Effect of RNase III on the Size of Bacteriophage T7 Lysozyme mRNA

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The size of lysozyme mRNA from T7-infected *E. coli* RNase III⁺ and RNase III⁻ strains was analyzed by sucrose gradient sedimentation, dimethylsulfoxide (Me₂SO) sucrose gradient sedimentation, and preparative gel electrophoresis. Each technique revealed a similar size distribution of multiple lysozyme mRNA's. Analysis by preparative gel electrophoresis of RNA extracted after infection of *Escherichia coli* B⁺ (RNase III⁺) separated lysozyme mRNA into six peaks of activity ranging in size from 0.2×10^6 to 1.9×10^6 daltons. Four well-resolved major peaks of activity were detected, having apparent molecular weights of approximately 0.61×10^6 , 0.76×10^6 , 0.92×10^6 , and 1.3×10^6 . A broad band of activity, with a molecular weight range from 0.2×10^6 to 0.37×10^6 , was also present, and a sixth peak of activity was sometimes observed that migrates with a mobility corresponding to a molecular weight of 1.9×10^6 . Judging from their molecular weight as estimated by electrophoresis, most, if not all, of the lysozyme mRNA's were polycistronic. The RNA extracted after infection of an RNase III⁻ host contained a more heterogeneous collection of lysozyme mRNA's. In addition to lysozyme mRNA activity on RNAs with molecular weights between 0.2×10^6 and 1.9×10^6 , RNA species with molecular weights estimated at 4×10^6 to 5×10^6 were also detected. The data indicate that RNase III processes at least some of the primary lysozyme transcripts. The multiple lysozyme mRNA's represent discrete RNA species rather than aggregates because analysis of the size of lysozyme mRNA under completely denaturing conditions, in Me₂SO, produced a similar size distribution of lysozyme mRNAs. Also, treatment of RNA with 90% Me₂SO, which separates the strands of a completely double-stranded RNA, did not significantly alter the electrophoretic mobility of the lysozyme mRNA.

During infection of *Escherichia coli* with bacteriophage T7, T7 DNA is transcribed by two different DNA-dependent RNA polymerases (1). The *E. coli* RNA polymerase functions first to transcribe the early region of the T7 DNA, ending at a termination signal in the DNA at 20.2 map units (15). The termination signal prevents read-through by the host polymerase into late genes (18). The early region is transcribed as one large RNA, which is processed by the host enzyme RNase III into five monocistronic messages and three initiation polyribonucleotides (2). One of these early messages codes for the T7 RNA polymerase, which functions to transcribe the remainder of the T7 genome as well as gene 1.3 (ligase), the last gene of the early region. The left-most initiation site for the viral polymerase is near 17 map units, to the left of gene 1.3 (16). Many late RNAs are synthesized by the viral RNA polymerase (20).

Some of the late RNAs, like the T7 early RNAs, seem to be products of RNase III cleavage. During infection of an RNase III⁻ host, fewer late RNAs are detected, and some of these are larger in size than the RNAs detected during infection of an RNase III⁺ host (3).

Further evidence for processing of late RNAs by RNase III comes from studies with in vitro-synthesized T7 RNA. In vitro transcription of T7 DNA by purified T7 RNA polymerase produces seven major RNA transcripts, which are similar in size to the late RNAs produced after infection of an RNase III⁻ host. Treatment of the RNA synthesized in vitro with purified RNase III produces RNAs more similar in size to the late RNAs extracted after infection of an RNase III⁺ host (3).

Mapping of the major in vitro late RNAs using exonuclease-digested T7 DNA, T7-T3 DNA hybrids, and in vitro translation placed most if not all of the major RNAs in the class III region of the T7 genome (4, 12). The late region of T7 codes for two classes of proteins; class II pro-

