# INHIBITION OF PHAGE MULTIPLICATION BY NITROFURYL-VINYL-QUINOLINE DERIVATIVES[1]

Akira Taketo, Hideo Sawada and Yasuyuki Takagi
Department of Biochemistry, Kanazawa University, Medical School, Kanazawa, Ishikawa, Japan.

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During the study of bacteriocidal action of nitrofuran compounds, evidence was obtained that (5-nitro-2-furyl)vinyl-quinoline and its derivatives (Miura, 1962) have the ability to inhibit the synthesis of DNA in Escherichia coli and to induce the development of active lambda phage from the prophage state in the lysogenic strain (Taketo et al), as reported with 3-amino-6-[(5-nitro-2-furyl)vinyl]-1,2,4-triazine (Panfuran) (Endo et al, 1963). Thus it seemed of interest to see whether these agents have effects on the virus multiplication. This communication shows that these compounds inhibit the reproduction of coliphage strikingly without suppressing the formation of bacterial and viral DNA.

The synthesis of DNA by T2r phage-infected or uninfected E. coli B in the presence of various concentrations of 4-amino-2-[(5-nitro-2-furyl)vinyl]-quinoline (4A-2Q-ran) is illustrated in Table I. Cells infected with phage produce DNA at the normal rate at a level of 0.1 μg/ml of the agent; this concentration is enough to depress the increase of DNA in uninfected organisms to 50 per cent of the control level. Thus it was

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shown that the impaired formation of DNA in uninfected bacteria in the presence of 4A-2Q-ran was significantly reversed upon infection with T2r phage, though the extent of reversal was low compared with that found in the case of mitomycin C (Sekiguchi et al, 1960).

Table I. DNA synthesis by T2r-infected and uninfected E. coli B cells in the presence of 4A-2Q-ran

| Time of incubation (min.) | Conc. of 4A-2Q-ran (μg/ml) | Uninfected cells: Relative amount of DNA | Uninfected cells: Increment of DNA* (%) | T2r-infected cells: Relative amount of DNA | T2r-infected cells: Increment of DNA* (%) |
|---|---|---|---|---|---|
| 0 | 0 | 100 | — | 100 | — |
| 30 | 0 | 135 | 100 | 192 | 100 |
| 30 | 0.05 | 132 | 91 | 194 | 103 |
| 30 | 0.1 | 116 | 47 | 186 | 94 |
| 30 | 0.5 | 99 | 0 | 129 | 32 |

Cells growing in a glucose-salts synthetic medium were harvested at the logarithmic phase of growth, resuspended in a salts-solution at a concentration of $9 \times 10^8$ cells/ml and divided into two portions. One was infected with T2r phage with a multiplicity of 4, and another was kept uninfected as control. After 10 minutes of incubation at 37°, they were resuspended respectively in the fresh glucose-salts synthetic medium containing 4A-2Q-ran at various levels described in the table, and then incubated at 37° for 30 minutes. The increment of DNA during incubation was determined by the method of Burton (1956), and that of the control, incubated in the absence of 4A-2Q-ran is represented by 100 (*).

Of great interest is the observation that phage reproduction was completely inhibited even in the presence of 0.05 μg/ml of 4A-2Q-ran, a concentration sufficiently low to permit the infected or uninfected bacteria to synthesize DNA at almost the same rate as in the control organisms (Table II). Similar results were obtained in experiments in which E. coli C, a fairly resistant strain to the agent, was used as the host strain in place of E. coli B, indicating the generality of

the phenomenon. Then the sensitivity of various coliphages to 4A-2Q-ran treatment was compared. It was noticed that reproduction of ϕX174 phage could proceed relatively unimpaired under the same conditions, although it was progressively reduced with increasing concentrations of the agent. This reduction was roughly comparable to the decrease of DNA synthesis in host cells, as was observed with mitomycin C (Taketo, 1963). The reproduction of T3 was also suppressed less by the addition of 4A-2Q-ran than that of T2r. The exposure of free T2r, T3 and ϕX174 phage particles to the agent did not cause any inactivation.

