ON THE ACTION MECHANISM OF MITOMYCIN C

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Since Shiba et al reported the anti-tumor antibiotic, mitomycin C (MC), to have selective inhibitory effects on the biosynthesis of bacterial DNA (Shiba et al, 1958), many investigations have been done. Among these studies, Reich et al reported that this antibiotic causes the rapid break down of bacterial DNA in vivo (Reich et al, 1960). We have also had the same result. In this paper we report studies on the mechanism of the action of MC, from the standpoint of the molecular mechanism.

Crystalline MC was obtained from the Kyowa Hakko Kogyo Co., Ltd., Tokyo. Determination of DNA was carried out by the method of Burton (Burton, 1955). Acid soluble deoxyribonucleosides were measured as thymidine by the microbiological assay (Hoff-Jørgensen, 1952). "Masked" deoxyribonucleosides were determined as deoxyribonucleoside (thymidine) after hydrolysis with venom phosphatase (Okazaki et al, 1958).

Transformation of biochemically deficient strains of Bacillus subtilis (tryptophan-less mutant) by DNA from nutritional independent strain was carried out according to the method of Spizizen (Spizizen, 1958). The strain of B.subtilis were obtained from Dr. Hiuga Saito, Institute of Applied Microbiology, University of Tokyo. DNase was isolated from Escherichia coli B with sonic oscillation (10 KC, 10 minutes) and then centrifugation (100,000 x g,

30 minutes). The supernatant liquid was used as enzyme source. The activity of DNase was determined by measuring the release of ultraviolet absorbing material and of acid-soluble deoxyribose. DNA was extracted from E.coli B with lysozyme treatment, salt extraction and repeated chloroform-octanol treatment.

The methods and results are as follows: E. coli B cultured with shaking at 37°C in glucose-Simmons medium, was harvested at the logarithmic phase of growth, then resuspended (optical density at 660 mμ = 0.3) in similar fresh medium in the presence of MC (1 μg/ml) and incubated at 37°C with shaking. Aliquots were taken at certain intervals, and the DNA and deoxyribonucleosides were measured. It is evident that MC breaks down bacterial DNA in vivo, and accumulates the "masked" deoxyribonucleosides in the medium (Fig. 1).

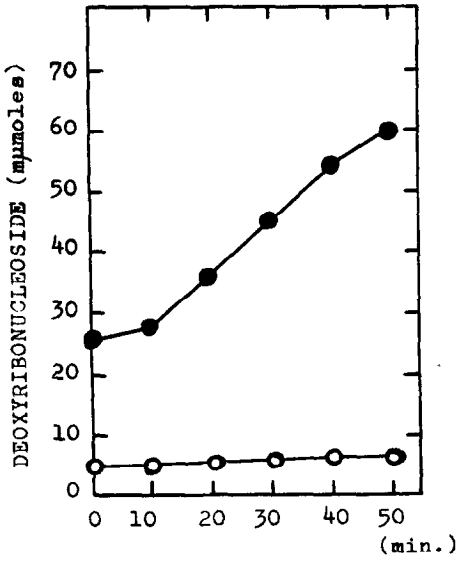

Fig.1. Accumulation of "masked" deoxyribonucleoside.

"Masked" deoxyribonucleoside (● - ●), Deoxyribonucleoside (o - o).

The transforming activity of the DNA was greatly impaired when the bacteria (B. subtilis, strain 116) were incubated with

MC (1 μg/ml) before the DNA was prepared. Although transformation occurred at the frequency of one in 10^4 with the intact transforming principle (20 μg DNA/ml), the frequency decreased to less than 10 % of this value in the course of incubation with MC (Fig. 2). Because transformation is known to be prevented by treatment with DNase, these results suggested that the MC-DNA was degraded by treatment of intact cells with MC. When DNA was treated with MC in salt solution at 0°C for 20 minutes and then washed repeatedly with ethanol, the activity of transforming principle was not impaired.