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teins, specified by genes 1.3 to 6, and class III proteins, coded for by genes 7 through 19. Even though the major in vitro late RNAs map in the class III region, translation of unfractionated in vitro RNA produces all of the late class II and class III proteins, which can be detected readily by gel analysis. This indicates the presence of minor class II RNA species in the in vitro RNA in addition to the seven major late class III RNAs. Furthermore, when the four largest in vitro RNAs are translated, the class II proteins 1.3 and 3.5 are synthesized (12). This indicates that there are minor RNAs coding for lysozyme which co-electrophorese with the major-band RNAs. Translation of the smaller in vitro RNA bands V and VI (molecular weights, 0.5×10^6 and 0.25×10^6 , respectively) does not produce lysozyme (gene 3.5 polypeptide), thus indicating the lack of RNAs of this size coding for lysozyme. Because small amounts of the class II RNAs are synthesized in vitro, they have not been mapped and their sensitivity to RNase III has not been tested. The evidence described in this paper suggests that RNase III does process a large precursor RNA into smaller lysozyme messages. However, either the RNase III defect in the strains studied is not complete or other enzymes can perform a similar cleavage.

MATERIALS AND METHODS

E. coli and bacteriophage strains. The bacterial and phage strains are described in the accompanying paper (6). The RNA of midi-variant MDV-1 (11) of bacteriophage Q β was obtained from F. Ryan.

Sucrose gradient sedimentation. The sucrose solutions were prepared by mixing 1 part concentrated buffer (1.1 M LiCl, 11 mM EDTA) with 10 parts 5.5 or 22% (wt/wt) sucrose solution. Linear sucrose gradients (36 ml, 5 to 20%) were constructed in diethyl pyrocarbonate sterilized polyallomer tubes utilizing a gradient maker and peristaltic pump. The gradients were stored at running temperature for over 3 h for temperature equilibration. RNA (0.5 to 1 mg in 0.5 to 1 ml of water) was carefully layered onto each gradient. A portion of the sample was mixed with sucrose solution, stored at running temperature for the duration of centrifugation, and processed with the gradient fractions as a control. Centrifugation was done in an International Preparative Centrifuge model LB-60 with an SB110 rotor at 25,000 rpm ($110,000 \times g$ maximum) at 4°C. The gradients were removed from the centrifuge tubes through a disposable micropipette inserted from the top to the bottom of the tube. A peristaltic pump attached to the micropipette moved the gradient from the tube through a flow cell in a Beckman Spectrophotometer and to a fraction collector. As the gradients were dispensed, the absorbance was monitored at 260 nm by the spectrophotometer and recorded on a Gilford Recorder. If the RNA was to be analyzed for lysozyme mRNA activity, the RNA in each tube was ethanol precipitated, dried, and dissolved in water for in vitro protein synthesis.

Sucrose gradient sedimentation in Me₂SO. The method used for sucrose gradient sedimentation was that of Strauss et al. (17). Dimethylsulfoxide (Me₂SO) stock solutions were prepared as follows. Approximately 30 ml of 25% Me₂SO-75% deuterated Me₂SO-10% sucrose-1 mM EDTA was prepared by mixing 7.5 ml of Me₂SO, 22.5 ml of deuterated Me₂SO, 3.0 g of RNase-free sucrose, and 0.3 ml of 4% EDTA. A volume of 30.3 ml of a nondeuterated solution of 100% Me₂SO and 1 mM EDTA was prepared by mixing 30 ml of Me₂SO and 0.3 ml of 4% EDTA. For 13-ml gradients, thin-wall nitrocellulose tubes (14.5 by 96 mm) were used. A 0 to 10% sucrose and 0 to 75% deuterated Me₂SO linear gradient was constructed by using a gradient maker and peristaltic pump. The gradients were poured and run at room temperature. The 0.5-mg sample of RNA (100 μ l) was layered onto the gradient after mixing with 100 μ l of Me₂SO and 200 μ l of dimethylformamide. Centrifugation was performed by an International Preparative Ultracentrifuge model LB-60 with an SB283 rotor at 40,000 rpm ($270,000 \times g$ maximum), at 25°C for 35 h. After centrifugation, the gradients were processed as described above.

Extraction of RNA from T7-infected cells. Extraction procedures are outlined in the accompanying paper (6).

RNA slab gel electrophoresis. The gel and buffer system was that of Loening (9). The slab gel apparatus has been described by Studier (19). RNA was subjected to electrophoresis at 50 V constant current for 4 h. Under these conditions, bromophenol blue migrated the full length of the 10-cm gel.

