been done for over 30 years. However, in the present note we wish to emphasize the quantity $n_3(t)$, which describes the arrival rate of phage at cell injection sites where they become bound, and to suggest that the prediction of observable effects (e.g., NMR, EPR, fluorescence, radioactive-labeled DNA detection) arising from virus interaction at the injection site may be a uesful way to distinguish between possible attachment mechanisms by comparison with experiment. To elaborate on this further, define a quantity:

$$\lambda(t) = \frac{n_3(t)}{N} \tag{7}$$

$$= m\frac{n_3(t)}{n_0} \tag{8}$$

which is the average number of phage over injection sites (state 3) on each cell at time t. The ratio $m = n_0/N$ is the multiplicity of infection (m.o.i). The use of first order kinetics restricts m to be not too large. The probability that a given cell has k phage in state 3 is:

$$p(k) = e^{-\lambda(t)} \cdot \frac{(\lambda(t))^k}{k!} \tag{9}$$

and at least 1 phage in state 3 is:

$$p(k\neq 0) = 1 - e^{-\lambda(t)} \tag{10}$$

A phage in state 3 leads to eventual infection of that cell. The sequence of events is pictured in the following way:

(i) Volume diffusion to the cell surface.
(ii) Surface diffusion or volume diffusion with encounter collisions to a DNA injection site, depending on the mechanism.
(iii) Arrival at the site and binding to it.
(iv) Injection of DNA at the site.
(v) Cell infection.

This sequence is based on one proposed in [2]. We now wish to exploit the result (10) with $\lambda(t)$ given by eq. (7) to predict the time course of effects which occur when stage (iii) of the above event sequence is reached. To be more specific, consider the emission of fluorescence which has been observed to occur from labeled cells following phage adsorption [3]. There the emission was attributed to structural transitions occurring in the outer membrane. The onset of any particular observable effect could in

principle occur during any one of the stages (ii)–(v) in the above event sequence. Lacking more definitive evidence, we hypothesize in this note that, for the case of fluorescence emission, a direct causal relation exists between the time of emission and the arrival of phage at the injection site. In this case, the fraction of labeled cells emitting fluorescence, $S(t)$, would simply be given by eq. (10), or:

$$S(t) = 1-e^{-\lambda(t-t_0)} \tag{11}$$

In eq. (11) we have anticipated a time delay t_0 following mixing, prior to the onset of fluorescence. We have also assumed that the second or subsequent phage arriving at an injection site on a given cell would produce no further fluorescence signal. Inserting eq. (8) into eq. (11) we have:

$$S(t) = 1-e^{-m\frac{n_3(t-t_0)}{n_0}} \tag{12}$$

for the predicted fluorescence signal as a function of the state-3 occupation number evolving in time. Measurement of phage adsorption on *Salmonella anatum* yields the data of fig.2, from which we find $k_1 \approx 1.4 \times 10^{-2}$ s^{-1}. The cell concentration in these experiments was $N = 2 \times 10^8$ cells/ml. We estimate k_2 from the observations of the diffusion of lipopolysaccharide on the surface of *Salmonella* [4]. This gives $k_2 \approx 1.9 \times 10^{-2}$ s^{-1}. The predicted fluorescence emission $S(t)$ is then shown in fig.3 for each of the two mechanisms, and for multiplicities of infection, m, of 1 and 10. The time delay t_0 is taken as 60 s. It is seen that the rate of emission is markedly different for the two mechanisms we have considered. This suggests that study of this effect could be a useful way to distinguish between possible attachment mechanisms. Indeed, preliminary fluorescence measurements [6] bear this out. The preliminary data, indicated by the shaded region, appear to favor the surface diffusion mechanism. Clearly, this conclusion depends on the sensitivity to the value of k_2 used: a higher value of k_2 would produce a faster rise of the fluorescence curve, tending to reduce the difference in the kinetics of the two mechanisms. It is found that k_2 can be increased by as much as a factor of 50 before the limit of distinguishability is reached, taking the experimental uncertainty of k_1 into account (~10%).

