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Early Molecular Events During the Interaction of Enveloped Riboviruses with Cells

II. A Kinetic Study

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Abstract. A kinetic model was constructed and partly solved to describe the migration of the fluorescence label 1,6-diphenylhexatriene (DPH) in both directions when enveloped viruses, labelled with DPH in their envelopes are in contact with unlabelled cells or cell labelled in their membranes are in contact with unlabelled enveloped viruses. The central assumption is that two types of receptor sites exist on the cell surface, i.e., physical adsorption sites (*P*-sites), available to all the viruses studied in these papers and binding sites (*B*-sites) available only to the viruses which penetrate into the specific cells.

The differential equations for the label migration, for different values of the ratio number of viruses number of sites were numerically solved, assuming different fractions of *P*- and *B*-sites.

The equations also describe, appropriately the mechanism of rapid label migration in the system and substantiate the magnitude "time of residence" of the nonpenetrating viruses adsorbed on the cell surface. The resulting curves match satisfactorily those for the label release by the viruses and account well for the steady state values of the kinetics of label migration in the virus-cell system.

Key words: DPH-migration – Virus adsorption – Virus penetration – Cells membranes.

Introduction

The penetration of enveloped viruses into cells is a multistage process [1, 2]. These stages occur with different rates [2] but, whatever the mechanism of the virus-cell interaction, the first stage taking place is the physical adsorption of the virus particles on the cell surface [2].

Detailed NMR [3] fluorescence and radioactivity investigations [4] have produced evidence regarding the kinetic aspects of the interactions occuring

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between a number of enveloped riboviruses and cells. The systems investigated [3, 4] consisted of the following viruses:
– Newcastle disease virus (NDV) – a paramyxovirus whose lipid envelope fuses with the cell membrane.
– The influenza virus – trypsinized ($N + T$) in order to cleave the virus glycoprotein spikes and make the virus infectious for chick embryo fibroblasts (CEC).
– The influenza virus, strain N, grown on egg chorioallantoic membranes, infectious for the chick embryo fibroblasts.
– The nontrypsinized influenza virus ($N - T$) noninfectious, i.e., nonpenetrating into the CEC.
– The Rous sarcoma virus (RSV) which was used with two CEC lines, one susceptible to this virus (C/E) and one nonsusceptible (C/B).

The study of the virus-cell interaction, by means of the fluorescence polarization degree measurements of diphenylhexatriene (DPH) embedded into the cell (or virus) membrane (envelope) lipids and of the time-resolved fluorescence measurements indicated, as a general conclusion, that the penetrating viruses were releasing the fluorescence label into the cells at rates identical or very close to those at which the label was released by the cells into the viruses, while the nonpenetrating viruses released the label into the cells, significantly faster than the cells released it into the viruses. The radioactivity measurements [4] indicated also significant differences in the rate of migration of cholesterol from the virus envelope into the cell membranes, between penetrating and nonpenetrating viruses.

In the present paper we construct and numerically solve a kinetic model of the system we have measured, which is based, as generally as possible on the kinetic mechanisms which may be reasonably expected to occur at a molecular level. We deal with a system which contains a total of V_0 viruses and C_0 cells, with S_0 adsorption sites.

We assume that these sites may be divided into two classes, of which αS_0 are P-sites where physical adsorption of viruses may take place; $(1 - \alpha)S_0$ are B-sites where binding of the penetrating viruses only may occur. Let s_P and s_b denote the numbers of vacant P- and B-sites respectively.

Of the V_0 viruses, let V_P^* be adsorbed at P-sites, V_b^* at B-sites and

$$V = V_0 - V_P^* - V_b^* , \tag{1}$$

to remain free in suspension. We also have

$$\alpha S_0 = s_P + V_P^* , \tag{2}$$

$$(1 - \alpha)S_0 = s_b + V_b^* . \tag{3}$$

Thus

$$V = V_0 - S_0 + s_P + s_b . \tag{4}$$

Abbreviations: B virus = penetrating virus; P virus = nonpenetrating virus; NDV = Newcastle disease virus; RSV = Rous sarcoma virus; CEC = chick embryo fibroblasts; $(N + T)$ = trypsinized influenza virus, strain N; $(N - T)$ = nontrypsinized influenza virus, strain N

