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Studies on an Endonuclease Activity Associated with the Bacteriophage T7 DNA-Membrane Complex

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Infection of *Escherichia coli* with bacteriophage T7 results in the formation of an endonuclease which is selectively associated with the T7 DNA-membrane complex. A specificity of association with the complex is indicated by the finding that the enzyme is completely resolved from a previously described T7 endonuclease I. When membrane complexes containing ^{3}H-labeled in vivo synthesized DNA are incubated in the standard reaction mixture a specific cleavage product is formed which is about one-fourth the size of T7 DNA. The endonuclease associated with the complex produces a similar cleavage product after extensive incubation with native T7 DNA or T7 concatemers. Degradation of concatemers occurs by a mechanism in which the DNA is converted to molecules one-half the size of T7. This product is in turn converted to fragments one-fourth the size of mature phage DNA. The endonuclease is not present in membrane complexes from uninfected cells or cells infected with gene 1 mutants. The enzyme activity is, however, present in cells infected with mutants defective in T7 DNA synthesis or maturation.

Several lines of evidence suggest that after infection of *Escherichia coli* with bacteriophage T7, the newly synthesized DNA becomes associated with host membrane material (5, 11). Evidence for this conclusion is suggested by the finding that replicating T7 DNA cosediments with host membranes in sucrose and cesium chloride density gradients (11). Furthermore, the sedimentation rate of the DNA is dependent on the integrity of membrane structure (11). We have also shown that the membrane complex contains a DNA synthesizing system which is capable of utilizing endogenous DNA as the template (5). One component of this system is the T7-induced DNA polymerase activity (7, 10). An analysis of the physical properties of the membrane-associated DNA labeled toward the end of the latent period indicates the presence of T7 concatemers (8) containing two to three genome equivalents linked together (5). It appears that the DNA present in the complex is metabolically active since in pulse-chase experiments a major portion of this DNA is used in the formation of mature virus particles (11). The information obtained thus far therefore suggests that host membranes may have an important function in the replication of T7 DNA and in the process of DNA maturation whereby T7 concatemers are converted to unit length molecules. Although the role of membranes in these processes is unknown some information concerning this role may be obtained by identifying certain enzymatic activities associated with DNA-membrane complexes and relating these activities to specific processes occurring during the metabolism of viral DNA. Since endonucleases may participate in the events occurring during DNA synthesis or maturation we have examined membrane complexes for the presence of this activity. The present report describes the identification of a T7 endonuclease which is selectively associated with the DNA-membrane complex. This activity which is completely resolved from T7 endonuclease I (2, 3, 12) is highly specific in its degradation of T7 DNA.

MATERIALS AND METHODS

Bacteria and phage. *E. coli* ER22 end I$^-$ (ATCC 27213), a nonpermissive host for amber mutants was used as host for T7 infection. *E. coli* 011' (ATCC 27214) was the permissive host for amber mutants. Bacteriophage T7 was obtained from F. W. Studier. Bacteriophage PM2 was obtained from C. C. Richardson.

Phage DNA. T7 and PM2 DNAs labeled with ^{3}H or ^{32}P were prepared as described previously (3, 4). Phage ϕX174 labeled with [^{3}H]thymidine was prepared as described by Sinsheimer (15).

Extraction of DNA. Phage DNA was extracted with phenol and dialyzed as described previously (3).

Preparation of cell lysates. *E. coli* ER22 (20 ml) was grown in T-Broth (10 g of tryptone broth, 5 g of

NaCl per liter) at 37 C to a density of 3.5×10^8 cells per ml. T7 was added at a multiplicity of 5.0, and at 10 min after infection the culture was mixed with an equal volume of a 20% sucrose solution containing 0.1 M Tris-hydrochloride (pH 8.0)-0.005 M ethylene glycol bis (β-aminoethyl ether)-*N,N'*-tetraacetic acid (EGTA) and centrifuged at 10,000 rpm for 10 min in the Sorvall SS-34 rotor. The cell pellet was suspended in 3.0 ml of 0.1 M Tris-hydrochloride (pH 8.0)-5 mM EGTA, and lysozyme (5 mg/ml) was added to a final concentration of 33 μg/ml. The solution was held for 90 min at 0 C during which time complete lysis occurred. In some experiments Brij 58 was added to a final concentration of 0.16% after the incubation with lysozyme. The addition of Brij had no effect on the levels of membrane-associated endonuclease activity.

