Infectivity of algal viruses studied by chlorophyll fluorescence

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Algal virus infection proceeds via the specific recognition of the host cell wall, penetration of the cell wall and transfer of genetic material into the cytoplasm of the host cell. This process is similar to that which occurs when bacteriophage infect bacteria so that techniques and concepts developed to study bacteriophage are applicable to algal virus studies. By measuring virus-induced changes in chlorophyll fluorescence we have redefined classical studies on the distribution of infectivity. We show that infectivity does not follow a Poisson distribution with a fixed mean, n. By analysing the infectivity of algal viruses over a broad range of virus:cell ratios we have obtained a corrected Poisson distribution that reflects the probability of multiple virus particles attached per cell and is equally applicable to algal viruses and bacteriophage.

Paramecium bursaria Chlorella virus-1 (PBCV-1) is the type member of a group of Chlorella viruses that infect Chlorella cells which are exsymbionts from Paramecium of North American, Japanese or European origin (Van Etten et al., 1991). Chlorella strain NC64A is host to viruses of North American and Asian origin and has all the features characteristic of eukaryotic plant cells (Van Etten et al., 1991). PBCV-1 is polyhedral, approximately 150–190 nm in diameter (Van Etten et al., 1982) and has a linear, non-permuted, double-stranded DNA genome of 333 kbp with covalently closed hairpin ends (Girton & Van Etten, 1987; Rohozinski et al., 1989).

The infection cycle is initiated by attachment of virus to the host cell and digestion of the cell wall. Subsequently, material from the core of the virion enters the cytoplasm and host protein and nucleic acid syntheses are then directed to virus replication (Van Etten et al., 1991). Since attachment of PBCV-1 to the algal cell wall is rapid and irreversible (Meints et al., 1983) it can be assumed that synchronous infection of the host cells occurs. Ultimately, 10 h after infection, progeny virus is released from the host cell by lysis (Van Etten et al., 1991).

Recently, it has been shown that the photosynthetic apparatus of the host is inhibited upon virus infection. This change in photosynthetic efficiency can be monitored directly by observing changes in modulated chlorophyll fluorescence (Seaton et al., 1995). Change in the coefficient of fluorescence photochemical quenching (qp) was measured as described earlier (Schreiber, 1986; Schreiber et al., 1986) using the methods of Seaton & Walker (1992) which were adapted for this particular system (Seaton et al., 1995).

qp is inversely related to the reduction status of Qa, the primary acceptor of photosystem II (PS II). The conditions of the assay were chosen so that only about 5% of the QA pool was initially reduced, thus maximizing the sensitivity of the system (Fig. 1). During the first 30 min of the infection cycle there is a lag phase of about 15 min, which represents the time taken for attachment to, and penetration of, the host cell wall by the virus and is quantified as AΔt. Following this there is a rapid drop in qp due to virus replication inducing changes in the pattern of energy flow through photosystems I and II. This change in energy flow can be observed as a depression in qp (Δqp), which reflects the increase in the reduction status of QA in PS II (Fig. 1).

Since we are dealing with a population of algal cells that are infected with virus, the magnitude of Δqp is a direct measure of the proportion of cells infected. When a saturating level of virus was used, a maximal change in qp was observed. By varying the amount of virus used for inoculum between zero and saturation, direct observations on the relationship between the amount of virus added and the number of cells that become infected can be established by observing the magnitude of the change in qp (Δqp). This phenomenon is illustrated in Fig. 1 where the drop in qp, with several different levels of virus is shown. The lag phase is essentially constant; however, the decline in qp is related to the amount of virus added.

These data can be plotted as the amount of virus added versus Δqp (Fig. 2). This graph shows the relationship between the number of infected and

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Fig. 1. Change in $q_p$ with different amounts of virus added to $1 \times 10^8$ algal cells in a volume of 2 ml. Amounts of virus used were (\(\triangle\)) no virus, (\(\square\)) 0·5 ml, (\(\blacktriangledown\)) 1·5 ml and (\(\blacktriangle\)) 6 ml of stock solution that had a titre of 4·6 x 10$^5$ p.f.u./ml calculated by the method of Van Etten et al. (1983). The insert shows how the values of $\Delta t$ and $\Delta q_p$ are calculated. $\Delta q_p$ is as the difference between the $q_p$ value at the time of inoculation ($t = 0$) and that 60 min later.

