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Dynamics of Double Stranded DNA Reptation From Bacteriophage

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Abstract

The dynamics of dsDNA release process from a phage head has been analyzed theoretically. The process was considered as dsDNA reptation through the phage tail. The driving force is assumed to be the decrease of the DNA globule free energy on its releasing from the head in the surrounding medium. The results of the equilibrium theory on an intraphage DNA globule were applied. Three possible sources of friction were examined. The first one is in the inner channel of the tail. The second is the friction of DNA segments in the whole globule volume, which is essential when the globule decondensation is a collective process, at simultaneous moving of all the turns (mechanism 1). The third is the globule friction with the capsid inner surface, that is most important when decondensation proceeds via the globule rotation as a whole spool (mechanism 2). Mechanism 1 would require a lot of time for ejection. Mechanism 2 would lead to different ejection dynamics of short- and long-tailed phages. Comparison of the theoretical results with the published experimental data argues in favor of mechanism 2.

Introduction

Study of the dynamics of cell infection by virus is of great importance not only for biology and medicine. The infection involves very interesting physical processes as well. Particularly, the nucleic acid release from the protein envelope, preceding the virus intracellular development, is an unusual example of globule-coil phase transition from the physical point of view. The very process of DNA motion, when it passes through the narrow phage tail channel, can be considered as a peculiar type of reptation (1).

In this paper we have attempted to analyze phenomenologically the basic features of the physical mechanism of the infectious process. We believe that investigation of the simplest physical mechanism of DNA transfer into a cell should be the first step

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of such an analysis, because (i) this mechanism takes place in some, if not in almost all, phages, and (ii) its investigation may prove useful in considering other possible cases.

In this paper the dynamics of infectious process for an important example of dsDNA and an icosahedral phage is analyzed. In the physical sense, this case is the simplest, since (i) the phage head is nearly spherical and (ii) dsDNA is a more uniform polymer (on a macroscopic scale) than ssDNA or RNA, and this gives grounds for a more universal description.

A lot of experimental observations concern the initial stages of bacteriophage infection, i.e. the processes, when the phage absorbs to bacterium exterior and injects the nucleic acid into the cytoplasm during several seconds. The nucleic acid ejection can also be triggered *in vitro* by the receptors isolated from host bacteria (2-5), the liposomes containing susceptible receptors (6,7), the bacterial membrane fragments, the spheroplastes (7-10) and the physical-chemical influences (11-14). In this case, the duration time depends essentially on the ejection medium state.

Many hypotheses of DNA release have been published since the discovery of this phenomenon. Their mere enumeration is spacious: the linear Brownian motion and a force exerted by oscillatory thermal movement of the part of DNA that has already entered the cell (15); the phage tail contraction and cost action like a hypodermic microsyringe (shell protein "pressure") (16-19); thermal agitation of the large molecules within the phage head ("internal pressure") (20); the presence of some cell wall compounds released after lytic enzyme action of the tail ("chemical control") (14); the repulsive forces between the negative charge on the coat protein and the negatively charged DNA (14); DNA thermal agitation within the head (21); electrostatic (21-23) or hydration forces (24) repulsion of intraphage DNA segments, packed into the phage head in a metastable state ("DNA pressure") (25); infected cell metabolic processes; the protonmotive force: a concentration gradient of ions from the inside to the outside of the cell (the chemical gradient, ΔpH), a membrane potential ($\Delta \psi$) (26-30); the use of the host transport mechanism (31); the "pilot" protein action (32) and the coupling with DNA transcription (33).

Quantitative calculations were carried out only for the DNA release by the Brownian motion (15) and the "internal pressure" (20) on the basis of a hydrodynamical approach. However, the former theory predicts a complete DNA discharge from the capsid during several hours while the second assumes non-real value of pressure in phage.

Thus, the general phenomenological approach seems to be useful. It should be based on the general concepts of intraphage DNA globular state and, as far as possible, it should be free of specific *ad hoc* suppositions on atomic scale forces in the system. Therefore, the "driving force" of the process under investigation might be simply free energy decrease of the globule. Such a theory is considered in this paper.