Centrifugation of cell lysates in sucrose gradients. Portions of the cell lysates (1.5 to 2.0 ml) were centrifuged through 24.5 ml of a 5 to 20% sucrose gradient which was formed over 6 ml of a 60% sucrose solution. The sucrose was prepared in 0.01 M Tris-hydrochloride (pH 7.6)-0.005 M β-mercaptoethanol. Centrifugation was for 30 min at 22,000 rpm and 5 C in the Spinco type SW27 rotor. Fractions of 1.0 ml were collected after puncture of the bottom of the tube with a 20-gauge needle.

Preparation of T7 concatemers. *E. coli* ER22 was grown in T-broth to a cell density of 3.8×10^8 cells per ml at 25 C. T7 *am* 10 (amber mutant in gene 19) (17) was added at a multiplicity of 5.0. At 26 min after infection during which time T7 DNA synthesis is at a maximum, [^{3}H]thymidine was added to a final concentration of 2 μCi/ml. At 28 min after infection cell lysates were prepared as described above with the addition of Brij 58. The lysates were centrifuged in sucrose gradients (11) and the DNA sedimenting to the sucrose shelf was dialyzed and extracted twice with phenol. The phenol-extracted DNA was extensively dialyzed against 0.01 M Tris-hydrochloride (pH 7.6)-10^{-4} M EDTA. The sedimentation properties of the isolated DNA are shown in Fig. 4.

Assay for endonuclease activity. Assay I. Hydrolysis of native DNA. This assay which was used to measure endonuclease activity towards native DNA has been previously described in detail (4). The assay utilizes the covalently closed, circular duplex DNA of PM2 phage as substrate, and measures the conversion of this DNA to a form which can be trapped on nitrocellulose membrane filters after heating at 100 C for brief time periods. Unless indicated otherwise the standard reaction mixture (0.2 ml) contained 0.6 mμmol of ^{3}H-labeled PM2 DNA (1.2×10^4 counts/min/mμmol), 50 mM Tris-hydrochloride (pH 8.0), 5.5 mM $MgCl_2$, 4.5 mM β-mercaptoethanol, 20 μg of bovine serum albumin. Enzyme was added and incubation was carried out at 37 C for 10 min. Just prior to the endonuclease assay the enzyme was diluted in a solution containing 0.01 M Tris-hydrochloride (pH 7.6)-0.01 M β-mercaptoethanol-10% glycerol-bovine serum albumin, 0.5 mg/ml.

Assay II. Hydrolysis of single-stranded DNA. Previous studies have shown that T7 endonucleases I can extensively degrade single-stranded DNA to acid-soluble material (3, 12). Other T7 endonuclease which have been reported exhibit only low levels of activity towards single-stranded DNA (13) or do not degrade this material at all (4). To test for the presence of T7 endonuclease I, the single-stranded circular DNA of ϕX174 was used as substrate, and its conversion to acid-soluble material was determined. The standard reaction mixture was as described above except that in place of PM2 DNA, ^{3}H-labeled ϕX174 DNA (1.6×10^5 counts/min/mμmol) was used as the substrate. After incubation for 15 min at 37 C, 2 μmol of EDTA, and 12 μg of salmon sperm DNA were added. Trichloroacetic acid was added to a final concentration of 6%, the tubes were held on ice for 15 min and thereafter centrifuged at 10,000 rpm for 15 min. The supernate was collected and the radioactivity was measured in a scintillation counter.

A unit of enzyme activity is defined as the amount of enzyme which can convert 1 mμmol of PM2 DNA in 10 min at 37 C to a form which can be trapped on nitrocellulose membrane filters, or as the amount of enzyme which can release 1 mμmol of ϕX174 DNA as acid-soluble material in 10 min at 37 C.

Other methods. Sedimentation of DNA in sucrose gradients was carried out as previously described (2). Fractions were collected from the bottom of the tube directly into scintillation vials containing glass fiber filter paper. After drying, the radioactivity was measured in a scintillation counter. Protein was determined by the procedure of Lowry et al. (9). The molecular weights of DNA were determined by sedimentation analysis (16) applying the equation of Burgi and Hershey (1).

