Effect of P22-mediated Receptor Release and of Phage DNA Injection on Cell Viability of Salmonella typhimurium

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SUMMARY

Infection with u.v.-inactivated P22 bacteriophage at multiplicities higher than 30 caused a decrease in Salmonella typhimurium viability without cell lysis. Neither the action of the endoglycosidase of the P22 virion on the lipopolysaccharide of S. typhimurium nor the concomitant release of cell wall components was responsible for m.o.i.-dependent cell death. Using both free P22 tails and u.v.-inactivated P22, we have shown that p9 tail protein activity has no effect either on the integrity of host cells or on cell viability. Our results show that cell death is due to the injection of the u.v.-inactivated P22 DNA.

Bacteriophage adsorption to host cells produces, in some cases, cell lysis without phage production or phage gene transcription. This phenomenon is responsible for the death of Escherichia coli cells infected at high multiplicity by T4 and T2 bacteriophages and their ghosts (Delbrück, 1940; Duckworth, 1970; Kao & McClain, 1980), and is caused by enzyme activities of the virion acting on the cell wall or cell membrane. Virion-dependent cell death without lysis has also been described in T7-infected cells (Haar et al., 1981). In previous work (Barbé et al., 1982) we demonstrated that infection of Salmonella typhimurium cells by u.v.-inactivated P22 bacteriophage causes cell death when the m.o.i. used is higher than 30. In this case lysis does not occur, but there are important modifications of cell volume that suggest some alterations in the cell wall. It is known that P22 virions have a glycosidase active on the lipopolysaccharide (LPS) of S. typhimurium and this activity is mediated by the p9 protein of the tail (Berget & Poteete, 1980). To evaluate the possible relationship between this degradation of receptor and cell death, we have investigated the effect both of tail-associated glycosidase activity and of phage DNA injection on both cell viability and cell integrity, using free P22 tails and u.v.-inactivated bacteriophage.

Free P22 tails were obtained by growing P22 DB1416 (a double amber mutant in genes 5 and 8) on a non-suppressor strain. The titration of tails was performed as previously described (Israel et al., 1967) with the P22 256 ts9.c2mh tail-less virion bacteriophage. P22 wild-type, the P22 mutant SieA− and phage F0 were also employed. The strains of S. typhimurium used were LT2 (wild-type), JL2744 (recA1 rpsL22 hisG), UA121 (thy), UA3090 (recA1 rpsL22 hisG thy), UA149 (as LT2 but lysogenic for P22), UA130 (as LT2 but lysogenic for P22 SieA−) and DB7004 (leu515 sup19).

Fig. 1 shows that the efficiency of wild-type P22 adsorption to LT2 cells decreased about 50-fold when 1000 tails/cell were simultaneously present in the medium. Thus, there was competition between the p9 proteins of both whole and defective virions for the primary receptor on the cell surface. Under the same conditions, the adsorption rate of phage F0 increased (Fig. 1), showing that the free tail-associated glycosidase activity released the external region of LPS, making the F0 core receptor more accessible. Thus, the primary recognition and degradation of the P22 receptor can proceed in the absence of P22 heads, whereas irreversible adsorption requires whole virions (Israel, 1976).
Fig. 1. Concentration of unadsorbed P22 particles in the presence (□) and in the absence (■) of 1000 tails/cell, and of unadsorbed F0 particles to intact LT2 cells (●), and to LT2 cells previously exposed to 1000 tails/cell for 10 min (○).

Fig. 2. Concentration of total (■, ●, △) and viable cells (□, ○, △) of an LT2 culture after exposure for 45 min to various concentrations of P22 free tails (■, □) or to u.v.-inactivated P22 bacteriophage at various multiplicities in the absence (●, ○) and in the presence of 1000 tails/cell (△, △). Total cell number was determined using a Coulter Counter as previously described (Barbé et al., 1982).

Fig. 2 shows cell viability and integrity after exposure of S. typhimurium LT2 to increasing tail concentration. There was no effect either on cell viability or on the total cell number 45 min after tail addition. Nevertheless, Fig. 2 also shows that infection with u.v.-inactivated P22 at high m.o.i. produced a dramatic decrease in cell viability but not in cell number. This decrease was delayed if high concentrations of free tails were present. Pyrimidine dimers seem not to be decisive elements, because bacteriophage inactivated with hydroxylamine produced the same effects. Moreover, these results suggest that p9-mediated LPS degradation is not responsible for m.o.i.-dependent cell death. The release of trisaccharides catalysed by p9 is probably a less harmful and more external phenomenon than the effect produced by the gp5 protein of T4 base-plates in E. coli cells infected at high multiplicity. In the latter case, there is also a release of cell wall components, not only sugars but also lipopolysaccharides (Loeb, 1974), which points out the greater effect of the enzyme activity of T4 proteins.

To test the possible roles of irreversible adsorption and phage DNA injection in the loss of cell viability, we used as the recipient a lysogen for P22 SieA- phage. In this strain, both reversible and irreversible P22 adsorption are normal, as well as DNA ejection from the head of superinfecting virions; however, the presence of SieA+ prophage prevents entry of exogenous DNA into the cytoplasm (Susskind et al., 1974). Fig. 3 shows that infection with P22 at high m.o.i. induced cell death in the UA130 (P22 SieA-) strain but not in UA149 (P22 SieA+). This supports the hypothesis that, in the S. typhimurium-P22 system, the loss of cell viability after infection at high m.o.i. requires the introduction of inactivated DNA into the cell. The greater decrease in the proportion of surviving cells of the strain harbouring P22 SieA- prophage is probably due to the SOS-dependent prophage induction that occurs when a high concentration of exogenous inactivated DNA is present in the cell (Barbé et al., 1983).

These results show that the enzyme activity of neither free tails nor P22 virions caused any decrease in the viability of infected cells even when an m.o.i. of 1000 was used. The fact that the
m.o.i.-dependent cell death required the presence of inactivated DNA inside the cell suggested a causal relationship between this phenomenon and a m.o.i.-dependent indirect SOS induction. Because of this, we studied the effect of infection with u.v.-inactivated particles on the mutant JL2744 recA1, in which the SOS system is not inducible (Guerrero & Barbé, 1982) because the RecA protein is not functional.

Fig. 3 shows that m.o.i.-dependent cell death was dramatically higher in the RecA− strain than in the wild-type. Degradation of bacteriophage and bacterial DNA in cultures infected with u.v.-inactivated P22 was also studied, and Fig. 4 shows that about 50% of bacteriophage DNA was degraded during the first 30 min in both RecA+ and RecA− strains. On the other hand, the cellular DNA of RecA− cells was partially degraded after infection, whereas that of RecA+ cells remained intact.

These results suggest that the death of infected RecA+ cells may be due to the induction of some SOS function such as the inhibition of cell division as a consequence of the degradation of inactivated phage DNA. Since the RecA− mutant can not induce the SOS system, its m.o.i.-dependent decrease in cell viability must be attributed to the degradation of undamaged cell DNA. In contrast, this degradation of cell DNA is not observed in RecA+ strain probably because an SOS function preserves chromosomal DNA from extensive degradation (Satta et al., 1979; Walker, 1984).

In the interaction between bacteria and phages two mechanisms are presently known which result in cell death without virion production: enzymic lysis from without and virion-dependent enzymic inhibition of major cell functions. The results presented here show that further mechanisms are possible which depend on phage DNA injection.

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REFERENCES


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