Preparative electrophoresis. The procedure for preparative electrophoresis is outlined in Hagen and Young (5).

The effluent from preparative electrophoresis was collected in disposable test tubes (13 by 100 mm). Fraction volumes of 1 ml were usually collected. To insure complete recovery of mRNA activity, carrier RNA and salt were added to the collection tubes prior to electrophoresis. The final concentration of carrier RNA, *E. coli* tRNA, or rRNA was 30 μ g/ml, and that of salt, ammonium, or sodium acetate was 0.2 M. The RNA in each fraction was precipitated with 2 volumes of 95% ethanol, and the fractions were stored overnight at -20°C. The RNA precipitate was collected and then washed with 95% ethanol by centrifugation for 0.5 h at 3,000 rpm in a Sorvall RC-3 centrifuge utilizing an HL-8 rotor.

RESULTS

Denaturation of RNA with Me₂SO. A treatment was sought which would completely disrupt intermolecular association between RNA molecules and which could be used on RNA prior to electrophoresis. The treatment had to be rigorous enough to assure complete denaturation, but gentle enough to preserve the integrity of the RNA for translation. Two reagents, formamide (14) and dimethylsulfoxide (17), were tested. As a control for denaturation, a completely double-stranded RNA, MDV-1

(midi variant of Q β [11]), with 218 base pairs and 71.2% guanine-plus-cytosine content was chosen. The rationale was that if a treatment could separate the strands of this very stable, completely double-stranded RNA, it would disrupt intermolecular associations between RNAs which were not completely double-stranded. Double-stranded MDV-1 was treated and subjected to electrophoresis on a 5% acrylamide slab gel to determine the efficiency of denaturation. Double-stranded MDV-1 was treated for 2 min with 90% formamide at 37 or 60°C or for 2 min with 90% dimethylsulfoxide at either 22, 37, or 60°C. Untreated RNA and RNA which had been incubated for 1 min in a boiling-water bath in 3

mM EDTA was also analyzed.

Treatment of double-stranded MDV-1 with 90% formamide did not separate the strands of the RNA at either 37 or 60°C. However, exposure of MDV-1 to 90% dimethylsulfoxide at all temperatures tested caused complete strand separation (Fig. 1). Incubation at 100°C in 3 mM EDTA also denatured MDV-1. However, this treatment also destroys most of the biological activity of lysozyme mRNA (Hagen, unpublished data).

Size of T7 lysozyme mRNA extracted after T7 infection of RNase III⁺ *E. coli*. Three different techniques were used to analyze the size of the lysozyme mRNA extracted from

FIG. 1. Denaturation of MDV-1 RNA with formamide, dimethylsulfoxide, and heat. ³H-labeled MDV-1 (10,000 cpm) with 20 μ g of carrier *E. coli* tRNA were ethanol precipitated, collected by centrifugation, and dried. The RNA was resuspended and treated in the manner indicated below; then 10 μ l of glycerol was mixed with 50 μ l of each sample, and 30 μ l of this mixture was applied to a 5% acrylamide, 10-cm slab gel. Electrophoresis was for 4 h at 50 V constant current. The gel was prepared for fluorography and exposed for 72 h at -70°C. The double-stranded MDV-1 migrated about 3.5 cm from the origin. No radioactivity was detected at the origin. RNA treatment (slot): (1) 3 mM EDTA; (2) 2 mM EDTA, boiled for 1 min and quenched in ice bath; (3) 90% formamide, 2 min, 35°C; (4) 90% formamide, 2 min, 60°C; (5) 90% Me₂SO, 2 min, 23°C; (6) 90% Me₂SO, 2 min, 37°C; and (7) 90% Me₂SO, 2 min, 60°C. The formamide and Me₂SO solutions were in 10 mM Tris (pH 7) and 3 mM EDTA.

T7-infected RNase III⁺ *E. coli* Bst. These were aqueous sucrose gradient sedimentation, sucrose gradient sedimentation in Me₂SO, and preparative gel electrophoresis. The results from these analyses have been consistent with one another. However, the sedimentation analysis did not afford the resolution provided by polyacrylamide gel electrophoresis.

