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Inhibition of DNA Replication Initiation by Aminoglycoside Antibiotics

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The reinitiation of DNA replication induced by a temperature shift in a *dnaC*(Ts) mutant of *Escherichia coli* was markedly inhibited by aminoglycoside antibiotics around the MIC in a short period. Protein synthesis continued for several minutes after the addition of aminoglycosides but was immediately blocked by chloramphenicol, suggesting that the inhibition of initiation of replication by aminoglycosides is not a secondary effect due to the interruption of protein synthesis. Aminoglycosides did not significantly affect RNA synthesis, suggesting that primer RNA synthesis for DNA initiation is not blocked by the agents. The lethal action of habekacin was observed simultaneously with the inhibition of DNA reinitiation. DNA elongation demonstrated with a *dnaE*(Ts) mutant or toluene-treated cells of a *polA* mutant was not significantly affected by aminoglycosides. The *oriC*-membrane complex formation was markedly interrupted by habekacin in the *dnaC*(Ts) mutant, and the in vitro reconstitution of the *oriC*-membrane complex was completely blocked by aminoglycosides. The present studies (i) show that aminoglycosides block initiation of DNA replication and (ii) suggest that the inhibition is caused by the interruption of *oriC*-membrane attachment.

Aminoglycoside antibiotics display pleiotropic effects on bacterial cells. The antibiotics induce various molecular events, including inhibition of functions of the ribosome and the plasma membrane (6, 8, 13). However, the specific reason for the lethal activity of aminoglycosides remains to be determined (see references 1, 12, 14, 30, 31, and 35 for reviews). We studied the mechanism of action and cellular uptake of habekacin, a new aminoglycoside (20, 32).

Freda et al. (9) first demonstrated rounded nucleoids in streptomycin-treated cells. We have further observed by electron microscopy that habekacin has a tendency to induce contraction or aggregation of the nucleoid (unpublished data). This observation has led us to study the effect of aminoglycosides on DNA synthesis by using various mutants of *Escherichia coli*. We have found that habekacin and related aminoglycosides inhibit reinitiation of replication and *oriC*-membrane attachment in a *dnaC* mutant. The results are presented in this publication. Hancock (14) proposed disruption of the *oriC*-membrane complex during DNA initiation as the mechanism of aminoglycoside killing of cells.

(A preliminary report has been published as a short communication [33].)

MATERIALS AND METHODS

Chemicals. Habekacin, dibekacin, kanamycin, and streptomycin were supplied by Meiji Seika Kaisha, Ltd., Tokyo, Japan, and chloramphenicol was supplied by Sankyo Co., Ltd., Tokyo, Japan. Gentamicin was a product of Schering Corp., Bloomfield, N.J. Rifampin was purchased from Lepetit S.p.A., Milan, Italy. [^{3}H]thymidine (25 Ci/mmol), [^{14}C]thymidine (497 mCi/mmol), [^{3}H]thymine (57 Ci/mmol), [^{3}H]uridine (44 Ci/mmol), [^{3}H]leucine (138 Ci/mmol), and [^{3}H]dCTP (19 Ci/mmol) were purchased from Amersham Japan, Tokyo, Japan.

Bacterial strains. A temperature-sensitive DNA initiation mutant of *E. coli* PC2 [*dnaC*(Ts) *leu thy dra rpsL*] (15) was provided by K. Nagai, and a temperature-sensitive DNA polymerase III mutant of *E. coli* E486 [F$^-$ *dnaE*(Ts) *thi thy rpsL*] (11) was provided by Y. Nishimura. A DNA polymerase I-deficient mutant of *E. coli* P3478 (*thy rha lac rpsL polI*) (19) was given to us by J. Cairns and stocked in our laboratory.

Media. *E. coli* PC2 and E486 were grown in a semisynthetic medium containing the following (per liter): glucose, 2 g; Casamino Acids, 4 g; thymine, 40 mg; NaCl, 0.54 g; KCl, 0.3 g; NH_4Cl, 1.1 g; $CaCl_2 \cdot 2H_2O$, 15 mg; $MgCl_2 \cdot 6H_2O$, 203 mg; $FeCl_3 \cdot 6H_2O$, 0.2 mg; KH_2PO_4, 87 mg; Na_2SO_4, 22.7 mg; and Tris hydrochloride (pH 7.5), 12.1 g. *E. coli* P3478 was grown in heart infusion broth (Difco Laboratories, Detroit, Mich.).

DNA initiation. DNA reinitiation was carried out with *E. coli* PC2, a *dnaC*(Ts) mutant, as described previously (33), except that the restrictive and permissive temperatures used were 38 and 28°C, respectively, and the incubation period at the restrictive temperature was 60 min. The modification resulted in better recovery of DNA replication after the shift back to the permissive temperature.

DNA elongation. An overnight culture of *E. coli* E486, the *dnaE*(Ts) mutant, grown at 30°C was diluted to approximately 6×10^7 cells per ml with the medium and incubated for 2.5 h. The culture was shifted to a restrictive temperature, 42°C, at a cell density of 4×10^8/ml, and then DNA replication was stopped immediately. After 10 min, the culture was shifted back to 30°C to allow DNA elongation.

DNA synthesis. The protein, RNA, and DNA synthesis experiments were performed by the method of Hanna and Carl (15). The rate of DNA synthesis was determined as the ratio of [^{14}C]thymidine incorporated by pulse-labeling to [^{3}H]thymine incorporated by steady-state labeling. Bacterial DNA was prelabeled with 1 μCi of [^{3}H]thymine per ml for 2.5 h at the permissive temperature. To 0.5 ml of the bacterial culture, 0.01 μCi of [^{14}C]thymidine was added, and the pulse was terminated by the addition of cold 5% trichloroacetic acid (TCA).