RESULTS

Distribution of T7-induced endonuclease activity after centrifugation of cell lysates in sucrose gradients. *E. coli* ER22 was infected with either T7 wild type or T7 *am* 29 an *amber* mutant in gene 3, the structural gene for T7 endonuclease I (2, 12). At 10 min after infection cell lysates were prepared and centrifuged in neutral sucrose gradients. Fractions from the gradient were assayed for endonuclease activity with PM2 and ϕX174 DNA as substrates. Centrifugation of a lysate from cells infected with T7 wild type reveals the presence of two peaks of activity when PM2 DNA is used as the substrate (Fig. 1b). The major portion of the activity is located near the top of the gradient, whereas a second activity sediments to the 60% sucrose shelf. The endonuclease located on the sucrose shelf exhibits sedimentation properties identical to a previously described T7 DNA membrane complex (5, 11). Under the conditions of the present experiments we find that the endonuclease and membrane-complex cosediment in neutral sucrose gradients. There is essentially no endonuclease activity located on the sucrose shelf when a lysate from uninfected cells is centrifuged in the sucrose gradient (Fig.

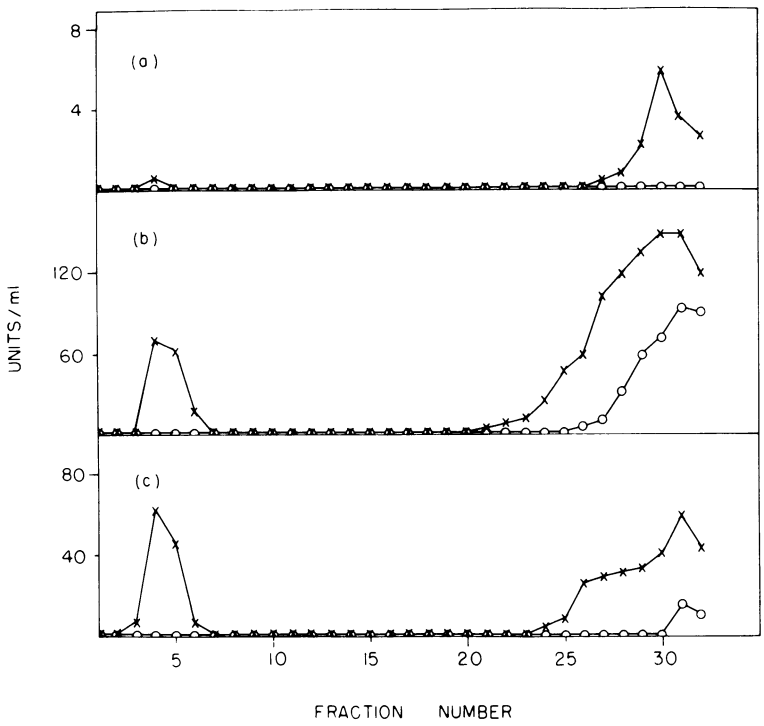

FIG. 1. *Distribution of endonuclease activity after centrifugation of cell lysates in sucrose gradients. E. coli ER22 was infected with either T7 wild type or T7 am 29 (gene 3 mutant), and at 10 min after infection cell lysates were prepared and centrifuged in neutral sucrose gradients. A lysate prepared from uninfected cells was also centrifuged under the same conditions. Fractions of 1.0 ml were collected after needle puncture of the bottom of the tube, and a portion from each fraction was used for determining the presence of endonuclease activity with PM2 (×) or φX174 DNA (O) as substrates. Panels a, b, and c show the results from uninfected cells, T7 wild-type infection, and T7 am 29 infection, respectively.*

1a). When φX174 DNA is incubated with the fast-sedimenting endonuclease from T7-infected cells there is no degradation as measured by the release of acid-soluble material (Fig. 1b). It is found, however, that the endonuclease present in the top fractions of the gradient is capable of degrading φX174 DNA with the release of acid soluble material (Fig. 1, panel b). Similar studies have been carried out with cells infected with T7 *am* 29, an amber mutant in gene 3. Under these conditions of infection the activity of the endonuclease present on the 60% sucrose shelf is essentially the same as that obtained after infection with T7 wild type. In contrast, the activity located near the top of the gradient is considerably reduced after infection with a gene 3 mutant (Fig. 1, panel c). The nature of the residual endonuclease present in the top fractions after infection with a mutant in gene 3 is not known at the present time. The results of these experiments suggest that a portion of the endonuclease activity induced after T7 infection cosediments with the T7-DNA membrane complex. This enzyme appears to be distinct from T7 endonuclease I (3, 12) which is located in the top fractions of the sucrose gradient. The ability to completely resolve these two endonuclease activities indicates that the fast-sedimentation rate of one of these enzymes is not due to mechanical trapping in the DNA-membrane complex.