Table II. Effect of 4A-2Q-ran on the reproduction of coliphage

| Conc. of 4A-2Q-ran | Relative increment of phage titer (% of control) | | | | |
|---|---|---|---|---|---|
| | T2r | | T3 | | ϕX174 |
| (μg/ml) | Exp.1 | Exp.2 | Exp.1 | Exp.2 | Exp.2 |
| 0 | 100 | 100 | 100 | 100 | 100 |
| 0.05 | 0 | 3 | 14 | 29 | 97 |
| 0.1 | 0 | 0 | 0 | 0 | 50 |
| 0.5 | 0 | 0 | 0 | 0 | 0 |

Cells harvested during the logarithmic phase of growth were resuspended in a salts-solution at a concentration of $5 \times 10^8$ cells/ml and infected with phage T2r, T3 or ϕX174. After incubation at 37° for 10 minutes (adsorption), aliquots of the infected culture were transferred into fresh glucose-salts synthetic medium (in the case of infection with ϕX174 phage, 0.5 mM $CaCl_2$ was supplemented) containing 4A-2Q-ran at various levels shown in the table. After shaking for 30 minutes at 37°, phage titer was assayed with addition of chloroform. Exp. 1 : host strain, E. coli B; multiplicity of infection (m.o.i.), 0.03. Exp. 2 : E. coli C; m.o.i., 0.05.

Similar antiphage activity was observed with other (5-nitro-2-furyl)vinyl-quinoline derivatives (Table III). However, Panfuran exhibited no specific inhibitory action on

the reproduction of T2r, T3 and ϕX174. It appears that the antiphage activity of nitrofuryl-vinyl-quinoline derivatives is not related to their inducing ability of lambda phage production nor to their inhibitory action on the synthesis of bacterial and viral DNA.

Table III. Effect of nitrofuryl-vinyl-quinoline derivatives on the reproduction of T2r phage

| Compound | Relative increment of phage titer (% of control) at the following concentration (μg/ml) | | | |
|---|---|---|---|---|
| | 0 | 0.05 | 0.1 | 0.5 |
| 2-(——)*-quinoline | 100 | 15 | 0 | 0 |
| 4-(——) -quinoline | 100 | 0 | 0 | 0 |
| 2-(——) -quinoline-N-oxide | 100 | 4 | 0 | 0 |
| 2-amino-4-(——) -quinoline | 100 | 16 | 2 | 0 |

E. coli B cells were infected with T2r with a multiplicity of 0.01. Other experimental conditions as described in Table II.
* (5-nitro-2-furyl)vinyl

The exposure of a culture of infected cells to 4A-2Q-ran during the first half of the latent period caused a profound inhibition of the reproduction of phage T2r. However when the agent was added later, the yield of phage was approximately equivalent to the number of phage particles already matured before the addition. The inhibitory effect of 4A-2Q-ran was not reversed by the presence of spermidine, putrescine or agmatine. Pretreatment of host bacteria with 4A-2Q-ran did not inhibit the reproduction of T2r.

These results lead to assumption that 4A-2Q-ran exerts its inhibitory action at the later stage of phage growth, such as the maturation. Certain acridine dyes including proflavine

are known to prevent the maturation process of phage units. Similar effects were reported with actinomycin (Nakata, 1962). Since proflavine and actinomycin react with DNA, the possibility of interaction of 4A-2Q-ran with DNA was tested, using the gel filtration method. T2r DNA, native and denatured, was incubated with 4A-2Q-ran in 0.1 M NaCl at 37° for 30 minutes, and passed through a Sephadex G-25 column. No evidence of reaction of the agent with DNA was obtained, because all of T2r DNA was eluted in the fraction moving at faster rate, while 4A-2Q-ran which was detected by optical density at 390 mμ appeared in later fraction. Furthermore, the infectivity of ϕX174 DNA was unaffected by *in vitro* treatment with 4A-2Q-ran nor with Panfuran.

These findings appear to provide evidence that the antiphage activity of (5-nitro-2-furyl)vinyl-quinoline derivatives is not due to the effect on host cells nor to the inhibitory action on DNA replication, but is attributed to the attack on some stage in the vegetative cycle of phage growth itself.

The apparent difference observed in effects of these compounds on the reproduction of various phages implies several possibilities concerning the mode of action, but precise elucidation must await further studies.

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## References

Burton, K., Biochem. J., 62, 315 (1956).

Endo, H., Ishizawa, M., Kamiya, T. and Kuwano, M., Biochim. Biophys. Acta, 68, 502 (1963).

Miura, K., Antimicrobial Agents and Chemotherapy, p.275 (1962).

Nakata, A., Biken's J., 5, 29 (1962).

Sekiguchi, M. and Takagi, Y., Biochim. Biophys. Acta, 41, 434 (1960)

Taketo, A., J. Biochem., 54, 520 (1963).

Taketo, A., Sawada, H. and Takagi, Y., in preparation