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Bacteriophage T4 Inhibits Colicin E2-Induced Degradation of *Escherichia coli* Deoxyribonucleic Acid[1]

I. Protein Synthesis-Dependent Inhibition

ROBERT L. SWIFT AND JOHN S. WIBERG

Department of Radiation Biology and Biophysics, University of Rochester School of Medicine and Dentistry, Rochester, New York 14642

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The deoxyribonucleic acid (DNA) of *Escherichia coli* B is converted by colicin E2 to products soluble in cold trichloroacetic acid; we show that this DNA degradation (hereafter termed solubilization) is subject to inhibition by infection with bacteriophage T4. At least two modes of inhibition may be differentiated on the basis of their sensitivity to chloramphenicol. The following observations on the inhibition of E2 by phage T4 in the absence of chloramphenicol are described: (i) Simultaneous addition to *E. coli* B of E2 and a phage mutated in genes 42, 46, and 47 results in a virtually complete block of the DNA solubilization normally induced by E2; the mutation in gene 42 prevents phage DNA synthesis, and the mutations in genes 46 and 47 block a late stage of phage-induced solubilization of host DNA. (ii) This triple mutant inhibits equally well when added at any time during the E2-induced solubilization. (iii) Simultaneous addition to *E. coli* B of E2 and a phage mutated only in gene 42 results in extensive DNA solubilization, but the amount of residual acid-insoluble DNA (20 to 25%) is more characteristic of phage infection than of E2 addition (5% or less). (iv) *denA* mutants of phage T4 are blocked in an *early* stage (endonuclease II) of degradation of host DNA; when E2 and a phage mutated in both genes 42 and *denA* are added to *E. coli* B, extensive solubilization of DNA occurs with a pattern identical to that observed upon simultaneous addition of E2 and the gene 42 mutant. (v) However, delaying E2 addition for 10 min after infection by this double mutant allows the phage to develop considerable inhibition of E2. (vi) Adsorption of E2 to *E. coli* B is not impaired by infection with phage mutated in genes 42, 46, and 47. In the presence of chloramphenicol, the inhibition of E2 by the triple-mutant (genes 42, 46, and 47) still occurs, but to a lesser extent.

Colicin E2 is a bactericidal protein that has a molecular weight of approximately 60,000 (12). It belongs to a class of antibiotics termed colicins, which kill sensitive strains of coliform bacteria by a variety of biochemical mechanisms (22, 24). Apparently, colicins do not traverse the bacterial plasma membrane (20, 25, 31), and yet they kill sensitive bacteria with single-hit kinetics (15, 26). Plausible modes of action of colicins have been proposed by Changeux and Thiéry (3), Luria (19), and by Nomura (22, 23). Although the basic mechanism that transmits the biological information inherent in the colicin molecule appears analogous for all colicins, the biochemical manifestations of the transmission are remarkably colicin-specific. For example, colicin E3 specifically inhibits protein synthesis (16, 24, 29), whereas colicins E1, K, I, and A interfere primarily with energy metabolism (6–8, 13, 19, 22, 24). The primary target in the case of colicin E2 appears to be deoxyribonucleic acid (DNA; reference 22), and Ringrose (27) distinguishes at least three target-specific, sequential events involved in the colicin E2-induced conversion of DNA to acid-soluble form. (i) Stage I

[1] A preliminary report of this work has appeared: Abstracts, 1970 Phage Meetings, Cold Spring Harbor Laboratory of Quantitative Biology, Cold Spring Harbor, N.Y. 11724.

occurs almost immediately after addition of colicin E2 to *Escherichia coli* and involves the introduction of single-strand nicks into the DNA. (ii) After a slight lag, stage II converts the DNA to double-stranded fragments; and (iii), still later, an exonuclease rapidly converts the double-stranded fragments to acid-soluble form. Increasing the multiplicity of colicin E2 increases the rate at which the DNA is degraded (24). Since chloramphenicol (CM) does not diminish the solubilization of DNA induced by colicin E2, it is apparent that preexisting *E. coli* nucleases are responsible for the observed activity (22).

