JOURNAL OF BACTERIOLOGY, Sept. 1979, p. 842-849
0021-9193/79/09-0842/08$02.00/0

Vol. 139, No. 3

Ribosomal Ribonucleic Acid Isolated from *Salmonella typhimurium*: Absence of the Intact 23S Species†

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Received for publication 6 July 1979

Ribonucleic acid (RNA) isolated by four distinct methods and from a variety of *Salmonella typhimurium* strains lacked intact 23S ribosomal RNA (rRNA). On sucrose gradients which minimize aggregation, the vast majority of *S. typhimurium* rRNA sedimented as a 16S peak with a 14S shoulder. RNA from this region of the gradient was resolved into three discrete bands by electrophoresis in formamide. Two very minor *S. typhimurium* RNA peaks were resolved at 21S and 10S on sucrose gradients, and each peak formed discrete bands in electrophoresis. It is concluded that if *S. typhimurium* does possess an intact 23S rRNA species, this species is extremely "labile." The absence of isolatable *S. typhimurium* 23S rRNA possibly reflected in vivo processing of the rRNA before isolation. Under certain conditions, *S. typhimurium* rRNA formed discrete aggregates which sedimented similarly to intact *Escherichia coli* 23S rRNA.

It is generally assumed that enteric bacteria contain 23S rRNA molecules consisting of single, continuously covalent polynucleotide chains (5, 19). In contrast, it is demonstrated here that RNA isolated from *Salmonella typhimurium* by four different procedures and from a variety of strains lacked a 23S species identifiable on sucrose gradients which minimize aggregation. The missing 23S species could be accounted for by recovery from the 14S–16S region of gradients of two RNA species with approximate molecular weights of 0.66×10^6 and 0.47×10^6. *S. typhimurium* may process its 23S rRNA, as do some other procaryotes (6, 10, 14, 15, 22) and eucaryotes (see references in 5, 19).

MATERIALS AND METHODS

Source of materials. [5,6-^{3}H]uridine (specific activity = 49.9 Ci/mmol) was purchased from ICN. [2-^{14}C]uridine (specific activity = 53.19 mCi/mmol) was purchased from New England Nuclear Corp. DNase I (RNase free; code, DPFF) and lysozyme (code, LYSF) were purchased from Worthington Biochemicals Corp. RNase-free sucrose (grade I) and diethylpyrocarbonate were purchased from Sigma Chemical Co. Michael D. Cole kindly provided an *E. coli* rRNA standard.

Bacterial strains, media, and growth conditions. Bacterial strains used in this study are listed in Table 1. The presence of genetic markers was confirmed. Bacteria were grown in Vogel-Bonner minimal (E) medium (28) or in nutrient broth (Difco Laboratories) as specified in the figure legends. Growth was monitored by a Klett-Summerson colorimeter equipped with a red (660-nm) filter.

RNA isolation. In the four methods given below, all glassware and solutions were treated by baking, autoclaving, or adding DEPC to inactivate any contaminating RNase.

(i) The standard "freeze-thaw" method of Forchhammer et al. (9) was used with the following modifications. First, 0.1% (wt/vol) 8-hydroxyquinoline (8-HQ) was sometimes added to the singly distilled phenol. Second, after three phenol extractions, the aqueous phase was first extracted with 2 volumes of chloroform/phenol (1:1), which had been saturated with 10 mM sodium acetate buffer, pH 5.0 (NaAc, pH 5.0), and then with 2 volumes of buffer-saturated chloroform/isoamyl alcohol (24:1). Third, after three ethanol precipitations, the RNA pellet was dissolved in TKE buffer (10 mM Tris, 0.33 M KCl, 1 mM EDTA [pH 7.3]) and filtered through a 0.45-μm type HA membrane filter (Millipore Corp.). The purified RNA was stored as an ethanol precipitate unless otherwise indicated. About 0.13 mg of RNA was recovered from a 10 ml bacterial culture "killed" at about 60 Klett units. Over 96% of the counts per minute of this purified RNA was retained on Whatman DE81 (DEAE) filter disks.

A modification of the standard freeze-thaw procedure was used to isolate aggregated rRNA (see Results). In this modification, Mg^{2+} ions (5 or 10 mM $MgCl_2$) were added to each step of the RNA isolation. Chelators like 8-HQ and EDTA were omitted from all extraction steps. The Millipore filtration buffer was 20 mM Tris–10 mM $MgCl_2$ (pH 7.5) instead of the TKE buffer. None of the solutions were treated with DEPC.