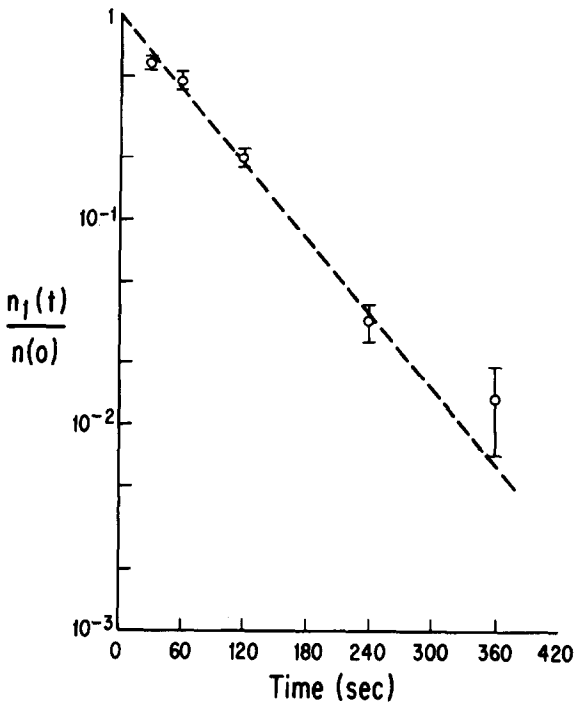

Fig.2. Measurement of the adsorption of phage ϵ_{15} on cells *Salmonella anatum*.

3. Simple 1-parameter model

In this section we analyze the first stage of the event sequence in greater detail, namely, the volume diffusion to the cell surface. Specifically, we consider a boundary value problem in which the cell, or radius R, is initially immersed in a sea of phage of uniform concentration. We assume the concentration at the cell surface $u(R,t)$ to be proportional to the phage arrival rate, i.e.:

$$u(R,t) \propto D \left\{\frac{\partial u(r,t)}{\partial r}\right\}_R \tag{13}$$

$$= \frac{D}{\beta} \left\{\frac{\partial u(r,t)}{\partial r}\right\}_R \tag{14}$$

Here D is the diffusion coefficient for phage in solution; the parameter β, inserted to complete the equality, evidently has dimensions of velocity. We shall think of β as providing a measure of the velocity in the vicinity of the cell surface. Solving this bound-

ary value problem [7] leads to a depletion of phage from solution that can be expressed in the form:

$$n_1(t) = n_0 \exp\{-4\pi DRf(\beta)Nt\} \quad (15)$$

where

$$f(\beta) = \frac{\beta}{\omega D} \quad (16)$$

and

$$\omega = \frac{D + \beta R}{DR} \quad (17)$$

ω has dimensions of inverse length. A fit to the experimental data of fig.2 yields an adsorption rate which is 0.63 ($=f(\beta)$) times the maximum rate ($4\pi DRN$), corresponding to a value $\beta \approx 4 \times 10^{-3}$ cm/s.

The exponent in eq. (15) is appropriate for characterizing the volume diffusion to the cell in only one of the two mechanisms we have considered here. It is not appropriate for the encounter mechanism, where the phage has a finite probability of escaping while diffusing near the cell surface. The phage eludes escape (adsorbs irreversibly) only if during its many collisions with the cell it arrives at an injection site by a direct hit and binds to it. Thus for the encounter picture the net flow of phage to the cell surface is decreased by a factor P_c, the capture probability. P_c is given by [8]:

$$P_c = 1 - P_{\text{escape}} \quad (18)$$

$$= 1 - \sum_{n=0}^{\infty} \left(1 - N_i \frac{\delta^2}{4R^2}\right)^n (P_\delta)^n (1 - P_\delta) \quad (19)$$

where N_i is the number of injection sites on the cell surface, δ the injection site radius, and P_δ the chance for a phage to collide with the cell when released from a point a distance $\sim\delta$ from it. In eq. (19) the first term in the summation is the probability of not hitting an injection site during a single collision and the index n is the number of collisions. The summation includes all possible number of collisions during a single encounter of the phage with the cell. Evaluation of this expression with P_δ calculated from the solution to the above boundary value problem [7] yields:

$$P_c = N_i \delta^2 (\omega R - 1)/\{4R^2(\omega\delta + 1) + N_i \delta^2 (\omega R - 1)\} \quad (20)$$

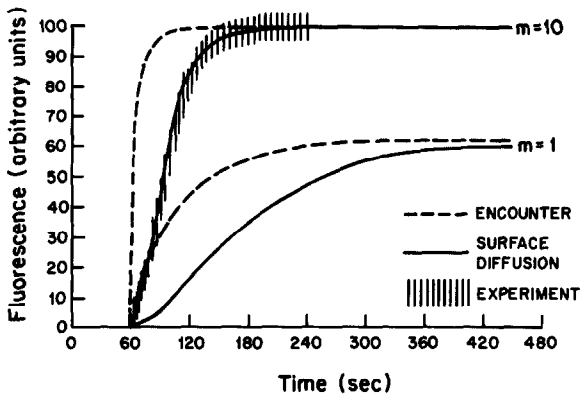

Fig.3. Prediction of the fluorescence emission for the two attachment mechanisms considered in the text. The shaded area indicates the results of preliminary measurements from adsorption of phage ϵ_{15} on ANS labeled *Salmonella anatum*.

$$= P_c(\beta) \quad (21)$$

This factor can now be inserted into eq. (15) to give, for the encounter mechanism:

$$n_1(t) = n_0 \exp\{-4\pi DRf(\beta)P_c(\beta)Nt\} \quad (22)$$

A fit to the adsorption data now gives $\beta \approx 3 \times 10^{-3}$ cm/s, about 100-times larger than for the surface diffusion picture.

