Induction of Phage Formation in Lysogenic *Escherichia coli* by Myxin

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The primary effect of the antibiotic myxin (1-hydroxy-6-methoxyphenazine-5,10 dioxide; Peterson, Gillespie & Cook, 1966) is the inhibition of DNA biosynthesis (Lesley & Behki, 1967) but synthesis of new DNA has been shown to occur in myxin-exposed cells following infection with T4 bacteriophage (Behki & Lesley, 1971). At a myxin concentration of 5 µg/ml, *Escherichia coli* are rapidly killed (50% survivors at 5 min), DNA synthesis is inhibited by 7 min, and intracellular DNA is extensively degraded (Lesley & Behki, 1967; Lesley & Behki, 1971). Agents that act as inducers of phage production in lysogenic bacteria appear to have in common the above three properties and the effect of myxin on the induction and development of active phage from the prophage state in lysogenic bacterium *E. coli* K12(λ)Y10 was therefore examined.

Escherichia coli K12(λ)Y10 cultures in nutrient broth were used in the logarithmic phase of growth at 2×10^8 cells/ml. Samples incubated with 0, 0.1, 0.5, 1.0 and 2.0 µg/ml myxin were aerated with vigorous shaking at 37 °C. The growth and lysis of the cultures were followed turbidometrically. All the cultures showed a logarithmic increase in extinction for about 60 min. Thereafter the turbidity of cultures exposed to 0.5, 1.0 and 2.0 µg myxin/ml rapidly decreased due to lysis of the cells and release of λ phage. After a further 60 min the extinction of these cultures decreased by 28, 62 and 68% respectively for the three myxin concentrations and after another 1 h they were almost clear. This characteristic growth pattern in the presence of myxin resembles very closely the reported growth curves after induction of lysogenic bacteria with appropriate doses of either ultraviolet light (Weigle & Delbrück, 1951), inducing chemical agents (Borek & Rockenbach, 1954; Gots, Bird & Mudd, 1955) or mitomycin C (Otsuji, Sekiguchi, Lijima & Takagi, 1959). Two non-lysogenic strains of *E. coli* tested did not exhibit this phenomenon with any concentration of myxin (up to 10 µg/ml.)

The effect of myxin on the intracellular multiplication of λ phage was, therefore, examined in *Escherichia coli* K12(λ)Y10. Cultures were exposed to different myxin concentrations and the number of viable cells and infective centres were titrated after 30 min. In the culture exposed to 0.5 µg myxin/ml the number of infective centres reached a maximum of about 25% of the original number of colony-formers present (Fig. 1). Under these conditions 40% of the original cells retained their colony-forming ability. At 2 µg myxin/ml, when almost all the treated bacteria were killed, 37% of the original number were titratable as infective centres. The induction of lambda phage was not severely inhibited with up to 10 µg myxin/ml. After full lysis of the cells the maximum yield of phage was found, with 2 µg myxin/ml, to be 6.6×10^9 /ml when titrated on the indicator strain K1T4.

Myxin can, therefore, be added to the list of agents that induce the production of bacteriophage in lysogenic bacteria. It is similar in its antimicrobial activity to mitomycin C (Reich, Shatkin & Tatum, 1961), streptonigrin (White & White, 1968) and nalidixic acid (Goss,

Fig. 1. Percent viable cells and infective centres with *Escherichia coli* K12 (λ)Y10 as a function of myxin concentrations. The data has been normalized by expressing the number of viable cells and infective centres as a percentage of initial number of colony-formers. $\circ$ — $\circ$, Viable cells; $\bullet$ — $\bullet$, infective centres.

Dietz & Cook, 1965) in that DNA synthesis is inhibited, intracellular DNA is degraded and cell death rather than bacteriostasis results. Structurally, these compounds differ significantly in that mitomycin C and streptonigrin are quinones, myxin is a phenazine derivative and nalidixic acid is a naphthyridine derivative (Leshner *et al.* 1962). Infectious T₄ particles are produced in the presence of myxin (Behki & Lesley, 1971) but not in the presence of mitomycin C. This indicates abnormal or biologically inactive bacteriophage DNA synthesis with the latter compound (Sekiguchi & Takagi, 1960). Myxin may be useful in studies of differential synthesis of DNA species in such systems.

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