(ii) The standard "hot sodium dodecyl sulfate" (SDS) method was a modification of the method used by Ikemura and Dahlberg (12). Labeled cultures (Fig. 1) were briefly chilled to about 25°C and centrifuged

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TABLE 1. *Bacterial strains*

Strain and genotype	Source/reference
E. coli K-12	
K1: W3110 *trpR trpE trpA lacZU118* Azi^r Val^r	Korn and Yanofsky (13)
S. typhimurium LT 2	
Ames wild type	Zinder and Lederberg (30)
ara-9: *his*$^+$ *ara-9*	Ely et al. (7)
DA11: *hisU1206*	Anton (1)
SB3095: *hisG46 fla-2055*	Ely et al. (7)
SB4047: *his*$^+$ *fla-2055*	SB3095 × ara-9 phage
TM150: *hisT1504 Δ(hisOGDCBH)2253 rna-1*[a]	Bruni et al. (4)
*S. typhimurium*LT7	
Wild type	Zinder and Lederberg (30)

[a] Formerly *rns-1* (23).

for 5 min at 19°C. The pellet was rapidly suspended with blending on a Vortex mixer in 0.5 vol of 96°C 30 mM Tris-0.1 M NaCl-1 mM EDTA, 0.5% SDS (wt/vol) (pH 7.3). After being swirled for 90 s longer at 96°C, the viscous lysate was poured directly into 2 volumes of cold, buffer-saturated phenol (no 8-HQ), containing 0.2 volume of 1.5 M NaAc (pH 5.0). The remaining extractions, ethanol precipitations, and filtration were carried out as described above in the standard freeze-thaw method, except that 8-HQ was omitted from all phenol extractions.

A modification of the hot SDS procedure was used to pulse label RNA (see Fig. 4A). After being labeled, the culture was poured directly into an equal volume of 96°C 4 mM Tris-1 mM EDTA-2% SDS (wt/vol) (pH 7.3). After being swirled for 90 s longer at 96°C, the mixture was poured into 0.5 volume of cold, buffer-saturated phenol (no 8-HQ). The pellet from the first ethanol precipitation was suspended in cold 0.4 M NaAc (pH 5.0), and the RNA was salt precipitated twice by the addition of 4 M NaCl (20). The remaining ethanol precipitations and filtration were completed as described above in the standard freeze-thaw method.

(iii) The "direct phenol" method was based on another procedure used by Ikemura and Dahlberg (12). Labeled cultures (Fig. 1) were added to a tube containing 2 volumes of cold, buffer-saturated phenol (no 8-HQ), 0.25 ml of 10% (wt/vol) SDS, and 1.0 ml of 1.5 M NaAc (pH 5.0). The remaining extractions, ethanol precipitations, and filtration were carried out as described above in the standard freeze-thaw method, except that 8-HQ was omitted from all phenol extractions.

(iv) The DEPC method of Summers (24) was followed without modification. Additional ethanol precipitations and a filtration were completed as described above.

Sucrose gradients. The standard sucrose gradient, which minimizes RNA aggregation, was prepared by the method of Ennis and Kievitt (8). Immediately before the gradients were loaded, unless otherwise noted, the samples were heated for 1 min at 80°C and then rapidly cooled to 25°C. The gradients were centrifuged in a Sorvall AH627 rotor (equivalent of SW27) at 25,500 rpm for 13 h at 19°C. Nearly 100% of the counts per minute initially loaded were recovered from each gradient.

Nonstandard ("aggregation") gradients (Fig. 2) were based on the procedures of Venetianer et al. (27). The gradient buffer was 0.1 M NaAc-5 mM $MgCl_2$ (pH 5.0). None of the gradient solutions were treated with DEPC. One-half of the sample was loaded directly onto a gradient in the cold. The other half was heated to 80°C for 1 min, chilled rapidly, and loaded onto a second gradient in the cold. The gradients were