Properties of membrane-associated endonuclease activity. In these experiments fractions 4 and 5 of the sucrose gradient shown in Fig. 1, panel b, were dialyzed and some properties of the endonuclease activity were determined with PM2 DNA as substrate. The enzyme exhibits maximum activity at pH 8.0 in 0.05 M Tris-hydrochloride buffer. At pH 7.5 and 8.5 the enzyme exhibits 85 and 60%, respectively, of the activity observed at pH 8.0. At pH 6.5 and 7.0 in 0.05 M potassium phosphate buffer the enzyme has 60 and 70%, respectively, of the activity at pH 8.0. Additional studies show that the enzyme has an absolute requirement for Mg^{2+} with maximum activity obtained

at 5.5 mM. In the absence of β-mercaptoethanol in the reaction mixture there is essentially no reduction in endonuclease activity.

Endonuclease activity after infection with T7 amber mutants. DNA membrane complexes were prepared from uninfected and cells infected with various T7 amber mutants (17) in genes necessary for DNA replication (genes 1–6) or maturation (genes 18–19). Endonuclease activity was determined with PM2 DNA as substrate. After infection with T7 wild type the specific activity of the endonuclease is about 35 times that from uninfected cells (Table 1). There is essentially no increase in activity after infection with gene 1 mutants. Since the gene 1 protein is a T7-specific RNA polymerase (6) whose function is necessary for the synthesis of most T7 proteins (17), these results provide additional evidence that the membrane-associated endonuclease is a phage-induced enzyme. The levels of activity after infection with all other mutants examined are however comparable to those obtained after T7 wild-type infection.

Degradation of in vivo synthesized T7 DNA associated with the DNA-membrane complex. In these experiments T7 DNA was labeled in vivo with [^{3}H]thymidine and the newly synthesized DNA associated with host membrane material was isolated as described in Materials and Methods. Isolation of the complex was carried out under conditions which provided maximum recovery of the membrane-associated endonuclease. The membrane complexes were incubated in the standard reaction mixture, and after incubation for various time periods the DNA was dissociated from the membrane by treatment with sarcosyl and Pronase. The DNA was thereafter analyzed in alkaline and neutral sucrose gradients. The results of an experiment in which the newly synthesized DNA was labeled after infection with T7 wild type is shown in Fig. 2. Prior to incubation in the standard reaction mixture the DNA sediments in alkaline sucrose as single strands about one-half the size of intact T7 DNA (Fig. 2a). In neutral sucrose most of the DNA sediments with the T7 marker although a considerable fraction sediments faster than the marker and may represent T7 concatemers (Fig. 3c). After a 40-min incubation period a limited number of endonucleolytic scissions have been introduced into the membrane-associated DNA. The products of the degradation process sediment in alkaline and neutral sucrose as symmetrical peaks having a size about one-fourth that of intact T7 DNA (Fig. 3b and d). When incubations are carried out for longer periods of time there are no additional breaks introduced into the T7 DNA molecules suggesting that the limit of reaction has occurred.

Hydrolysis of native T7 DNA. In these experiments the membrane-associated endonuclease was isolated after centrifugation of a lysate from gene 3-infected cells. The fast-sedimenting material was dialyzed and the action of the endonuclease on native T7 DNA was examined. The degradation products of this reaction were examined in neutral and alkaline sucrose gradients (Fig. 3). After a 30-min incubation period most of the treated DNA is fragmented after centrifugation in alkaline (Fig. 3b) but not in neutral sucrose (Fig. 3a), indicating that degradation has occurred predominantly by the introduction of single-strand breaks. The size of the major fragments formed in alkaline sucrose are about one-third the size of intact single-stranded T7 DNA. After an 80-min incubation period additional endonucleolytic scissions have been introduced into the DNA and some fragmentation can be observed after neutral sucrose centrifugation. The slower sedimenting material (fraction 13, Fig. 3c) which is about one-fourth the size of intact native T7 DNA is similar in size to the cleavage product formed when membrane

TABLE 1. *Membrane-associated endonuclease activity after infection with T7 amber mutants*[a]

Mutant	Gene	Endonuclease act (units/mg of protein)
Uninfected		2.5
T7	Wild type	90
am 193	1	3.0
am 29	3	85
am 64, 29	2, 3	75
am 29, 20	3, 4	77
am 29, 28	3, 5	86
am 29, 147	3, 6	80
am 182	18	66
am 29, 10	3, 19	68

[a] *E. coli* ER22 (10 ml) was grown at 37 C in T-Broth to a cell density of 3.8×10^8 cells per ml and infected with T7 amber mutants at a multiplicity of 5.0. At 10 min after infection the cells were collected and lysed and after incubation with lysozyme, Brij 58 was added to 0.16%. The lysate was centrifuged at 15,000 rpm for 30 min, and the supernate was discarded. The pellet was gently suspended in 1.0 ml of 0.01 M Tris-hydrochloride (pH 7.6)-5 mM β-mercaptoethanol and recentrifuged. The pellet was taken up in the same buffer as before and used for endonuclease determinations. The amount of endonuclease activity associated with the pellet fraction from a wild-type infection is essentially the same as that present in membrane complexes isolated after centrifugation of lysates in sucrose gradients.