Earlier we have proposed (34) the equilibrium theory of the dsDNA intraphage globule state; the theory have been formulated in terms of statistical physics of macromolecules (1). It has been shown there that the DNA translocation both into bacteriophage and

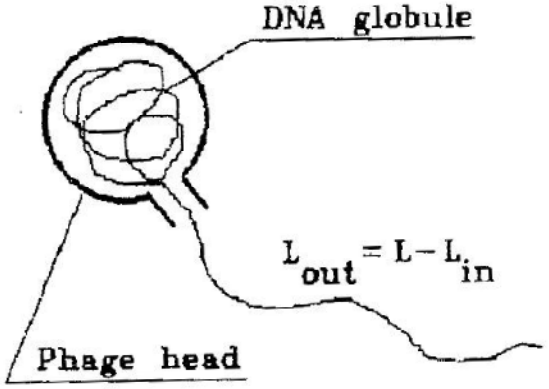

Figure 1: Schematic representation of DNA ejection from a bacteriophage.

from can be thermodynamically favorable at certain protein coat dimensions and the solution "quality" within and outside the phage (on the concept of solvent quality in polymer physics see (1)). The results of this work allow us to propose the theory of the ejection process for the case when ejection has no special highly specific mechanism and is the result of free energy decrease of the system. As emphasized in (34), in this case the only mechanism of DNA movement is its reptation along its own axis through the tail inner channel. The aim of the present paper is the investigation of the dynamics of this process.

Dynamic Models

Investigating the thermodynamic equilibrium states of dsDNA with the phage head, we considered the model of bacteriophage with the "open" DNA "portal" (34). It was shown that under definite conditions the equilibrium of the phage genome corresponds to its partial arrangement both within the protein shell (L_{in}) and in the environment (L_{out}) see (Figure 1). According to (34), the DNA equilibrium is governed by the difference in solvent qualities for DNA inside and outside the phage head. This difference can be characterized by the value of τ_{eff}, an analog of Flory-Huggins parameter χ (35); τ_{eff} was defined in (34) as the difference between energies per one persistent length of the DNA inside and outside the phage head. When τ_{eff} decrease to τ_{eff}^{prep} (τ_o designated as in (34)), the process of partial DNA globularization, i.e. its sucking into the head and condensation, appears to be thermodynamically favorable. However, it is known that after DNA packaging, the head is completed by serveral proteins which can prevent the DNA release through the portal (36). Moreover, the tail, that can be closed for phage genome by special proteins, adjoins to a finished head (12,36). These proteins can be removed both by the interaction with suitable receptors *in vivo* and *in vitro* and by some physical and chemical actions *in vitro*. Thus, the globular state of the intraphage DNA, equilibrated in a closed head, can become non-equilibrium after phage "unplugging", which, consequently, can trigger the DNA release from the shell. In other words, when the DNA portal is opened, the value of τ_{eff} can differ essentially from the value of τ_{eff}^{prep}. Therefore, the deviation

from equilibrium at the initial state of the DNA release can be characterized by the value of $\Delta\tau = \tau_{eff} - \tau_{eff}^{prep}$. Thus, it is of interest to examine the dynamics of DNA reptation caused by the difference of ejection solvent conditions from those of solvent packaging ($\Delta\tau > 0$).

As the only possible mechanism of DNA movement is its reptation through the phage tail channel, the value of L_{in} (t) (the length of the DNA remaining in the capsid up to the time t) is the only necessary generalized coordinate. Therefore, the theoretical solution of the task boils down to the obvious equation:

$$-\frac{dF}{dL_{in}} = \xi \frac{dL_{in}}{dt} \qquad [1]$$

where F is the system free energy analyzed in (34), $(-dF/dL_{in})$ is the reptation "driving force", dL_{in}/dt is the velocity of the process and ξ is a friction coefficient; diffusion and inertial terms can be neglected as it is clear from simple estimations.

