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Bacterial Lysis by Phage—A Theoretical Model

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The similarity to materials corrosion is invoked to develop a model for phage-infected bacterial lysis based on the statistics of extremes. The importance of cell size, envelope thickness and lysozyme eclipse time on the final probability distribution of lysis is considered. Experiments are suggested to test the model.

Introduction

Kinetics of bacterial lysis by bacteriophages was actively studied in the dawn of molecular biology (Adams, 1959; Cairns *et al*., 1966). Thus e.g. Delbrück (1940) distinguished "lysis from within" (LI) from "lysis from without" (LO) and Doermann (1952) induced premature, artificial lysis. The "lysis inhibition" (LIN) phenomenon (Hershey, 1946; Bode, 1967), whereby an additional infection (termed "super-infection") by the same phage delays the lysis process, was a significant discovery. Streisinger *et al.* (1961) identified and purified the T4 lysozyme, and demonstrated it to be essential for T4-induced cell lysis. Very little has been published on phage-induced lysis since the 1970s (e.g. Karam *et al*., 1994; Wang *et al*., 1996), probably due to the rising interest in molecular biology and the difficulties in cloning the genes encoding lysozyme, designed to kill the host bacterium.

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The comprehensive review article of Young (1992) summarized all knowledge accumulated during over 50 years about phage-induced cell lysis, emphasizing molecular aspects. A full understanding of the fundamentals of the lysis process, whether theoretically or experimentally, is still missing. Thus, neither the measurement of the systematic chemical processes occurring at the cell envelope due to the presence of lysozyme nor extensive temporal measurements of the initiation or termination of lysis in these systems were carried out.

We develop here a simple model, based on the statistics of extremes, to gain better understanding and to mainly focus attention on the relevant problems, which should be addressed. It does not pretend to be an ultimate model and should be considered as a first attempt to treat this facinating problem. We are aware that for many phages "no lysozyme is elaborated, and in others the accumulation of lysozyme activity is irrelevant to the actual scheduling of lysis" (Young, 1992). Since "the molecular basis of phage lysis is still largely a mystery" (Young, 1992), we approach the problem from a physical viewpoint. The term

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"lysozyme" in this article could thus be taken as the limiting factor in a complex mechanism, such as holins, resulting in cell lysis (e.g. Josslin, 1971; Dressman & Drake, 1999; Loessner *et al.*, 1999).

The Model

We consider infection by a single phage only and therefore treat neither LIN nor LO. Following infection by the phage (at a time we designate $t = 0$), a certain period (ξ) elapses before the phage starts producing lysozyme (e.g., Champe, 1963). Assuming that for $t > \xi$ the lysozyme production rate (β) is constant in a specific bacterium/phage system under any particular conditions, the amount of lysozyme in the bacterium at time t is $\beta(t - \xi)$. If this amount is equally distributed throughout the cell volume V, and if the effective amount attacking the envelope is concentrated within a layer of thickness θ off its surface area S, then this effective amount is given by

$$\frac{\beta(t-\xi)\theta S}{V}. \tag{1}$$

The effective amount per unit area is therefore $\beta\theta(t - \xi)/V$. The corrosive influence on a unit area y by the effective lysozyme is proportional to the effective amount times the period of time this amount operated on the envelope:

$$y \sim \int_{\xi}^{t} \frac{\beta\theta}{V}\,\mathrm{d}t'\,(t - t') \quad \text{or}$$

$$y = A'\frac{\theta\beta}{2V}(t-\xi)^2 = A\frac{\beta}{V}(t-\xi)^2, \tag{2}$$

where A', A are constants.