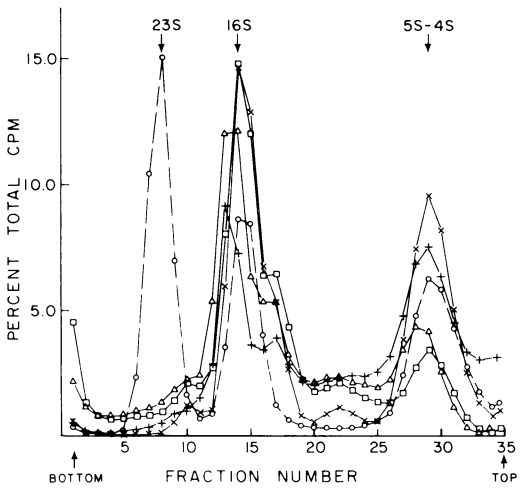

FIG. 1. *Sucrose gradient profiles for S. typhimurium strain TM150 RNA isolated by the standard freeze-thaw method (×), the direct phenol method (+), the hot SDS method (□), and the DEPC method (△). E. coli strain K1 RNA (- -○- -) was coextracted with the S. typhimurium RNA by the freeze-thaw method. For the coextraction, E. coli strain K1 was grown with shaking at 37°C in minimal (E) medium + 0.4% glucose + 0.49 mM tryptophan + 0.26 mM histidine. After exponential growth was established (≅ 10 Klett units) [^{3}H]uridine was added to 10 μCi/ml and growth was continued to approximately 30 Klett units. At the same time, S. typhimurium strain TM150 was grown with shaking at 37°C in minimal (E) medium + 0.4% glucose + 0.26 mM histidine. After exponential growth was established (≅ 13 Klett units), [^{14}C]uridine was added to 1 μCi/ml and growth was continued to 60 Klett units. The contents of the separately labeled flasks were poured into the same "kill" flask, and total RNA was isolated by the freeze-thaw method (see Materials and Methods). For the other three RNA isolations, strain TM150 was grown with shaking at 37°C in minimal (E) medium + 0.4% glucose + 0.26 mM histidine. At 60 Klett units, [^{3}H]-uridine was added to 50 μCi/ml. Growth was continued for 3 more min. Unlabeled uridine was then added to 0.2 mg/ml. RNA was isolated by procedures described in the text. The RNA isolated by the direct phenol and hot SDS methods originated from the same culture. In all cases, RNA was analyzed on 5 to 20% sucrose gradients under standard conditions (see text). At least 7.2 × 10^6 cpm of total RNA was analyzed per gradient. Positions in the gradients indicated by the arrows were based on sedimentation of the E. coli RNA.*

centrifuged in an AH627 rotor at 25,500 rpm for 13 h at 4°C. The counts per minute recovered from the gradient containing the unheated sample equaled those recovered from the gradient containing the heated sample.

Isotope counting. Gradient fractions were generally counted by adding 0.25 ml of each gradient fraction and 0.25 ml of water to 5.0 ml of Packard Instagel. For doubly labeled samples (^{14}C and ^{3}H), the ^{14}C spillover into the ^{3}H channel was 8.4% of the total ^{14}C disintegrations per minute, and no changes in quenching were detected across the sucrose gradients.

Polyacrylamide gel electrophoresis in formamide. RNA was electrophoresed on 4% polyacrylamide gels in formamide by a modification of the method used by Honjo et al. (11). The following modifications were made. (i) During polymerization, the upper surface of the gels was covered by gel buffer which had been diluted to 70% formamide by the addition of water (gel buffer = 20 mM sodium phosphate, pH 6.5-buffered 99% formamide). (ii) Before sample application, the gel was overlaid with 20 mM sodium barbital buffer (pH 6.5) in water. The reservoir buffer was 20 mM sodium phosphate buffer (pH 6.5) in water. (iii) Ethanol-precipitated RNA was dried under vacuum at 25°C and resuspended in sample buffer containing 5 mM sodium barbital, pH 6.5-buffered 99% formamide. (iv) Electrophoresis was started at 50 V at 25°C. After 30 min, the voltage was raised to 100 V. (v) The gels were stained for 1 h at 25°C in 0.2% (wt/vol) methylene blue–0.2 M sodium acetate–0.2 M acetic acid. The gels were destained in water.

The gels were scanned at 570 nm by using a Gilford spectrophotometer equipped with a linear transport attachment. Peak areas were approximated by multiplying the height times the width at half height. Migration distances were measured on the scan from the top of the gel. Molecular weights were approximated from a semi-logarithmic plot, using the standards listed.