It has been observed that colicins K and E3 interfere with replication of phage T4, whereas colicin E2 does not; this was interpreted to mean that T4 DNA is spared from the nucleolytic activities induced by colicin E2 (22). We show here that, even in the presence of CM, infection with phage T4 partially inhibits the E2-induced nuclease activity and that the inhibition is virtually complete if protein synthesis is not blocked. These observations may explain why phage T4 can replicate successfully in colicin E2-treated bacteria.

MATERIALS AND METHODS

Bacteriophage and bacterial strains. *E. coli* K-12 strain W3110, Sm^r, (col E2-P9) was obtained from M. Nomura via Sankar Adhya. Since frequent reference will be made to specific mutants of phage T4D, the following shorthand terminology will be employed. T4 *amber* (*am*) mutants are those conditional lethal mutants that are suppressed by *E. coli* CR63 but not by *E. coli* B (5). Specific *amber* mutants of phage T4 will be referred to by gene number or symbol in brackets: *am*N55x5 (deoxycytidylate hydroxymethylase) = [42]; *am*B14x5 and *am*A456x5 (presumptive deoxyribonuclease) = [46] and [47], respectively; S112x5 (endonuclease II) = [*denA*]. The designation x5 indicates that these mutants have been purified genetically (34). All other bacteriophage and bacterial strains used, as well as the procedures for the construction of multiple mutants, the preparation of phage stocks, and the assay procedures for viable phage and bacteria have been described previously (17, 18, 34–36).

Growth and dilution media. All bacteria were grown and infected in GCA, the glycerol-Casamino Acids medium of Fraser and Jerrel (9). Dilution broth is nutrient broth (Difco) in 0.5% NaCl. The composition of the M9 buffer solution is 49 mM Na_2HPO_4, 22 mM KH_2PO_4, 19 mM NH_4Cl, 8.6 mM NaCl, 0.1 mM $CaCl_2$, and 1 mM $MgSO_4$.

Chemicals. Unlabeled deoxythymidine (dT) and deoxyadenosine (dA) were purchased from Calbiochem. [*Methyl-*^{3}*H*]-dT (3.0 Ci/mmole) was obtained from Schwarz BioResearch, Inc. CM was a generous gift from Parke Davis and Co. *O*-nitrophenyl-beta-D-galactopyranoside and isopropyl-beta-D-thiogalactopyranoside were obtained from Mann Research Laboratories. Deuterium oxide was purchased from BioRad Laboratories. Bovine serum albumin (BSA; fraction V) was purchased from Armour Pharmaceutical Co.

Radioisotopic labeling and determination of the breakdown of DNA. The bacterial DNA was labeled by the procedure described by Kutter and Wiberg (17, 18). The degradation of DNA was determined by measuring the residual ^{3}H-dT in cold trichloroacetic acid-insoluble material by using the variation of Bollum's filter paper technique (1) described by Hercules et al. (11). Degradation measured this way is termed "solubilization" in this paper.

Purification of intact phage. To eliminate contamination of the concentrated phage stocks by phage ghosts (DNA-less phage), a portion of the concentrated stock was layered onto a 4.8-ml linear gradient (0 to 99%) of deuterium oxide and water in 0.1 M sodium-potassium phosphate buffer (*p*H 7.7) and centrifuged at 32,000 × *g* for 20 min at 4 C in a Spinco SW39 rotor. After centrifugation, the bottom of the centrifuge tube (cellulose nitrate, Beckman) was punctured, and 28 fractions of 15 drops each were collected. Fractions 1 to 12, containing "ghost-free" intact phage, were pooled and dialyzed against M9 buffer. The resulting phage are designated "purified" with respect to phage ghosts which sedimented only as far as fraction 16 in the linear gradient. The purified intact phage were titered by plaque-forming ability as well as by the method of Duckworth and Bessman (4), which is based on the ability of intact phage and phage ghosts to inhibit β-galactosidase induction with comparable efficiency. The titer of plaque-formers matched precisely the particle titer based on the inhibition of β-galactosidase induction.