Denote by X the maximal depth to which the action of the lysozyme has penetrated after time t for a unit of activated area. The statistics of X, being the statistics of maxima, follows the "statistics of extremes" (Gumbel, 1958; Castillo, 1988): the distribution of X can assume only one of the three families of probability distributions, namely Gumbel, Weibull or Frechet. Many studies have concluded (Shibata, 1994, 1996, and references therein) that the maximum pit depth for corrosion follows the Gumbel probability distribution. We hypothesize that the action of a lysozyme on a surface is similar to the corrosion action of chemicals on materials. Thus, the cumulative distribution function (cdf) of X is given by

$$F(x) = \mathrm{Prob}\{X < x\} = \mathrm{e}^{-e^{-(x-\lambda/\gamma)}}, \tag{3}$$

where λ is the mode of the distribution (in our case, it is a kind of threshold), and γ is a scaling parameter, to be discussed below.

Denoting by d the thickness of the envelope, the probability that the area be pierced is

$$1 - F(d) = 1 - \mathrm{e}^{-e^{-(d-\lambda)/\gamma}}. \tag{4}$$

For a larger area the perforation probability is obviously greater. Thus, consider an area that is T times larger (T unit areas). Since each portion of the surface behaves independently, the cdf becomes

$$F_T(x) = F^T(x) = \mathrm{e}^{-e^{-z}}, \tag{5}$$

where $z = (x - \lambda)/\gamma - \ln T$, and the probability of piercing becomes

$$1 - F_T(d) = 1 - \mathrm{e}^{-e^{-u}}, \tag{6}$$

where $u = (d - \lambda)/\gamma - \ln T$.

Consider now the scaling parameter γ. It is conceivable that γ should be proportional to the corrosive influence on a unit area y. We therefore assume

$$\gamma = B\frac{\beta}{V}(t-\xi)^2, \tag{7}$$

where B is a constant.

Discussion

The "lysozyme eclipse time" (LET) ξ must bear a relation to the phage eclipse time. They obviously are of the same order of magnitude, but no direct measurement has been published as to the LET's exact magnitude. Two conditions must be fulfilled for the beginning of lysozyme production: all its constituent ingredients must be ready and there has to be an initiation command, which most likely is genetically regulated (Young, 1992).

Lysozyme production rate β is probably proportional to the phage production rate α (Hadas *et al.*, 1997; Rabinovitch *et al.*, 1999). In the *Escherichia coli*/T4 system (to be published elsewhere) α is proportional to the total ribosomal activity in the infected cell (*Ract*, calculated from the data in Bremer & Dennis, 1996). *Ract*, in turn, is proportional to $M^{1.64}$ (where M is cell mass); since cellular density (M/V) is essentially constant (Rosenberger *et al.*, 1978), in this case, β should change approximately as $V^{5/3}$. Hence, for this system, the corrosive influence on a unit area y is proportional to $V^{2/3}(t-\xi)^2$. Note that the dependence on cell size of this y, and therefore of γ, is peculiar to a living system such as the *E. coli*/T4. No such dependence exists for metal corrosion.

Equations (6) and (7) constitute a system whereby the bacterial survival probability under phage lysozyme attack can be anticipated. We urge experimentalists to perform measurements with this interesting system. Several remarks are in order:

(a) The system contains the following parameters that should be obtained via such experiments: d—the effective thickness of the envelope, ξ—the LET, and the constants λ and B.

(b) A typical measurement should consist of the number of bursting bacteria $N(t)$ of a certain size as a function of time, where the initial number is N_0. The value of $N(t)/N_0$ should behave as $1-F_T(d)$. Therefore, the value of $\ln[-\ln|N(t)/N_0|]$ should behave like $(d-\lambda)/\gamma(t)-\ln T$, whence values of $d-\lambda$, T, and B should be gleaned. Changing cell size should add a corroborating measurement. Note that since d is not directly measurable (see below), it is only the value $d-\lambda$ that can be obtained.