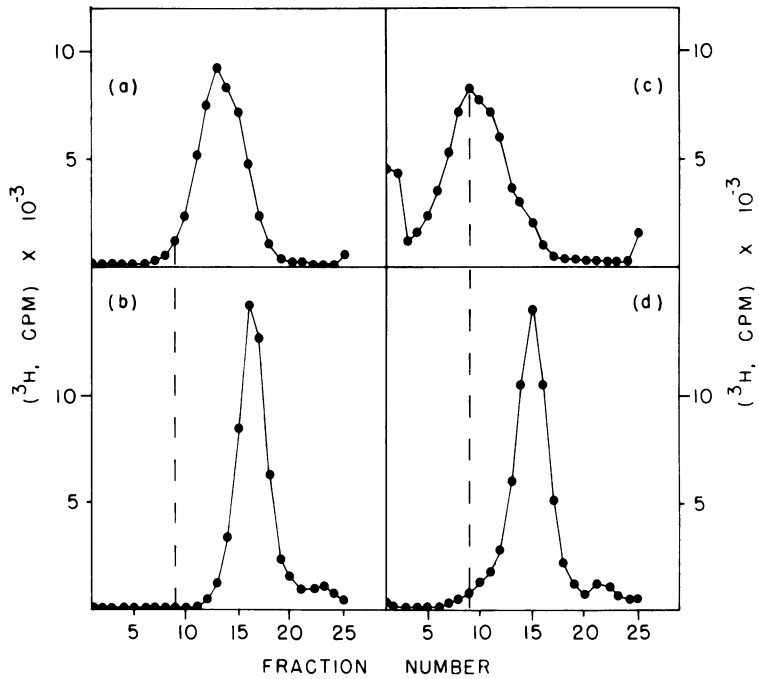

FIG. 2. *Sedimentation analysis of the products formed after degradation of membrane associated T7 DNA. E. coli ER22 was grown in T-Broth at 25 C to a density of 3.8 × 10^8 cells per ml. The cells were infected with T7 wild type at a multiplicity of 5.0 and at 26 min after infection [^{3}H]thymidine was added to a final concentration of 2 μCi/ml. At 28 min after infection the cells were lysed and the DNA-membrane complex was isolated. About 80% of the ^{3}H-labeled T7 DNA sedimented to the 60% sucrose shelf. The fast-sedimenting DNA containing the associated endonuclease activity was dialyzed for 5 h against 0.01 M Tris-hydrochloride (pH 7.6)-0.01 M β-mercaptoethanol-0.1 mM EDTA-10% glycerol at 2 C. Control experiments have shown that the endonuclease activity associated with the complex at 28 min after infection at 25 C is about the same as that observed with complexes isolated at 10 min after infection at 37 C. The dialyzed membrane complex (0.3 ml) was mixed with an equal volume of a reaction mixture to give the concentration of constituents described in Materials and Methods. Incubation was for 40 min at 37 C. At the end of the incubation 12 μmol of EDTA were added followed by the addition of sarcosyl NL-97 and Pronase to final concentrations of 0.25% and 125 μg/ml, respectively. The solution was incubated for 20 min at room temperature and thereafter was divided into two 0.2-ml portions. For the control sample incubation in the reaction mixture was not carried out, but the samples were otherwise treated the same as those incubated in the reaction mixture. Samples to be centrifuged in alkaline sucrose were made 0.1 N in NaOH prior to centrifugation. Centrifugation was in the Spinco type SW 50.1 rotor at 40,000 rpm for 4 h. Panels a and b, alkaline sucrose; panels c and d, neutral sucrose. Panels a and c are the controls, b and d samples were incubated in reaction mixture. The dotted line indicates the position to which marker T7 DNA sediments.*

complexes containing endogenous DNA are incubated in the reaction mixture (Fig. 2).