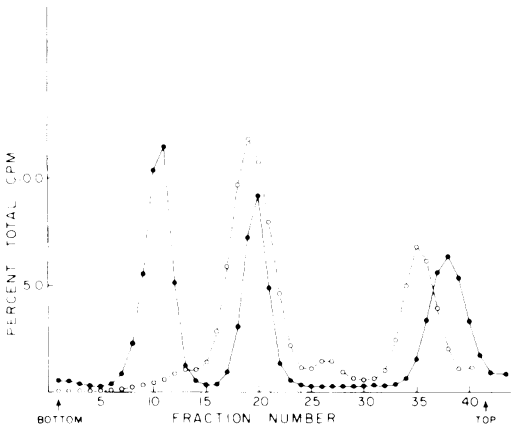

FIG. 2. *Aggregation of rRNA into faster-sedimenting species. Strain SB4047 was grown with shaking at 37°C in minimal (E) medium + 0.4% glucose. At about 50 Klett units, [^{3}H]uridine was added to 50 μCi/ml, and growth was continued 3 min longer. Unlabeled uridine was then added to 0.2 mg/ml, and growth was continued another 30 min. RNA was isolated by a modification of the freeze-thaw method which included Mg^{2+} ions in all solutions and omitted chelators and DEPC from all steps (see text). The RNA sample was divided in half. One-half was loaded directly onto a nonstandard (aggregation) gradient in the cold. The other half was first heated to 80°C for 1 min, rapidly chilled to 4°C, and loaded onto a second nonstandard (aggregation) gradient (see text). Each gradient contained 9.8 × 10^{6} cpm of ^{3}H. The offset in the 5S–4S region of the gradients was due to a draining artifact. The nonstandard (aggregation) gradient was not calibrated with E. coli RNA. Symbols: ●, unheated RNA; ○, heated RNA.*

RESULTS

Absence of intact *S. typhimurium* 23S rRNA. Typical sedimentation patterns for *E. coli* and *S. typhimurium* steady-state RNA on 5 to 20% sucrose gradients are shown in Fig. 1. In one of the experiments shown, separate cultures of *E. coli* strain K1 and *S. typhimurium* strain TM150 (RNase I$^-$) were labeled during exponential growth with [^{3}H]uridine or [^{14}C]uridine, respectively. The two cultures were added to the same "kill" flask, and their RNA was coextracted by the standard freeze-thaw procedure outlined in Materials and Methods.

Distinct, sharp peaks sedimenting at 23S, 16S, and 5S–4S were present for *E. coli* RNA. In contrast, material sedimenting at 23S was absent from the *S. typhimurium* profile. In addition to the 5S–4S peak, three distinct peaks were present. The vast majority of rRNA sedimented as a peak in the 16S region of the gradient with a shoulder at about 14S. Very small peaks reproducibly appeared at about 10S and 21S.

Minor modifications of the standard freeze-thaw procedure (like extraction temperature, omission of chelators, and inclusion of Mg^{2+} ions) resulted in the same sedimentation pattern for *S. typhimurium* RNA as depicted in Fig. 1 (data not shown). RNA was also extracted from strain TM150 by three other distinct procedures (Fig. 1). These procedures included a variation of the direct phenol method, a variation of the hot SDS method, and Summers DEPC method (see Materials and Methods for details). All three of these additional RNA isolation procedures resulted in essentially the same sedimentation profile for *S. typhimurium* RNA as the standard freeze-thaw procedure (Fig. 1).

The relative yield of higher-molecular-weight rRNA varied with the isolation procedure. For example, RNA isolated by the direct phenol method had a significantly reduced amount of 16S region rRNA relative to 5S–4S RNA (Fig.

1). However, the sedimentation pattern characteristic of *S. typhimurium* rRNA was still obvious. The hot SDS procedure yielded the highest ratio of higher-molecular-weight rRNA to 5S–4S RNA. The apparently complete lysis afforded by the hot SDS method (as judged by turbidity and viscosity) made the loss of an *S. typhimurium* 23S rRNA molecule by trapping during extraction seem unlikely.

Independence from strain and growth conditions. To examine whether the *S. typhimurium* rRNA sedimentation profile was dependent on genetic background or growth conditions, RNA was extracted from a set of nonisogenic *S. typhimurium* strains (Fig. 1 to 3; unpublished data). RNA isolated by the standard freeze-thaw method from the Ames LT2 wild-type strain again lacked 23S rRNA (data not shown). RNA isolated from an LT7 wild-type strain, which had a completely different origin from that of the LT2 strains (Table 1), was indistinguishable on high-resolution gradients from RNA isolated from LT2 strain TM150 (see Fig. 1).

RNA also was isolated by the DEPC method from strain DA11 after a 30-min incubation at 43°C, followed by a 10-min pulse of [^{3}H]uridine at 43°C (data not shown). This RNase P mutant strain (2) accumulated precursor tRNA molecules which sedimented in the 14S–4S region of the sucrose gradient. Relative synthesis of large rRNA also seemed to be slowed in strain DA11 at high temperature. However, 23S rRNA again was not detectable in this probable RNase P mutant strain.

