^{35}S INCORPORATION INTO BACTERIAL RNA

George L. Eliceiri
Department of Pathology
St. Louis University School of Medicine
St. Louis, Missouri 63104

Received December 7, 1970

SUMMARY

When Escherichia Coli MRE600 was grown in the presence of $Na_2{}^{35}SO_4$ as the only source of sulfur in the medium, ^{35}S was incorporated into 4S RNA. ^{35}S incorporation could not be detected in the ribosomal RNA species: 5S, 16S and 23S.

Cotter and Gratzer (1) have recently published data which were interpreted as evidence of the presence of sulfur in the ribosomal RNA (rRNA) of E. coli MRE600, in the form of 16 4-thiouridylic acid residues per ribosome. They found that when bacterial rRNA was exposed to N-ethylmaleimide-1-^{14}C, the number of counts retained in RNA was equivalent to 16 thiol groups per ribosome, or about one thionucleotide per 300 nucleotides. By reacting rRNA with 5,5'-dithiobis-2-nitrobenzoic acid (DTNB), using the color value found for the reaction of DTNB with 4-thiouracil, again they calculated a concentration of one thiol group in 300 nucleotides. From spectral studies they concluded that rRNA contained 4-thiouridylic acid and that its concentration was one residue per 300 nucleotides.

The presence of sulfur in mammalian 4S RNA has been reported recently (2), but sulfur could not be detected in any other RNA species, including the rRNA's. Some preliminary experiments are presented here, that seem to contradict the conclusions of Cotter and Gratzer.

MATERIALS AND METHODS

E. coli MRE600 (a generous gift from Dr. Walter Gilbert, Harvard University) was grown in C medium (3) supplemented with 15 mM glutamate. A one-liter flask with 200 ml of medium was inoculated with 8 ml of an overnight culture and 0.64 mC of $Na^{35}SO_4$ (New England Nuclear, specific activity: 275 mC/mMole) was added. The cells were grown for 6 hours (to late log phase), and then spun for 10 minutes at 13,000 x g. An aliquot of 0.63 g of wet cells was suspended in 3 ml of RSB buffer (0.01 M Tris-HCl, 0.01 M NaCl, 0.0015 M $MgCl_2$, pH 7.4) and 0.1 mg of bentonite was added. The cells were lysed by passing them through a French pressure cell at less than 12,000 p.s.i., and the lysate spun down at 13,000 x g for 10 minutes. Sodium dodecyl sulfate was added to the supernatant to a final concentration of 0.5% and this preparation was phenol-extracted three times, by shaking for 15 minutes at room temperature. The aqueous layer was precipitated with ethanol.

The conditions for polyacrylamide gel electrophoresis, ultraviolet scanning, and radioactivity assay were as described before (2), except that the gels, frozen with dry ice, were fractionated with a Mickle gel slicer, and the slices incubated overnight with 0.05 ml of NCS (Amersham Searle) and 0.35 ml of toluene.

RESULTS AND DISCUSSION

Figure 1B shows a 10% polyacrylamide gel electrophoresis pattern of RNA from E. coli grown in the presence of $Na_2{}^{35}SO_4$. A peak of ^{35}S coincides with the optical density of 4S while no radioactive peak can be detected migrating with 5S. When a duplicate sample of this RNA preparation was analyzed in a 2.4% gel electrophoresis, no ^{35}S peaks could be found migrating with 16S or 23S.

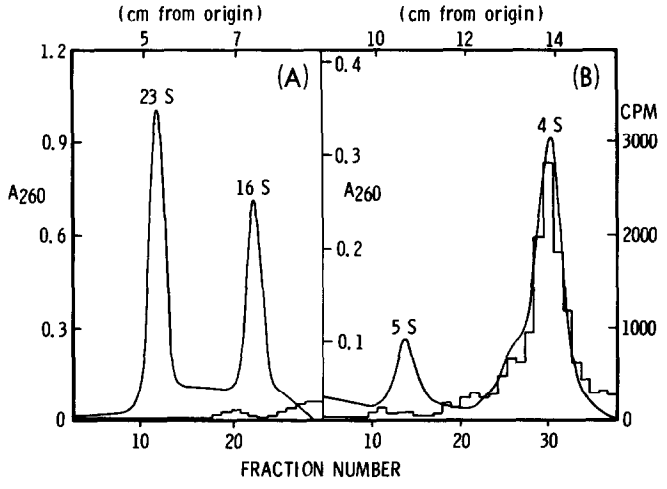

Fig. 1. Polyacrylamide gel electrophoresis of RNA extracted from E. coli MRE600 which had been grown in the presence of $Na^{35}SO_4$. The conditions of isotope incorporation and RNA extraction are described in Methods. In A, electrophoresis was carried out for 4 hours at 6 mA per gel, in a 2.4% polyacrylamide gel that was 9 cm long. In B, electrophoresis was carried out for 14 hours at 6 mA per gel, in a 10% polyacrylamide gel that was 23 cm long. Similar aliquots of an RNA preparation were applied on each gel (approximately 1.5×10^4 cpm of ^{35}S, 0.08 mg of RNA). All the optical absorbance is due to the RNA preparation, no carrier or markers were added. Absorbance, recorder tracing; ^{35}S cpm, bar diagram.

When another aliquot of this RNA preparation was fractionated on a sucrose gradient, the specific activity (trichloroacetic acid-precipitable counts) of 4S RNA was found to be about 760 cpm/μg. This specific activity seems to be completely due to incorporation of ^{35}S per se into RNA, rather than incorporation as ^{35}S-amino acids, because this specific activity was not affected by "stripping" the preparation (incubating in 1 M Tris-HCl, pH 9, for 45 minutes at 37°C). Taking into account the specific activity of the isotope preparation used, and the sulfate concentration in the medium, the specific activity of sulfate in the medium was estimated to be 3.74×10^7 cpm per μmole. Based on these specific activities, the sulfur concentration in 4S RNA

should be approximately 0.5 mole of sulfur per mole of 4S RNA. This figure is quite similar to values which can be estimated from the literature. Lipsett (4) calculated that there is one molecule of 4-thiouridylic acid per 140 nucleotides in E. coli 4S RNA, and the data of Wong et. al. (5) show that the majority of sulfur in E. coli 4S RNA is present as 4-thiouridylic acid.

If rRNA contains one atom of sulfur per 300 nucleotides, its specific activity should be about one half that of 4S RNA. If the proportion of thionucleotides is similar in all rRNA species, one would expect in Figure 1 a peak of about 9×10^3 cpm in 23S, 6×10^3 cpm in 16S, and 1×10^3 cpm in 5S.

In Figure 1 it would have been possible to detect 200-250 cpm per RNA peak. This would be approximately equivalent to one thiol group per 4 molecules of 23S, 3 molecules of 16S, or 12 molecules of 5S, respectively. Therefore, it seems that E. coli MRE600 rRNA does not contain 4-thiouridylic acid at a concentration of one thionucleotide per 300 nucleotides. It seems unlikely that another thionucleotide was present at this concentration but was degraded during handling. Of the many thionucleotides found in 4S RNA, none has been found to undergo significant degradation while in polynucleotide linkage under conditions of low temperatures and neutrality.

ACKNOWLEDGEMENT

Supported by grant GB-17546 from the National Science Foundation.

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Editor's Note: When this manuscript was originally received a paper containing similar conclusions was in press [BBRC 41 (1970) 1328].