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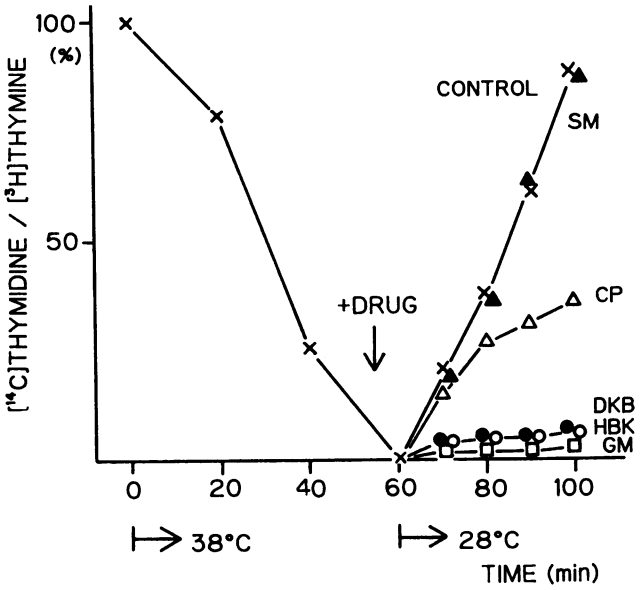

FIG. 1. Effects of aminoglycosides and chloramphenicol on DNA reinitiation in a *dnaC*(Ts) mutant. Drug abbreviations: HBK, habekacin; DKB, dibekacin; GM, gentamicin; SM, streptomycin; CP, chloramphenicol. *E. coli* PC2 was grown at 28°C and shifted to 38°C at the logarithmic phase of growth (4×10^8 cells per ml). After 60 min, the culture was shifted back to 28°C. The antibiotic was added to the cells 5 min before the return to 28°C. The rate of DNA synthesis was measured as described in Materials and Methods.

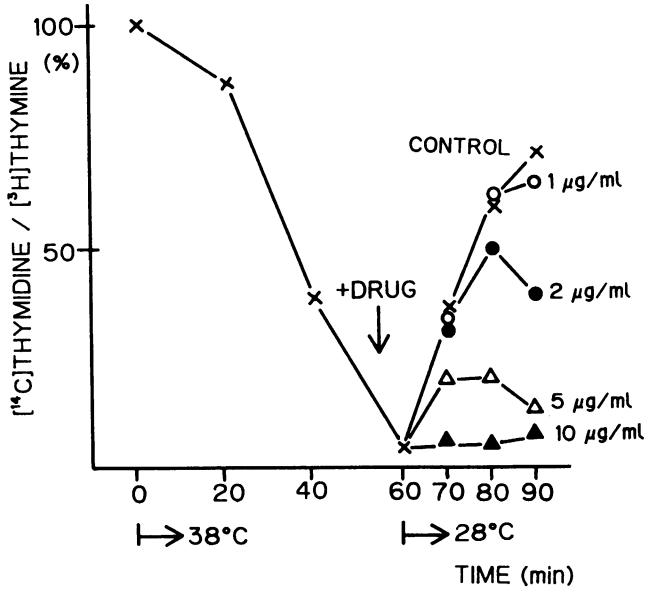

FIG. 2. Effect of habekacin on DNA reinitiation in a *dnaC*(Ts) mutant: dependency on antibiotic concentrations. *E. coli* PC2 was cultured as described in the legend to Fig. 1. The indicated concentration of habekacin was added to the culture 5 min before the return to 28°C.

Protein synthesis. Protein synthesis was measured by incorporation of [^{3}H]leucine into hot TCA-insoluble materials.

RNA synthesis. The rate of RNA synthesis was measured as the rate of incorporation of [^{3}H]uridine into the TCA-precipitable fraction after a 2-min pulse. The cells were put into cold 5% TCA immediately after the uridine uptake to prevent ribonuclease action.

Cell viability. Serial 10-fold dilutions of a culture of *E. coli* PC2 were plated on heart infusion agar supplemented with 10 μg of thymine per ml. The colonies formed after overnight incubation at 28°C were counted.

DNA synthesis in toluene-treated cells. DNA synthesis was carried out in toluene-treated cells by the method of Burger (4). Exponentially growing cells of *E. coli* P3478 were treated with 1% toluene in 50 mM Tris hydrochloride buffer (pH 7.5). The reaction mixture (0.2 ml) contained 50 mM Tris hydrochloride (pH 7.5), 5 mM $MgCl_2$, 1 mM ATP, 80 mM KCl, 0.1 mM dithiothreitol, 0.02 mM dTTP, 0.02 mM dCTP, 0.02 mM dGTP, 0.02 mM dATP, 5 μCi of [^{3}H]dCTP per ml, and 10^{10} toluene-treated cells per ml. The reaction was terminated by the addition of TCA, after incubation at 30°C for 15 to 30 min.

***oriC*-membrane complex.** The culture of *E. coli* PC2 was pulse-labeled with [^{3}H]thymidine (1 μCi/ml) for 3 min after the shift back to 28°C in the reinitiation experiment described above. Habekacin (20 μg/ml) was added 5 min before the shift back. The cells were disrupted with a French pressure cell, and the membrane fraction was analyzed by 30 to 50% sucrose density gradient (containing 1 mM EDTA) centrifugation.