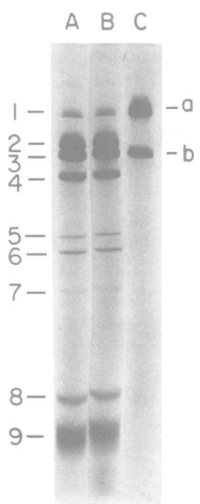

FIG. 3. *Electrophoresis on 4% polyacrylamide gels in buffered formamide of S. typhimurium RNA which showed sucrose gradient profiles identical to those depicted in Fig. 1. Strains TM150 and LT7 wild type were grown separately in minimal (E) medium + 0.4% glucose + 0.49 mM tryptophan + 0.26 mM histidine at 37°C with shaking. At about 60 Klett units, RNA was isolated by the freeze-thaw method and electrophoresed as described in the text. After electrophoresis, the bottoms of the gels were cut in the middle of the tracking dye. The gels were stained with methylene blue. (A) 10 μg of strain TM150 RNA; (B) 10 μg of LT7 RNA; (C) 5 μg of E. coli standard RNA. Band identities are described in the text.*

RNA isolated by the DEPC method from strain TM150, which had been labeled throughout exponential growth in Difco nutrient broth (data not shown), showed the same rRNA profile as RNA isolated from strain TM150 grown in minimal (E) glucose medium (Fig. 1). Finally, RNA isolated by the freeze-thaw method from LT2 strain SB4047 showed the same rRNA sedimentation profile as RNA isolated from other *S. typhimurium* strains (data not shown).

Aggregation of *S. typhimurium* rRNA. Earlier studies had reported detection of *S. typhimurium* 23S rRNA by methyl albumin chromatography (16) and by sucrose gradient analysis (27). To reconcile our findings with those of these studies, we repeated the RNA isolation and sucrose gradient analysis under basically the same conditions of an earlier study (27). Half the sample was loaded directly on the gradient in the cold, as was done in the earlier study (27). The other half was heated to 80°C for 1 min, rapidly chilled to 4°C, and then loaded on an identical gradient. The gradient containing the heated sample (Fig. 2) resembled the gradients run under standard conditions which minimize RNA aggregation (Fig. 1). The gradient containing the unheated sample showed a discrete rRNA aggregate which was previously assumed to be intact 23S rRNA (27).

Little if any aggregation was present in either heated or unheated samples under standard gradient conditions in an analogous experiment using RNA isolated by the standard freeze-thaw method (data not shown). Therefore, heating of the RNA could not account for the *S. typhimurium* rRNA sedimentation profile.

Discrete *S. typhimurium* rRNA molecules by gel electrophoresis. *S. typhimurium* strain TM150 RNA, *S. typhimurium* LT7 wild-type RNA, and the *E. coli* standard rRNA were analyzed by polyacrylamide gel electrophoresis in formamide (gels A, B, and C, respectively, in Fig. 3). The *E. coli* rRNA standard contained only 1.1×10^6-dalton rRNA (23S species; band a, Fig. 3) and 0.55×10^6-dalton rRNA (16S species; band b, Fig. 3). A very faint band, which possibly resulted from autolysis, was also present (position 7, Fig. 3). The gel electrophoresis pattern for RNA isolated from *E. coli* strain K1 showed little if any autolysis product and closely

resembled the electrophoresis profile depicted in Fig. 3C for the standard *E. coli* rRNA (data not shown).

S. typhimurium RNA was present as a series of discrete molecular species. The gel patterns were indistinguishable for the two *S. typhimurium* strains. Bands 3, 7, 8, and 9 corresponded to 0.55×10^6-dalton rRNA (16S), a possible low-level autolysis product also seen in the *E. coli* standard, 0.035×10^6-dalton rRNA (5S), and tRNA, respectively. Most of the remaining rRNA was contained in two heavy-staining bands (bands 2 and 4, Fig. 3), which together with band 3 must constitute the 16S peak and 14S shoulder observed on sucrose gradients (Fig. 1). From the gradient analysis, RNA species corresponding to the 21S and 10S peaks were expected. It is reasonable to assume that faint band 1 was the 21S species and that faint bands 5 and 6 made up the 10S peak. RNA isolated from *S. typhimurium* strain SB4047 by both the standard freeze-thaw method and a variation of the hot SDS method, which included a high salt precipitation of the rRNA, showed the same formamide gel pattern as depicted in Fig. 3 (data not shown).