Analysis by sucrose gradient sedimentation of RNA extracted 10 min after T7 infection of *E. coli* Bst revealed a polydisperse pattern of lysozyme mRNA activity of apparent molecular weight ranging from 0.13×10^6 to 1.9×10^6 (Fig. 2). The one-point peak at 0.13×10^6 daltons is probably not significant. Prominent peaks of lysozyme mRNA activity were found at 0.44×10^6 , 0.60×10^6 , 0.74×10^6 , and 1.1×10^6 daltons. A shoulder on the high-molecular-weight side of the 1.1×10^6 -dalton peak extends out to about 1.9×10^6 daltons. These molecular weights were calculated by estimating the S values of the messenger peaks from a plot of S value versus fraction number of *E. coli* RNA markers and converting S values into molecular weight using the equation $s_{20,w} = 0.98 \times 10^{-2} M_w^{0.56}$ derived by Kurland (7).

To show that the broad distribution of lysozyme mRNA activity detected by sucrose gradient sedimentation was not due to intermolecular aggregation, RNA extracted 8 min after infection of *E. coli* Bst was analyzed by sucrose gradient sedimentation in Me₂SO. Judging from the absorbance profile of 4S, 16S, and 23S RNA, the resolution of the Me₂SO sucrose gradient (Fig. 3) was quite poor. When the gradient was analyzed for lysozyme mRNA activity, a polydisperse pattern of lysozyme mRNA was observed. Part of the activity sedimented as a low-molecular-weight shoulder on the 16S *E. coli* rRNA. The majority of the activity cosedimented with the 16S and 23S *E. coli* rRNA with a small amount of activity sedimenting more rapidly than the 23S rRNA. Calculation of molecular weights of these species was not possible due to the poor resolution of 16S and 23S *E. coli* rRNA. However, comparison with the analysis by aqueous sucrose gradient sedimentation showed a general agreement on the size of lysozyme mRNA.

To provide better resolution of the lysozyme mRNA's, RNA was fractionated by preparative gel electrophoresis. The superior resolution of this technique is obvious, as evidenced by the separation of the marker RNAs in Fig. 4. The mRNA molecular weights were determined from a standard plot of logarithm molecular weight versus 1/fraction number, which yields a straight line for RNAs with molecular weights between 0.55×10^6 and 1.8×10^6 (Fig. 5). The

FIG. 2. Analysis of T7 lysozyme mRNA by sucrose gradient sedimentation. RNA applied to the sucrose gradient was extracted from T7L-infected *E. coli* Bst at 10 min after infection as described in reference 5. The conditions of the infection were as follows. Cell density at the time of infection was 4×10^8 /ml, multiplicity of infection was 5, the medium was amino acid-supplemented M9, and the incubation temperature was 30°C. RNA (1 mg) was applied to a 5 to 20% sucrose gradient in 1 ml of water. Centrifugation was for 18 h at 25,000 rpm at 4°C in a Beckman L2B Centrifuge equipped with an SW27 rotor. Fractions of 1 ml were collected. tRNA (100 µg; Sigma) 0.1 ml of 2 M sodium acetate (pH 5.2), and 2 ml of 95% ethanol were added to each tube. The solutions were thoroughly mixed by blending in a Vortex mixer and stored at -20°C overnight to allow precipitation of the RNA. The precipitated RNA was collected by centrifugation in a clinical centrifuge at full speed for 15 min. The supernatant was decanted, and the RNA was washed by resuspension in 2 ml of 70% ethanol followed by recentrifugation. The RNA pellet was dried and suspended in 40 µl of water just before the assay for messenger activity. Each fraction was assayed for lysozyme mRNA activity by *in vitro* protein synthesis using an S-30 extract prepared from *E. coli* MRE600. The incubation period for the lysozyme filter assay was 1 h. Details of procedures are found in (6). (○) Absorbance at 260 nm; (×) lysozyme mRNA activity.

fractionation of RNA extracted 14 min after T7 infection of *E. coli* Bst (Fig. 6) revealed the existence of six peaks of lysozyme mRNA activity. More activity has been observed in the 1.9×10^6 -molecular-weight peak in other analyses (Fig. 7). The broadness of the peak of low-molecular-weight lysozyme mRNA activity compared with the marker RNAs suggests that it may be composed of several different-sized RNAs.