Hydrolysis of T7 concatemers. Previously we have shown that a considerable fraction of the in vivo synthesized DNA associated with membrane complexes from T7-infected cells exists as concatemeric material containing two to three genome equivalents linked together (5, 11). These results suggested the possibility that enzyme activities may be associated with host membranes which have a role in the maturation process. We have therefore carried out studies to examine the action of the membrane-associated endonuclease on isolated T7 concatemers. DNA membrane complexes were isolated from cells infected with an amber mutant in gene 3 and thereafter extensively sheared by passage through a needle. The solution was centrifuged, the supernate was collected, and the pellet containing membrane material was suspended in 0.01 M Tris-hydrochloride (pH 7.6)-0.01 M β-mercaptoethanol-10% glycerol. These two fractions were analyzed for endonuclease activity with PM2 DNA as substrate. It was found that of the total activity about 80% was located in the supernate. Many attempts to further purify the activity present in the supernate have been unsuccessful due to the extreme instability of the endonuclease activity. The concatemers to be used as the substrate in these

experiments were isolated from cells infected with an amber mutant in gene 19 (17) (see Materials and Methods). After infection with a mutant in gene 19 T7 DNA is synthesized at a rate comparable to that observed during a wild-type infection (17). However, in the absence of the gene 19 product concatemers are formed but are not converted to the size of mature phage DNA (14, 17). The results of an experiment in which the supernate and pellet fractions were incubated with isolated T7 concatemers are shown in Fig. 4. The concatemers sediment in neutral sucrose 1.2 to 1.5 times as fast as the T7 marker (Fig. 4a). The isolated DNA therefore seems to consist of T7 concatemers containing two to three genome equivalents linked together. The unit length molecules appear to be held together by non-covalent linkages since centrifugation in alkaline sucrose converts the concatemers to slower sedimenting structures which are equal to, to one-third the size of phage T7 DNA (data not shown). Incubation of the concatemers with either the supernate or pellet fraction results in the conversion of the DNA to a form which sediments slightly slower than the T7 marker (Fig. 4b and c). Although the product is somewhat heterogeneous the major fractions have a molecular weight of about 13×10^6 to 16×10^6. Mature, phage DNA has a molecular weight of 26×10^6 (16). Additional experiments have been carried out in which the supernatant fraction was incubated with the concatemers for various time periods and the cleavage products were analyzed in neutral sucrose gradients (Fig. 5). After a 10-min incubation period a considerable fraction of the concatemers have been converted to a form which sediments close to the T7 marker (Fig. 5b). Continuing the incubation period for 20 and 40 min results in the formation of fragments which are one-half and one-fourth, respectively, the size of T7 DNA. Further incubation does not change the sedimentation rate of the cleavage product shown in Fig. 5d. A