We can expect

$$\frac{ds_P}{dt} = - k_a s_P V + k'_P V^*_P \,, \tag{5}$$

and

$$\frac{ds_b}{dt} = - k_a s_b V + k'_b V^*_b \,, \tag{6}$$

to describe the adsorption kinetics. Eliminating V, V^*_P, V^*_b we obtain

$$\frac{ds_P}{dt} = - k_a s_P (V_0 - S_0 + s_P + s_b) + k'_P(\alpha S_0 - s_P) \,, \tag{7}$$

$$\frac{ds_b}{dt} = - k_a s_b (V_0 - S_0 + s_P + s_b) + k'_b[(1 - \alpha) S_0 - S_P] \,, \tag{8}$$

where k_a, k'_P, and k'_b are rate constants whose value we discuss presently. It is convenient to define the following dimensionless quantities

$$\xi = k_a S_0 t \,,$$

$$\sigma_P = s_P / s_0 \,,$$

$$\sigma_b = s_b / S_0 \,,$$

$$R_P = k'_P / k_a S_0 \,,$$

$$R_b = k'_b / k_a S_0 \,,$$

$$\nu_0 = V_0 / S_0 \,, \tag{9a–f}$$

so that

$$\frac{d\sigma_P}{d\xi} = - \sigma_P (\nu_0 - 1 + \sigma_P + \sigma_b) + R_P(\alpha - \sigma_P) \,, \tag{10}$$

$$\frac{d\sigma_b}{d\xi} = - \sigma_b (\nu_0 - 1 + \sigma_P + \sigma_b) + R_b(1 - \alpha - \sigma_b) \,. \tag{11}$$

We note that at steady state, Eqs. (10, 11) yield the Langmuir adsorption isotherm. The kinetics of label transfer is evidently more complicated. Clearly, fast transfer can only take place between adsorbed viruses and the cells to which they are attached [4]. It is likely that two mechanisms operate here. In the case of nonpenetrating viruses, transfer takes place by reversible diffusion from the labelled lipid bilayer to label-free one. Labels move also from the penetrating

viruses by a similar process prior to the penetration of these viruses into the host cells. To formulate the kinetics, let $L(t)$ be the total number of labels in the cell membranes and let $V_b^*(n, t)$ and $V_P^*(n, t)$ be the numbers of adsorbed viruses of B and P type which contain n labelled molecules. Finally let $V(n, t)$ be the number of free viruses which contain n labels. The mechanism above may be thus represented by the infinite reaction scheme

$$V_P^*(n, t) \underset{k'_P(n)}{\overset{k_P(n)}{\rightleftharpoons}} V_P^*(n-1, t) + L(t) \quad (n = 0, 1, \ldots,) ,$$

and

$$V_b^*(n, t) \underset{k'_B(n)}{\overset{k_B(n)}{\rightleftharpoons}} V_b^*(n-1, t) + L(t) \quad (n = 0, 1, \ldots,) .$$

Naturally, interchange of adsorbed and free nonpenetrating viruses occurs simultaneously. We shall assume that

$$k_P(n) = nk_\pi ,$$

$$k_B(n) = nk_\beta ,$$

$$k'_P(n) = k'_\pi ,$$

$$k'_B(n) = k'_\beta . \tag{12a–d}$$

Also, the relations

$$\sum_{n=0}^{\infty} V_P^*(n, t) = V_P^*(t) , \tag{13}$$

$$\sum_{n=0}^{\infty} V_b^*(n, t) = V_b^*(t) , \tag{14}$$

$$\sum_{n=0}^{\infty} V(n, t) = V(t) , \tag{15}$$

$$\sum_{n=0}^{\infty} n[V_P^*(n, t) + V_b^*(n, t) + V(n, t)] + L(t) = L_0 , \tag{16}$$

must hold, where L_0 is the total number of labelled molecules.

We can write the differential equations corresponding to the above reaction scheme and the simplification (12) as

$$\frac{dV_P^*(n, t)}{dt} = k_a S_0 V(n, t) - k'_P V_P^*(n, t) + (n+1)k_\pi V_P^*(n+1, t) + k'_\pi L(t) V_P^*(n-1, t) - nk_\pi V_P^*(n, t) - k'_\pi L(t) V_P^*(n, t) , \tag{17a}$$

and

$$\frac{dV_b^*(n,t)}{dt} = k_a S_0 V(n,t) - k_b' V_b^*(n,t) + (n+1)k_\beta V_b^*(n+1,t) + k_\beta' L(t) V_b^*(n-1,t) - nk_\beta V_b^*(n,t) - k_\beta' L(t) V_b^*(n,t)\,, \tag{17b}$$

for $n = 0, 1, \ldots, \infty$, where the fourth terms of both equations are absent in the case $n = 0$.