However, in subsequent experiments (e.g., Fig. 7) these peaks were not resolved, and a broad region of activity was observed with an apparent

FIG. 3. Analysis of lysozyme mRNA by dimethylsulfoxide sucrose gradient sedimentation. RNA which had been extracted from T7L-infected *E. coli* Bst as described in reference 5 was fractionated. A 0.5-mg sample of RNA was layered onto a 0 to 10% sucrose and 0 to 75% deuterated Me₂SO linear gradient. The procedures for centrifugation and for collection of the gradient are described in the text. tRNA (50 µg; Sigma), 0.05 ml of 2 M sodium acetate (pH 5.2), and 1 ml of 95% ethanol were added to each 0.5-ml fraction. The fractions were blended in a Vortex mixer, and the RNA was allowed to precipitate overnight at -20°C. The RNA was collected by centrifugation and washed twice with 70% ethanol. The lysozyme mRNA activity in each fraction was determined as in Fig. 2. (○) Absorbance at 260 nm; (×) lysozyme mRNA activity.

FIG. 4. Preparative electrophoresis of *E. coli* and rat marker RNAs. A 100-µg sample of *E. coli* RNA and 75 µg of rat RNA were ethanol precipitated and resuspended in 90% Me₂SO. The RNA was subjected to electrophoresis on a 1.5-cm-diameter, 4.6-cm-long, 2.1% acrylamide gel, which was poured at the same time as the gel in Fig. 6. Electrophoresis was carried out as outlined in the text.

average molecular weight of 0.75×10^6 . The apparent molecular weights of four of these peaks of lysozyme mRNA activity agree with the analysis in which lysozyme mRNA activity was measured by detection of radioactive gene 3.5 polypeptide on sodium dodecyl sulfate-polyacrylamide gels (C. Pachl and E. T. Young, *J. Mol. Biol.*, in press). By the latter technique, four peaks of activity were detected having molecular weights of 0.23×10^6 , 0.89×10^6 , 1.3×10^6 , and 1.9×10^6 .

The same RNA extracted 14 min after T7 infection of *E. coli* Bst was refractionated later by preparative electrophoresis using a longer 2.1% acrylamide gel. Better recovery of the messenger activity was obtained by collecting the eluted RNA into test tubes containing carrier RNA. The analysis (Fig. 7) showed lysozyme mRNA activity eluting in fractions corresponding to molecular weight ranges of 0.21×10^6 to 0.39×10^6 , 0.65×10^6 to 0.9×10^6 , and 1.3×10^6 . The increased recovery of mRNA activity in this

FIG. 5. Plot of log molecular weight versus $1/\text{fraction number}$ for *E. coli* and rat RNAs. The $1/\text{fraction number}$ for each RNA of Fig. 4 was determined and plotted against the log molecular weight of the RNA. The molecular weight values were as follows: *E. coli* 4S RNA, 0.025×10^6 ; 16S RNA, 0.55×10^6 ; 23S RNA, 1.1×10^6 ; rat 18S RNA, 0.70×10^6 ; and 28S RNA, 1.8×10^6 (9).

experiment confirmed the presence of a small amount of activity associated with an RNA having an apparent molecular weight of 1.9×10^6 . The peaks in the two lower-molecular-weight regions were not as well resolved as in the analysis of Fig. 6.

Size of T7 lysozyme mRNA extracted from T7-infected RNase III⁻ *E. coli*. To determine whether T7 lysozyme mRNA is transcribed as a larger precursor RNA which is processed by RNase III, RNA was extracted from two RNase III⁻ hosts after infection with T7. The RNase III⁻ hosts, *E. coli* AB301-105 and BL107, are described in the accompanying paper (6). The molecular weights of the lysozyme mRNA's were determined by sucrose gradient sedimentation and preparative electrophoresis. RNA extracted 14 min after infection of *E. coli* AB301-105 was analyzed by sucrose gradient sedimentation (Fig. 8) and preparative acrylamide gel electrophoresis (Fig. 9). Lysozyme mRNA activity was detected in the usual low-molecular-weight regions of 0.2×10^6 to 0.4×10^6 and 0.5×10^6 to 1×10^6 . However, a large proportion of the activity was larger than 10^6 daltons, with 60% of the activity migrating in this region as determined by the gel analysis. RNAs as large as 3×10^6 daltons as measured by sedimentation analysis or as large as 5×10^6 daltons as measured by electrophoretic analysis

