EFFECT OF STREPTOVARICIN ON RNA SYNTHESIS IN PHAGE T4-INFECTED ESCHERICHIA COLI

Satoshi Mizuno and Kazuo Nitta

Department of Antibiotics, National Institute of Health,

Shinagawa-ku, Tokyo, Japan

Received March 6, 1969

As reported in our previous papers (Mizuno et al., 1968a; Mizuno et al., 1968b; Umezawa et al., 1968; Nitta et al., 1968), streptovaricin, rifamycins and rifampicin, which have structures resembling one another, inhibit initiation of RNA synthesis in E. coli, presumably binding to the RNA polymerase, but do not inhibit the RNA synthesis by RNA polymerase obtained from a resistant mutant of E. coli. Hartmann et al. (1967) also reported a similar observation on inhibition of RNA polymerase by rifamycins. Recently, it was proved by Wehrli et al. (1968) that E. coli RNA polymerase forms a stable complex with rifampicin but the enzyme obtained from a rifampicin-resistant mutant of E. coli does not form such a complex with the antibiotic. Genetic analysis made by Yura and Igarashi (1969) suggested that the RNA polymerase in streptovaricin-resistant mutants of E. coli would be structurally modified. These findings indicate that these antibiotics might be useful to study the role of E. coli RNA polymerase in transcription of phage genes in bacteriophage-infected E. coli.

We will report in the present communication the effect of streptovaricin (SV) on pulse labeled RNA synthesis in SV-sensitive and resistant E. coli at various times after phage T4-infection. The results obtained indicate that phage RNA synthesis in SV-sensitive E. coli is inhibited by SV at any time after infection, but that in SV-resistant cells it is not inhibited at any time.

MATERIALS AND METHODS

The SV-sensitive E. coli B which had been maintained in our laboratory

was completely inhibited by 20 μg/ml of SV. The SV-resistant mutant employed was obtained by plating 10^9 cells of the sensitive E. coli B on broth-agar plates containing SV (1,000 μg/ml). The mutant grew in a plate containing 1,000 μg/ml of SV or rifampicin. Bacteriophage T4D was kindly supplied by Dr. J. Tomizawa of our institute. ^{3}H-uridine (2.7 C/mM) was obtained from the Radiochemical centre.

SV-sensitive or resistant mutant cells were grown with aeration at 37° in Tris-glucose-casamino acids medium (Nomura et al., 1962) and log phase cells were employed for the experiments. These log phase cells (5×10^8 cells/ml of the sensitive cells and 3.5×10^8 cells/ml of the resistant cells) were directly infected in the growth medium with phage T4D at a multiplicity of 10 (zero time) in the presence of L-tryptophan (50 μg/ml) and incubated at 37°. The number of uninfected cells 5 min after the addition of phage was no more than 0.5 % of the number of infected cells. SV (20 μg/ml) was added to the infected cells 5, 10, 15, 20 and 25 min after the infection, and at various times (Figure 1) after the addition of SV, 40-second pulse labeling was carried out with ^{3}H-uridine

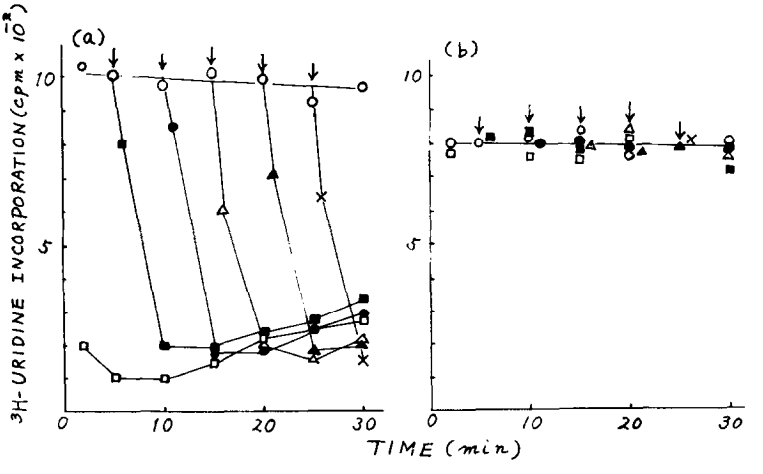

Fig. 1. Effect of streptovaricin on the incorporation of ^{3}H-uridine in phage T4-infected streptovaricin-sensitive E. coli (a) and resistant E. coli (b). Phage T4D infection was made at zero time in both control and test cultures. In test cultures, streptovaricin (20 μg/ml) was added at the times indicated by arrows. (O) Control, (□) Streptovaricin was added at zero time, (■) 5 min., (●) 10 min., (△) 15 min., (▲) 20 min., (×) 25 min.