(c) The bacterial cell size appears twice in the equations. On the one hand, it appears in β/V [of eqn (7)] which, for the *E. coli*/T4 case, is proportional to $V^{2/3}$ or to the surface area of the cell. On the other, it occurs in the T of eqn (6). Both effects increase the bursting probability, as is seen by the following argument. In this case, eqns (6) and (7) yield for a constant time $1-F_T=1-\mathrm{e}^{-Te^{-a/S}}$, where $a=(d-\lambda)/B(t-\xi)^2$ is a constant. Measuring S in units of a we get

$$F_T=\mathrm{e}^{-S'e^{-1/S'}} \tag{8}$$

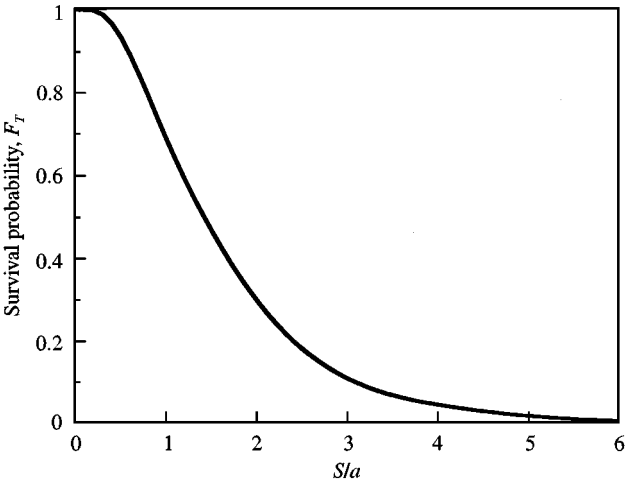

FIG. 1. Expected survival probability for the system *E. coli*/T4 (according to eqn 8) as a function of S/a (see Discussion).

where $S'=S/a$. Figure 1 depicts this survival probability F_T as a function of S/a. Note the very steep decrease of the survival probability for $S>1$. As time proceeds, a decreases, hence, for the same S, S/a increases and we move to the right in Fig. 1 (i.e. survival drops).

(d) Evidently, the best type of measurement is the one which would have directly measured the $F(z)$ distribution, namely a measurement of the distribution of "pitting depths" for an equal time operation of lysozyme on a set of equal area envelope samples. Obtaining these cdf's as a function of time would directly lead to the determination of $\gamma(t)$ for the temporal and size dependencies. Such experiments are the standard ones carried out in corrosion studies but do not seem feasible for phage action on bacteria. For example, an empirical $t^{3.4}$-dependence of the depths for super heater and economizer tubes of boilers was obtained (Fig. 4 in Shibata, 1994). Our prediction for bacteriophage/bacteria [eqn (7)] is, however $(t-\xi)^2$.

(e) Bacteria are maintained intact protected from lysis (due to their high intracellular turgor pressure; Scheie, 1973) by the rigid murein in their envelope (Höltje, 1998). This structure has been considered to be only one layer thick (Zaritsky *et al.*, 1979); thus, apparently, an adapted version of the materials corrosion analogy proposed here should have been used.

However, an intensive ultrastructural investigation (Hobot *et al*., 1984) discovered that the murein is more hydrated than had previously been assumed, and seems to fill the entire space between the inner and outer membranes in the form of a "periplasmic gel". Thus, no modification is required. This concept has recently been given another twist on theoretical grounds (Dmitriev *et al*., 1999). Thus the endolysin of T4, encoded by the late gene *e*, accumulates but does not degrade the murein layer of the envelope until released into the so-called periplasmic space. This is allowed by a regulated pore (holin), encoded by another late gene *t* (Josslin, 1971).

In the model elaborated by holin workers (Bläsi *et al*., 1999; Dressman & Drake, 1999; Loessner *et al*., 1999; Young, 1992), a high-order oligomer slowly assembles in the cell membrane, and when one or a few oligomers are completed, endolysin passes and lysis occurs. Thus, the rate-limiting factors would include the viscosity of the membrane (Zaritsky *et al*., 1985; Parola *et al*., 1990), the rate of synthesis of holin subunits and the resulting intra-membrane concentration.

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