were detected. It should be noted that the molecular weight determinations above 2×10^6 involve extrapolation from lower-molecular-weight markers and are thus only estimates of the true molecular weight. Similar analysis by sucrose gradient sedimentation of RNA extracted 14 min after infection of *E. coli* BL107 (6) resolved lysozyme messenger peaks of 2×10^6 and 4.7×10^6 daltons. The RNA from T7-infected BL107 also displayed the usual low-molecular-weight pattern (6). Sixty-seven percent of the lysozyme mRNA activity sedimented with an apparent molecular weight greater than 10^6 .

DISCUSSION

The size of lysozyme mRNA from T7-infected *E. coli* was analyzed by aqueous sucrose gradient sedimentation, sucrose gradient sedimentation in Me₂SO, and preparative gel electrophoresis. Multiple-lysozyme mRNA's were detected, as reported previously (5). The size of the lysozyme mRNA's, as estimated by these three techniques, was very similar.

The most reliable estimates of the molecular weight and the best resolution of the lysozyme mRNA's were obtained by preparative gel electrophoresis. One such analysis of RNA after T7 infection of *E. coli* Bst (RNase III⁺) separated lysozyme mRNA into six peaks of activity ranging in size from approximately 0.2×10^6 to 1.9×10^6 daltons (Fig. 6). Four well-resolved major peaks of activity were detected at 0.61×10^6 , 0.76×10^6 , 0.92×10^6 , and 1.34×10^6 daltons. A broad band of activity was observed between 0.2×10^6 and 0.37×10^6 daltons, possibly composed of several differently sized RNAs. The sixth peak of activity, at 1.9×10^6 daltons, was more reliably detected in a subsequent experiment (Fig. 7). Therefore, during infection of *E. coli* Bst (RNase III⁺) the coding capacity for lysozyme is carried on a multiplicity of differently sized mRNA's. Most if not all of these RNAs are polycistronic, since a monocistronic message of 0.13×10^6 daltons is sufficient to code for lysozyme. It is difficult to determine with certainty the number of distinct RNAs present that contain lysozyme coding sequences. The analyses that appear to provide the best resolution (e.g., Fig. 6) suggest at least six, but this resolution is not always obtained, and it may be that the RNA migrates and elutes from the gel more heterogeneously than the stable *E. coli* species. This could lead to the appearance of more species than are actually present. The analysis by Pachl and Young (in press), using the synthesis of radioactive gene 3.5 (lysozyme) polypeptide as an indication of lysozyme mRNA activity, revealed four species

FIG. 6. Analysis of T7 lysozyme mRNA by preparative electrophoresis. The RNA for this analysis was extracted from T7L-infected *E. coli* Bst at 14 min after infection. The method of extraction was the hot sodium dodecyl sulfate-phenol chloroform method of Hagen and Young (6). A 200- μ g sample of the RNA was ethanol precipitated and resuspended in 90% Me₂SO. The sample was subjected to preparative electrophoresis on a 1.5-cm-diameter, 4.6-cm-long, 2.1% acrylamide gel at a constant current of 15 mA. The RNA was eluted from the bottom of the gel at a flow rate of 0.1 ml/min, and 1.0-ml fractions were collected. The absorbance of the effluent was continuously monitored by an Altex UV monitor and recorded by an Omniscribe chart recorder (Houston Instruments). The RNA in each 0.5-ml fraction was precipitated by the addition of 25 μ g of *E. coli* tRNA, 0.05 ml of 2 M ammonium acetate (pH 5.2), and 1.25 ml of 95% ethanol. After storage at -20°C overnight, the precipitate was collected and washed by centrifugation at 3,000 rpm for 1 h. The supernatant was decanted, and the precipitate was dissolved in 40 μ l of water and translated directly in a cell-free protein synthesizing system prepared from *E. coli* MRE600, 0.1 ml total volume. After a 20-min incubation at 37°C , the reaction was stopped with the addition of 1 ml of 0.1 M ammonium acetate and 100 μ g of chloramphenicol per ml. The fractions were blended in a Vortex mixer and assayed for lysozyme activity by the filter assay with an incubation period of 2 h. The recovery of lysozyme mRNA activity was 21%. The molecular weights of the lysozyme mRNA's were determined from a plot of marker RNA molecular weights versus 1/fraction number (Fig. 5) with the marker RNAs electrophoresed on a separate gel poured at the same time (Fig. 4). (—) Absorbance at 254 nm; (x) lysozyme mRNA activity.