RESULTS

Effects of aminoglycosides on reinitiation of DNA replication. *E. coli* PC2, a *dnaC*(Ts) mutant, was grown to the logarithmic phase at a permissive temperature (28°C) and transferred to a restrictive temperature (38°C). When the mutant was shifted back to 28°C, reinitiation of DNA synthesis occurred. The rate of DNA replication was observed as the ratio of DNA pulse-labeled with [^{14}C]thymidine to DNA steady-state-labeled with [^{3}H]thymine. The organism was resistant to streptomycin but sensitive to habekacin, dibekacin, and gentamicin (data not shown). The reinitiation

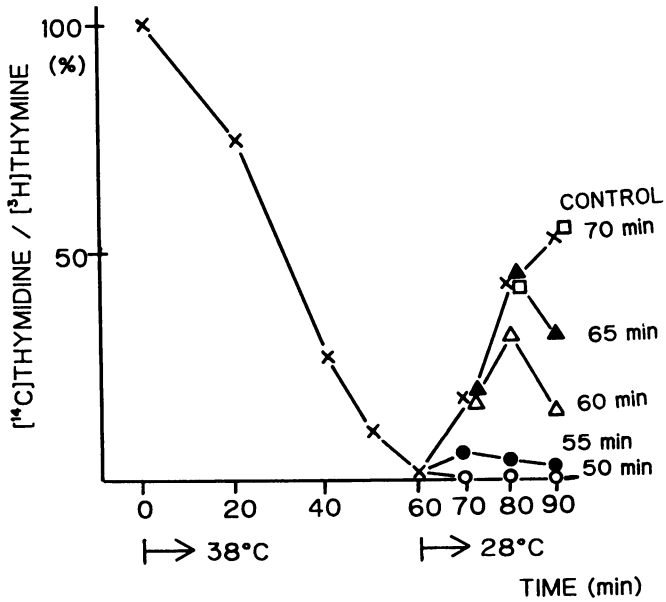

FIG. 3. Effect of habekacin on DNA reinitiation in a *dnaC*(Ts) mutant: dependency on time of antibiotic addition. *E. coli* PC2 was cultured as described in the legend to Fig. 1. Habekacin (10 μg/ml) was added to the culture at the indicated time after the initial temperature shift to 38°C.

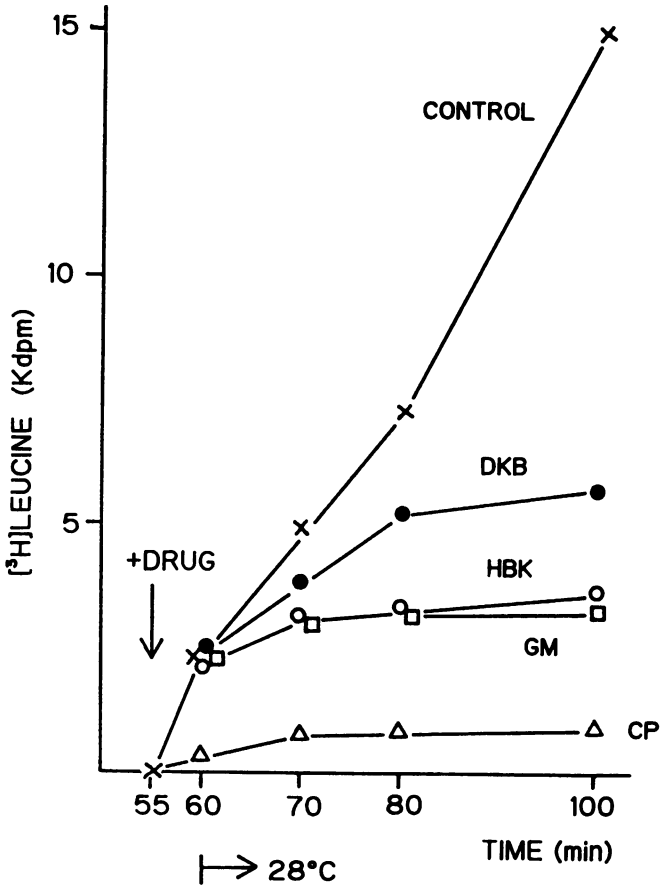

FIG. 4. Effects of aminoglycosides and chloramphenicol on protein synthesis in a *dnaC*(Ts) mutant. *E. coli* PC2 was cultured as described in the legend to Fig. 1. The antibiotic and [^{3}H]leucine (10 μCi/ml) were added to the culture 5 min before the return to 28°C. Drug concentrations (micrograms per milliliter): chloramphenicol (CP), 100; habekacin (HBK), 10; dibekacin (DKB), 10; gentamicin (GM), 10.

was almost completely inhibited by habekacin, dibekacin, and gentamicin when the antibiotic (concentration, 10 μg/ml) was added 5 min before the temperature was returned to 28°C, but initiation was not inhibited by streptomycin under those conditions (Fig. 1). Chloramphenicol (100 μg/ml), an inhibitor of protein synthesis, did not significantly affect the reinitiation, suggesting that the inhibition of reinitiation by the aminoglycosides is not due to the suppression of protein synthesis (15). Streptomycin did not inhibit the reinitiation, presumably because the cellular transfer did not occur, owing to a loss of ribosomal affinity for streptomycin in *rpsL* mutants (2).

The dependency of the inhibition of DNA reinitiation on antibiotic concentration was studied with habekacin (Fig. 2). Habekacin completely blocked the reinitiation at a concentration of 10 μg/ml and moderately inhibited it at 5 μg/ml. The MIC was 6 μg/ml under the same conditions (data not shown). The results suggest a certain relationship of the inhibition of DNA reinitiation to the antibacterial activity.