Fig. 2. Mathematical relationship between $\Delta q_p$ and amount of virus added (x). The fitted curve was described by the general equation $\Delta q_p = a(1 - e^{-bx})$ where $a$ is the maximum observed value of $\Delta q_p$ and $b$ is calculated from the fitted curve.

uninfected cells when various amounts of virus are added. Thus, at saturating levels of virus, the maximum $\Delta q_p$ (0·59) represents all $1 \times 10^8$ cells being infected. When non-saturating levels of virus are used, the ratio of $\Delta q_{p,max}$ to observed $\Delta q_p$ is essentially a measurement of the ratio of infected cells to the total number of cells. Under conditions where non-saturating amounts of virus are used, the condition of random infection exists, and the chance of any host cell binding 0, 1, 2, 3 or more virus particles is given by the Poisson series. This feature of infectivity has been described for coliphage by Ellis & Delbrück (1939) and is a feature of any host–pathogen system where specific attachment of multiple infection units is possible. However, in the case of bacteriophage, at high levels of infection, spontaneous cell lysis may occur, limiting observations to very high dilutions of phage relative to bacteria. In the case of algal cells, the rigid cell wall and relatively large size of the host cell allow observations to be made at saturating levels of virus without the complication of spontaneous lysis. Furthermore, algal cells can potentially adsorb 5000 PBCV-1 particles per cell (Van Etten et al., 1991) since the cell surface is apparently saturated with binding sites. The algal cell system can thus be used to study infectivity across a large range of virus concentrations including saturation levels, which could not be studied with any other known system.

The observed relationship between $\Delta q_p (y)$ and volume of virus-containing solution added (x) as in Fig. 2 can be described by the general equation

$$y = a(1 - e^{-bx})$$

(1)

where $a$ equals the observed maximum value of $\Delta q_p$ (in this case 0·592). The value of $b$ is estimated by fitting the theoretical curve to the observed data and in this case $b$ is 0·639. By allowing $a$ in equation (1) to equal 1·0 (i.e. $\Delta q_{p,max}/\Delta q_{p(max)} = 1·0$) then any calculated value of $y$ will fall between 0 and 1 and be equivalent to $\Delta q_p/\Delta q_{p(max)}$ as shown in Fig. 3(a). This can then be treated as the fraction ($P$) of infected cells and (1–$P$) the proportion of healthy cells $P_o$ (Fig. 3a). Alternatively, $P_o$ can be calculated directly from the data as $(\Delta q_{p(max)} - \Delta q_p)/\Delta q_{p(max)}$ which is equivalent to $(\Delta q_{p(max)}/\Delta q_{p(max)}) - (\Delta q_p/\Delta q_{p(max)}) = 1 - P$

Under the assumption of Ellis & Delbrück (1939) that, at any fixed quantity of virus, $P_o$ and $P$ are given by the Poisson distribution, it follows that:

$P_o =$ proportion of healthy cells

$= P$ (no virus particle attached to an individual cell)

$= e^{-n}$

(2)

and

$P =$ proportion of infected cells

$= P$ (at least one particle attached to an individual cell)

$= \sum_{r=1}^{n} n^r e^{-n} (r!)-1$

$= 1 - e^{-n}$

(3)

where $n$ is the average number of virus particles attached per cell.
depends on the amount of virus \( (x) \). However, their experiment does not allow different levels of \( x \). Therefore, the relationship between \( n \) and \( x \) could not be explored. The amount of virus \( (x) \) can be expressed as a volume of virus-containing solution or as plaque forming units (p.f.u.). However, traditional plaque assays are inherently inaccurate (as demonstrated below) so we have chosen to use volume.

