MEMBRANE ALTERATION INDUCED BY T7 VIRUS INFECTION

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1. Introduction

To survive and multiply successfully in a host cell, an infecting virus has to induce immediate changes. In coli virus T7 infections a mechanism involving the viral M gene product avoids the digestion of its DNA by host restriction nucleases [1,2]. The same gene product is also responsible for the killing of the host cell [3] for ion efflux after infection [4] and for exclusion of competing viruses [5]. Previously, we have discussed the evidence indicating that these events are based on a very early membrane alteration induced by the virus [4]. Here we present direct evidence for just such a rapid membrane alteration after T7 infection, using pyrene as a fluorescence probe in the cell membrane.

2. Materials and methods

The bacterial strains and the virus mutants used have been described in refs [4] and [6]. The fluorescence probe laser technique was performed as described in ref. [7].

3. Results and discussion

In a suspension of cells pyrene is exclusively dissolved in the hydrophobic part of the cell membrane. There it can be excited by a short pulse of laser light and the decay rate of the molecules in the excited state can subsequently be followed by rapid fluorescence spectroscopy [7]. The inserts in fig.1 show oscillographic tracings of the decay of the pyrene fluorescence, monitored at 380 mm (A) before and (B) immediately after, T7 infection. The sharp downward deflection of the signal occurring at the same time as the laser pulse is mainly a result of scattered laser light being picked up by the photo-detection system. In a control experiment using a suspension of bacteria and viruses without pyrene, the detection system recovered from the overshoot due to this scatter in 150 ns after the laser pulse (not shown). Thereafter the heights of the signals accurately reflect the intensity of pyrene fluorescence as a function of time. In the left part of fig.1 the logarithm of the emission intensity is plotted versus time. The linearity of the semilogarithmic plots demonstrates that the fluorescence decay follows first-order kinetics. From the slopes of these lines we obtained values for the half-lives of pyrene, prior to and after infection, of 140 ns and 90 ns, respectively. This reduction reflects an alteration in the physical state of the bacterial membrane after T7 infection. Similar results were found with the bacterial strains *E. coli* B_{s-1}, *E. coli* BB and *E. coli* 802.

The half-life of the pyrene fluorescence is influenced by the fluidity of the hydrophobic membrane bilayer and by the permeability of the membrane to agents which quench pyrene [8]. The influences of these two parameters on the reduction of the half-life of pyrene after T7 infection were investigated by measurements in the presence and absence of quenching molecules. The reduction of the half-life without quenchers after T7 infection was from 140 ns to 120 ns (not shown) and this implies that membrane fluidity is increased. The bigger effect shown in fig.1 was due to

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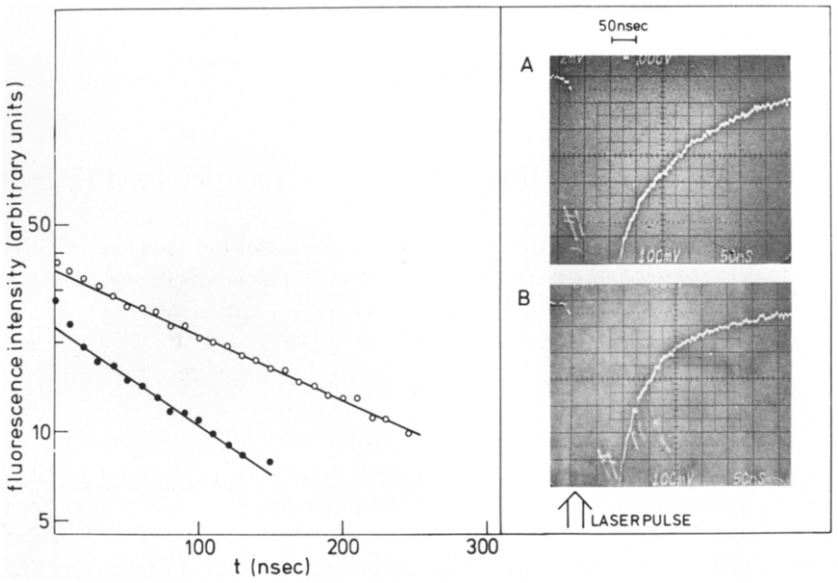