The nature of friction, i.e. the determination of the ξ value, is a key problem. There are the following possible hypotheses: (A) friction takes place only in the tail channel, so $\xi = \text{const}(L_{in})$; (B) all the intraphage DNA is involved in friction, $\xi \sim L_{in}$; C) only one DNA layer is involved in friction with the inner surface of capsid, $\xi \sim L_{sur}$. In this expression, L_{sur} is the total length of macromolecule links placed in a "spool" surface layer; it was discussed in (34). Let's consider all these hypotheses.

A. The coefficient of the DNA thread friction with the inner surface of the tail core can be estimated using the known solution of the hydrodynamical problem concerning the cylinder movement along the axis of the tube filled by a liquid (37):

$$\xi = \eta_A \frac{2\pi H_t}{ln(D_t/d)} \equiv \xi_A = \text{const}, \qquad [2]$$

where H_t and D_t are the channel length and diameter, respectively, d is the dsDNA diameter, η_A is the effective viscosity of the liquid filling the channel.

B. The "mobile" model of the intraphage DNA organization during ejection (38) assumes that the mutual arrangement of the DNA links, remaining in the capsid, changes, i.e. the contacting DNA parts can slide along each other within the condensate. In this case, the friction coefficient is estimated from the above hydrodynamic considerations, but here we deal with the reptation of the whole DNA with length of L_{in} along the tube of d_{eff} diameter, formed for every DNA part by neighboring parts:

$$\zeta = \eta_B \frac{2\pi H_t}{ln(d_{eff}/d)} \equiv \xi_B L_{in} \sim L_{in}. \qquad [3]$$

C. If DNA globule is rotating as a whole during the intraphage nucleic acid release, the coefficient of friction between the globule external and capsid internal surfaces can be estimated by the known solution of the hydrodynamic problem on relative

rotation of two coaxial cylinders, the clearance between them is filled by a liquid (37):

$$\xi = \eta_C S/\Delta \equiv \xi_C L_{sur} \sim L_{sur} , \qquad [4]$$

where Δ is the clearance thickness (it is assumed to be close to $(d_{eff} - d)/2$), S_{sur} is the DNA globule with the capsid wall contact surface, L_{sur} is the length of DNA forming the globule surface layer.

All three above mentioned friction mechanism do work simultaneously, i.e.:

$$\xi = \xi_A + \beta \xi_B L_{in} + \gamma \xi_C L_{sur} , \qquad [5]$$

where β and γ coefficients depends on the release of the DNA from the condensed globule. Here various hypotheses are possible and discussed in literature: from the DNA collective motion in the whole globule volume ("the mobile ejection model" for which $\beta = 1$ and γ is near to zero; let's refer this mechanism as 1) to the globule rotation as a whole spool ("the whole spool rotation model", in this case $\gamma = 1$ and β is negligible; mechanism 2). In all cases there is friction in the tail. This friction is proportional to the tail length and can be more or less essential depending on this length.

Results and Discussion

Let's discuss the solution character of kinetic equation [1]. Of interest is the situation when at the initial moment the whole DNA is in globular state inside the capsid (the initial condition is $L_{in} = L$ when $t = 0$), and the system parameters, $\Delta\tau$ in the first place, satisfy the thermodynamic advantage of the DNA coil state outside the head. The process is triggered at $t = 0$ by the phage "uncorking" reactions or the hollow tail tube contact with a good solvent for DNA, etc.

First, let's consider the simplest case in the absence of the DNA globule interactions with the capsid inner surface, i.e. when $\sigma = 0$ in designations of (34), and τ_{eff} - the "ejection advantage" - is sufficiently great. In this case, the process "driving force" $(-dF/dL_{in})$ does not practically depend on L_{in} up to an almost complete release of DNA (except the last turn). That is why equation [1] predicts virtually a complete DNA release from the head during some finite time, but not exponential approach to the equilibrium state. For the above-mentioned cases A, B and C, the calculation of this time results in:

$$t_A \simeq \xi_A (l/kT) L / \Delta\tau, \qquad t_B \simeq \xi_B (l/kT) L^2 / (2\Delta\tau),$$

$$t_C \sim \xi_C (l/kT) L L_{sur} / \Delta\tau .$$

where l is the DNA persistent length; kT is the temperature in energetic units; L_{sur} is the length of the DNA occupying the globule surface layer at $t = 0$ and $\Delta\tau = \tau_{eff} - \tau_{eff}^{prep}$ can be referred as an effective "overheating". These results and the t_C estimation with additional assumptions that $\eta_A \approx \eta_B \sim \eta_C$, $D_t \approx d_{eff} \approx (2\Delta + d)$ and $\Delta/d \ll 1$ lead to following relations:

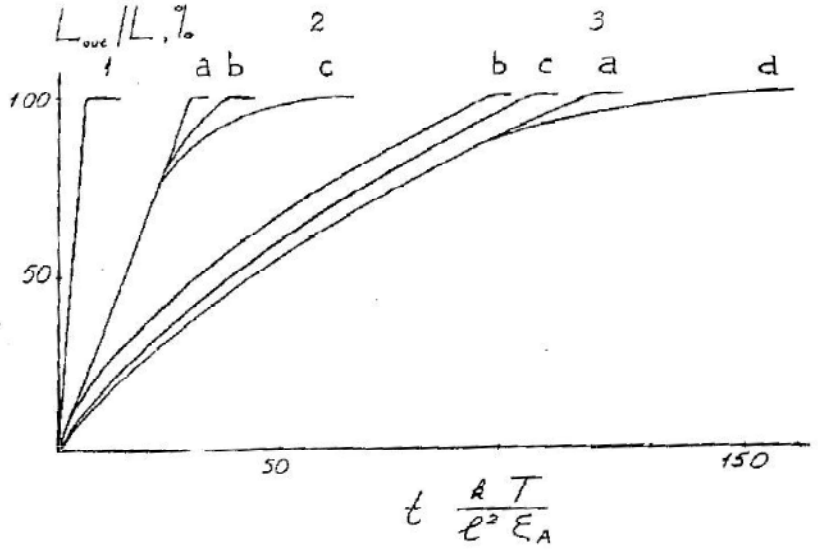

Figure 2: Time dependence of DNA molecule output for the model A.

1. $\Delta\tau = 100$ $-20 < \sigma < +20$
2. $\Delta\tau = 20$ a) $\sigma = 0$; b) $\sigma = +10$; c) $\sigma +15$
3. $\Delta\tau = 5$ a) $\sigma = 0$; b) $\sigma = -25$; c) $\sigma -5$; d) $\sigma +5$

$$t_A / t_B \approx 2 H_t / L; t_B / t_C \sim \pi L / L_{sur} \sim R / d; t_A / t_C \sim \pi H_t / L_{sur}$$

According to eq. [5] the friction mechanism with the largest coefficient and, consequently, requiring the longest time plays a basic role. Since usually for the dsDNA bacteriophages $H_t / L < 10^{-2}$, $\pi H_t / L_{sur} < 1$ and $R / d > 10$, on the above simplest assumptions when $\sigma = 0$, i.e. DNA does not interact with capsid inner walls, we draw a conclusion that: mechanism B which qualitatively corresponds to the so-called "mobile ejection" model (38) is the principal one, mechanism C is the second on significance, and mechanism A is the largest essential in the examined simplest assumptions.

The interaction of DNA with the capsid inner surface can essentially change the situation. It is qualitatively clear that in case of attraction ($\sigma > 0$), the viscosity η_C can be large enough, so time t_C and, in general, the role of mechanism C increase essentially. On the contrary, in the case of repulsion ($\sigma < 0$) the role of mechanism C is decreased and mechanism A can compete with it, especially when the phages have long tails.

The results of equation [1] numerical solutions are presented in Figures 2-4. (Calculations have been made with parameters of phage $S_d = R / l = 0{,}64$; $R / d = 16$, $N = 600$ (39,40)).