The reinitiation of DNA replication was markedly prevented by habekacin (10 μg/ml), when added to the culture 5 or 10 min before the shift back to the permissive temperature, but it was not significantly affected when the drug was added simultaneously or after the shift back (Fig. 3). Preincubation for 5 min may be required for sufficient cellular uptake of habekacin (20).

Effects on protein synthesis in DNA reinitiation. [^{3}H]leucine incorporation was rapidly blocked by chloramphenicol (100 μg/ml) in a simultaneous experiment with temperature shifts as described above. Habekacin, dibekacin, and gentamicin (10 μg/ml) inhibited protein synthesis after a lag of several minutes (Fig. 4). The lag periods for the inhibition of leucine uptake and for the inhibition of DNA reinitiation were similar, suggesting that both of these steps are dependent upon a common prerequisite, presumably aminoglycoside transport. The results also suggest that the prevention of DNA reinitiation by aminoglycosides is not a secondary effect due to the inhibition of protein synthesis, because a more potent inhibitor of protein synthesis, chloramphenicol, did not significantly affect DNA reinitiation.

Effects on RNA synthesis in DNA reinitiation. The effects of habekacin and gentamicin on RNA and DNA syntheses were compared with those of chloramphenicol and rifampin in an experiment on DNA reinitiation (Fig. 5). Habekacin and gentamicin (10 μg/ml) both completely blocked DNA reinitiation without significantly affecting RNA synthesis. In a

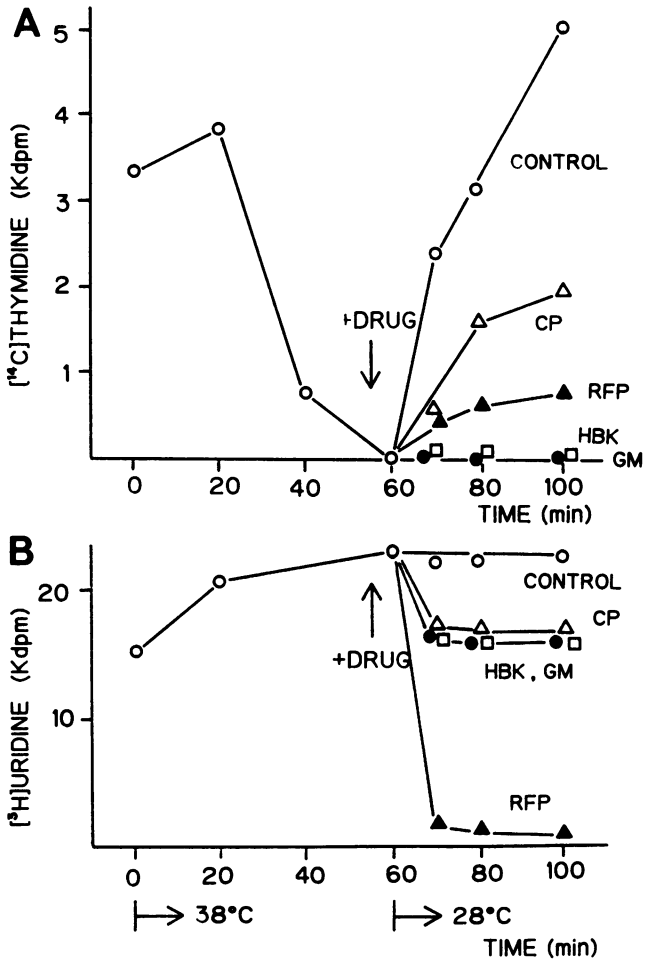

FIG. 5. Effects of aminoglycosides, rifampin, and chloramphenicol on DNA (A) and RNA (B) synthesis in a *dnaC*(Ts) mutant. Drug abbreviations and concentrations except rifampin (RFP, 100 μg/ml) are described in the legend to Fig. 4, and *E. coli* PC2 was cultured as described in the legend to Fig. 1. The antibiotic was added to the culture before the return to 28°C.

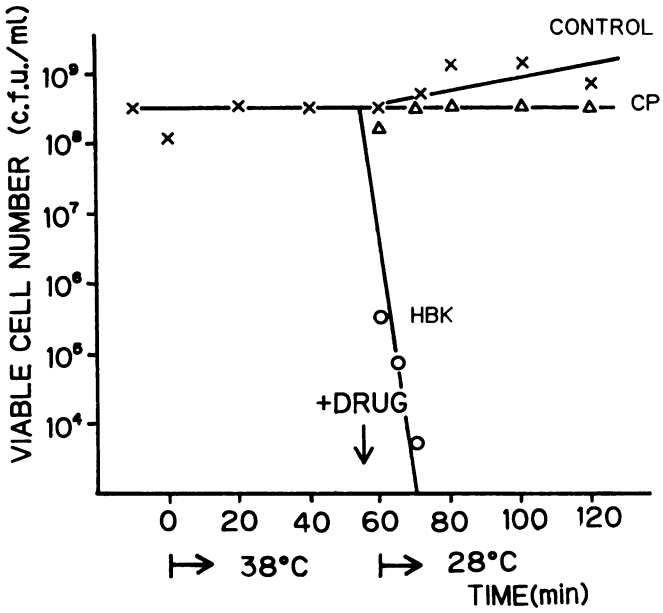

FIG. 6. Effects of habekacin and chloramphenicol on cell viability. *E. coli* PC2 was cultured as described in the legend to Fig. 1, and drug abbreviations and concentrations were as described in the legend to Fig. 4.

simultaneous experiment, rifampin (100 μg/ml), an inhibitor of RNA polymerase, markedly prevented both RNA synthesis and DNA reinitiation, suggesting that the interruption of DNA reinitiation is due to the inhibition of primer RNA synthesis. Chloramphenicol did not significantly block RNA synthesis and DNA reinitiation. The results suggest that