Fig.1. T7 induced half-life reduction of the pyrene singlet excited state. *E. coli* 834 were grown in M9 medium (6 g Na_2HPO_4, 3 g KH_2PO_4, 0.5 g NH_4Cl, 10^{-4} M $MgSO_4$, 1% glucose) to an optical density at 600 nm of 0.4. At 30°C pyrene was added to a concentration of 1×10^{-5} M and potassium iodide to a concentration of 2×10^{-2} M. The pyrene in uninfected cells (A) or in cells 1 min after infection with T7 wild type with a multiplicity of infection of 10 (B) was excited with a laser pulse of 30 ns at 347.1 nm from a frequency doubled ruby laser, manufactured by Korad. The oscillographic traces of the decay of the pyrene excited state as monitored at 380 nm and 30°C are shown in A and B. A semilog plot is demonstrated in the left half of the figure (o——o) calculated from A, (•——•) calculated from B. Time-zero corresponds to 150 ns after the deflection on the oscillographic trace, at which time the overshoot due to scatter was completed.

the additional action of quenching I^--ions and this further reduction demonstrates that the permeability of the membrane is also increased.

A quantitative measure for the influence of the quenching molecules is given by the quenching constant:

$$k_q = \frac{\ln 2}{[Q]} \left[\frac{1}{\tau} - \frac{1}{\tau_0} \right]$$

$[Q]$ = concentration of the quenching agent, τ = half-life in the presence of quencher, τ_0 = half-life without quencher). This constant was calculated for I^-. Under the experimental conditions used, this quencher did not alter the half-life of the pyrene excited state in the uninfected cell membrane ($k_q < 10^7$ M^{-1} s^{-1}) but had a strong influence after T7 infection which is demonstrated by a quenching constant of 1×10^8 M^{-1} s^{-1}.

As a control, pyrene in a suspension of T7 virion particles in buffer did not give rise to a fluorescence signal when excited with a laser pulse (not shown). A possible influence of pyrene, and/or the solvent acetone or the quenching agent on viral development was ruled out by comparison of the eclipse period and burst size with and without the different agents (not shown).

The change in the half-life time of the singlet excited state of pyrene occurred immediately after infection with T7 virus and was restored later during virus development (fig.2). In the presence of potassium cyanide the half-life of the pyrene fluorescence decay did not change (fig.2). Thus, the reduction of the half-life is not due to adsorption of the virus particles but is caused by a later process, e.g., by the injection of the DNA into the cell.

The early region of the T7 genome carries genes for control functions [9]. We tested whether one of these genes might be responsible for the membrane alteration detected by the pyrene fluorescence technique and indeed found that, only deletions in the M gene

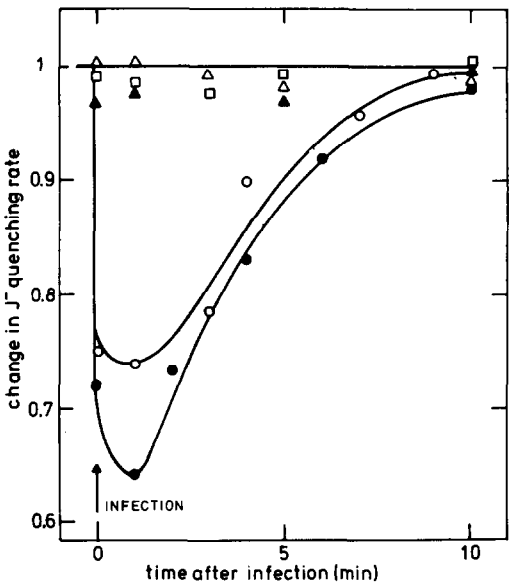

Fig.2. Membrane alterations with various T7 mutants. The half-lives of the pyrene singlet excited state at various times after T7 infection were determined essentially as described in fig.1 and compared with the half-life in uninfected cells. The multiplicity of infection was 10. (△——△) Uninfected *E. coli* 834. (●——●) T7 wild type. (○——○) T7 H280 (infection with T7 LG3 gave a similar result). (□——□) amH28OD1. (▲——▲) T7 H280 in the presence of KCN (4 mM).

