Heterogeneous anomalous diffusion of a virus in the cytoplasm of a living cell

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Abstract The infection pathway of a virus in the cytoplasm of a living cell is studied from the viewpoint of diffusion theory, based on a phenomenon observed by single-molecule imaging. The cytoplasm plays the role of a medium for stochastic motion of a virus contained in an endosome as well as a free virus. It is experimentally known that the exponent of anomalous diffusion fluctuates in localized areas of the cytoplasm. Here, generalizing the fractional kinetic theory, such fluctuations are described in terms of the exponent locally distributed over the cytoplasm and a theoretical proposition is presented for its statistical form. The proposed fluctuations may be examined in an experiment of heterogeneous diffusion in the infection pathway.

Keywords Fluctuations • *HeLa* cell • Adeno-associated virus • Endosome • Infection pathway • Fractional kinetic theory

1 Introduction

In recent years, an exotic phenomenon has experimentally been observed by making use of the technique of real-time single-molecule imaging in the infection pathway of adeno-associated viruses in the cytoplasm of a living *HeLa* cell [1, 2]. In each experiment, a virus solution of low concentration, in which the virus is labeled with a fluorescent dye molecule, was added to a culture medium of the living cells. Then, the trajectories of the fluorescent viruses in the cytoplasm were observed. The experiments show that the

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adeno-associated virus exhibits stochastic motion inside the cytoplasm in two different forms: one is in the free form and the other is the form contained in the endosome (i.e., a spherical vesicle).

Let $\overline{x^2}$ be the mean square displacement in stochastic motion. In general, it scales for large elapsed time, t , as

$$\overline{x^2} \sim t^\alpha. \quad (1)$$

Normal diffusion reads $\alpha = 1$ whereas $0 < \alpha < 1$ ($\alpha > 1$) corresponds to subdiffusion (superdiffusion). The result of experimental observation shows that the trajectory of the virus exhibits not only $\alpha = 1$ but also $0 < \alpha < 1$ in the form of (1). However, what is truly remarkable is the fact [1] that, in the case of subdiffusion, α fluctuates between 0.5 and 0.9, depending on the localized areas of the cytoplasm. This manifests the *heterogeneous* structure of the cytoplasm as a medium for stochastic motion. It is noted [1] that this heterogeneity is not due to the forms of existence of the virus (i.e., being free or contained in the endosome). Thus, this phenomenon is in marked contrast to traditional anomalous diffusion [3] discussed for physical systems, such as particle motion in a turbulent flow [4], charge carrier transport in amorphous solids [5], the flow of a contaminated vortex in a fluid [6], chaotic dynamics [7], porous glasses [8], and so on. The experimental observation may be due to the technique of single-molecule imaging. In more recent literatures [9–11], the analysis of trajectories observed by such a technique has been discussed for systems showing anomalous diffusion.

The experimental result mentioned above poses a novel and interesting problem for the physics of diffusion. On the other hand, in biology, it is essential to understand the virus infection process for both designing an antiviral drug and developing efficient gene therapy vectors. It is therefore of obvious importance to investigate the virus infection pathway from a physical viewpoint. In fact, the intracellular dynamics of viruses is studied based on a stochastic approach in the literatures [12, 13].

In this paper, we study the infection pathway of the adeno-associated virus in the cytoplasm of the living *HeLa* cell by generalizing the traditional theory of anomalous diffusion, based on the phenomenon observed by single-molecule imaging. We regard the cytoplasm as a medium for stochastic motions of both the free virus and the virus contained in the endosome. Then, we imaginarily divide the medium into many small blocks. In other words, a block is identified with a localized area of the cytoplasm. This procedure seems to be necessary when the infection pathway of the virus in the entire cytoplasm is considered. The mean square displacement of the virus does not always show normal diffusion and/or subdiffusion with a fixed exponent, since the virus in a given localized area moves to neighboring ones before reaching the nucleus of the cell [1]. Thus, the exponent, α , in (1) locally fluctuates from one block to another in the cytoplasm. Furthermore, we consider that this fluctuation varies slowly over a period of time, which is much longer than the time scale of the stochastic motion of the virus in a localized area of the cytoplasm. It is therefore assumed that there is a large time-scale separation in the infection pathway. For the virus in each block, we apply the fractional kinetic theory, which generalizes Einstein's approach to Brownian motion [14]. Generalizing the traditional fractional kinetic theory, we describe the local fluctuations of the exponent, α . We propose the statistical form of fluctuations from the experimental data. We then show that the proposed form of fluctuations can be derived by the maximum entropy principle [15].