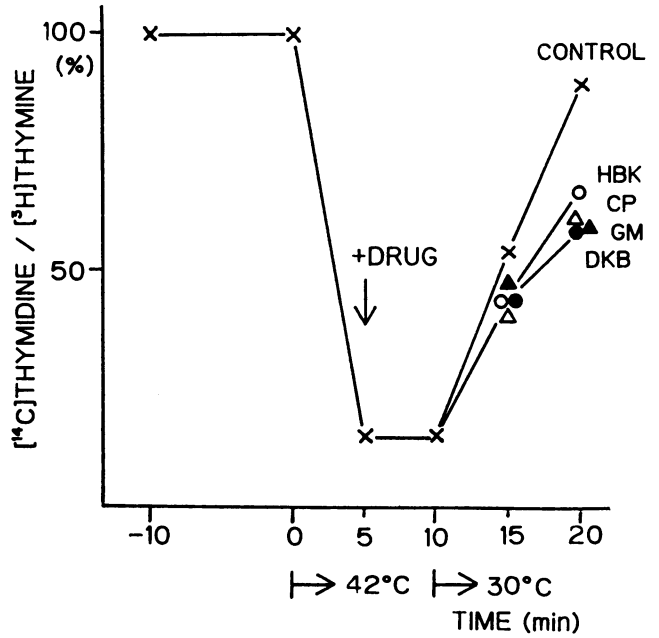

FIG. 7. Effects of aminoglycosides and chloramphenicol on DNA elongation in a *dnaE*(Ts) mutant. Drug abbreviations and concentrations were as described in the legend to Fig. 4. *E. coli* E486 was cultured as described in Materials and Methods. The antibiotic was added to the culture 5 min before the return to 30°C.

TABLE 1. Effect of habekacin on DNA synthesis in toluene-treated cells of *E. coli* P3478

Incubation time (min)	Habekacin added (μg/ml)	[^{3}H]dCMP incorporated [dpm (%)]
15	0	2,400 (100)
	10	2,300 (96)
	100	2,400 (100)
30	0	4,100 (170)

aminoglycosides do not significantly affect primer RNA synthesis in the initiation of DNA replication.

Effect on cell viability. The colony-forming ability of the *dnaC*(Ts) mutant treated with habekacin (10 μg/ml) was studied simultaneously in an experiment on DNA reinitiation

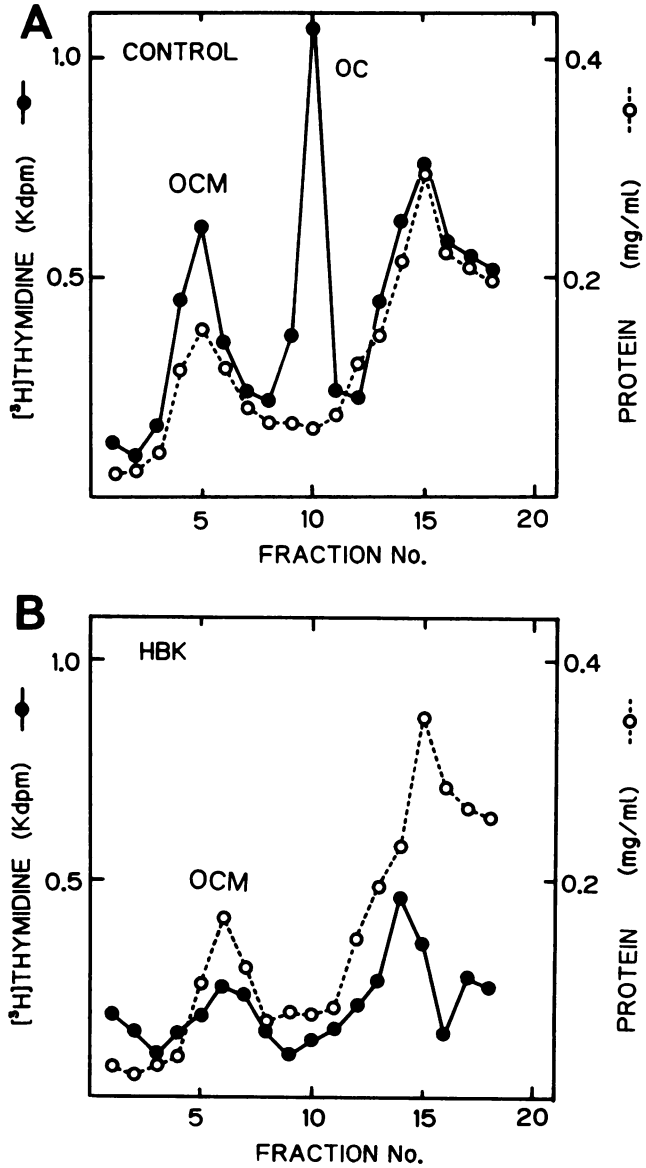

FIG. 8. Effect of habekacin on *oriC*-membrane attachment in a *dnaC*(Ts) mutant. The membrane fraction of *E. coli* PC2, with (B) or without (A) treatment by habekacin (HBK, 20 μg/ml), which was added 5 min before the reinitiation, was analyzed by 30 to 50% sucrose density gradient sedimentation (3 h).