Preparation and titering of colicin E2. A 25-ml culture of *E. coli* W3110, Sm^r, (E2-P9) was grown to a concentration of 5 × 10^8/ml in GCA medium. The culture was chilled and then irradiated with ultraviolet light (254 nm) to a survival of approximately 20% with a Mineralight UVS-12 lamp (Ultraviolet Products, Inc., San Gabriel, Calif.). The irradiated culture was transferred to an actinic-glass shake flask and incubated at 30 C on a gyratory shaker (New Brunswick Scientific Co., Inc.) for 3 hr. The culture was then shaken with chloroform and allowed to stand overnight at 4 C. Bacterial debris was removed by centrifugation at 5,000 × *g* for 15 min. The titer of this crude preparation of colicin was determined by measuring the fraction of *E. coli* B survivors after incubation with various concentrations of colicin at 37 C for 10 min in the presence of 200 μg of CM/ml. The killer titer was determined from the bacterial survivor values by using the Poisson distribution.

When a large number of samples was to be titered for E2, an adaptation of the colorimetric method of Shannon and Hedges (30) to the Technicon Autoanalyzer was used.

Measurement of the effect of phage infection on the adsorption of E2 to E. coli B. *E. coli* B was grown to a concentration of 5 × 10^8 cells per ml. The culture was resuspended in the same volume of chilled GCA medium containing BSA at a final concentration of 2

mg/ml. The BSA prevents nonspecific inactivation of colicin E2 during incubation at 37 C (21). Portions of the resuspended culture were prewarmed for 5 min at 37 C on a gyratory shake bath before infection with phage [42, 46, 47] at a multiplicity of 5 or with a sham equivalent to the volume of GCA medium used for infection. The latter sham addition provides the control uninfected culture. After 10 min of incubation at 37 C, portions of the cultures to be used for the preparation of supernatant fluids were removed and chilled. To the remaining portions of the uninfected or [42, 46, 47]-infected cultures, E2 was added to give a final concentration of 2×10^{10} killer particles per ml. Shaking was continued at 37 C. At 2-min intervals, 1-ml samples were withdrawn and immediately centrifuged at 150,000 × *g* for 1 min in an Eppendorf centrifuge, model 3200. The supernatant fluid was then decanted into a test tube and chilled on ice. The entire sampling procedure required 90 sec to complete. All sampling was done in a 5 C cold room. Supernatant fluids were prepared from the uninfected and [42, 46, 47]-infected cultures collected just prior to the addition of E2 by centrifugation at 10,000 × *g* for 30 min at 4 C. These supernatant fluids were tested for "apparent" adsorption of E2 in a manner identical to that used for the cultures. Unadsorbed colicin E2 was assayed by the automated colorimetric bioassay described above. *E. coli* B/4 was used as the sensitive bacterium in the bioassay to preclude any killing by unadsorbed phage.

RESULTS

Inhibition of colicin E2-induced DNA solubilization by phage infection. (i) Simultaneous infection with T4 mutants defective in genes 42, 46, and 47. Amber mutants of phage T4D that are defective in genes 46 or 47, or both, fail to degrade the DNA of the nonpermissive host *E. coli* B to acid-soluble form (34). In addition, phage DNA synthesis virtually ceases at about 15 min after infection at 37 C (5, 36). When the present studies were begun, it was not clear to what extent the failure to degrade host DNA contributed to the arrest of phage DNA synthesis and the markedly reduced phage production by these mutants. [Recently, mutants in a gene far removed from 46 and 47 have been isolated (11, 33) that produce normal or near-normal yields of phage, despite their inability to degrade host DNA.] During an attempt to answer this question by testing whether E2 could phenotypically "rescue" mutants in genes 46 and 47, we observed that the DNA solubilization normally induced by E2 was virtually abolished in *E. coli* B infected with these phage mutants. This effect is shown in Fig. 1: when [42, 46] and E2 are added to *E. coli* B simultaneously, the extensive solubilization of DNA normally induced by E2 was slowed drastically; the mutation in gene 42 was included to prevent phage DNA synthesis, which otherwise