Fortunately, we do not need to know the individual quantities, $V_P^*(n,t)$ and $V_B^*(n,t)$, but instead only the total number of labels in cells $L(t)$ and in viruses

$$L_0 - L(t) = \sum_{n=1}^{\infty} n[V_B^*(n,t) + V_P^*(n,t) + V(n,t)]\,, \tag{18}$$

which suggests that we multiply Eq. (17) by n and sum over all values of it. We must also have that

$$\frac{dL(t)}{dt} = k_\pi \sum_{n=1}^{\infty} n V_P^*(n,t) + k_\beta \sum_{n=1}^{\infty} n V_b^*(n,t) - L(t)\left\{k_\pi' \sum_{n=0}^{\infty} V_P^*(n,t) + k_\beta' \sum_{n=0}^{\infty} V_b^*(n,t)\right\}. \tag{19}$$

Again it is useful to define the following dimensionless quantities

$$\Gamma_B = \left[\sum_{n=1}^{\infty} n V_b^*(n,t)\right]/L_0\,, \tag{20a}$$

$$\Gamma_P = \left[\sum_{n=1}^{\infty} n V_P^*(n,t)\right]/L_0\,, \tag{20b}$$

$$l(t) = L(t)/L_0\,, \tag{20c}$$

$$T_B' = k_\beta'/k_a\,, \tag{20d}$$

$$T_P' = k_\pi'/k_a\,, \tag{20e}$$

$$T_B = k_\beta/k_a S_0\,, \tag{20f}$$

$$T_P = k_\pi/k_a S_0\,, \tag{20g}$$

after rearrangement, we find

$$\frac{dl}{d\xi} = T_B\Gamma_B + T_P\Gamma_P - T_B' l(1 - \alpha - \sigma_B) - T_P' l(\alpha - \sigma_P)\,, \tag{21}$$

$$\frac{d\Gamma_P}{d\xi} = \sigma_P - \sigma_P\Gamma_B - \Gamma_P(\sigma_P + R_P + T_P) - l[\sigma_P - T_P'(\alpha - \sigma_P)]\,, \tag{22}$$

and

$$\frac{dI_B}{d\xi} = \sigma_B - \sigma_B\Gamma_P - \Gamma_B(\sigma_B + R_B + T_B) - I[\sigma_B - T'_B(1 - \alpha - \sigma_B)] , \tag{23}$$

where the dimensionless time $\xi = k_a S_0 t$ and R_P, R_B are given in Eq. (9a–f). Although Eqs. (21–23) are linear they involve the unknown solutions σ_P, σ_B of the nonlinear Eqs.(10, 11). Thus, even though it is possible to make some analytic progress with the equations, the resulting formulae are extremely complicated and thus not of much use.

Instead, we have followed the course of numerically integrating the Eqs. (10, 11, 21–23) using a 4th order Runge-Kutta method, treating the constants which appear as adjustable, within the following constraints: whereas the B-viruses, once absorbed, are very unlikely to desorb, the viruses adsorbed at P-sites only have a much shorter average residence time. This implies that R_P is very much greater than R_B. The constant ν_0 will naturally depend on the ratio number of viruses: number of sites, virus dimensions and cell dimensions.

If D_v is the diameter of a virus and D_c that of a cell, and there are respectively V_0 viruses and C_0 cells, we can estimate ν_0 as

$$\nu_0 = \frac{D_v^2 V_0}{4 D_c^2 C_0} , \tag{24}$$

if we assume that particle dimensions are the limiting factors. Equation (24) thus gives an upper limit on the value of ν_0. The parameter α is of course completely unknown, although it must be appreciable or else no difference would be observed between penetrating and nonpenetrating viruses. On the other hand it cannot be too large (close to unity) or else only the label molecules present in the penetrating viruses first adsorbed on the cell surface would be transfered, i.e., a small fraction of the total label concentration.