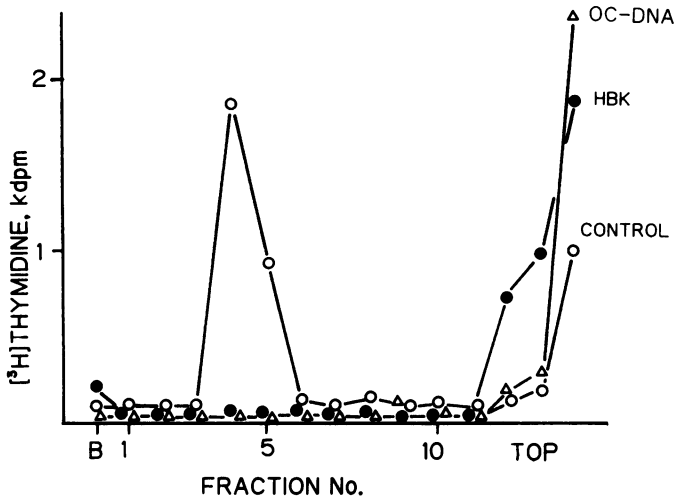

FIG. 9. Effect of habekacin (HBK, 20 μg/ml) on reconstitution of the *oriC*-membrane complex. A mixture of membrane and *oriC* DNA of *E. coli* PC2 was treated and analyzed as described in the legend to Fig. 8. OC-DNA, *oriC* DNA without membrane.

(Fig. 6). Habekacin killed the cells within several minutes, but chloramphenicol prevented cell growth without showing a lethal effect. The inhibition of DNA reinitiation seemed to be related to the bactericidal action, because both occurred simultaneously within a short period after the addition of habekacin. However, the precise relationship remains to be determined.

Effects on DNA elongation. As reported previously (33), habekacin as well as chloramphenicol does not significantly affect DNA elongation of the *dnaC*(Ts) mutant at a restrictive temperature.

The effect of aminoglycosides on DNA elongation was further studied with a *dnaE*(Ts) mutant, *E. coli* E486, in which the α subunit of DNA polymerase III is thermosensitive (19). A logarithmically growing culture was shifted from a permissive temperature (30°C) to a restrictive temperature (42°C), kept at 42°C for 10 min, and shifted back to 30°C. The antibiotics were added 5 min before the shift back. Habekacin, dibekacin, gentamicin, and chloramphenicol did not significantly affect DNA elongation (Fig. 7).

DNA synthesis was not significantly affected by habekacin at a concentration of 10 or 100 μg/ml in toluene-treated cells of a *polA* mutant, *E. coli* P3478, which presumably lacks DNA repair and initiation (4) (Table 1).

The above results suggest that aminoglycosides do not significantly affect DNA elongation.

Effect on *oriC*-membrane complex formation. In the initiation of replication, a DNA region around the initiation point, *oriC*, may bind to the cell membrane and the attachment may be mediated by several proteins (21, 24). The *oriC*-membrane complex is partially dissociated into a membrane and an origin complex, consisting of *oriC* DNA (DNA carrying the *oriC* gene) and proteins, in EDTA solution, which prevents the degradation of DNA (21, 22).

The effect of habekacin on *oriC*-membrane complex formation in vivo was studied by using the *dnaC*(Ts) mutant, which was treated by a shift back to a permissive temperature, pulse-labeling with [³H]thymidine, cell disruption, and EDTA-sucrose density gradient sedimentation. Formation of the *oriC*-membrane and the origin complex was significantly blocked by habekacin (20 μg/ml) (Fig. 8). The inhibition of the latter was more marked than that of the former. Since the origin complex is released from the *oriC*-membrane complex in the presence of EDTA, the results suggest that habekacin interrupts the *oriC*-membrane attachment.

Effects on reconstitution of the *oriC*-membrane complex in vitro. The *oriC*-membrane complex obtained by the above procedure was separated into *oriC* DNA and membrane fractions by CsCl density gradient centrifugation (16). The *oriC* DNA and the membrane fractions were mixed together, and the reconstituted *oriC*-membrane complex was analyzed by sucrose density gradient sedimentation (21). Habekacin (20 μg/ml) completely blocked formation of the *oriC* DNA-membrane complex (Fig. 9). The reconstitution was also inhibited by streptomycin, gentamicin, and kanamycin but not by chloramphenicol (data not shown).

DISCUSSION

The present studies have revealed that aminoglycosides inhibit initiation of DNA replication without affecting DNA elongation. The inhibition is not a secondary effect due to the interference with protein or RNA synthesis but may be due to the interruption of DNA-membrane attachment.

The role of the plasma membrane in the initiation of DNA replication has not been well established. The absence of DNA initiation in toluene- or phenethyl alcohol-treated cells supports the assumption that the membrane participates in the initiation of replication (4, 5, 17a, 26, 27, 34). The *dnaC* product is involved in the attachment of the nucleoid to the membrane (28). On the other hand, the results of in vitro experiments with *oriC* plasmids oppose this assumption (10, 23). However, the membrane may be required for efficient initiation of replication of the *E. coli* chromosome. Our results seem to support the hypothesis that the attachment of *oriC* DNA to the membrane is required for initiation of chromosome replication.

Aminoglycosides show pleiotropic effects, including inhibition of ribosomal functions, membrane damage, and suppression of DNA initiation. However, a general inhibition of protein synthesis and an ability to cause codon misreading are not the specific reasons for the lethality (2, 14). For instance, chloramphenicol and most other ribosomal inhibitors have a bacteriostatic effect, and ethionine, an amino acid analog (25), is a strong inducer of misreading but does not show a killing effect in 1 h.