The 16S- and 23S-like peaks from the non-standard (aggregation) gradients shown in Fig. 2 were analyzed by gel electrophoresis in formamide (data not shown). In this single instance, the RNA from the pooled peak fractions had undergone some random autolysis during storage at −20°C in the gradient buffer which lacked SDS. Nevertheless, it was clear from the gels that: (i) bands 2, 3, and 4 (Fig. 3) were the predominant species in the heated sample 16S peak (fractions 16 through 22); (ii) bands 2 and 4 were the predominant species in the unheated sample 23S-like peak (fractions 8 through 12), whereas band 3 was substantially missing; and (iii) band 3 was the predominant species in the unheated sample 16S peak (fractions 18 through 21), whereas bands 2 and 4 were substantially missing.

Scanning of the gels in Fig. 3 and molecular weight calibration using the 1.1×10^6-dalton (23S), 0.55×10^6-dalton (16S), 0.035×10^6-dalton (5S), and 0.027×10^6-dalton (tRNA) RNA species indicated the following tentative molecular weights for the *S. typhimurium* bands: Band 1, 0.96×10^6; band 2, 0.66×10^6; band 4, 0.47×10^6; band 5, 0.25×10^6; and band 6, 0.20×10^6. Clearly, the molecular weights of the species in band 2 + band 4, band 1 + band 5, and band 1 + band 6 approximate 1.1×10^6, which is the molecular weight of intact *E. coli* 23S rRNA. However, band 1 and band a (23S) ran so very close together that this assignment of molecular weights is tentative.

The areas of the scanned peaks were also approximated. The methylene blue stain did not bind strictly according to RNA concentration as judged by comparing two gels containing different amounts of *E. coli* standard rRNA. With this reservation, bands 2 and 3 contained nearly equimolar amounts of rRNA, whereas band 4 contained about 70% of the molar amount in band 2. Likewise, bands 1, 5, and 6 contained nearly equimolar amounts of RNA. By this highly approximate analysis, band 2 or band 3 contained as much as a 10- to 15-fold molar excess over band 1, 5, or 6.

Absence of detectable precursor rRNA after pulse labeling. Two approaches have been used in other studies to definitively demonstrate in vivo processing of rRNA (6, 15; see references in 5). The first approach depended on accumulation of precursor rRNA molecules in certain RNase-mutant strains (5). As described above (Fig. 1; unpublished data), mutations in the genes coding for RNase I and RNase P, which are the only two types of endoribonuclease mutations known so far in *S. typhimurium*, did not lead to the appearance of a high-molecular-weight precursor rRNA.

The second approach utilized pulse labeling to show the presence of rapidly labeled, high-molecular-weight precursor molecules which then could be chased into lower-molecular-weight rRNA end products (6, 15). Consequently, we pulse labeled RNA in two strains of *S. typhimurium* and extracted the RNA by two different procedures (Fig. 4). Figure 4A shows the RNA isolated from strain SB4047 after a 35-s pulse of [^{3}H]uridine. The pulse-labeled RNA sedimented as a broad peak between 16S and 5S (3). Slight shoulders were present in the 21S and 16S regions of the gradient. No peak of faster-sedimenting precursor rRNA was detected. For comparison, Fig. 4A shows the steady-state *E. coli* rRNA profile obtained by essentially the same variation of the hot SDS method used to isolate the pulsed *S. typhimurium* RNA.

RNA was additionally isolated by the standard freeze-thaw method from strain TM150 after 1- and 2-min pulses of [^{3}H]uridine (Fig. 4B). Likewise, RNA was isolated from strain SB4047 after a 3-min pulse (data not shown). For comparison, a profile of the absorbance at 260 nm (A_{260}) of the 1-min pulse sample is shown (Fig. 4B). Although accumulation of pulse label into the 16S and 5S–4S regions of the gradient had occurred, no faster sedimenting precursor, especially in the 23S region of the gradient, was resolved above the labeling background (Fig. 4B).