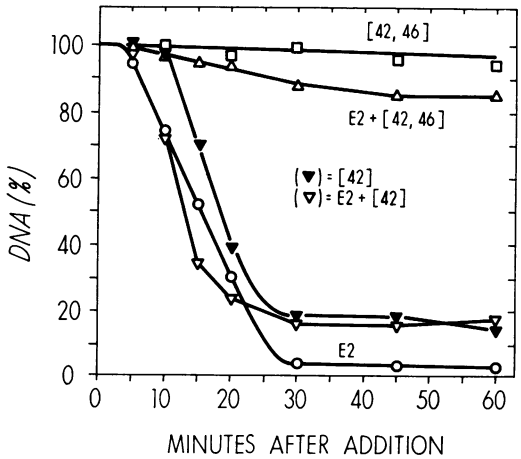

FIG. 1. *Inhibition of colicin E2-induced DNA solubilization in E. coli B by [42, 46] and [42]; simultaneous addition of colicin E2 and phage. E. coli B (at 4.8 × 10^8 cells/ml), prelabeled with ^{3}H-dT as described in Materials and Methods, was treated at 37 C with colicin E2 (multiplicity of 200) or purified phage (multiplicity of 5), or both. Samples were taken at the indicated times for determination of acid-soluble ^{3}H.*

would mask DNA solubilization by allowing reincorporation of ^{3}H-dT from host DNA into phage DNA. An identical inhibition of E2 was observed whether genes 46, 47, or both were mutated (*data not shown*). In contrast, the simultaneous addition of E2 and [42] results in extensive DNA solubilization (Fig. 1), but this may be attributable to phage-directed enzymes since [42] by itself gives extensive DNA solubilization. Note that the solubilization of DNA begins 5 to 6 min earlier than with [42] alone; this argues that E2 is active at least initially in spite of the phage. Conversely, the amount of residual acid-insoluble DNA (20 to 25%) is more characteristic of phage infection than of E2 addition (5% or less).

(ii) Delayed infection with [42, 46, 47]. The preceding results suggest that even though E2 may induce deoxyribonuclease activity in [42]-infected *E. coli* B, this activity is subsequently diminished by a phage-directed factor. Conceivably, the inhibitory factor may block continued induction by E2 or directly inhibit the induced deoxyribonuclease activity. If the inhibition affects only induction, one would expect to observe progressively less inhibition as the interval between E2 addition and phage infection is increased; solubilization of DNA by E2 alone rapidly becomes trypsin-irreversible, implying that induction is only a transient event (14, 27). Conversely, if the inhibitory factor directly inactivates E2-induced deoxyribonuclease activity, the

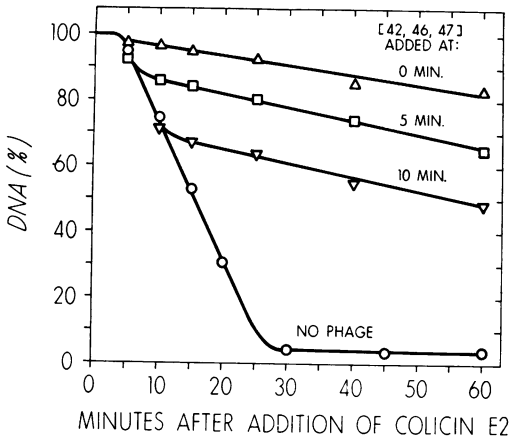

FIG. 2. *Effect of delaying addition of phage on inhibition of colicin E2-induced DNA solubilization in E. coli B by [42, 46, 47]. Procedures and conditions were the same as for Fig. 1 except that the concentration of bacteria was 5.6 × 10⁸ cells/ml.*

