T4 Phage Mutant Deficient in Lysis-from-Without

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SUMMARY

T4 phage mutants unable to cause lysis-from-without were isolated. The mutant phages did not induce lysis-from-without in the crude lysate, but when isolated from the lysate they induced the normal extent of lysis-from-without. The mutant phages exhibited nearly the same amount of lytic activities on cell walls as did the wild type.

INTRODUCTION

The cells of *Escherichia coli* infected with T-even phage are immediately dissolved without viral production when the multiplicity of infection is greater than 20 to 30 phages per cell (lysis-from-without). It was assumed that lysis-from-without is induced by phage lysozyme which is carried in the phage tail structure, but it was demonstrated that lysis-from-without is also induced by T4 phage devoid of lysozyme activity [1,2]. These results might suggest the possible presence of an enzyme different from phage lysozyme. It was reported that T4 phage carries a cell wall degrading enzyme which is different from phage lysozyme [2,3].

This prompted the author to search for a mutant unable to cause lysis-from-without. The function of this phage enzyme may be elucidated by study with mutants.

The purpose of this communication is to report the presence of T4 phage mutants which are deficient in lysis-from-without.

MATERIALS AND METHODS

Bacteria and phage strains; *E. coli* B and bacteriophage T4B were mostly used in this study.

Mutagenesis; *E. coli* B cells (5×10^8 cells/ml) grown in Glucose-Simmonds medium were transferred to the same amount of medium deprived of glucose and sodium glutamate, and the cell suspension was supplemented with 20 μg/ml of L-tryptophan and infected with T4B at a multiplicity of 0.3. After 5 min incubation under vigorous aeration at 37°C, 30 μg/ml of 1-methyl-3-nitro-1-nitrosoguanidine was added to the cell suspension and further incubated under aeration for 15 min. The culture was subsequently diluted hundred times with Glucose-Simmonds medium, and kept at 37°C for

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more 40 min and then phage growth was terminated by addition of a drop of chloroform.

Selection of phage unable to cause lysis-from-without; The mutagenized phage was plated onto agar seeded with *E. coli* B and each isolated plaque was propagated in *E. coli* B. The phage lysate was added to *E. coli* B broth culture (5×10^8 cells/ml) at the multiplicity of infection above fifty. After 10 min incubation at 37°C, the turbid cultures were selected and the phages were further examined for their lysis-from-without.

Test for lysis-from-without; After addition of phage lysate to *E. coli* B broth suspension (5×10^8 cells/ml), the change of turbidity of the mixture was followed using a Klett-Summerson photoelectric colorimeter (660 mμ) at 37°C.

Assay of enzymatic activities on cell walls; The assay of cell wall degrading enzyme and the preparation of altered phage (disrupted by freezing and thawing) are described previously [2].

RESULTS AND DISCUSSION

When T4B was mutagenized as described in METHODS, 5.6 per cent *r* mutants were observed. This may suggest the appearance of mutant phage is 5.6/4 per cent in each cistron. Lysis-from-without deficient mutants were selected as described in METHODS, and 6 variants were obtained from 1276 plaques tested. These will be tentatively referred to T4B1 mutants in the meaning of lysis-defective.

As shown in Fig. 1, T4B wild phage ($T4B1^+$) lyses the host cells when the multiplicity of infection is higher than about 25 ; The higher the multiplicity, the more rapid the lysis. In contrast to this wild type, $T4B1_5$ does not induce lysis even if the multiplicity of infection is much higher (up to 200 of multiplicity of infection). Fig. 2 shows the extent of lysis-from-without induced by the phage mutant as a function of multiplicity of infection. It can be seen that T4B1 mutants are difficult to induce lysis-from-without but at a much higher multiplicity of infection lysis takes place to some extent, and the multiplicity at which lysis is induced varies in each mutant; T4B1 mutants are partially deficient in lysis-from-without.

The mutant phages were characterized as follows; They are inactivated by anti-T4 serum. They can not adsorb to *E. coli* B/4. Their plaque morphology can not be discriminated from that of wild type. They induce "lysis inhibition[4]".