having molecular weights of about 0.25×10^6 , 0.8×10^6 , 1.3×10^6 , and 1.9×10^6 . These values are similar to those reported here. Whether the two smallest message species will require purification and further analysis by electron microscopy.

The possibility that multiple lysozyme mRNA peaks are caused by RNA aggregation was investigated by using the organic solvent Me₂SO. Strauss et al. (17) demonstrated that Me₂SO prevents interactions between RNAs that would allow formation of RNA aggregates. The size of lysozyme mRNA was analyzed by Me₂SO sucrose gradient sedimentation (Fig. 3). Even though the resolution of the Me₂SO sucrose gradient was quite poor, a general qualitative comparison can be made between the results of the Me₂SO sucrose gradient analysis and the analysis by preparative electrophoresis. On this basis the size distribution of the lysozyme mRNA was similar. In addition to this analysis, RNA was treated with 90% Me₂SO prior to analysis by preparative electrophoresis. This

treatment separated the strands of a very stable, completely double-stranded RNA of 218 base pairs. Therefore, it should prevent base pairing or stacking interactions between T7 lysozyme mRNA and other RNAs of the infected cell. This treatment did not alter the observed size distribution of the lysozyme mRNA's.

It is clear that RNase III does have an effect on the size of lysozyme mRNA. RNA extracted after infection of an RNase III⁻ host contained a more heterogeneous collection of lysozyme mRNA's, and some of these lysozyme mRNA's were much larger in size than those found after infection of an RNase III⁺ host. Specifically, 60 to 67% of the lysozyme mRNA from RNase III⁻ infections was larger than 10^6 daltons (Fig. 9; Fig. 9 in [6]) while only 10% of the lysozyme mRNA was of that size after infection of an RNase III⁺ host (Fig. 6). Lysozyme mRNA molecules with an apparent molecular weight of 4×10^6 to 5×10^6 were found in significant amounts after infection of host cells lacking RNase III (Fig. 9; Fig. 9 in [6]) whereas there

FIG. 7. Analysis of T7 lysozyme mRNA by preparative electrophoresis. The RNA of this analysis was extracted from T7L-infected *E. coli* B⁺ at 14 min after infection as in Fig. 6. A 200- μ g sample of the RNA was fractionated and assayed for lysozyme mRNA activity as described in Fig. 6 with the following differences: the 2.1% acrylamide gel was 6 cm long, and the RNA of the entire 1-ml fraction was precipitated with 30 μ g of carrier tRNA, 0.1 ml of 2 M ammonium acetate (pH 5.0), and 2.5 ml of 95% ethanol. The tRNA and ammonium acetate were added to the tubes prior to fraction collection. The RNA precipitate was collected and washed by centrifugation at 3,000 rpm for 0.5 h at 4°C; 107% of the lysozyme mRNA activity was recovered. The molecular weights of the lysozyme mRNA's were determined from a plot of the log of the molecular weight versus 1/fraction number. Marker RNAs used for this purpose were *E. coli* RNAs as well as ¹⁴C-labeled Q β MDV-1 (74,000 molecular weight) and *E. coli* rRNA precursor (2×10^6 molecular weight) electrophoresed in the same gel. (—) Absorbance at 254 nm; (x) lysozyme rRNA activity.