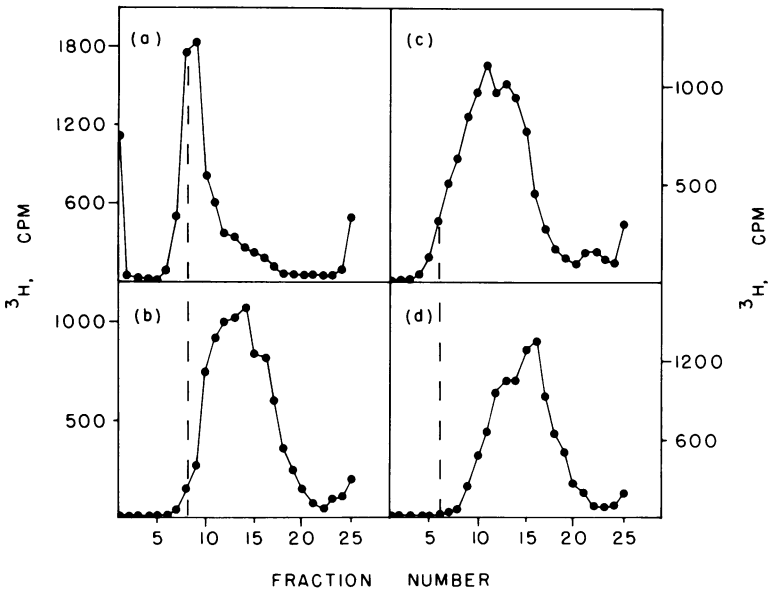

FIG. 3. *Sedimentation analysis of the action of the membrane-associated endonuclease on native T7 DNA. The membrane-associated endonuclease was isolated from lysates of cells infected with T7 am 29. The fractions from the sucrose gradient containing the fast-sedimenting endonuclease were dialyzed against 0.01 M Tris-hydrochloride (pH 7.6)-0.01 M β-mercaptoethanol-0.1 mM EDTA-10% glycerol for 5 h at 2 C. Portions of the dialyzed enzyme were added to a standard reaction mixture (0.2 ml) containing native ^{3}H-labeled T7 DNA. Incubation was carried out for 30 min (panels a and b) or for 80 min (panels c and d). At the start of the incubation 1 unit of enzyme was added, and after 20 min of incubation an additional 0.5 units were added. Identical incubations were carried out in the absence of added enzyme. At the end of the incubation the reaction was stopped by the addition of 4 μmol of EDTA. Those reaction mixtures to be analyzed in alkaline sucrose were made 0.1 N in NaOH prior to centrifugation. Sedimentation was carried out in the Spinco type SW 50.1 rotor in either neutral (panel a and c) or alkaline (panel b and d) sucrose gradients at 40,000 rpm for 4.5 h. The dotted line indicates the position to which [^{32}P]T7 DNA sediments.*

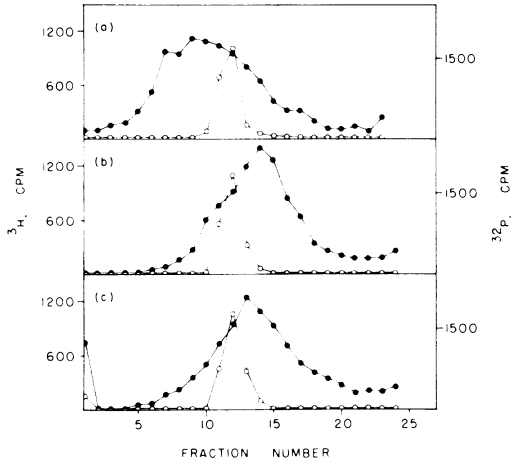

FIG. 4. *Sedimentation analysis of the action of membrane-associated endonuclease on T7 concatemers. The membrane-associated endonuclease was prepared as described in Fig. 3. The dialyzed material was centrifuged at 15,000 rpm for 30 min and the supernate was collected. The pellet was suspended in 1 ml of 0.01 M Tris-hydrochloride (pH 7.6)-0.01 M β-mercaptoethanol-10% glycerol (TMG) and was sheared five times by passage through a 25-gauge needle. The solution was centrifuged as above and the two supernates were pooled and thereafter concentrated in a dialysis bag covered with Sephadex G-200. The pellet (membrane fraction) obtained from the centrifugation was suspended in 1.0 ml of TMG. One unit of enzyme activity from either the supernate or membrane fraction was added to the standard reaction mixture (0.2 ml) containing the ^3H-labeled T7 concatemers prepared as described in Materials and Methods. Incubation was carried out for 30 min at 37 C. At the end of the reaction 2 μmol of EDTA were added and the solutions were centrifuged in the Spinco SW 50.1 rotor at 20,000 rpm for 15 h in a neutral sucrose gradient. (a) Control DNA, incubated in the absence of enzyme; (b) supernatant fraction added; (c) membrane fraction added. Symbols: ●, [^3H]DNA; ○, ^{32}P-T7 DNA marker.*

similar limit digest product is also observed after incubating the pellet fraction with the concatemers.

DISCUSSION

The results of the present study suggest that infection with T7 results in the formation of a specific endonuclease which is associated with the T7 DNA-membrane complex. The association appears to be selective since the enzyme is completely resolved from T7 endonuclease I (3, 12). Although other T7-induced endonuclease activities have been recently described (4, 13) their relation to the membrane-associated enzyme is difficult to determine since mutants in these activities have not been isolated. It appears that a major portion of the endonuclease present in the complex is associated with the