In our experiment we denote the volume of virus added in microlitres by \( x \) and allow it to vary. In the general case where the amount of virus per unit volume is constant but unknown, we are interested in estimating the number of virus particles per unit of virus solution. For this, we first establish that the average number of particles attached per cell is directly proportional to the virus volume, i.e. \( n \propto x \). In particular, if \( b \) denotes the constant of proportionality, we establish that

\[
 n(x) = bx
\]

where we write \( n(x) \) rather than \( n \), to emphasize dependence of the average number of virus particles attached per cell on the amount of virus solution. This relationship, together with the knowledge of the total number of cells, gives us the number of virus particles per unit volume for a given virus solution.

To make it precise, let \( P(x) \) denote the proportion of infected cells and \( P_0(x) = 1 - P(x) \) be the proportion of healthy cells when \( x \) is the volume of virus solution added. Then using equations (2)–(4)

\[
 P_0(x) = e^{-bx}
\]

and

\[
 P(x) = 1 - e^{-bx}
\]

Experimental values of \( P_0(x) \) and \( P(x) \) are plotted against \( x \) in Fig. 3(a), and Fig. 3(b) demonstrates the linear relationship between \( x \) and the natural logarithm of \( P_0(x) \), i.e. \( \ln P_0(x) = -bx \), for some value of \( b \). We confirm this relationship and our estimate of \( b \) (from equation 1) using linear regression theory as discussed in the statistics’ literature (e.g. Neter et al., 1985).

Since we have a fixed number of cells, \( c \), per experiment and we know the relationship between the amount of virus added (volume \( x \)) and the number of uninfected cells (equation 5) we can calculate the amount of virus particles added in any volume \( x \) \( (V(x)) \) using the equation as previously discussed

\[
 V(x) = n(x) \cdot c = bx \cdot c
\]

By dividing by the volume \( x \) we can calculate the number of virus particles per unit volume (\( V \))

\[
 V = \frac{V(x)}{x} = \frac{bx}{x} \cdot c = b \cdot c
\]

Substituting our observed value of 0.639 for \( b \) and since we know the number of cells \( (c) \) in our experiment to be \( 1.6 \times 10^8 \) we calculate \( V \) to be \( 1.02 \times 10^8 \) virus particles/\( \mu \)l.

Using a standard plaque assay (Van Etten et al., 1983) an estimate of \( 4.6 \times 10^7 \) p.f.u./\( \mu \)l was obtained. The difference between the observed viral titre using chlorophyll fluorescence and the plaque assay is due to plating efficiency which is equal to 0.45 in this case. The concept of plating efficiency and its relationship to the accuracy of plaque assays has previously been described by Ellis & Delbrück (1939).

By observing the changes in chlorophyll fluorescence induced by an algal virus, and relating these changes to virus concentration, a powerful method of studying the relationship between a plant virus and its host has been developed.

Our observations indicate that algal viruses behave in a manner analogous to bacteriophage; however, we can make direct observations of viral infectivity over a large range of virus:host ratios that could not easily be achieved with bacteriophage-based systems. With classical bacteriophage work saturating amounts of phage are not used because the standard experimental methodology requires a small number of phage particles to be added to
a large number of bacteria (Ellis & Delbrück, 1939; Stent, 1963) so that supposedly a maximum of one phage attaches per cell. The requirement of one phage per cell is critical to the assumption that infectivity follows a Poisson distribution. However, in reality the criterion of one infectious unit attaching per cell is never met. Where a very low phage:bacteria ratio is used, statistical variation may interfere with accurate titre determination. Our data show that assumptions based on the use of the Poisson distribution to model the interactions between phage or viruses and their hosts are basically correct. However, caution must be used when using the Poisson mean (n) as an estimate of virus titre because n is a function of the amount of virus added (x) and, since n is not necessarily equal to x, the underlying relationship between n and x must be determined empirically before an accurate estimate of virus or phage titre can be obtained. Using the methods described in this paper the classical approach of measuring virus titre established by Ellis & Delbrück (1939) and Delbrück & Luria (1943) can be modified so that accurate estimates of titre can be obtained.

References


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