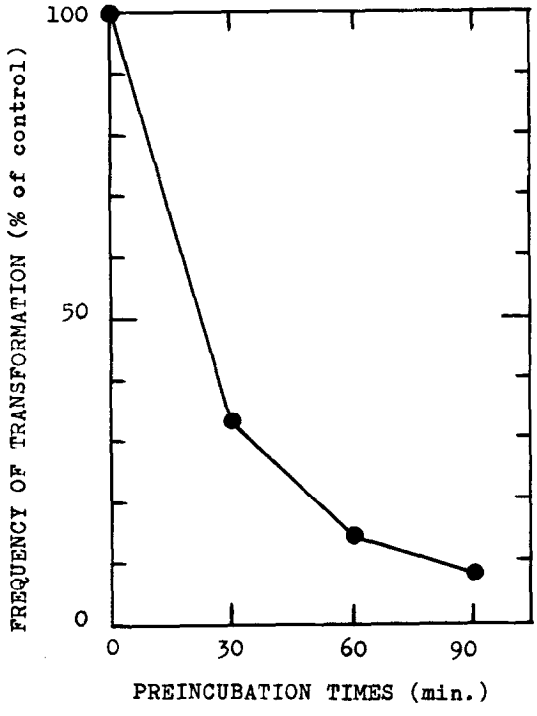

Fig.2. Time course of impairment of transformating DNA by MC treatment of whole cells.

MC alone had no effect on DNA and also no effect on DNase activity *in vitro*.

When E. coli B cells were preincubated with MC for 30 minutes the specific activity of DNase extracted from the cells was elevated up to 180 % (Table 1).

Table 1. DNase activities of E. coli B

Systems	DNase activities
With untreated enzyme	
+ none	100
+ MC (50 μg)	100
+ protamine sulfate (100 μg)	28
+ protamine sulfate (100 μg) + MC (50 μg)	30
With MC-pretreated enzyme	
+ none	180
+ protamine sulfate (100 μg) + MC (50 μg)	47
Without enzyme	
+ MC (200 μg)	0

incubated for 30 minutes at 37°C with 20 ug of DNA and 10^{-2}M of $MgSO_4$ in veronal buffer, pH 7.4.

In a preliminary experiment with the cell-free system the incorporation of H^3-thymidine into DNA was not inhibited even at high concentration of MC. This finding indicates that MC has no effects on the activities of deoxyribonucleotide kinase and polymerase.

In a recent report Amos demonstrated that protamine sulfate protected degradation of RNA by ribonuclease (Amos, 1961). He also found the degradation of DNA by DN ase to be protected by protamine sulfate. We also observed that protamine sulfate inhibited the cell-free DNase activity of E. coli B *in vitro* (Table 1), and to restore the impaired DNA synthesis *in vivo* (20 μg/ml) (Fig. 3).

These results suggested that MC exerts it actions on DNA at the level of the DNase activity.

References

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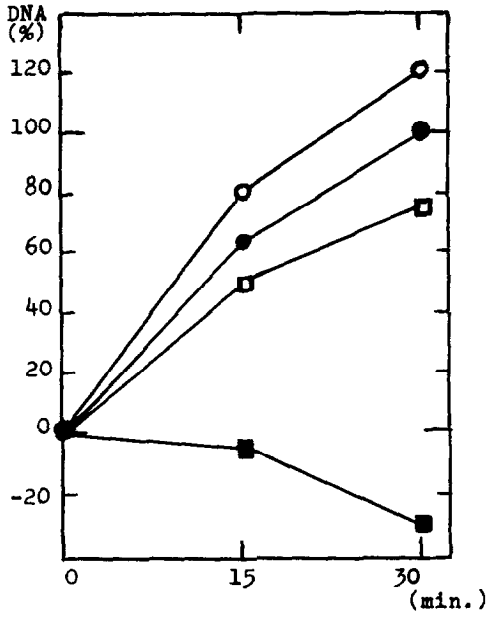

Fig.3. DNA synthesis of E. coli B under the influence of MC and/or protamine sulfate.

● Control (no addition)
■ + MC (1 μg/ml)
○ + Protamine sulfate (20 μg/ml)
□ + MC (1 μg/ml) + Protamine sulfate (20 μg/ml)

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