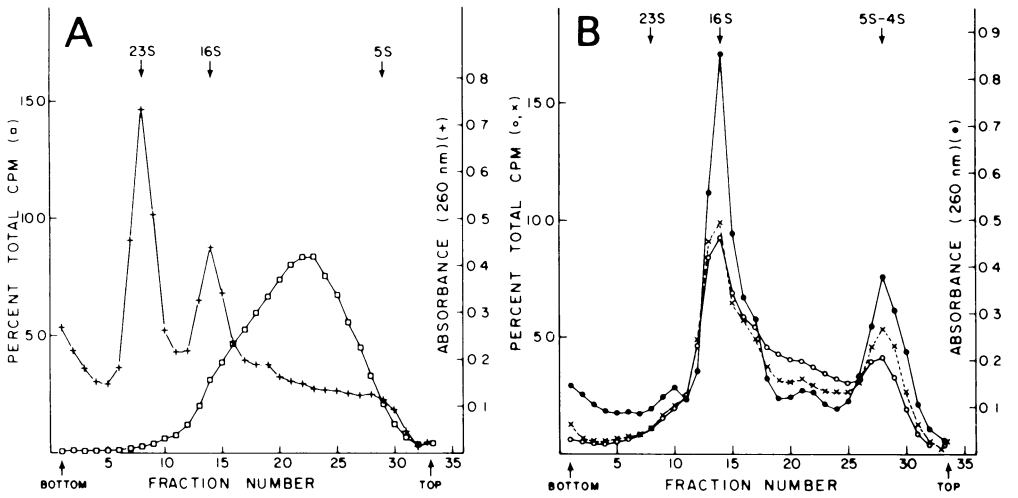

FIG. 4. *Sucrose gradient profiles of pulse-labeled RNA. (A) Strain SB4047 RNA pulsed for 35 s and isolated by the hot SDS method* (□). *A 40-ml culture of strain SB4047 was grown with shaking at 37°C in minimal (E) medium + 0.4% glucose + 0.26 mM histidine. At 50 Klett units, the cells were centrifuged at 12,100 × g for 5 min at 22°C. The pellet was suspended in 10 ml of fresh 37°C medium. After exponential growth was reestablished, the culture was pulsed with 50 μCi of [^{3}H]uridine per ml for 35 s. The RNA was isolated by the pulse variation of the hot SDS procedure given in the text. The RNA (2.3 A_{260} units and 5.9 × 10^6 cpm) was analyzed on a standard sucrose gradient. RNA was also isolated from E. coli strain K1 by a variation of the standard hot SDS method, which included two high salt precipitations (see text). The E. coli RNA (3.3 A_{260} units) served as a marker (+). (B) Strain TM150 RNA pulsed for 1 min* (○) *or 2 min* (×) *and isolated by the freeze-thaw method. Strain TM150 was grown with shaking at 37°C in minimal (E) medium + 0.4% glucose + 0.26 mM histidine. At 60 Klett units, [^{3}H]uridine was added to 50 μCi/ml and growth was continued for either 1 or 2 min. The RNA was isolated by the freeze-thaw method. All steps in the isolation were completed in the cold, and 8-HQ was omitted. The RNA was analyzed on standard sucrose gradients (see text). The separate gradients contained either 4.4 A_{260} units and 2.2 × 10^7 cpm of RNA from the 1-min pulse or 4.6 A_{260} units and 3.8 × 10^7 cpm of RNA from the 2-min pulse. The A_{260} profile of the RNA from the 1-min pulse was also recorded* (●). *Positions in the gradient were calibrated by sedimentation of E. coli RNA.*

DISCUSSION

RNA isolated from *S. typhimurium* lacked intact 23S rRNA (Fig. 1, 2, and 4). On sucrose gradients which minimize aggregation, the vast majority of the *S. typhimurium* rRNA sedimented as a sharp, but broad peak at about 16S. In most experiments, a 14S shoulder was resolved from the 16S peak. The rRNA in the 16S peak and 14S shoulder was resolved into three discrete bands by gel electrophoresis in formamide (Fig. 3). Besides 0.55 × 10^6-dalton rRNA (16S species), two other major rRNA species, which most likely originated from the larger ribosomal subunit, were found with approximate molecular weights of 0.66 × 10^6 and 0.47 × 10^6. Preliminary analysis indicated that the three major bands were present in nearly equimolar amounts.

Extensive chemical or physical degradation of *S. typhimurium* rRNA seems very unlikely. A reasonably normal amount of intact *E. coli* 23S rRNA was co-isolated with *S. typhimurium* RNA (Fig. 1). *S. typhimurium* RNA was isolated by four distinct methods (Fig. 1 and 2) and analyzed by two gradient procedures (Fig. 1 and 2), which presented a variety of chemical conditions. Unlike the case for *E. coli* rRNA (17), the *S. typhimurium* RNA sedimentation profile under conditions which minimize aggregation was independent of the presence of Mg^{2+} ion in the first steps of the extraction (Fig. 1 and 2). Heating of RNA samples before gradient analysis also could not account for the absence of 23S rRNA from the *S. typhimurium* RNA sedimentation profile. The discrete *S. typhimurium* rRNA bands in gel electrophoresis ruled out random chemical or shear degradation (Fig. 3).

Enzymatic degradation of an *S. typhimurium* 23S rRNA species during isolation is more difficult to completely rule out. Pulse labeling failed to reveal in vivo processing of a high-molecular-weight precursor into the lower-molecular-weight rRNA end products (Fig. 4). However, the same basic result was obtained by using different RNA isolation procedures (Fig. 1 and 2). Notably, the direct phenol and hot SDS methods offered essentially instantaneous cel-

lular lysis with few intermediate steps (Fig. 1). Loss of an *S. typhimurium* 23S rRNA molecule by trapping during extraction also was unlikely (Fig. 1).