If, as stated earlier, we think of the parameter β as providing a measure of the phage velocity near the cell, we can estimate β from the relation $x^2 \sim Dt$ where x denotes spatial displacement. For $x \sim 10^{-4}$ cm, $D \sim 10^{-7}$ cm^2/s, this gives the time t required for a phage to diffuse a distance of the order of cell dimensions as $t \sim 10^{-1}$ s, or a 'velocity', $v \sim x/t \sim 10^{-3}$ cm/s. This simple argument suggests β should be of the order of 10^{-3} cm/s, favoring the surface diffusion mechanism for phage attachment.

4. Summary

We have applied a 3-state model of the bacteriophage adsorption process to predict the time course of possible observable effects arising from virus interaction at cell injection sites. Two specific mechanisms leading to virus attachment at these sites are con-

sidered (surface diffusion and encounter mechanisms). It is suggested that comparison of such predictions with experimental observations may be a useful way to distinguish between possible attachment mechanisms. As an example, the model is applied to the ϵ_{15}-*Salmonella anatum* system. The rate of emission of fluorescence is predicted, using experimentally determined adsorption constants. Comparison of this rate with the experimental rate indicates that a distinction between mechanisms does appear possible; indeed, preliminary data, for ϵ_{15} on *Salmonella anatum*, favors a surface diffusion mechanism.

A more detailed analysis of the volume adsorption is made using a diffusion model with a boundary condition containing one free parameter. This parameter, β, is evaluated from the experimental adsorption rate yielding markedly distinct values for the two attachment mechanisms considered. Interpreting β physically from dimensional arguments as the phage velocity near a cell, a simple order of magnitude estimate of this quantity (β) from the time required for a phage to diffuse a distance of a cell diameter also supports a surface diffusion mechanism.

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[4] Mühlradt, P. F., Menzel, J., Golecki, J. R. and Speth, V. (1974) Eur. J. Biochem. 43, 533–539.
The surface diffusion coefficient in this reference is determined to be $D_2 \approx 3 \times 10^{-13}$ cm²/s at 37°C. We have assumed in using this number that the phage, on colliding with the cell surface, locks efficiently onto the lipopolysaccharide molecules of the outer membrane and is transported with these molecules in their surface motion. The rate constant k_2 is given by a simple dimensional argument as $k_2 = (b\Delta^2/D_2)^{-1}$ where the constant b has been obtained from a calculation similar to one in [5], and Δ is the average spacing in the lattice of injection sites, assumed for *Salmonella* to be $\Delta \approx 1.2 \times 10^{-5}$ cm. This corresponds to $N_i \approx 400$ sites on the surface. The radius δ of each injection site is assumed to be $\delta \approx 1.5 \times 10^{-4}$ cm. About 5% of the total surface area of the cell is covered by injection sites.

[5] Adam, G. and Delbrück, M. (1968) in: Structural Chemistry and Molecular Biology (Rich, A. and Davidson, N. eds) pp. 198–215, Freeman, USA.

[6] Bayer, M. H. (1978) private communication. These measurements were obtained from the adsorption of phage ϵ_{15} on ANS-labeled *Salmonella anatum*, and done with a multiplicity of infection $m = 10$. This work to be published elsewhere.

[7] The spatial and temporal variation of phage concentration is given by:

$$u(r,t) = u_0 - u_0 \frac{\beta}{\omega D}\frac{R}{r}\left\{\Phi\left(\frac{r-R}{\sqrt{4Dt}}\right) - \exp\left(\omega(r-R)+\omega^2 Dt\right)\cdot\Phi\left(\frac{r-R}{\sqrt{4Dt}}+\omega\sqrt{Dt}\right)\right\}$$

where

$$\Phi(x) \equiv \frac{2}{\sqrt{\pi}}\int_x^\infty e^{-\xi^2} d\xi$$

and u_0 is the initial uniform concentration. Details will be published elsewhere.

[8] Berg, H. C. and Purcell, E. M. (1977) Biophys. J. 20, 193–219.