2 Stochastic motion of the virus

Let us start our discussion with the motion of the virus in a one-dimensional block (i.e., a segment). To describe it, we consider the following evolution equation based on the scheme of continuous-time random walks [16]:

$$f(x, t) dx = dx \int_{-\infty}^{\infty} d\Delta \int_0^t d\tau f(x + \Delta, t - \tau) \phi_{\tau}(\Delta) \psi(\tau) + \delta(x) R(t) dx, \quad (2)$$

where $f(x, t) dx$ is the probability of finding a virus in the interval $[x, x + dx]$ at time t . The first term on the right-hand side describes all of the possible probabilities that the virus moves into the interval from outside or stays in the interval. Then, the second term is a partial source guaranteeing the initial condition $f(x, 0) = \delta(x)$ from which the condition $R(0) = 1$ follows. $\phi_{\tau}(\Delta)$ is the normalized probability density distribution for a displacement, Δ , in a finite time step, τ . This distribution is sharply peaked at $\Delta = 0$ and fulfills the condition, $\phi_{\tau}(\Delta) = \phi_{\tau}(-\Delta)$. τ in (2) is treated as a random variable following the normalized probability density, $\psi(\tau)$, which satisfies $\psi(0) = 0$. $R(t)$ describes a time-dependent partial source and is connected to $\psi(\tau)$ through the relation $R(t) = 1 - \int_0^t d\tau \psi(\tau)$, which comes from the normalization condition for $f(x, t)$. In particular, in the deterministic case, $\psi(\tau) = \delta(\tau - \tau_0)$, (2) becomes reduced to the basic equation in Einstein's approach to Brownian motion [14] after the replacement, $t \rightarrow t + \tau_0$.

Now, an origin of subdiffusion found in the experiments may not be in $\phi_{\tau}(\Delta)$ but in $\psi(\tau)$. Accordingly, we assume in what follows that $\phi_{\tau}(\Delta)$ is actually independent of time steps: $\phi_{\tau}(\Delta) = \phi(\Delta)$. To see how (2) leads to subdiffusion, we here employ the Laplace transform of (2) with respect to time:

$$\tilde{f}(x, u) = \int_{-\infty}^{\infty} d\Delta \tilde{f}(x + \Delta, u) \phi(\Delta) \tilde{\psi}(u) + \delta(x) \frac{1 - \tilde{\psi}(u)}{u}, \quad (3)$$

where $\tilde{f}(x, u)$ and $\tilde{\psi}(u)$ are the Laplace transforms of $f(x, t)$ and $\psi(\tau)$ respectively, provided that $\mathcal{L}(g)(u) = \tilde{g}(u) = \int_0^{\infty} dt' g(t') e^{-ut'}$. Then, we require $\tilde{\psi}(u)$ to have the following form:

$$\tilde{\psi}(u) = \tilde{\psi}_{\alpha}(u) \sim 1 - (su)^{\alpha}. \quad (4)$$

Here, s is a characteristic constant with the dimension of time. This characteristic time is indicative of the time at which the virus is displaced. We also impose the condition that $\psi(\tau)$ has a divergent first moment, so that the exponent α is in the interval $(0, 1)$. Equation (4) implies that $\psi(\tau)$ decays as a power law, $\psi(\tau) \sim s^{\alpha} / \tau^{1+\alpha}$, for a time step τ longer than s . Later, we shall show how the traditional fractional kinetic theory [17] is derived from (3) and (4). The mean square displacement of the virus turns out to have the form in (1), reproducing the behavior observed in a localized area of the cytoplasm. In particular, in the present theory, the case of normal diffusion is realized in the limit $\alpha \rightarrow 1$.

3 Fluctuations of the exponent of anomalous diffusion

Now, the virus moves from one block to another and in such a process the exponent α locally fluctuates over the cytoplasm. We shall therefore develop a generalized fractional kinetic theory, in which this fluctuation is incorporated. To do so, it is essential to clarify the statistical property of the fluctuations. According to the experiment [1], the trajectories of 104 viruses were analyzed. 53 trajectories among them exhibit $\alpha = 1$ in (1) and another 51 show α varying between 0.5 and 0.9. Besides this fact, no further information is available about the weights of $\alpha \in (0.5, 0.9)$. Although α for the virus contained in the endosome might be different from that for the free virus in general, we here assume that the exponents found in both the free and endosomal forms differ from each other only slightly. The virus tends to reach the nucleus of the cell. Due to this tendency, an exponent near $\alpha = 0$ may seldom be realized. On the other hand, normal diffusion is often the case. From these considerations of the experimental data, we propose an exponential form of fluctuations:

$$P(\alpha) \sim e^{\lambda\alpha} \quad (5)$$

with a positive constant λ .

