Mode of Action of Clostocin 0 : Part 2. On the Synthesis of Nucleic Acid in Sensitive Bacteria

Kato, Fumio
Laboratory of Applied Microbiology, Faculty of Agriculture, Kyushu University

Ogata, Seiya
Laboratory of Applied Microbiology, Faculty of Agriculture, Kyushu University

Yoshino, Sadazo
Laboratory of Applied Microbiology, Faculty of Agriculture, Kyushu University

Hayashida, Shinsaku
Laboratory of Applied Microbiology, Faculty of Agriculture, Kyushu University

他

https://doi.org/10.5109/23660

出版情報：九州大学大学院農学研究院紀要. 22 (3), pp.145-152, 1978-07. Kyushu University
バージョン：
権利関係：

J. Fac. Agr., Kyushu Univ., 22, 145 152 (1978)

Mode of Action of Clostocin 0

Part 2. On the Synthesis of Nucleic Acid in Sensitive Bacteria

Fumio Kato*, Seiya Ogata, Sadazo Yoshino, Shinsaku Hayashida and Motoyoshi Hongo**

Laboratory of Applied Microbiology, Faculty of Agriculture, Kyushu University 46-02, Fukuoka 812

(Received November 4, 1977)

An inducible bacteriocin clostocin 0 from *Clostridium saccharoperbutylacetonicum* (ATCC 13564) inhibited the both biosyntheses of DNA and RNA in sensitive organism. Especially, the biosynthesis of messenger RNA was most strongly affected and the biosynthesis of 23 S ribosomal RNA was also affected by clostocin 0 infection. It also seemed that the release of 23 S and 16 S ribosomal RNA from the cellular membrane system easily occurred in clostocin O-infected organism.

INTRODUCTION

Clostocin 0 is a phage tail-like bacteriocin produced by *Clostridium saccharoperbutylacetonicum* (Ogata *et al.*, 1972), and inhibits the macromolecule biosynthesis of sensitive organism (Kato *et al.*, 1976) staying at the cell surface (Ogata *et al.*, 1976). But, it has been reported that some low molecular bacteriocins, such as colicins, can penetrate through the cell wall and cytoplasmic membrane into cytoplasma of sensitive organism.

Action of large molecular bacteriocin, such as clostocin 0, may be explained by Nomura's hypothesis which suggests that there may be a specific relationship between bacteriocin particles and cytoplasmic membrane of sensitive organism and bacteriocin transmits its effect through a specific transmission system of cytoplasmic membrane to the final target in the infected organism (Nomura, 1964). It is very interesting theme to know the first target of action of large bacteriocin and transmission system.

As previously reported, clostocin 0 has a restricted receptor site (Ogata *et al.*, 1976) and inhibits the biosyntheses of macromolecules of infected organism, especially the biosynthesis of nucleic acids (Kato *et al.*, *1977)*. In this paper, we attempt to know the first target of clostocin 0 and to note the behavior of messenger RNA (m-RNA), because its turnover is the fastest in various nucleic acids.

* Present address: Department of Agricultural Chemistry, Saga University, Saga
** Present address: Department of Applied Microbial Technology, The Kumamoto Institute of Technology. Kumamoto

MATERIALS AND METHODS

Organisms

The bacterial strains used were N 1-4 (ATCC 13564) and No. 8 of *Clostridium saccharoperbutylacetonicum* for producing and sensitive strains of clostocin 0, respectively (Ogata et al., 1976).

Media and cultural conditions

The organisms were grown at 30°C under reduced atmospheric pressure (5 to 10 mmHg) in TYA medium (Ogata and Hongo, 1974) or CA-MM (Kato *et al., 1976).* TYA medium was used as preculture. To obtain a young exponentially growing culture, fresh CA-MM was inoculated with sufficient organism to produce an optical density (OD) of 0.15 at 660 nm, and unless otherwise mentioned the culture was incubated until its OD_{660} became 0. 3. Then, the culture was divided into 2 parts, and the incubation was followed at 30°C with bubbling N_2 gas. One part was added 30units of clostocin 0, and the other was added heated clostocin 0 (100°C, for 10 min) for control. At 5 min after clostocin 0 infection ^3H labeled compound was added both cultures, and a portion of cultures was taken out to fractionate the bacterial nucleic acid at suitable intervals.

Fractionation of RNA and DNA

The bacterial RNA and DNA were fractionated by modified STS procedure (Mizuno and Whitely, 1968).