The enzymatic activities on cell walls in mutant phage were examined as described previously [2]. As shown in Fig. 3, $T4B1_5$ carries nearly the same level of enzymatic activities as the wild type. There are three different pH optima for the cell wall degradation; pH 7 (peak I), pH 5 (peak II) and pH 3.5 (peak III). Peak I represents phage lysozyme [2]. The function of the other peaks are not known. The presence of peak II and III activities in T4B1 mutants does not support the prediction that lysis-from-without is brought about by phage enzyme [2].

Fig. 4 shows that $T4B1_5$ induces the normal extent of lysis-from-without when the phage particles are isolated from the lysate. This may be taken to indicate that some substance related to lysis-from-without which might be present in the lysate may be

mutated in this strain. There are several lines of evidences suggesting that antilytic metabolic system is induced soon after the T-even phage infection. Bacteria in which intracellular T2 phage development has already progressed for a few minutes are resistant to lysis-from-without[5]. Lysis-from-without can be brought about at a lower multiplicity of infection when potassium cyanide or chloramphenicol is added to the host cell suspension prior to phage infection (unpublished results). The unstable property of T4B1 mutants in inducing lysis-from-without makes difficult the further characterization of T4B1 mutants. It might be necessary to examine the mutants in relation to such a mechanism. The presence of such mutants reflects an aspect of complicated mechanism of lysis-from-without. Although the presence of enzymatic activities on cell walls in T4B1 mutants does not support the postulated function of the phage enzyme (peak II or/and peak III), there remains a possibility that true lysis-deficient mutant might be isolated as a conditionally lethal mutant.

The author wishes to express his gratitude to Drs. Y. Takagi and M. Sekiguchi for their interest and encouragemennt throughout the work of this study.

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LEGENDS to Figures.

Fig. 1. Lysis-from-without induced by $T4B1^+$ and $T4Bl_5$.

The phage lysate was added to *E. coli* B broth suspension (5×10^8cells/ml) at a multiplicity of infection as indicated in the Figure, and the change of turbidity of the mixture was followed using a Klett-Summerson photoelectric colorimeter (660 mμ) at 37°C.

Fig. 2. Lysis-from-without induced by T4B1 mutants as a function of a multiplicity of infection.

The lysis-from-without was measured as described in the METHODS, and the extent of lysis-from-without was expressed by the reduced turbidity (Klett readings) per min.

Fig. 3. The degradation of C^{14}-labelled cell walls by altered $T4B1^+$ and $T4Bl_5$ as a function of pH of the assay mixture.

The preparation of altered phage and the assay of the cell wall degrading enzymes are described previously[2].

The incubation mixture (1 ml total vol.) contained 0.2 ml of C^{14}-labelled cell wall preparation (4000 counts/min), 0.2M buffer and 2×10^{10} altered phage particles, and incubated for 60 min at 37°C. Buffer used: ○—○, sodium acetate; ●—●, potassium phosphate.

Fig. 4. Lysis-from-without induced by isolated $T4Bl_5$.

After removal of unlysed cells and debris by low-speed centrifugation (3000×g, 20 min), the lysate was centrifuged at 20000×g for 60 min. The sedimented phage particles were resuspended in the original volume of broth. This washing cycle was repeated and the phage particles were finally suspended in broth. The isolated phage particles were tested for lysis-from-without. $T4B1^+$ and $T4Bl_5$ in the lysates were also tested as references.

