NOTES

INDUCTION OF COLICIN E_1 SYNTHESIS BY NEOCARZINOSTATIN AND BLEOMYCIN

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The synthesis of certain colicins is induced by mitomycin C (MTC)[1] and ultraviolet light (UV).[2] During the course of investigation of the mechanism of the induction of colicin E_1, we found that neocarzinostatin (NCS) and bleomycin A_2 (BLA_2) were as effective as MTC and UV on the active colicin synthesis. The action of both compounds is known to be much the same in terms of the selective inhibition of DNA synthesis *in vivo* and *in vitro* and the concomitant degradation of DNA in the susceptible bacteria.[3~6]

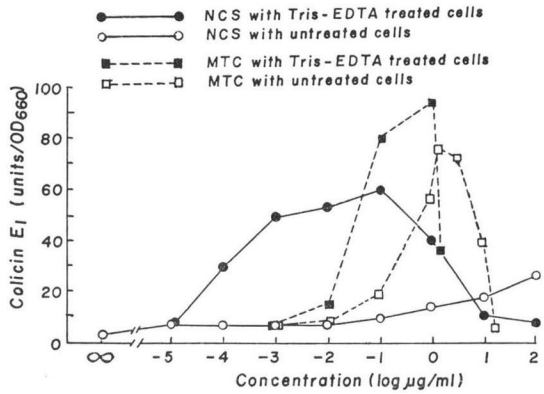
With regard to the chemical nature of the compounds, NCS is an acidic polypeptide with a molecular weight of 10,000,[7] while BLA_2 is a basic glycopeptide with a molecular weight of 1,300.[8] It is surprising that such large macromolecules, especially NCS, should induce colicin synthesis. The induction of colicin synthesis should somehow involve the function of the cell membrane, since JACOB *et al.*[9] have postulated that plasmid DNA is attached to the cell membrane. This paper describes the effective concentrations of these drugs for induction and the effect on viability of the host cell.

Escherichia coli K-12 Y 20 (Col E_1) was grown to the log phase with constant bubbling in the minimal media (M-9) supplemented with vitamin-free casamino acids (0.15 %), thiamine (0.001 %) and glucose (0.4 %). The cells were collected and incubated with the inducing agent in the fresh media without aeration at 37°C.[10] The cells were disrupted with sonic oscillation before the colicin assay. Colicin assays, lacunae counts and Tris-EDTA treatment were made as previously described.[11]

In Figs. 1 and 2 are shown the effects of varying concentrations of the inducing agents on the colicin E_1 synthesis. Without the Tris-EDTA treatment, a procedure to promote the membrane permeability by liberating a part of lipopolysaccharide from the surface of the bacterial cells,[12] NCS had little inducing activity, whereas MTC and BLA_2 showed active induction without the treatment. On treatment with Tris and EDTA, however, even relatively low concentrations of NCS (1~100 ng/ml) could induce synthesis of colicin E_1. Requirement of this treatment for susceptibility of *E. coli* to NCS has been shown by ONO and ISHIDA.* MTC was effective at a narrower concentration range and the Tris-EDTA treatment made the cells more sensitive to the agent. BLA_2 showed no optimum in the dose-response curve within the concentrations tested and the sensitivity increased to some extent by the treatment (Fig. 2). Thus variations in the effective concentrations of these inducing agents may be due to their differences in accessibility to the target of induction.

Fig. 1. Effect of concentrations of NCS and MTC on the colicin E_1 synthesis.

Log phase cells of *E. coli* Y 20 (Col E_1) were incubated with varying concentrations of the drugs for one hour at 37°C. Colicin E_1 produced was expressed as units per absorbancy at 660 nm of the incubation mixture.

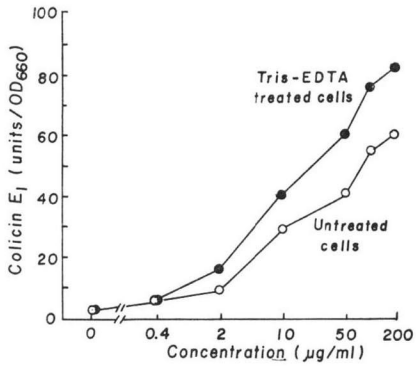


The inducing effects of these drugs were irreversible. When NCS or BLA_2 was removed

* Y. ONO and N. ISHIDA, report at the 39th annual meeting of Japanese Biochemical Society, Kyoto, 1966.

Fig. 2. Effect of concentrations of BLA_2 on the colicin E_1 synthesis.

The cells of *E. coli* Y 20 (Col E_1) were incubated with varying concentrations of BLA_2 for one hour at 37°C. Colicin E_1 produced was expressed as units per absorbancy at 660 nm of the incubation mixture.

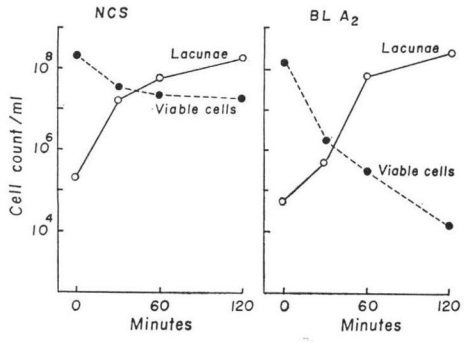


after 30 minutes of incubation by filtration with Milipore HA filter, the production of colicin E_1 thereafter closely paralleled that obtained without removal of the drug. The irreversibility was also observed in the phage induction by bleomycin.[13]

It has been reported that the viable cells decrease as the lacunae counts increase during the colicin E_1 induction by MTC.[14] We, therefore, analyzed the lethality of the host cell upon induction by NCS and BLA_2 at concentrations optimal for the colicin synthesis. As shown in Fig. 3, BLA_2 decreases the viable cell counts markedly to less than 1 % the original number during 30 minute-incubation, whereas NCS gives less effect on the viability of the host cell. On the other hand, the time course of the lacunae formation was rather faster with NCS than with BLA_2. Under conditions for optimal induction with these agents, the host cells retained about 90 % of their ability to synthesize DNA without the agents, as judged by the incorporation of ^{3}H-thymine into acid-insoluble materials. In contrast, the extensive degradation of the host DNA to relatively large fragments was observed on incubation with BLA_2. Cells of Y 20 (Col E_1) were grown for three generations in the media containing ^{3}H-thymine (6μ Ci/ml) and deoxyadenosine (250 μg/ml). After washing the log phase cells, incubation was carried out for 30 minutes with the inducing agent and the cleared lysates were prepared as described previously.[11] About 50 % of the radioactivity of the host DNA was recovered as the acid-insoluble material in the lysate of the cells incubated with BLA_2, whereas only 5 % of the radioactivity was observed in the lysate after incubation with NCS. Under these conditions, the acid-insoluble radioactivity due to colicin E_1 DNA is less than 0.5 % of the host DNA.

Fig. 3. Kinetics of lacunae formation and loss of viability during induction of colicin E_1 by NCS and BLA_2.

NCS (0.1 μg/ml) was added to the cells of *E. coli* Y 20 (Col E_1) pretreated with Tris-EDTA and incubated at 37°C. BLA_2 (100μ g/ml) was added to the untreated cells.



The mechanism whereby these agents induce the colicin synthesis is still unknown. The above findings, however, at least suggest that the disturbance in the DNA metabolism of the host cell is not a prerequisite to the induction. Taking into account the macromolecular nature of some of the inducing agents, it is rather attractive to speculate that these agents attack the bacterial membrane as a first target and the subsequent effects on the membrane activities such as growth and division of the cell pave the way to the colicin induction.

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