Fluorescence Changes of a Membrane-Bound Dye During Bacteriophage T5 Infection of Escherichia coli

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The fluorescence intensity of membrane-bound N-phenyl-1-naphthylamine increases dramatically when T5 bacteriophage infect colicin Ib plasmid-containing hosts. This dramatic increase is not seen during normal infections or in infections wherein either the plasmid or the phage contain mutations which allow productive infection to occur. Two smaller increases in fluorescence intensity are seen, however, in all T5 infections in which the characteristic two-step injection of DNA can proceed.

When bacteriophage T5 infects Escherichia coli, it initially injects only 8% (the first-step transfer) of its DNA into the cell. New proteins coded for by this DNA must be synthesized before the second-step DNA transfer can occur. After this period of pre-early protein synthesis and second-step DNA entry, the infection proceeds unremarkably; early proteins are synthesized, phage DNA replication occurs, late proteins are synthesized, and progeny phage are released. If, however, the E. coli contains the colicinogenic factor ColIb, the infection is abortive (24, 30). The phage adsorb normally, and first-step transfer of the T5 genome, pre-early protein synthesis, and second-step DNA transfer all proceed as usual (23, 30). At about 10 to 12 min after infection, however, many cellular functions including RNA and protein synthesis stop abruptly and simultaneously (12, 23). It has been hypothesized that membrane depolarization is the cause of this abortive response (12) and that the colicin Ib protein itself may be responsible (22). This communication provides evidence that membrane depolarization does, indeed, occur during the abortive T5 infection of ColIb-containing cells. In addition, we have noted two earlier indications of changes in the cell membrane that we believe to be associated with the two-step injection of the DNA molecule.

Previously, we have described alterations in the ability of T5-infected ColIb+ hosts to accumulate various substances (12). Net uptake of both proline and glutamine decreases markedly at nearly the same time as macromolecular synthesis stops. In a normal infection, there is a transient inhibition that reverses itself between 5 and 10 min postinfection. The inhibition of amino acid accumulation in the abortive infection is not due merely to inhibition of protein synthesis, since chloramphenicol did not diminish the accumulation (11, 12). Uptake of thio-β-methylgalactoside, furthermore, was also inhibited (5). Net uptake of α-methylglucoside, unlike that of proline, glutamine, and thio-β-methylgalactoside, is stimulated during both a normal and an abortive infection (12), but the stimulation of uptake of this compound is far greater in the abortive infection. Each of the substances tested for transport is taken up by a different mechanism. Proline, glutamine, and thio-β-methylgalactoside transport systems require an "energized" membrane but all have different types of carriers (2, 3, 28), whereas α-methylgalactoside transport is driven directly by phosphoenolpyruvate (18). Interestingly, both uncouplers of oxidative phosphorylation and colicin proteins—E1, K, Ia, and Ib—which all cause membrane deenergization, cause a similar pattern of changes in uptake abilities (2, 3, 8, 16, 17, 21). The changes in transport activity during the abortive infection suggested, then, that the membrane was deenergized during the abortive infection at about the same time as macromolecular synthetic processes cease. Furthermore, the transient inhibition of proline and glutamine uptake, as well as that of thio-β-methylgalactoside (5) seen in a normal infection, indicated that membrane changes may also be occurring even in a productive infection.

This report deals with changes in fluorescence intensity of a membrane-bound fluorescent probe, N-phenyl-1-naphthylamine (NPN), during T5 infections. The fluorescence intensity emitted by NPN or other fluorescent dyes increases when bacteria are treated with uncouplers of oxidative phosphorylation or with colicins E1, K, or Ia in the presence of these lipophilic probes (4, 14, 25, 27). The time course of this change appears to correlate well with the observed membrane deenergization, as determined by inhibition of active transport (14) or decrease in ATP level (27). Fluorescence may
not correlate with the state of membrane energization in every situation, however (7). Nonetheless, by measuring a number of fluorescence parameters under a variety of conditions, it has been hypothesized that agents which cause membrane deenergization cause structural changes in the outer membrane of the cell, allowing an increased amount of NPN to move into the lipophilic environment of the inner membrane (14). Since we believe (12) the mechanism underlying abortive T5 infections is quite similar to that thought to underlie the killing action of the group of colicin proteins listed above (20, 21, 29), we wished to see whether NPN fluorescence would substantially increase between 10 and 15 min after infection of ColIB-containing cells—at the same time membrane deenergization is hypothesized to occur. In addition, we wished to see whether we could attribute two lesser changes in fluorescence to events related to the two-step transfer of the phage DNA.