The constants T_P, T'_P, T_B, T'_B must now be chosen. Since the kinetics of transfer when the cells are initially labelled are fairly independent of the nature of the viruses, we feel justified in assigning

$$T'_B = T'_P . \tag{25}$$

However, since the rate constant for the transfer of label molecules from virus to cell appears to be rather greater for nonpenetrating viruses, we might suspect that

$$T_P > T_B , \tag{26}$$

although this phenomenon may only be a reflection of the much longer residence time of B-viruses on the B-sites. It now remains to specify the connection between fluorescence experimental measurements and the various kinetic parameters we wish to calculate. Let P_v be the initial measured polarization degree when all labels are in viruses and P_c that when they are all in cells. At subsequent times

the measured value $P(t)$ will reflect the relative amounts of labels in both environments. We might therefore write:

$$P(t) = \left[P_c L(t) + P_v \left\{ \sum_{n=1}^{\infty} [n V_n(t) + n V_n^*(t)] \right\} \right] / L_0$$

$$= P_v + (P_c - P_v) l(t) \,. \tag{27}$$

Using the value [4] of $P_v = 0.260$ and $P_c = 0.190$ for the $(N + T)$, $(N - T)$, CEC system, the differential equations were solved numerically for

$$\alpha = 0.7 \,,$$

$$R_P = 100 \,,$$

$$R_B = 0.001 \,,$$

$$T_P = T_B = 20 \,,$$

$$T'_P = T'_B = 1 \,,$$

for different values of the quantity ν_0, two such curves being shown in Figures 1 a and b. Although these curves do not match the experimental ones [4] precisely, we observe the following broad features:

(i) The resulting half-life times for the label transfer from the P-viruses to the cells are shorter than those derived from the computed ones for the B-viruses, in agreement with the experiment.

(ii) When the ratio no. of viruses : no. of sites is unity there is an inversion of this relation in the computed curves, which appears also to be the actual case.

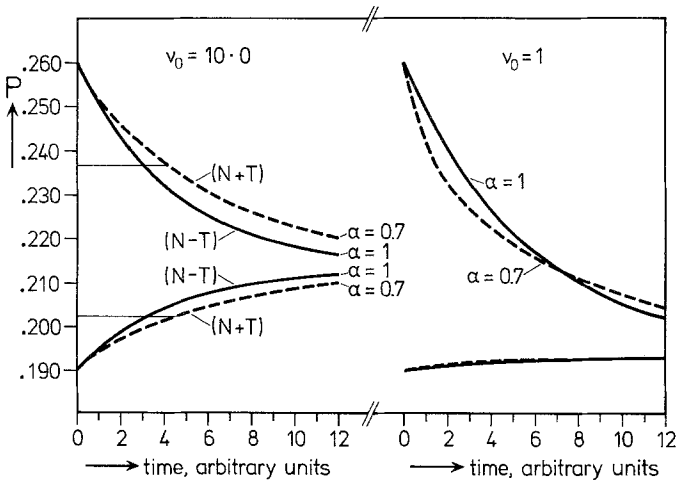

Fig. 1. Computed curves obtained from the numerical solutions of the kinetic equations for the label migration in the system. $(N + T)^*$–CEC, $(N - T)^*$–CEC, (N/T), $(N - T)$–CEC*. The asterisk stands for labelled P-fluorescence polarization degree of the label

(iii) As the equations predict there is a unique steady state whatever the direction of label migration if ν_0 is constant in the forward and backward experiments. However, experimentally in some cases, different steady state were measured as a result of the fact that the precision of determining the number of viral particles is low and ν_0 was not consequently the same in both cases.

These results indicate that the physical model used is in broad agreement with the actual, quite complex system investigated. The equations derived, which account satisfactorily for the differences in label release by the two type of viruses (penetrating (B) and nonpenetrating (P) viruses) cannot, obviously, take into account all the processes of intracellular distribution of the label. This is the reason why the best agreement is obtained for the release of the label by the viruses – where the label cannot be located anywhere but in the lipid envelope, whereas in the case of the labelled cells, where the dye is distributed among the various membranes of the cell, a rigorous description of the label movement within the cells is extremely difficult. However, we can conclude that coupling between adsorption at the different type of sites and label transfer can account for the observed kinetic behaviour, and note that no simple first order reaction could account for the different half-life times measured for label migration in the forward and reverse reactions.

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