Prepration of phenol-extractable RNA

Twenty ml of culture was taken out at suitable interval to extract with phenol containing sodium dodecyl sulfate (SDS) (Okamoto et al., 1962 ; Imamoto, 1969) and bentonite (Frankel-Conrat *et al., 1961)* to inhibit the activities of RNase and nuclease. Extracted RNA was precipitated by 95 % of ethyl alcohol (Forchhammer and Kjeldgaard, 1967; Mizuno et al., 1969). This procedure was repeated twice, as shown in Fig. 1.

Sucrose density gradient centrifugation of phenol-extractable RNA

The sucrose gradients were 5 to 20 % sucrose (Merck, Ltd.) in 0.02 M tris-HCl buffer containing 0.02 M NaCl. A 0.5 ml of phenol-extractable RNA solution was applied on the prepared gradients and was centrifuged at 23,000 rpm for 15 hr (Itoh *et al., 1968;* Muto, 1970) with SW 41 Ti rotor using ultracentrifuge (Beckman SPINCO, model L 3-50). Then, the solution was divided into 30 fractions (6 drops/tube) by the dropcounter (LKB 7000, ULTRORAC). Each fraction was measured the extinction at 260 nm using minicell by a photoelectric colorimeter (Spectrophotometer Hitachi 124).

Isotopes

^3H-labeled uridine and thymidine were purchased from Daiichi Radioisotope Lab., Ltd..

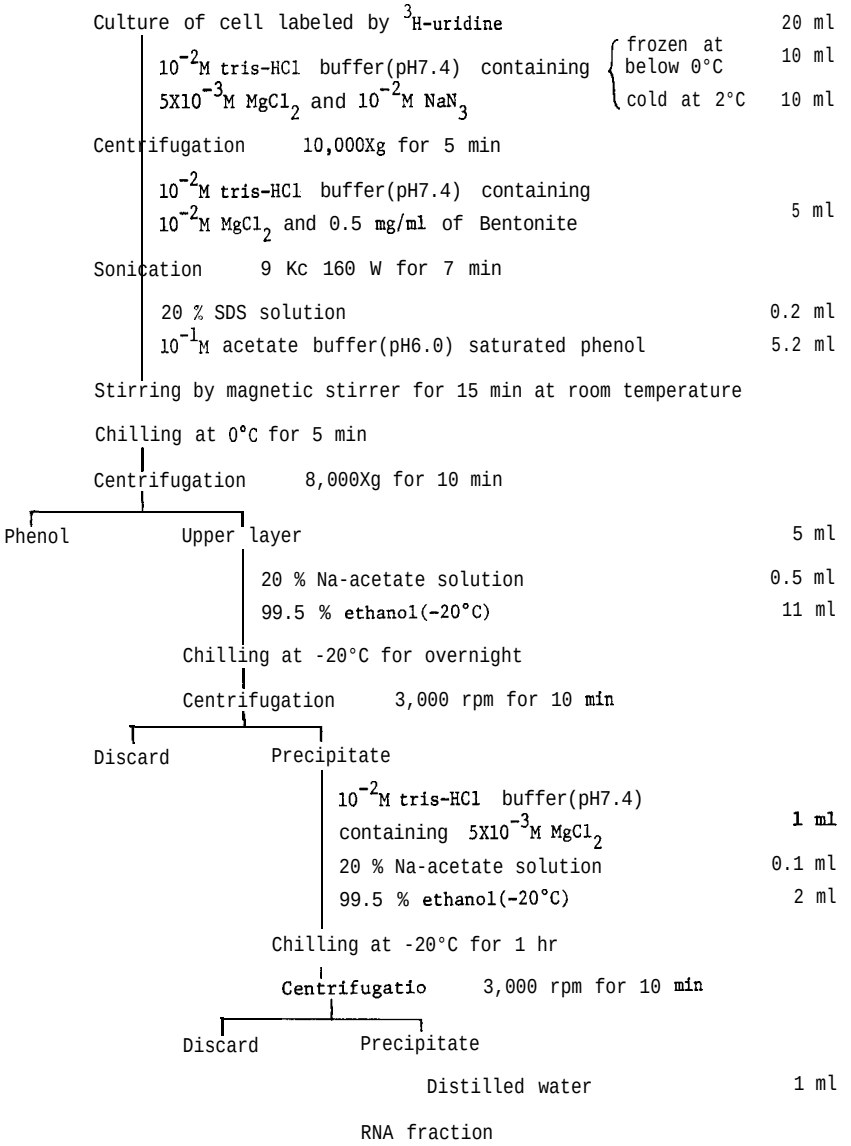

Fig. 1. Preparation of phenol-extractable RNA.