The same RNA sedimentation profile was obtained from a variety of nonisogenic *S. typhimurium* strains (Fig. 1 and 2; unpublished data). Significantly, strain DA11 contained the temperature-sensitive *hisU1206* mutation which most likely results in defective RNase P (2), whereas strain TM150 contained an *rna* mutation which greatly decreases levels of RNase I (4). Other endoribonuclease mutations are currently unknown in *S. typhimurium* (23). However, it might be expected that certain other endoribonucleases like RNase III, which is absolutely Mg^{2+} ion dependent (21, 25), would be inhibited by the high EDTA concentrations initially present in the hot SDS and DEPC methods used to isolate RNA from strain TM150 (Fig. 1). Although total RNase levels can change under different growth conditions (29), the RNA sedimentation profile was the same from strain TM150 grown in nutrient broth (data not shown) or grown in minimal (E) glucose medium (Fig. 1).

Therefore, if *S. typhimurium* does possess a 23S rRNA species, this species is clearly labile. Furthermore, the above results suggest that the absence of an intact *S. typhimurium* 23S rRNA species might reflect the in vivo state before isolation and possible in vivo processing. In these studies, ribosomes were never isolated as a preliminary step because of the increased chance for rRNA degradation during ribosome isolation. However, Wehr (29) parenthetically described a gel electrophoresis pattern for rRNA extracted from the isolated ribosomes of another *S. typhimurium rna* mutant strain which seems to be very similar to the gel electrophoresis pattern depicted in Fig. 3. The discrete aggregation of *S. typhimurium* rRNA molecules in the presence of Mg^{2+} ions (Fig. 2) and the preliminary electrophoretic analysis of the discrete aggregates suggest that the two major non-5S rRNA species might be noncovalently associated in the larger ribosomal subunit.

In addition to the major rRNA peak, two very minor *S. typhimurium* RNA peaks were resolved at about 21S and 10S on sucrose gradients (Fig. 1). The 21S peak was resolved by gel electrophoresis into one band with a tentative molecular weight of 0.96×10^6, whereas the 10S peak was resolved by gel electrophoresis into two bands with approximate molecular weights of 0.25×10^6 and 0.20×10^6 (Fig. 3). The three minor gel bands were present in nearly equimolar amounts, and the major gel bands were present in at least a 10-fold molar excess over these minor bands. Lack of resolution of the 21S and 10S species during pulse experiments (Fig. 1 and 4) probably reflected high mRNA background levels in these regions of the gradients.

The identity of the minor *S. typhimurium* RNA species was not further studied. However, the preliminary results of Wehr, using isolated ribosomes (29), indicates that the three minor RNA species contained in the 21S and 10S gradient peaks are also probably of ribosomal origin. The 21S and 10S species could reflect degradation of *S. typhimurium* 23S rRNA, or they could indicate a second mode of in vivo processing from heterogenous rRNA cistrons. That the 21S species was always obtained in minor quantities irrespective of the RNA isolation condition (Fig. 1 and 2) seems to support processing rather than degradation.

Reasons for cleavage of the non-5S rRNA in the larger ribosomal subunit of some procaryotes and some eucaryotes remains obscure even in the most studied systems (6, 15, 19). Presumably, the cleavage plays some role in ribosome assembly, maturation, or conformation (15). rRNA lability of the type demonstrated here in *S. typhimurium* might be more widespread in procaryotes than previously imagined. The tendency of rRNA to form discrete aggregates under certain conditions (Fig. 2) obscured the absence of isolatable 23S rRNA in *S. typhimurium*. Interestingly, intact 23S rRNA was reported as having been isolated from *S. typhosa*, although details of the RNA isolation procedure were not given (26).

ACKNOWLEDGMENTS

I thank R. G. Martin, C. T. Wehr, and C. Yanofsky for supplying bacterial strains, G. Ketner, M. Rhoades, and E. Weinberg for access to equipment, Philip E. Hartman for support and encouragement, and G. Chester for glassware and media preparation. Michael D. Cole supplied an *E. coli* rRNA standard and helped perform the gel electrophoresis.

This work was supported by Public Health Service grant AI01650 from the National Institute of Allergy and Infectious Diseases (to P.E.H.). M.E.W. was a trainee on Public Health Service grant HD139 from the National Institute of Child Health and Human Development.

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