Fig. 1

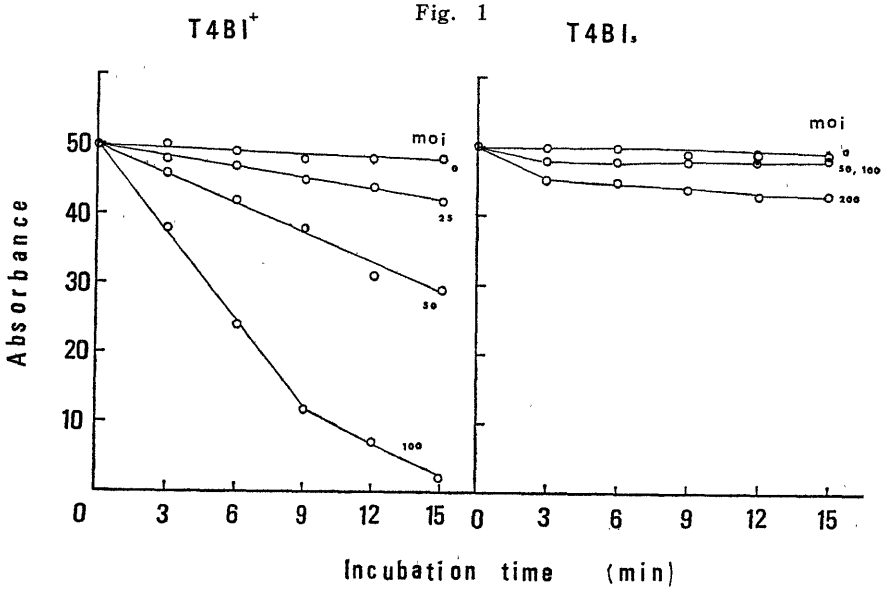


Fig. 2

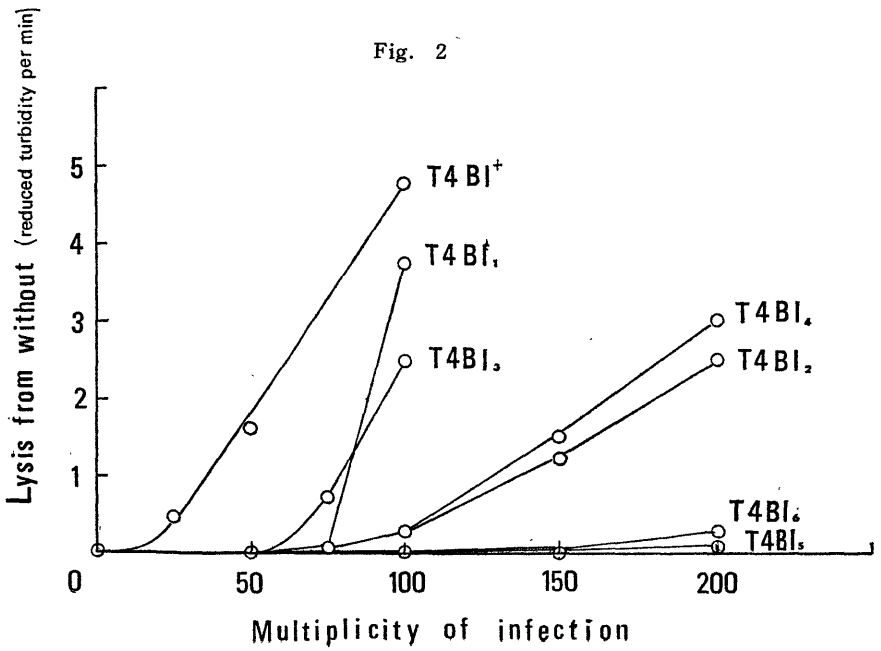
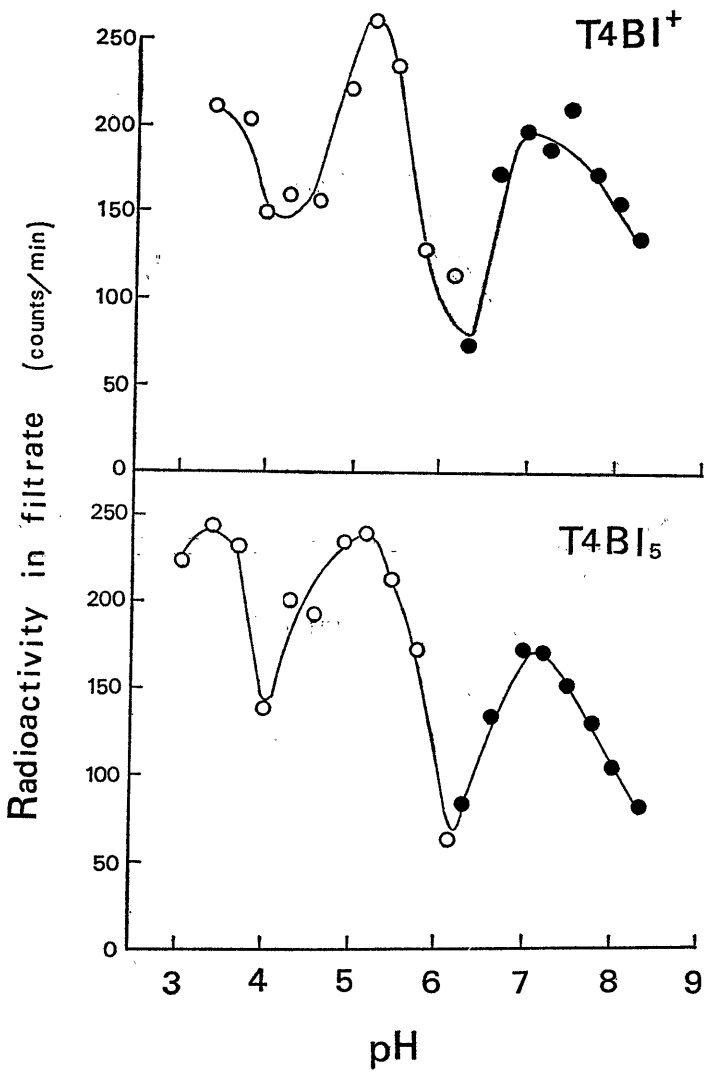
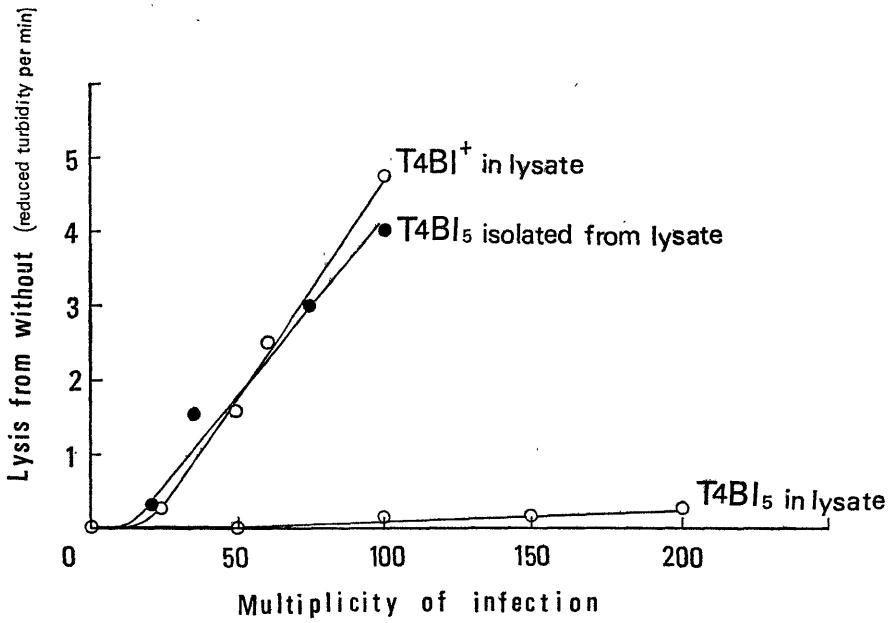


Fig. 3


T4BI+
T4BI5
Radioactivity in filtrate (counts/min)
250
200
150
100
50
0
3
4
5
6
7
8
9
pH

Eig. 4


Lysis from without (reduced turbidity per min)
5
4
3
2
1
0
50
100
150
200
Multiplicity of infection
T4BI+ in lysate
T4BI5 isolated from lysate
T4BI5 in lysate