Measurement of radioactivity

Radioactivity was measured by a liquid scintillation counter (Beckman, model LS-250). The scintillation fluid consisted of 6 g of PPO (2, 5-Diphenyl-oxazole, Beckman, Ltd.) in 500g of toluene.

RESULTS

Incorporation of ^{3}H into DNA fraction clostocin O-infected organism

The incorporation of ^{3}H-thymidine into DNA fraction was measured as shown in Fig. 2. It was observed that its incorporation into DNA fraction was strongly inhibited by clostocin O infection. This result agrees very closely with that obtained by the experiment using ^{32}P, described in the preceding paper (Kato *et al.*, 1978).

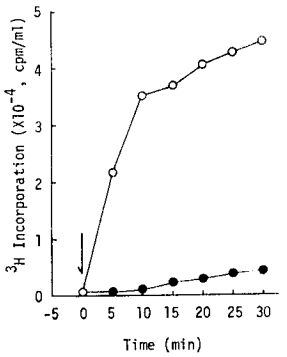

Fig. 2. Incorporation of ^{3}H-thymidine into DNA fraction. The sensitive organism was anaerobically incubated at 30°C with bubbling N_2 gas. At 5 min after infection of 30 units of clostocin O, ^{3}H-thymidine (final concentration of 0.5 μCi/ml) was added to the culture (shown by arrow). A portion of the culture was taken out at suitable intervals to fractionate with modified STS procedure. A 0.2 ml of the DNA fraction was measured radioactivity by a liquid scintillation counter. ○, control (normal organism) ; ●, clostocin O-infected organism.

Incorporation of ^{3}H into RNA fraction of clostocin O-infected organism

The incorporation of ^{3}H-uridine into RNA fraction was measured at 1 min intervals. As shown in Fig. 3, the ^{3}H incorporation into RNA fraction of normal organism finished within 3 min after ^{3}H addition. On the other hand, ^{3}H incorporation into RNA fraction of clostocin O-infected organism was depressed in the early time after ^{3}H addition. This result indicates that clostocin O inhibits the incorporation of ^{3}H-uridine into the RNA which has the fastest turnover. When the labeling time is short, it is said that the almost all radioactivity would detect in m-RNA. To clear the inhibition of clostocin O on the biosynthesis of m-RNA, the incorporation of ^{3}H-uridine into phenol-extractable RNA will be measured in the next part.

Incorporation of ^{3}H-uridine into phenol-extractable RNA

As it is generally said that bacterial m-RNA is labeled by added isotope within a short time such as 1 to 2% of bacterial generation time. So, the sampling time was set up in short time, 30, 60, 120 and 240 sec after the addi-

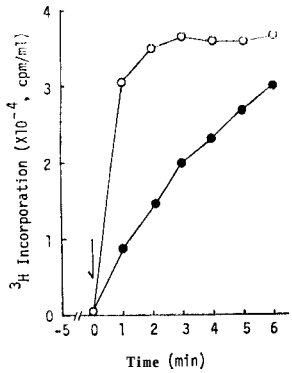

Fig. 3. Incorporation of ^{3}H-uridine into RNA fraction. Cultural and experimental conditions, and symbols are the same as described in Fig. 2. Arrow indicates the time of addition of ^{3}H-uridine (final concentration of 0. 5 μCi /ml).

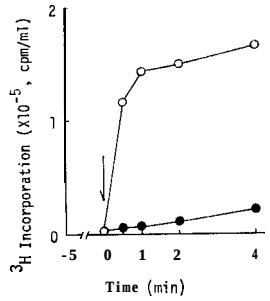

Fig. 4. Incorporation of ^{3}H-uridine into phenol-extractable RNA. Cultural condition and symbols are the same as described in Fig. 2. Arrow indicates the time of addition of ^{3}H-uridine (final concentration of 0.5 μCi/ml). Twenty ml of culture broth was used for the preparation of phenol-extractable RNA, as described in Fig. 1. A 0. 1 ml of the RNA solution was measured radioactivity by a liquid scintillation counter.

tion of ^{3}H-uridine. RNA was extracted by phenol method as described in Materials and Methods, and precipitated with alcohol and then dissolved in 1 ml of distilled water. The incorporation into phenol-extractable RNA was strongly inhibited as shown in Fig. 4. It is clear that the main action of clostocin 0 toward RNA biosynthesis must occur m-RNA biosynthesis. The inhibition of incorporation of ^{3}H-uridine by clostocin 0 is stronger in phenol-extractable RNA than in RNA fraction of modified STS procedure. This result indicates that clostocin 0 may inhibit the polymerization of mononucleotides.