Now, we show that the distribution in (5) can be derived by the maximum entropy principle. To do so, let us recall that the cytoplasm is regarded as a medium being imaginarily divided into many small blocks with different values of the exponent. In other words, the cytoplasm is viewed as a collection of these blocks. Therefore, from this viewpoint, considering distinct collections in terms of the local fluctuations of the exponent, we evaluate the entropy associated with the fluctuations of the exponent for a block in the medium.

We form all of the distinct collections by constructing the blocks, since there is no information available about how the exponent is locally distributed over the medium. The fluctuations of the exponent in a given collection are statistically equivalent to those in other collections but are locally not. In the case of discrete values of the exponent, the total number of the distinct collections, G , is given by, $G = N! / (n_{\alpha_1}! n_{\alpha_2}! \cdots n_{\alpha_A}!)$, where N is the total number of blocks in the medium, while n_{α_i} ($i = 1, 2, \dots, A$), satisfying $\sum_{i=1}^A n_{\alpha_i} = N$ (with A being the number of different exponents found in the medium), stands for the number of blocks with exponent, α_i . Here, $N!$ is divided by each of the $n_{\alpha_i}!$'s to eliminate the collections, which are equivalent to a given collection in terms of the local fluctuations of the exponent.

Let us now evaluate the quantity $(\ln G)/N$, as the entropy associated with the fluctuations of the exponent for a block in the medium. It turns out that this quantity can be approximately expressed by the Shannon entropy, $(\ln G)/N \cong -\sum_{i=1}^A P_{\alpha_i} \ln P_{\alpha_i}$, where $P_{\alpha_i} = n_{\alpha_i}/N$ is the probability of finding the exponent α_i in a given block. In deriving this expression, assuming that N and n_{α_i} are large since the medium is divided into many blocks, Stirling's approximation (i.e., $\ln(M!) \cong M \ln M - M$ for large M) was employed. Thus, in the case of continuous values of the exponent, the quantity corresponds to the following form of the Shannon entropy:

$$S[P] = - \int_0^1 d\alpha P(\alpha) \ln P(\alpha). \quad (6)$$

We shall derive the distribution in (5) based on the maximum entropy principle with the Shannon entropy in (6). The situation we consider here is the case when information is only

available about the statistical property of the fluctuations of the exponent. In this case, not only the normalization condition but also the expectation value of α should be constrained:

$$\int_0^1 d\alpha P(\alpha) = 1, \quad (7)$$

$$\int_0^1 d\alpha P(\alpha) \alpha = \bar{\alpha}. \quad (8)$$

Accordingly, we maximize $S[P]$ with respect to $P(\alpha)$ under these constraints:

$$\delta_P \left\{ S[P] - \kappa \left(\int_0^1 d\alpha P(\alpha) - 1 \right) + \lambda \left(\int_0^1 d\alpha P(\alpha) \alpha - \bar{\alpha} \right) \right\} = 0, \quad (9)$$

where κ and λ are the Lagrange multipliers associated with the constraints in (7) and (8), respectively, and δ_P denotes the variation with respect to $P(\alpha)$. The solution of (9) is given by $\hat{P}(\alpha) \sim e^{\lambda\alpha}$, the distribution in (5).

Thus, we see that the proposed distribution in (5) can be derived by the maximum entropy principle with the Shannon entropy in (6). A crucial point here is that the cytoplasm is regarded as a medium being imaginarily divided into many small blocks. This offers a discussion for the entropy associated with the fluctuations of the exponent.

Closing this section, we mention the following point for the organization of the cytoplasm. In the experiments [1, 2], it is pointed out that the nature of subdiffusion is due to obstacles in the cytoplasm. Accordingly, this may indicate that the statistical distribution of the obstacles over the cytoplasm is given by the distribution in (5).