Bryan and Kwan (2) have proposed, as a model of aminoglycoside uptake and killing, that lethality results from the cumulation of the effects on the cytoplasmic membrane or from a gradual disruption of membrane integrity. The present results seem to be in accordance with their model. Based on the present studies, we propose the following mechanism of aminoglycoside transport and lethality. The energy-independent phase (ionic binding) and the energy-dependent phases I and II are involved in the process of cellular uptake of aminoglycosides. Energy-dependent phase II is initiated and accelerated by binding to ribosomes. Aminoglycosides bind to polysomes and disturb the ribosomal cycle and protein synthesis, accelerating the rate of transfer to ribosomes. The initial damage to the membrane occurs as part of the transport process, particularly energy-dependent phase II, and consequently blocks initiation of DNA replication through interruption of the *oriC*-membrane attachment, resulting in perturbation of cell division. The sequential events of membrane damage and inhibition of DNA initiation and protein synthesis may progress synergis-

tically, resulting in cell death. Alternatively, the membrane damage may not be repaired without protein synthesis, and the resulting inhibition of replication initiation may be irreversible, leading to cell death.

Since the *dnaC*(Ts) mutant recovers viability by a shift back from a restrictive temperature to a permissive temperature, the inhibition of replication initiation may be reversible (15). However, the synergism of membrane damage, subsequent interruption of DNA initiation, and inhibition of protein synthesis may exert an irreversible effect, leading to cell death, as described above. The membrane damage results in a loss of permeability control for various materials (bases, nucleotides, β-galactoside, etc.) and bleb formation (17, 32).

It was noted in the present experiment that DNA synthesis is resistant to streptomycin in the streptomycin-resistant mutant, owing to ribosomal alteration. The cells may lose their ability to take up streptomycin and, subsequently, their ability to show membrane disruption induced by the drug transfer because of a deficiency of energy-dependent phase II (2, 3). Alternatively, protein synthesis resistant to streptomycin may repair the membrane damage.

Another hypothesis for cell killing by aminoglycosides is that the inhibition of DNA replication may induce SOS functions and result in cell death, as in the case of nalidixic acid. Nalidixic acid exerts its killing effect by the SOS response (7, 18). Nalidixic acid inhibits DNA elongation by forming a complex with DNA and gyrase or topoisomerase. The inhibition induces the SOS reaction and consequently blocks cell division, leading to cell death. Both aminoglycosides and nalidixic acid are lethal and inhibit DNA replication and cell division. However, the mode of action of aminoglycosides seems to differ from that of nalidixic acid in several ways. (i) Nalidixic acid requires protein synthesis for the lethal action, and the SOS reaction needs newly synthesized protein to block cell division (12). In contrast, aminoglycosides show a killing effect in the absence of protein synthesis. For instance, aminoglycosides kill auxotrophs in amino acid starvation and in the presence of puromycin, an inhibitor of protein synthesis (29, 36). (ii) Nalidixic acid blocks both initiation and elongation of DNA replication, but aminoglycosides selectively inhibit DNA initiation. (iii) Nalidixic acid needs 30 to 60 min to exhibit a significant lethal effect, but aminoglycosides kill bacteria within several minutes (12).

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LITERATURE CITED

1. **Bryan, L. E.** 1982. Bacterial resistance and susceptibility to chemotherapeutic agents, p. 95, 146–159. Cambridge University Press, Cambridge.
2. **Bryan, L. E., and S. Kwan.** 1983. Roles of ribosomal binding, membrane potential, and electron transport in bacterial uptake of streptomycin and gentamicin. Antimicrob. Agents Chemother. **23**:835–845.
3. **Bryan, L. E., and H. M. Van den Elzen.** 1976. Streptomycin accumulation in susceptible and resistant strains of *Escherichia coli* and *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. **9**:928–938.
4. **Burger, R. M.** 1971. Toluene-treated *Escherichia coli* replicate only that DNA which was about to be replicated *in vivo*. Proc. Natl. Acad. Sci. USA **68**:2124–2126.
5. **Burger, R. M., and D. A. Glaser.** 1973. Effect of nalidixic acid on DNA replication by toluene-treated *Escherichia coli*. Proc. Natl. Acad. Sci. USA **70**:1955–1958.
6. **Choi, E. C., T. Nishimura, and N. Tanaka.** 1980. Mutational alterations of either large or small ribosomal subunit for the kanamycin resistance. Biochem. Biophys. Res. Commun. **94**:755–762.
7. **Drlica, K.** 1984. Biology of bacterial deoxyribonucleic acid topoisomerases. Microbiol. Rev. **48**:273–289.
8. **Dubin, D. T., R. Hancock, and B. D. Davis.** 1963. The sequence of some effects of streptomycin in *Escherichia coli*. Biochim. Biophys. Acta **74**:476–489.
9. **Freda, C. E., M. M. K. Nass, and S. S. Cohen.** 1968. T6r$^+$-induced proteins and nucleic acids in *Escherichia coli* infected in the presence of streptomycin. J. Bacteriol. **96**:1382–1399.
10. **Fuller, R. S., J. M. Kaguni, and A. Kornberg.** 1981. Enzymatic replication of the origin of the *Escherichia coli* chromosome. Proc. Natl. Acad. Sci. USA **78**:7370–7374.
11. **Gefter, M. L., Y. Hirota, T. Kornberg, J. A. Wechsler, and C. Barnoux.** 1971. Analysis of DNA polymerase II and III in mutants of *Escherichia coli* thermosensitive for DNA synthesis. Proc. Natl. Acad. Sci. USA **68**:3150–3153.
12. **Goss, W. A., W. H. Deitz, and T. M. Cook.** 1964. Mechanism of action of nalidixic acid on *Escherichia coli*. J. Bacteriol. **88**:1112–1118.
13. **Hancock, R. E. W.** 1981. Aminoglycoside uptake and mode of action—with special reference to streptomycin and gentamicin. I. Antagonists and mutants. J. Antimicrob. Chemother. **8**:249–276.
14. **Hancock, R. E. W.** 1981. Aminoglycoside uptake and mode of action—with special reference to streptomycin and gentamicin. II. Effects of aminoglycosides on cells. J. Antimicrob. Chemother. **8**:429–445.
15. **Hanna, M. H., and P. L. Carl.** 1975. Reinitiation of deoxyribonucleic acid synthesis by deoxyribonucleic acid initiation mutants of *Escherichia coli*: role of ribonucleic acid synthesis, protein synthesis, and cell division. J. Bacteriol. **121**:219–226.
16. **Hendrickson, W. G., T. Kusano, H. Yamaki, R. Balakrishnan, M. King, J. Murchie, and M. Schaechter.** 1982. Binding of the origin of replication of *Escherichia coli* to the outer membrane. Cell **30**:915–923.
17. **Iida, K., and M. Koike.** 1974. Cell wall alterations of gram-negative bacteria by aminoglycoside antibiotics. Antimicrob. Agents Chemother. **5**:95–97.