The extracted RNAs which were taken out at 30, 60 and 240 sec, were applied on the sucrose gradient centrifugation at 23,000 rpm for 15 hr, and then fractionated into 30 fractions. We measured the extinction at 260nm and the radioactivity of each fraction. As shown in Fig. 5, every sample had 3 peaks

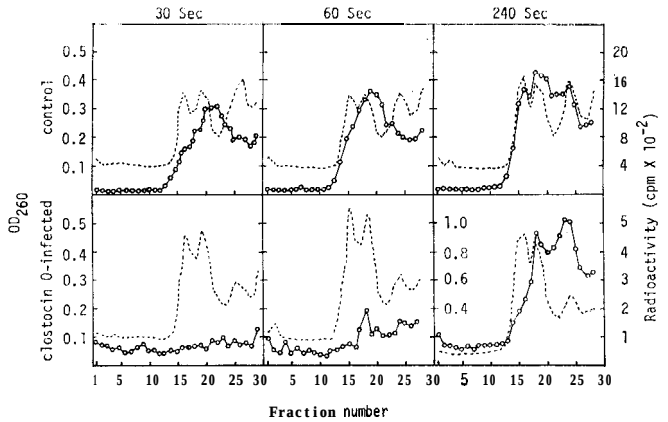

Fig. 5. Sucorse gradient sedimetation pattern of phenol-extractable RNA. The sample described in Fig. 4 was analysed by the sucrose density gradient sedimentation technique. A 0. 5 ml of sample was layered on top of 11 ml sucrose gradient (5 to 20 %) containing 10^{-2} M tris-HCl buffer (pH 7.4) and 10^{-2} M NaCl. Centrifugation was performed at 23,000 rpm for 15 hr. The upper tier is the samples of control (normal organism), and the lower tier is the samples of clostocin O-infected organism. ·····, OD_{260} ; o o, radioactivity.

of OD_{260}, these peaks were considered as the RNA which had the sedimentation constant of 23 S, 16 S and 4 S, from left to right in the figures, respectively (Gros et al., 1961). At 30 sec after the addition of ^{3}H-uridine, there was no incorporation of radioactivity in the sample of clostocin O-infected organism, but in the case of control, the peak of radioactivity was observed at the position between 16 S and 4 S RNA. This peak represents m-RNA, and it is clear that the biosynthesis of m-RNA is inhibited by clostocin 0 infection. At 60 sec, the peak of m-RNA in control stayed at same position. In the case of clostocin O-infected organism, the incorporation of radioactivity into phenol-extractable RNA was hardly observed. At 240 sec, the peaks of radioactivity in control coincided in position with those of 23 S, 16 S and 4 S RNA. In the case of clostocin O-infected organism, small peaks of radioactivity were observed at the positions of 16 S and 4 S RNA, but there was no peak of radioactivity at the position of 23 S RNA; clostocin 0 inhibited the incorporation of "H-uridine into 23 S RNA. Also, the amounts of 16 S and 23 S RNA of clostocin O-infected organism were larger than of normal organism, so they may be liable to release from the cytoplasmic membrane and easily extracted as a result of infection of clostocin 0.

DISCUSSION

Clostocin 0 affected the biosynthesis of nucleic acid in the sensitive organism. The incorporation of ^{32}P and ^{3}H-thymidine into DNA fraction were strongly inhibited by clostocin 0 infection. The incorporation of ^{32}P into RNA

fraction was strongly inhibited, but that of ^{3}H-uridine was gradually increased in depressed rate as time passed. This difference may be caused by the kind of compound; inorganic phosphate and nucleoside. Figs. 4 and 5 indicate that clostocin 0 strongly inhibits the incorporation of ^{3}H-uridine into high molecular RNA; clostocin 0 inhibits the polymerization.

The remarkable effect of clostocin 0 was detected at first in the biosynthesis of m-RNA. Little is known about the effect of large bacteriocin on m-RNA. However, it is reported that ghosts of phage T4 inhibit immediately the biosynthesis of protein in sensitive organism, but does not affect on m-RNA (Fukuma and Kaji, 1972). So, it is specific for clostocin 0 to inhibit the biosynthesis of m-RNA. The causal relation between the inhibition of DNA biosynthesis and that of m-RNA biosynthesis is not clear at present time. But it is clear that the polymerization to high molecular nucleic acid, such as m-RNA and DNA, is specifically inhibited by clostocin 0 infection. Clostocin 0 may inhibit the biosynthesis of m-RNA as the first target and also inhibit polymerization of DNA and other RNA. Moreover, from the result in Fig. 5, we noticed that the ribosomal RNA released easily from the cytoplasmic membrane system as the secondary effect of clostocin 0 infection. The amounts of 23 S and 16 S RNA of clostocin O-infected organism were larger than that of uninfected organism, and increased with the passage of time. The both of 23 S and 16 S RNA of clostocin O-infected organism may be liable to be released from the cytoplasmic membrane, and they may be easily extracted.