In case A the shape of $L_{out} = L - L_{in}$ dependence on time t curve is changed from the

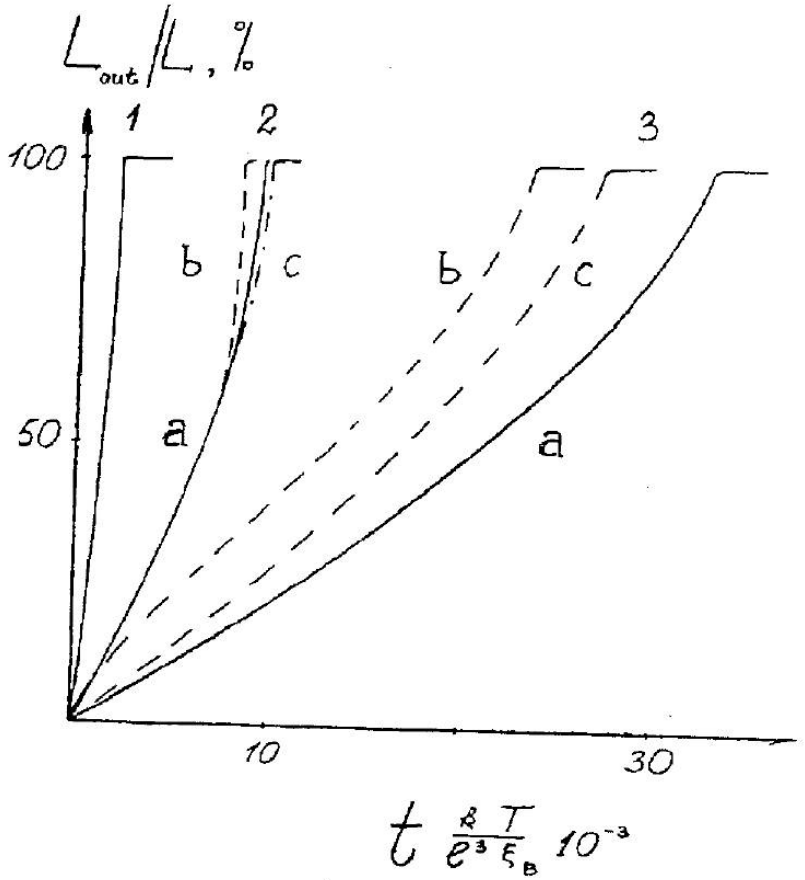

Figure 3: Time dependence of DNA molecule output for the model B.

1.	$\Delta\tau = 100$	$-20 < \sigma < +20$
2.	$\Delta\tau = 20$	a) $\sigma = 0$; b) $\sigma = -25$; c) $\sigma + 15$
3.	$\Delta\tau = 5$	a) $\sigma = 0$; b) $\sigma = -25$; c) $\sigma - 5$

exponential on "weak overheating" (i.e., $\tau_{eff} \cong \tau_{eff}^{prep}$ as the DNA reptation rate decreases during its release from the capsid) to a practically linear one corresponding to the constant reptation velocity while τ_{eff} is large enough (Figure 2).

In case B, the reptation rate is almost constant when τ_{eff} is close to τ_{eff}^{prep}, whereas at large τ_{eff} it gradually increases approximately proportional to L_{in}^{-1} (Figure 3).

The results of model C (a "rotating spool") are intermediate between the above discussed ones. To receive them, the dependence of the DNA surface layer length L_{sur} on the whole intraphage DNA length L_{in} should be taken from our previous work (34). The character of this dependence changes on σ variation, therefore the shape of the kinetic curves depends essentially on σ (Figure 4).