(5 μC/ml). The effect of SV on RNA synthesis in SV-sensitive and SV-resistant cells both of which had been infected with the phage was determined by measuring the amount of ^{3}H-uridine incorporation in the 5% trichloroacetic acid insoluble fraction of the cells. The acid insoluble radioactivity was determined by a liquid scintillation spectrometer (Beckman LS-200B).

RESULTS AND DISCUSSION

The effect of SV was investigated on ^{3}H-uridine incorporation in phage T4-infected SV-sensitive or resistant E. coli in 40-second pulse labeling (Figure 1). The results indicate that the pulse labeled RNA synthesis in the SV-sensitive cells is markedly inhibited by SV (20 μg/ml) whenever it is added after the phage infection (Figure 1(a)). In SV-resistant cells no appreciable effect of SV is shown in the pulse labeled RNA synthesis at any times examined (Figure 1(b)). RNA synthesis after the phage infection in phage-sensitive cells is known to be directed by phage DNA (Stent, 1963), and therefore these results indicate that SV inhibits phage-directed RNA synthesis in SV-sensitive cells but not in SV-resistant cells. In another experiment, the lysis of SV-resistant cells infected with phage T4 in the presence of SV (50 μg/ml) was observed after about four hours incubation at 37° with aeration. As reported in a previous paper (Nitta et al., 1968), the resistant cells do not inactivate SV, since incubation of SV with extract of the resistant cells does not reduce the inhibitory effect of SV on RNA polymerase of the sensitive cells.

Two conclusions can be made on the basis of the present experimental results. One is that during both the early and the late times of the phage growth, E. coli RNA polymerase itself functions in phage RNA synthesis. The other is that if E. coli RNA polymerase is modified by the phage infection, the structural characteristic of the host enzyme in relation to SV is conserved in the modified RNA polymerase.

It has been reported that products of RNA synthesis in in vitro on phage DNA template by E. coli RNA polymerase are mostly the molecular species of early RNA and do not contain appreciable quantities of late RNA (Khesin et al.,

1962; Geiduschek et al., 1966), but addition of the T4 gene 55 product allows E. coli RNA polymerase to transcribe late regions of T4-DNA in vitro (Snyder and Geiduschek, 1968). The informations obtained from these in vitro studies support our considerations on the enzyme in the phage DNA transcription based on in vivo experimental results. It has also been demonstrated by electrophoresis on polyacrylamide gel that E. coli RNA polymerase is modified by the infection with phage T4, although the modification has not been demonstrated by immunochemical methods (Walter et al., 1968). The modified enzyme is considered to conserve the structural characteristic which interacts with SV and probably has connection with the initiation of RNA polymerization.

ACKNOWLEDGMENTS

We wish to thank Dr. J. Tomizawa for useful advice and for the kind supply of phage T4D, and Dr. H. Umezawa for discussion and encouragement.

REFERENCES

Geiduschek, E. P., Snyder, L., Colvill, A. J. E., and Sarnat, M., J. Mol. Biol., 19, 541 (1966).
Hartmann, G., Honikel, K. O., Knüsel, F., and Nüesch, J., Biochim. Biophys. Acta, 145, 843 (1967).
Khesin, R. B., Shemiakin, M. F., Gorlenko, Zh. M., Bogdanova, S. L., and Afanaseva, T. P., Biokhimia, 27, 1092 (1962).
Mizuno, S., Yamazaki, H., Nitta, K., and Umezawa, H., Biochim. Biophys. Acta, 157, 322 (1968a).
Mizuno, S., Yamazaki, H., Nitta, K., and Umezawa, H., Biochem. Biophys. Res. Commun., 30, 379 (1968b).
Nitta, K., Mizuno, S., Yamazaki, H., and Umezawa, H., J. Antibiotics (Tokyo), 21, 521 (1968).
Nomura, M., Okamoto, K., and Asano, K., J. Mol. Biol., 4, 376 (1962).
Snyder, L., and Geiduschek, E. P., Proc. Natl. Acad. Sci. U. S., 59, 459 (1968).
Stent, G. S., Molecular Biology of Bacterial Viruses, W. H. Freeman and Company, San Francisco and London, 1963, page 139.
Umezawa, H., Mizuno, S., Yamazaki, H., and Nitta, K., J. Antibiotics (Tokyo), 21, 234 (1968).
Walter, G., Seifert, W., and Zillig, W., Biochem. Biophys. Res. Commun., 30, 240 (1968).
Wehrli, W., Knüsel, F., Schmidt, K., and Staehelin, M., Proc. Natl. Acad. Sci. U. S., 61, 667 (1968).
Yura, T., and Igarashi, K., Proc. Natl. Acad. Sci. U. S., in press (1969).

ADDENDUM

After this manuscript had been submitted to the editor we heard from Dr. Geiduschek that two papers, one by Dr. Haselkorn and the other by Dr. Geiduschek, were accepted in Nature in which using rifamycin an essentially identical conclusion was made.