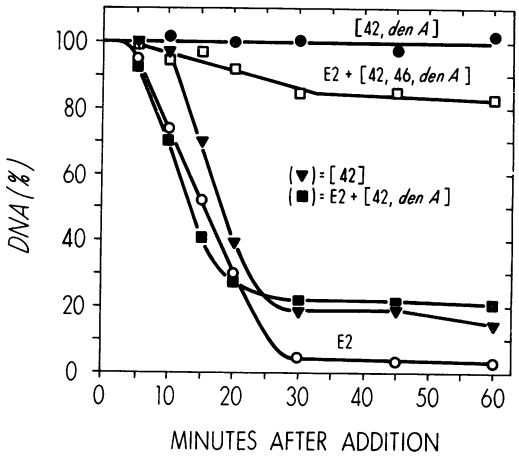

FIG. 3. *Inhibition of colicin E2-induced DNA solubilization in E. coli B by [42, denA] and [42, 46, denA]; simultaneous addition of colicin E2 and phage. Procedures and conditions were identical to those described in Fig. 1.*

inhibition should be independent of the interval between E2 addition and phage infection. As shown in Fig. 2, when [42, 46, 47] infection of *E. coli* B occurs either 0, 5, or 10 min after E2 addition, the extent of inhibition is virtually identical. However, under comparable conditions, when trypsin (2 mg/ml final concentration) is added 10 min after E2 (no phage present), there is no effect on subsequent solubilization of DNA (*data not shown*). We conclude, therefore, that the phage-directed inhibition is acting specifically on the expression of deoxyribonuclease activity; whether it also affects induction of deoxyribonucleases by E2 is not revealed by these experiments.

(iii) Simultaneous infection with [42, denA]. To observe phage-specific modifications of E2-induced DNA solubilization, one must use phage mutants unable by themselves to solubilize host DNA. Although [42, 46, 47] satisfies this criterion, the pleiotropic phenotype of [46] and [47] mutants and our poor understanding of the function of these genes creates uncertainty as to whether it is phage infection in general, or the gene 46-47 defect specifically, that is inhibitory to E2. It would be informative, therefore, to test other phage mutants unable to solubilize host DNA but that are defective in genes other than 46 or 47. Recently, such mutants, in a gene designated *denA*, have become available (11, 33). Mutants in this gene are defective in the production of T4-endonuclease II (28) and, unlike [42, 46, 47] which degrades host DNA to double-stranded fragments having a molecular weight of 10^5, they cause very little fragmentation of host DNA. As shown in Fig. 3, [42, *denA*] alone fails to solubilize *E. coli* B DNA. However, when E2 and [42, *denA*] are added simultaneously to *E. coli* B, extensive solubilization of DNA occurs, and the pattern of solubilization is nearly identical to that observed with simultaneous addition of E2 and [42] (Fig. 1). Note that phage [42, 46, *denA*] behaves like [42, 46] (Fig. 1, 3), indicating that the gene 46 mutation is epistatic to the *denA* mutation in this regard. The preceding results indicate that simultaneous addition of E2 phenotypically suppresses mutations in gene *denA* but not genes 46 and 47.

(iv) Delayed addition of colicin E2 to [42, denA]-infected E. coli B. Since we already have evidence that infection with [42] does not immediately inhibit E2 (Fig. 1), it seemed possible that the failure to observe inhibition upon simultaneous addition of E2 and [42, *denA*] to *E. coli* B could be ascribed to this apparent lag in onset of inhibition. If true, then delaying the addition of E2 for some time after infection with [42, *denA*] should reveal the inhibition. This prediction is verified by the experiment shown in Fig. 4A: if [42, *denA*] is added to *E. coli* B 5 min before E2, a substantial decrease in the rate of solubilization of DNA is observed; the effect is even more pronounced when [42, *denA*] precedes E2 by 10 min. When [42] is added 5 or 10 min before E2, no diminution of the rate of solubilization of DNA is observed (Fig. 4B). These results suggest that the E2-induced phenotypic suppression of *denA* mutants is inhibitable by phage infection but that the inhibition requires a finite interval to become fully developed.