Clostocin 0 inhibits the biosynthesis of m-RNA and DNA, staying at the surface of sensitive organism. Therefore, clostocin 0 may transmit its effect through a specific transmission system of cytoplasmic membrane to the final target in the infected organism. We are interested in the transmission system and wish to develop the study to elucidate the structure and function of biomembrane.

ACKNOWLEDGEMENTS

This work is Part IX of Bacteriocins of Nonpathogenic *Clostridium* Species and was supported in part by a Grant-in-Aid of Scientific Research from the Ministry of Education of Japan.

REFERENCES

Forchhammer, J. and 0. Kjeldgaard *1'367* Decay of messenger RNA in *vivo* in a mutant of *Escherichia coli 15. J. Mol. Biol., 24* :459–470

Frankel-Conrat, H., B. Singer and A. Tsugita 1961 Purification of viral RNA by means of bentonitc. *Virology*, 14 : 54-58

Fukuma, I. and A. Kaji 1972 Effect of bacteriophage ghost infection on protein synthesis in *Escherichia coli. J. Virol.*, 10 : 713–720

Gros, F., H. Hiatt, W. Gilbert, C. G. Kurland, R. W. Risebrough and J. D. Watson 1961 Unstable RNA revealed by pulse labelling of *Escherichia coli. Nature 190* : *581-585*

Imamoto, F. 1969 Intragenic initiation of transcription of the tryptophan operon in

Escherichia coli fullowing dinitruphenol treatment without tryptophan. *J.* Mol. *Biol.*, 43 : 51-69

Itoh, T., E. Otaka and S. Osawa 1968 Release of ribosomal proteins from *Escherichia coli* ribosomes with high concentrations of lithium chloride. *J. Mol. Biol.*, 33 : 109-122

Kato, F., S. Ogata and M. Hongo 1976 Killing activity of clostocin 0. *Agric. Biol. Chem.*, 40 : 1107-1111

Kato, F., S. Ogata and M. Hongo 1977 Action of an inducible hacteriocin clostocin 0 on the macromolecular syntheses of *Clostridium sacchatopevbutylacetonicum*. *Agric. Biol. Chem.*, **41** : 1883-1888

Kato, F., S. Yoshino, S. Ogata, K. H. Choi and S. Hayashida 1378 Mode of action of clostocin 0. Part 1. On the macromolecular synthesis in sensitive bacteria. *J. Fac. Agr., Kyushu Univ.*. *22* : *135* -145

Mizuno, S.. H. Eguchi, K. Yano and H. Yamaguchi 1969 Counting of ^{32}P-labeled rihonuclcic acid utilizing Cerenkov radiation. *Radioisotope, 18* : 19-25

Mizuno, S. and H. Ii. Whitely 1968 Nuclear fraction of *Bacillus subtilis* as *a* template for ribonucleic acid synthesis. *J. Bacteriol.*, 95 : 1221-1237

Muto, A. 1970 Nuclcotide distribution of *Eschcrichia coli* 16 S ribosomal ribonucleic acid. *Biochemistry, 9* : *3683* -3694

Nomura. M. 1964 Mechanism of action of colicins. *Proc. Natl. Acad. Sci. USA*, *52* : *1514 1521*

Ogata, S. and M. Hongo 1974 Lysis induced by sodium ion and its relation to lytic enzyme systems in *Clostvidium saccharoperbutylacetonicum*. *J. Gen. Microbiol.*, *81* : *315* -323

Ogata, S.. F. Kato and M. Hongo 1976 Study on adsorption of inducible bacteriocin clostocin 0 toward sensitive bacteria. *Agric. Biol. Chem.*, 40 : 1093-1099

Ogata, S., 0. Mihara, Y. Ikeda and M. Hongo 1972 Inducible phage tail-like particles of *Clostvidium saccharoperbutylacetonicum* and its related strains. *Agric. Biol. Chem.*, *36* : 1413-*1421*

Okamoto, K., Y. Sugino and M. Nomura 1962 Synthesis and turnover of phage messenger RNA in *Eschevichia coli* infected with bacteriophage T4 in the presence of chloromycetin. *J.* Mol. *Biol.*, 5 : 527-534