FIG. 8. Analysis of lysozyme mRNA from T7-infected *E. coli* AB301-105 (RNase III⁻) by sucrose gradient sedimentation. RNA applied to the gradient was extracted from T7L-infected *E. coli* AB301-105 (RNase III⁻) at 14 min after infection at 30°C by the hot sodium dodecyl sulfate-phenol chloroform method. A 200- μ g sample of the RNA was applied to the gradient in 200 μ l of water. Centrifugation was for 15 h at 25,000 rpm in an SB110 rotor at 4°C. Fractions of 1.6 ml were collected. The RNA in each tube was ethanol precipitated with the aid of 60 μ g of carrier rRNA. Lysozyme mRNA activity was determined by *in vitro* protein synthesis using a cell-free extract of *E. coli* MRE600. The lysozyme filter assay was for 1 h. (—) Absorbance at 260 nm; (x) lysozyme mRNA activity.

FIG. 9. Analysis of lysozyme mRNA from T7-infected *E. coli* AB301-105 (RNase III⁻) by preparative electrophoresis. A 200- μ g sample of RNA, which had been extracted by the hot sodium dodecyl sulfate method from T7-infected *E. coli* AB301-105 at 14 min after infection, was treated with 90% Me₂SO and subjected to preparative electrophoresis in a 4.8-cm-long, 1.5-cm-diameter, 1.9% acrylamide gel. The RNA was eluted, and the absorbance was monitored and recorded. The RNA in the 2-ml fractions was ethanol precipitated with the aid of 60 μ g of carrier *E. coli* rRNA per fraction (RNA was added to the tubes prior to fraction collection). The RNA was assayed for lysozyme mRNA activity by *in vitro* protein synthesis using an *E. coli* MRE600 S-30 extract. The lysozyme filter assay was incubated for 1.5 h; 119% of the activity applied to the gel was recovered. The molecular weights of the lysozyme mRNA's were determined from a standard plot of log molecular weight versus 1/fraction number of *E. coli* 4S, 16S, and 23S RNA, and 30S rRNA precursor electrophoresed in the same gel. (—) Absorbance at 254 nm; (x) lysozyme mRNA activity.

were no messages of this size detected in the presence of RNase III (Fig. 6 and 7).

There is reasonable agreement between the size of some of the lysozyme mRNA species we have detected after infection of an RNase III⁻ host and the size of *in vitro* RNAs which code for lysozyme. Niles and Condit (12) and Golomb and Chamberlin (4) have synthesized *in vitro* RNA with T7 DNA and purified T7 RNA polymerase. The major *in vitro* RNAs map in the class III region. However, there are minor RNAs that code for class II proteins which co-electrophorese with some of these major band RNAs. RNA in bands I, II, III, and IV (molecular weights of 3.1×10^6 to 5.2×10^6 , 2.6×10^6 to 4×10^6 , 1.9×10^6 , and 0.9×10^6 , respectively) code for two class II proteins, 1.3 (ligase) and 3.5 (lysozyme) (12). We have detected lysozyme mRNA's of all these sizes after infection of an RNase III⁻ host. Lysozyme mRNA's larger than

2×10^6 daltons have not been detected in RNase III⁺ infection, whereas small amounts of RNA of about 0.9×10^6 and 1.9×10^6 daltons have been observed (Fig. 6 and 7). Translation of bands V and VI *in vitro* RNA (0.26×10^6 and 0.51×10^6 daltons, respectively) does not produce lysozyme (12). Contrary to this observation, there was always lysozyme mRNA activity of that molecular weight present in RNA extracted after infection of RNase III⁺ *E. coli* strains. These observations suggest that high-molecular-weight precursors to lysozyme mRNA, lysozyme mRNA's of 2×10^6 or larger, are substrates for RNase III. The lysozyme mRNA's of less than 0.5×10^6 daltons are probably not generated by RNase III because they are present in both RNase III⁺ and RNase III⁻ infections. However, since lysozyme mRNA's of less than 0.5×10^6 daltons are not synthesized *in vitro* by purified T7 RNA polymerase, they may

be generated by cleavage by nuclease(s) other than RNase III. Alternatively, undetected residual RNase III activity in the strains studied could generate the small lysozyme transcripts.

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