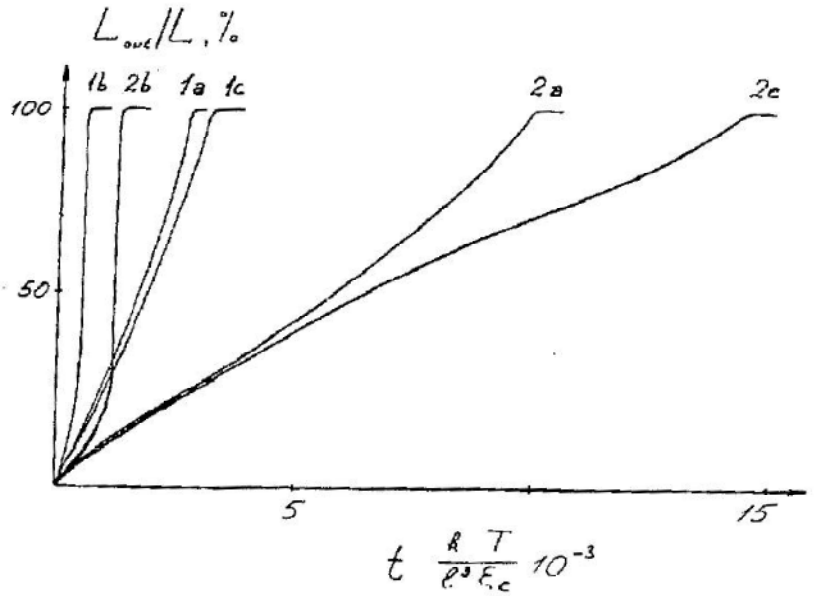

Figure 4: Time dependence of DNA molecule output for the model C.
1. $\Delta\tau = 20$ a) $\sigma = 0$; b) $\sigma = -5$; c) $\sigma = +5$
2. $\Delta\tau = 5$ a) $\sigma = 0$; b) $\sigma = -5$; c) $\sigma = +5$

The ejection time absolute values depend on the d / D_p, d / d_{eff} ratios and the effective viscosity values which are not strictly determined and, moreover, can be different for various phages. Therefore, we can estimate them only approximately. Supposing that η = 0,1-0,01 poise, D_t = 30-50 Å, H_t = 200-2000 Å, d = 20-25 Å, we obtain the following results. For a complete DNA release from the phage head, the necessary average effective "overheating" must be $\Delta\tau \sim 1, \sim 10$ in cases A, B, and C, respectively.

A special situation can take place when mechanism 2 is realized for long-tailed phages (with large enough H_t / R values) if there is repulsion ("negative affinity") of DNA from the capsid inner surface (sufficiently large $|\sigma|$). In this case, the DNA reptation dynamics will be essentially conditioned by the friction in the tail axial channel and practically will not depend on the decondensation dynamics of the intraphage DNA. Then both for long- and short-tailed phages the difference in the external and internal solution conditions required for rapid genome ejection will not exceed $\Delta\tau \sim 1$.

Conclusions

The dynamics of DNA release has been studied in a number of experimental papers. An interesting and important peculiarity is that the linear or exponential curves analogous to those in Figure 1 are observed for phages T2, T4, T5, λ and *No* 1

Bac.mycoides (2-5,11,13,14,29,41) whereas the curve for phage S_d (11) is practically similar to the ones in Figures 2 or 3. This fact is in a good qualitative agreement with the theory, so far as phages of the first group have long tails and, consequently, mechanism A is important for them but not for the short tailed phages like the S_d one.

There are also data on the dependence of the release time on temperature (14,29,31,41,42) and solution composition (14). The time varies from some seconds to hours in the temperature range 50°C - 4°C. Such dispersion can not be simply explained by the temperature inverse proportion of the viscosity, friction or diffusion coefficient, etc. In our theory this dependence is naturally explained by the phase character of the globule-coil transition which determines the sharp dependence of the system properties on τ_{eff} which in its turn depends on temperature.

Summarizing the qualitative conclusions of the theory, it should be noted that very large ("non-economical") difference inside and outside of the phage solution is required for the DNA release in an acceptable time by mechanism 1 which is analogous to the "mobile ejection" mechanism. On the other hand, in the second mechanism ("the whole spool rotation" type) the virus "pre-ejection modification", being connected with the capsid inner surface "negative affinity" to the DNA, would promote rapid ejection of the nucleic acid. This event would also initiate the DNA globule ("spool") rotation. If this hypothesis is correct, the ejection drag will be either the DNA globule friction on the capsid inner surface or the friction in the tail core one. Consequently, the situations for long- and short-tailed phages will be different and the time dependence of the length of the DNA releasing part will be approximately close to that represented in Figures 1 or 3. This difference has been observed experimentally (11).

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