Lack of effect of [42, 46, 47] on colicin E2 adsorption. It seemed possible that the inhibition

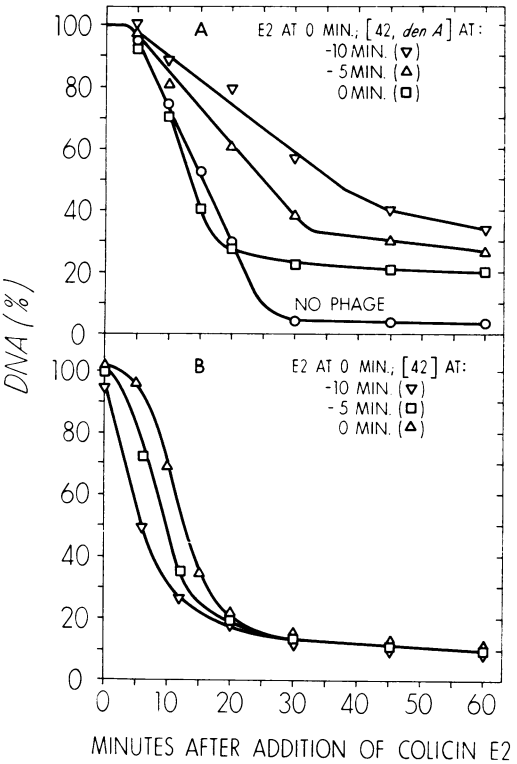

FIG. 4. *Effect of delaying addition of colicin E2 on the inhibition of colicin E2-induced DNA solubilization in E. coli B by [42, denA] (part A) or [42] (part B). Procedures and conditions were the same as for Fig. 1 except that the concentration of bacteria was 4.5 × 10^8 cells/ml.*

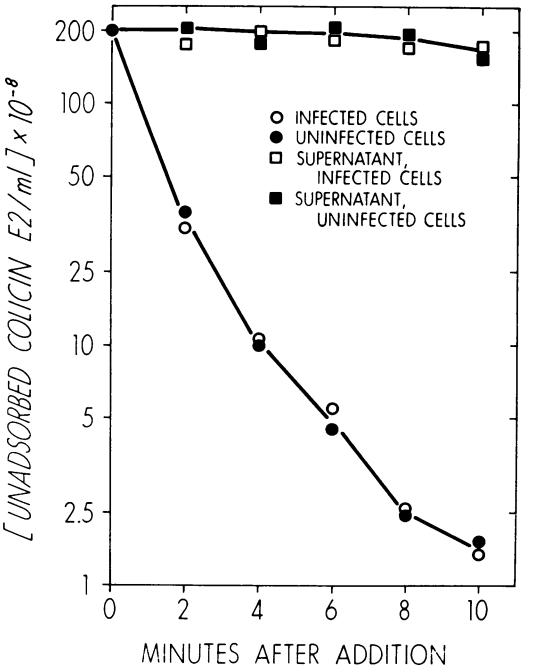

FIG. 5. *Comparison of rates of adsorption of colicin E2 to uninfected and [42, 46, 47]-infected E. coli B. Procedures and conditions are described in the Materials and Methods section. Each experiment was performed in duplicate; the values plotted are averages.*

by [42, 46, 47] could result from an alteration in the adsorption of E2 to *E. coli* B. To test this, we compared rates of adsorption of E2 to uninfected and [42, 46, 47]-infected *E. coli* B. During the course of this investigation, it was observed that E2 is rapidly inactivated at 37 C in GCA medium and in the supernatant fluids from both the uninfected or phage-infected *E. coli* B. It was necessary, therefore, to stabilize the colicin against this inactivation by adding 2 mg of BSA per ml of culture or supernatant fluid (21). The presence of BSA did not alter the inhibition of E2-induced DNA solubilization by [42, 46, 47] (*data not shown*). As shown in Fig. 5, there was negligible loss of E2 during incubation for 10 min at 37 C in BSA-supplemented supernatant fluids from either uninfected or [42, 46, 47]-infected *E. coli* B. It is also clear from Fig. 5 that cells of *E. coli* B infected for 10 min at 37 C with [42, 46, 47] adsorb E2 just as rapidly as do uninfected cells. Therefore, the T4 mutant [42, 46, 47] cannot be inhibiting E2 by interfering with its adsorption to the cell. This experiment does not eliminate the possibility that after initial adsorption of E2, T4 causes its desorption in an inactive form.