showed no half-life alteration, whereas those in other early T7 genes (H280: deletion in kinase gene, LG3: deletion in ligase gene and 1.1 gene, not shown) reduced the pyrene fluorescence half-life to the same values as did T7 wild type (fig.2). Gene expression of the late region of T7 gave no additional signals, as is demonstrated by the comparison amH28OD1 and am[1] H28OD1 and H280 and T7 wild type, respectively.

We have previously demonstrated that T7 virus excludes heterologous and homologous super-infecting viruses and that only M^--mutants are disturbed in this exclusion phenomenon [5]. Thus, we expected that a super-infecting virus should not further reduce the decay time of pyrene fluorescence, except if the first virus was a M^--mutant. This was indeed the case. When cells were infected with T7 H280 under the experimental conditions described in fig.1 then 3 or 10 minutes later super-infected with T7 wild type, no additional decrease of pyrene half-life was observed. After preinfection with T7 M^- (amH28OD1) super-infection with T7 wild type reduced the lifetime of the excited pyrene from 140 ns to 90 ns (not shown). Exclusion of co-infecting viruses was also demonstrated in an examination of the effect of changes in multiplicity of infection on pyrene fluorescence decay. The half-life was not further reduced by increasing the effective multiplicity of infection to values higher than 3 (at this multiplicity 95% of the cells are infected). Thus the exclusion phenomenon can be demonstrated at the level of the membrane alteration described here.

The pyrene fluorescence technique enabled us to measure membrane changes immediately after infection of *E. coli* with the virus T7. The results demonstrate an involvement of the viral M gene in this membrane alteration. Thus we describe here a novel function in which the M gene product is involved. It is likely that the different effects caused by the M protein [1–5] are the result of one primary event – the membrane alteration described here.

References

[1] Studier, F. W. (1975) J. Mol. Biol. 94, 283–295.
[2] Schweiger, M., Hirsch-Kauffmann, M., Ponta, H., Pfennig-Yeh, M. and Herrlich, P. (1975) FEBS Symposium 39, 55–68.
[3] Herrlich, P., Rahmsdorf, H. J. and Schweiger, M. (1974) Adv. in the Biosciences 12, 523–537.
[4] Ponta, H., Altendorf, K. H., Schweiger, M., Hirsch-Kauffmann, M., Pfennig-Yeh, M. and Herrlich, P. (1976) Molec. Gen. Genet. in press.
[5] Hirsch-Kauffmann, M., Pfennig-Yeh, M., Ponta, H., Herrlich, P. and Schweiger, M. (1976) Molec. Gen. Genet. in press.
[6] Ponta, H., Rahmsdorf, H. J., Pai, S. H., Hirsch-Kauffmann, M., Herrlich, P. and Schweiger, M. (1974) Mol. Gen. Genet. 134, 281–297.
[7] Cheng, S., Thomas, J. K. and Kulpa, C. F. (1974) Biochemistry 13, 1135–1139.
[8] Grätzel, M. and Thomas, J. K. (1973) J. Amer. Soc. 95, 6885–6890.
[9] Schweiger, M., Herrlich, P., Rahmsdorf, H. J., Pai, S. H., Ponta, H. and Hirsch-Kauffmann, M. (1974) in: Lipmann Symposium, Energy, Regulation and Biosynthesis in Molecular Biology (Richter, D. ed) pp. 547–563, Walter de Gruyter, Berlin.