17a. **Lark, K. G., and C. Lark.** 1966. Regulation of chromosome replication in Escherichia coli: a comparison of the effects of phenethyl alcohol treatment with those of amino acid starvation. J. Mol. Biol. **20**:9–19.

18. **Little, L. W., and D. W. Mount.** 1982. The SOS regulatory system of *Escherichia coli*. Cell **29**:11–22.
19. **Lucia, P. D., and J. Cairns.** 1969. Isolation of an *E. coli* strain with a mutation affecting DNA polymerase. Nature (London) **224**:1164–1166.
20. **Matsunaga, K., T. Nishimura, and N. Tanaka.** 1984. Bacterial uptake of habekacin, a novel aminoglycoside antibiotic. J. Antibiot. **37**:592–601.
21. **Nagai, K., W. Hendrickson, R. Balakrishnan, H. Yamaki, D. Boyd, and M. Schaechter.** 1980. Isolation of a replication origin complex from *Escherichia coli*. Proc. Natl. Acad. Sci. USA **77**:262–266.
22. **Nicolaides, A. A., and I. B. Holland.** 1978. Evidence for the specific association of the chromosomal origin with outer membrane fractions isolated from *Escherichia coli*. J. Bacteriol. **135**:178–189.
23. **Nüssein-Crystalla, V., and U. Scheefer-Borchel.** 1979. *In vitro* replication of a DNA fragment containing the vicinity of the origin of *E. coli* DNA replication. Mol. Gen. Genet. **169**:35–40.
24. **Olsen, W. L., H.-G. Heidrich, K. Hannig, and P. H. Hofschneider.** 1974. Deoxyribonucleic acid-envelope complexes isolated from *Escherichia coli* by free-flow electrophoresis: biochemical and electron microscope characterization. J. Bacteriol. **118**:646–653.

25. **Pine, M. J.** 1978. Comparative physiological effects of incorporated amino acid analogs in *Escherichia coli*. Antimicrob. Agents Chemother. **13**:676–685.
26. **Richardson, A. G., and F. R. Leach.** 1969. The effect of phenethyl-alcohol on *Bacillus subtilis* transformation. I. Characterization of the effect. Biochim. Biophys. Acta **174**:264–275.
27. **Richardson, A. G., D. L. Pierson, and F. R. Leach.** 1969. The effect of phenethylalcohol on *Bacillus subtilis* transformation. II. Transport of DNA and precursors. Biochim. Biophys. Acta **174**:276–281.
28. **Ryder, O. A., and D. W. Smith.** 1975. Properties of membrane-associated folded chromosome of *E. coli* related to initiation and termination of DNA replication. Cell **4**:337–345.
29. **Stern, J. L., H. D. Barner, and S. S. Cohen.** 1966. The lethality of streptomycin and stimulation of RNA synthesis in the absence of protein synthesis. J. Mol. Biol. **17**:188–217.
30. **Tanaka, N.** 1975. Aminoglycoside antibiotics, p. 340–364. *In* J. W. Corcoran and F. H. Hahn (ed.), Antibiotics. III. Mechanism of action of antimicrobial and anticancer agents. Springer-Verlag KG, Berlin.
31. **Tanaka, N.** 1982. Mechanism of action of aminoglycoside antibiotics, p. 221–266. *In* H. Umezawa and I. R. Hooper (ed.), Aminoglycoside antibiotics. Springer-Verlag KG, Berlin.
32. **Tanaka, N., K. Matsunaga, A. Hirata, Y. Matsuhisa, and T. Nishimura.** 1983. Mechanism of action of habekacin, a novel amino acid-containing aminoglycoside antibiotic. Antimicrob. Agents Chemother. **24**:797–802.
33. **Tanaka, N., K. Matsunaga, H. Yamaki, and T. Nishimura.** 1984. Inhibition of initiation of DNA synthesis by aminoglycoside antibiotics. Biochem. Biophys. Res. Commun. **122**:460–465.
34. **Treick, R. W., and W. A. Konetzka.** 1964. Physiological state of *Escherichia coli* and the inhibition of deoxyribonucleic acid synthesis by phenethyl alcohol. J. Bacteriol. **88**:1580–1584.
35. **Wallace, B. J., P. C. Tai, and B. D. Davis.** 1979. Streptomycin and related antibiotics, p. 272–303. *In* F. E. Hahn (ed.), Antibiotics. I. Mechanism of action of antibacterial agents. Springer-Verlag KG, Berlin.
36. **Yamaki, H., and N. Tanaka.** 1963. Effects of protein synthesis inhibitors on lethal action of kanamycin and streptomycin. J. Antibiot. **16**:222–226.