Effect of CM. It has been assumed so far that the T4-inhibition of E2-induced DNA solubilization is dependent on protein synthesis. To determine to what extent protein synthesis is required, we tested for the inhibition in the presence of CM. Although CM does not alter the solubilization of DNA induced by E2 (22), it completely blocks the phage-directed solubilization if added before or shortly after infection (2; Fig. 6). Therefore, when E2 and phage are added to *E. coli* B in the presence of CM, solubilization of DNA can be attributed solely to E2. There is clearly some inhibition of E2-induced solubilization that is insensitive to CM (Fig. 6), and the extent of this inhibition is identical whether the phage tested was [42], [42, 46], [42, 46, 47], [42, *denA*], or [42, 46, *denA*]. Since the inhibition by [42, 46] is much greater in the absence of CM, the difference must be due to a mechanism that is CM-sensitive. We have also observed that highly purified phage ghosts (DNA-less phage particles) are capable of inhibiting E2 (*data not*

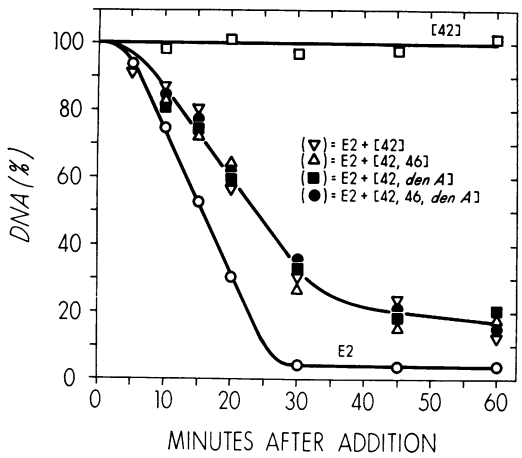

FIG. 6. *Inhibition of colicin E2-induced DNA solubilization in E. coli B by phage infection in the presence of chloramphenicol (CM). Conditions were identical to those described in Fig. 1 except that the concentration of bacteria was 5.2 × 10⁸ cells/ml and CM was added to all cultures to a concentration of 400 μg/ml 5 min before addition of colicin E2 or phage, or both. Note that DNA solubilization by [42] alone is abolished by this level of CM.*

shown), perhaps by a mechanism similar to that involved in the inhibition observed in the presence of CM by intact phage. Further details concerning the inhibition by intact phage in the presence of CM and by ghosts will be presented is a subsequent report.

DISCUSSION

We have observed that infection of *E. coli* B with [42, 46, 47] virtually abolishes the solubilization of bacterial DNA induced by E2, and that the inhibition appears to consist of both CM-sensitive and -insensitive mechanisms; we do not know that the CM-insensitive mode of inhibition is operative in the absence of CM. Infection of *E. coli* B with [42, 46, 47] in the absence of CM does not interfere with adsorption of E2 to *E. coli* B (Fig. 5). The present results do not allow us to distinguish between the two most likely general mechanisms of action of the inhibitory factor: (i) direct action at the membrane, e.g., to reverse the presumed conformational change induced by E2, which, in turn, would reverse the activation of the nucleases. Such a mechanism would have to differ, at least in some respects, from that operating when trypsin is used to remove E2, since trypsinization does not stop solubilization once it has begun (14, 27), whereas [42, 46, 47] does (Fig. 2) or (ii) direct inhibition of the net nucleolytic activity induced by E2.