The bacteria and phage used have been fully described elsewhere (12). In brief, RM 42, RM 43, and RM 39 are isogenic strains of E. coli W3110 obtained from Richard Moyer. RM 42 contains no plasmid; RM 43 contains the colicinogenic factor ColIB-P9, whereas RM 39 contains a mutant ColIB factor which allows wild-type T5 to replicate. T5am16d and T5h12− were the gift of D. J. McCrorquodale. T5am16d has an amber mutation in the A1 gene and therefore cannot effect second-step transfer (15, 19). T5h12− has a mutation in the A3 gene and can replicate in ColIB-containing hosts (1).

Fluorescence intensity was measured by using a Perkin-Elmer MPF-2A scanning fluorimeter equipped with a temperature-controlled chamber. The output of the instrument was corrected for wavelength-variable response by means of a rhodamine B quantum counter, and its monochromators were calibrated against the xenon line emission spectrum.

There is an increase in fluorescence (Fig. 1a) at about 2 to 3 min after initiation of T5 infections of RM 42, RM 43 (ColIB), and RM 39 (ColIB h−, also designated as pha− [22]). Another increase is observed in all cases at about 6 to 7 min after infection. Only during T5 infections of RM 43 (ColIB) is there a third rise, this one coming at about 12 min into the infectious process. Only a very slight rise in intensity is seen after 12 min during T5h12− infections of RM 43 (ColIB) (Fig. 1b). This minimal rise is consistent with the 50% plating efficiency of the mutant on RM 43 (ColIB), relative to that on strains lacking the ColIB factor (12). That the third change in emitted fluorescence occurs at 12 min into the abortive infection and does not occur if the abortive response is circumvented by means of phage or plasmid mutations is consistent with our prediction. It provides additional evidence indicative of membrane deenergization at the time when the other abnormalities of abortive infection appear.

We also measured fluorescence changes when T5am16d infected RM 42, RM 43 (ColIB), and RM 39 (ColIB h−) (Fig. 1c). It can be seen that, even without the contribution of early or late gene expression, a marked increase in fluorescence intensity occurs at about 12 min after infection of a restricting ColIB+ host. This finding provides additional evidence that no early or late phage protein synthesis is necessary to produce abortive infection. It is interesting also to note that during T5am16d infections of RM 42, RM 43 (ColIB), or RM 39 (ColIB h−), in which second-step transfer does not occur, the increase in intensity usually seen at about 5 min after infection is lacking. The same is true for infections with a T5 mutant deficient in the A2 gene function (data not shown)—a mutant which is likewise unable to effect second-step transfer. These findings are consistent with the thesis (13) that the early biphasic pattern of fluorescence is due to membrane changes related to the two steps of DNA transfer which occurs in wild-type infections. This interpretation agrees with the original observations and interpretations of Hantke and Braun (13) who have studied the fluorescence of 8-anilino-1-naphthalene sulphonate after T5 infection, although later work from the Braun laboratory indicates that the second fluorescence rise occurs only under anaerobic conditions (26; V. Braun and E. Oldmixon, J. Supramol. Struct., in press). Because our cells are fully exposed to air at all times during the fluorescence measurements, we do not know the cause of this difference.

To eliminate the possibility that a substance released from the abortively infected cells caused an increase in fluorescence yield, we spun down the cells at 20 min after T5 infection of ColIB-containing hosts. The fluorescence intensity emitted from the supernatant was not significantly different from fresh medium containing NPN (data not shown), indicating that the changes are, indeed, caused by membrane-bound NPN.

The fluorescence changes noted in abortive T5 infections join the growing list of physiological changes that occur both during abortive T5 infections (22) and during cell killing by colicin proteins E1, K, Ia, and Ib (17, 20, 21). Additionally, several other abortive systems—T7 infections of F+ E. coli (6), T4II infections of λ lysogens (10), T5 infections of P2 lysogens (8), and certain infections of P22 (sieB+) lysogens of...
Fig. 1. Fluorescence intensity of NPN during T5, T5am16d, and T5h12⁻ infections of E. coli. Bacteria were grown in synthetic medium to a concentration of $6 \times 10^8$ cells per ml and infected with phage at a multiplicity of infection of 5. (Further details of the infection process are outlined in reference 12). Infected cells were suspended in growth medium which was prewarmed to 37°C and contained 10 μM NPN. A 3-ml portion of the infected cells was then placed in the 37°C chamber of the fluorimeter, and the fluorescence intensity was measured continuously. Excitation wavelength was 352 nm with a band width of 5 nm; emission wavelength was 410 nm with a band width of 10 nm. (a) The infecting phage is T5; (b) phage is T5h12⁻; (c) phage is T5am16d. Symbols: ●, host is RM 42; ○, host is RM 43 (ColIb); ▲ host is RM 39 (ColIb h⁻) (also called pha⁻[22]).
Salmonella typhimurium (31) strongly resemble abortive T5 infections. It might be that a similar mechanism involving changes of the host membrane is operable in each case.

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