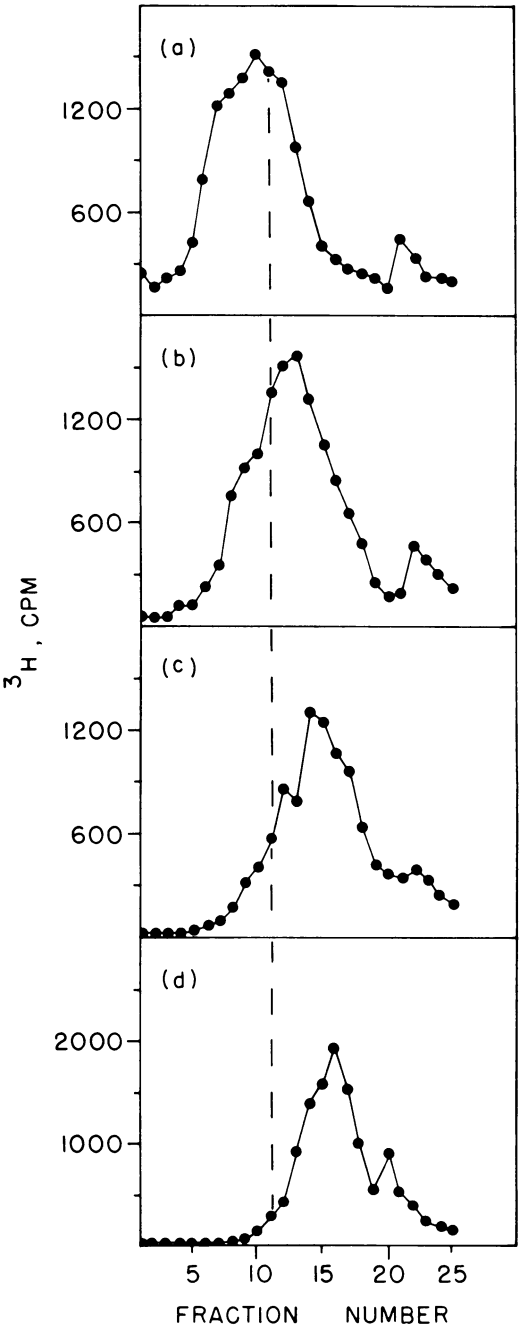

FIG. 5. *Analysis of concatemer degradation after incubation with the membrane-associated endonuclease for varying time periods. E. coli ER22 was infected with T7 am 29 and at 10 min after infection (37 C) a cell lysate was prepared as described in Table 1. The lysate was centrifuged at 15,000 rpm for 30 min, and the supernate was discarded. The pellet was suspended in 2 ml of TMG and recentrifuged. The pellet was taken up in 1.0 ml of TMG, sheared five times by passage through a 25-gauge needle, and thereafter was*

DNA and not the membrane itself since shearing results in the release of the enzyme from the membrane material. However, a significant fraction of the endonuclease remains associated with the membranes after extensive shearing. The relation of this activity to the membrane structure remains to be determined.

The action of the endonuclease on the various T7 DNA substrates examined appears to be highly specific since after prolonged incubation periods only a limited number of endonucleolytic scissions are introduced into the DNA molecules. This specificity is clearly demonstrated when isolated membrane complexes containing in vivo synthesized DNA are incubated in the reaction mixture. The limit digest of this reaction consists of fragments about one-fourth the size of T7 DNA. Fragments of similar size are also observed after incubation of the endonuclease with native T7 DNA or T7 concatemers. The mechanism for this specificity is unknown, but with concatemers as substrate there seems to occur a sequential cleavage resulting first in the formation of molecules which are close to the size of mature phage DNA. The initial cleavage product is converted to half molecules and this material is converted to fragments one-fourth the size of T7 DNA. From the present data it can not be determined if the products are formed as a result of double-strand breakage or single-strand breaks introduced at points in the molecule which increases the fragility of the DNA causing it to undergo fragmentation in neutral sucrose. Furthermore, it is also possible that the formation of the specific cleavage products are due to both endo- and exonucleolytic digestion of the DNA substrates. If this is the case it would appear that the exonuclease is highly specific in its degradative action since during the extensive incubation periods used only minor amounts of acid-soluble material can be detected. During a period of limited digestion the membrane-associated endonuclease can cleave T7 concatemers at points close to a unit length molecule. Whether this reaction bears any relation to events occurring in vivo during the maturation process remains to be determined. However, the finding that the levels of endonuclease present in isolated complexes from cells infected with either a maturation defective mutant or T7 wild type are similar seems to argue against this possibility.

We have also observed that the membrane-associated endonuclease can be separated from T7 endonuclease I by direct centrifugation of lysates in a low-speed centrifuge. The ability to rapidly isolate the membrane-associated enzyme from wild-type infected cells should facilitate a search for a mutant defective in the synthesis of this endonuclease activity.

centrifuged at 15,000 rpm for 30 min. The supernate was collected and portions (2.0 units of enzyme) were incubated with the [^{3}H]T7 concatemers in the standard reaction mixture for various time periods. At the end of the reaction 2 μmol of EDTA were added and the solutions were centrifuged in the Spinco SW50.1 rotor at 20,000 rpm for 15 h in neutral sucrose gradients. (a) Control; (b) 10 min incubation; (c) 20 min incubation; (d) 40 min incubation.

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