That the inhibition of E2 by [42, 46, 47] observed in the absence of CM is not unique to mutants defective in genes 46 and 47 is indicated by two lines of evidence. First, E2 by itself normally solubilizes greater than 95% of the DNA of *E. coli* B. However, upon co-addition with [42], the amount of residual acid-insoluble DNA (20 to 25%) is more characteristic of phage infection than of E2 addition (Fig. 1). Second, the mutant [42, *denA*], which is wild-type with respect to genes 46 and 47, also causes measurable inhibition of E2 provided that it is added 5 or 10 min before the colicin (Fig. 4A). Although the extent of this inhibition (phage added 10 min before E2) is less than that produced by [42, 46, 47] (Fig. 2), it is still greater than the inhibition produced by an equal multiplicity of [42, *denA*] in the presence of CM (Fig. 6). This indicates that a CM-sensitive function of phage infection possesses an inhibitory capacity which is independent of lesions in genes 46 and 47. Whether [42, 46, 47] possesses an augmented capacity to inhibit, relative to [42, *denA*], or simply allows a more unambiguous observation of the protein synthesis-dependent inhibition remains unclear. A possible explanation of an augmented inhibitory capacity could be that failure to express genes 46 or 47 results in an *active* interference with certain nuclease activities. By analogy, Goldmark and Linn have shown that extracts from *rec* B and *rec* C strains of *E. coli* are not only deficient in an adenosine triphosphate-dependent deoxyribonuclease, but inhibit the corresponding activity in *rec*$^+$ or *rec* A strains as well (10). Hence, it is not entirely unlikely that mutations in genes 46 and 47, which may specify subunits of a deoxyribonuclease, might impose an analogous inhibition on unrelated deoxyribonucleases of *E. coli* B.

Another possible explanation for the greater inhibition by [42, 46, 47] compared with [42, *denA*] is the following. It is clear from the observations by Ringrose (27) that stage I activity (introduction of single-strand nicks into the DNA) induced by E2 is required only transiently to produce appropriate substrate for other deoxyribonucleases. Consequently, inhibition of stage I activity *after* its product has been produced should not diminish subsequent steps dependent on unrelated deoxyribonucleases. Conversely, stage III activity (conversion of double-stranded fragments of DNA to acid-soluble material) would be needed continuously for complete solubilization since it presumably is the terminal activity involved in the solubilization of DNA. We propose that when E2 and [42, *denA*] are added simultaneously to *E. coli* B, stage I activity may be virtually complete before inhibition

by the phage has fully developed. Since [*denA*] apparently is defective only in a function equivalent to stage I activity, the remaining phage-directed deoxyribonucleases would be available to convert the substrate derived from stage I activity to acid-soluble form. When [42, *denA*] is added 5 or 10 min before E2, presumably allowing time for inhibition even of E2-induced stage I, progressively less substrate becomes available for the phage-directed exonucleases. However, in the case of [42, 46, 47], the deficient phage-directed activity may actually be this terminal exonuclease. Accordingly, in spite of the presence of appropriate substrate from the E2-induced stage I, the phage-directed exonucleases would not be available to convert it to acid-soluble form. Consequently, inhibition of E2-induced exonuclease by [42, 46, 47] would result in diminished solubilization regardless of when the inhibition occurred.

The preceding interpretation assumes that the inhibition of E2 observed in the absence of CM is directed at both endonucleolytic and exonucleolytic stages. This possibility currently is being investigated directly by means of zone-sedimentation studies in sucrose gradients, and results will be reported in a subsequent publication.

It is interesting that Tanner and Oishi (32) have recently reported that infection of *E. coli* with phage T4 rapidly diminishes an adenosine triphosphate-dependent deoxyribonuclease and that this inhibition does not occur in the presence of CM. The deoxyribonuclease investigated by Tanner and Oishi is equivalent to the activity reported missing in *rec B* and *rec C* mutant strains of *E. coli* (10). Since E2 is capable of inducing DNA solubilization in these strains (14), it is clear that inhibition of this adenosine triphosphate-dependent nuclease does not account entirely, if at all, for the diminution of E2-induced DNA solubilization by phage infection. It appears likely, therefore, that several other *E. coli* deoxyribonucleases may be subject to inhibition by phage infection.

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