4 Theory for the infection pathway of the virus in the cytoplasm

Next, we formulate the generalized fractional kinetic theory based on the distribution in (5). α slowly varies locally but is approximately constant while the virus moves through the localized areas in the entire cytoplasm. So, the effective distribution of the time step in the Laplace space appears as a superposition of $\tilde{\psi}_\alpha(u)$ with respect to $P(\alpha)$:

$$\tilde{\psi}(u) = \int_0^1 d\alpha P(\alpha) \tilde{\psi}_\alpha(u). \quad (10)$$

Substituting (10) into (3), expanding $\tilde{f}$ up to the second order of Δ , and neglecting the term $\langle \Delta^2 \rangle \int_0^1 d\alpha P(\alpha) (su)^\alpha$ (u being small in long time behavior) with $\langle \Delta^2 \rangle \equiv \int_{-\infty}^{\infty} d\Delta \Delta^2 \phi(\Delta)$, we find

$$\tilde{f}(x, u) = \frac{\langle \Delta^2 \rangle}{2 \int_0^1 d\alpha P(\alpha) (su)^\alpha} \frac{\partial^2 \tilde{f}(x, u)}{\partial x^2} + \delta(x) \frac{1}{u}. \quad (11)$$

Equivalently, performing the inverse Laplace transform of (11), we obtain the following generalized fractional diffusion equation:

$$\int_0^1 d\alpha P(\alpha) s^{\alpha-1} {}_0D_t^{-(1-\alpha)} \frac{\partial f(x, t)}{\partial t} = D \frac{\partial^2 f(x, t)}{\partial x^2}, \quad (12)$$

where D is the diffusion constant calculated as $D = \langle \Delta^2 \rangle / (2s)$ and the fractional operator $\mathcal{L}({}_0D_t^{-\alpha} g(x, t))(u) = u^{-\alpha} \tilde{g}(x, u)$ is used. For the virus in [17] a given local block,

the distribution of fluctuations is taken to be $P(\alpha) = \delta(\alpha - \alpha_0)$ in (12). This leads to the traditional fractional kinetic theory [17] after applying the operator, $(\partial/\partial t)_0 D_t^{-\alpha_0}$ to (12), as mentioned earlier.

Equation (12) is the main result of our theory describing the infection pathway of the virus in the entire cytoplasm.

We make a comment on the motion of the virus in the infection pathway. For it, we characterize the motion of the virus by mean square displacement. Using (11), the mean square displacement of the virus in the Laplace space is calculated as

$$\mathcal{L}(\overline{x^2})(u) \sim \frac{\ln(e^\lambda s u)}{u(e^\lambda s u - 1)}. \quad (13)$$

Accordingly, the mean square displacement is given by

$$\overline{x^2} \sim \ln\left(\frac{t}{e^\lambda s}\right) + \exp\left(\frac{t}{e^\lambda s}\right) E_1\left(\frac{t}{e^\lambda s}\right) + \gamma, \quad (14)$$

where $E_1(t) = \int_t^\infty dt' t'^{-1} e^{-t'}$ is an exponential integral [18] and Euler's constant is introduced as $\gamma = -\int_0^\infty dk e^{-k} \ln k$. For a large elapsed time t much longer than $e^\lambda s$, the mean square displacement in (14) behaves as

$$\overline{x^2} \sim \ln\left(\frac{t}{e^\lambda s}\right) + \gamma, \quad (15)$$

where the second term in (14) was neglected since it tends to decrease as t becomes very large [18]. Equation (15) implies that the motion of the virus shows logarithmic behavior. If the behavior of the virus in (15) can experimentally be observed, it is anticipated that the existence of a large time-scale separation may be a possible mechanism in the infection pathway.

It should be recalled that the adeno-associated virus is in the cytoplasm, freely as well as in the form being contained in the endosome. Theoretically, the discrimination between them could result in different values of the diffusion constant D , as performed in the experiments [1, 2]. The experiments study the characterization of the motion of the virus not only in the cytoplasm but also in the nucleus of the living cell. In order to examine if the theory formulated here for heterogeneous anomalous diffusion reproduces the experimental results, detailed information about the heterogeneity of diffusion in the cytoplasm is necessary.

5 Conclusions

We have studied the infection pathway of an adeno-associated virus in the cytoplasm of a living *HeLa* cell from the viewpoint of diffusion theory, based on a phenomenon observed by single-molecule imaging. We have regarded the cytoplasm as a medium for stochastic motions of both the free virus and the virus contained in the endosome. Dividing the cytoplasm into many small virtual blocks, we have described the local fluctuations of the exponent of subdiffusion of the virus. We have assumed slow variation of the exponent and proposed the form of its statistical distribution based on the maximum entropy principle. Then, we have formulated the generalized fractional kinetic theory by introducing heterogeneity of diffusion. It is of extreme interest to examine if the proposed fluctuation

distribution in (5) is realized in an experiment of heterogeneous diffusion in the infection pathway under the same experimental conditions.

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