ENZYMATIC DEGRADATION OF RIBOSOMAL RNA IN ISOLATED PURIFIED RIBOSOMES

W. Szer

Department of Biochemistry
New York University School of Medicine
New York, New York

Received April 25, 2969

The relatively easy susceptibility of bacterial ribosomal RNA, particularly 23S RNA, to degradation has been observed earlier (1). The cause of the degradation remained unclear and either enzymatic or nonenzymatic processes have been implicated by previous authors (see ref. 2 for extensive literature).

The present communication describes the enzymatic degradation of r-RNA* - intra- and extra-ribosomally - in the absence of added nucleases. Highly purified ribosomal preparations (washed twice with 1M NH_4Cl and DEAE-cellulose fractionated, ref. 3) from the RNase I^- and polynucleotide phosphorylase$^-$ E. coli strain Q_{13} were used. The most prominent feature of r-RNA degradation in intact 70S ribosomes kept at 0° for 15-30 days is the almost complete disappearance of 23S RNA with the concomitant formation of 16S RNA in the larger ribosomal subunit. If freshly isolated 70S ribosomes are fractionated into subunits and kept at 0°, the degradation goes further and both 23S and 16S RNA's are "halved". However, when subunits are immediately resolved into core particles and split protein in 5M CsCl (4) their respective RNA's are preserved undegraded in the core particles for at least 3 months at 0°. The split protein

*Abbreviations: r-RNA, ribosomal RNA; t-RNA, transfer RNA; SDS, sodium dodecyl sulphonate.

fraction from either subunit exhibits residual endonuclease activity toward 16S and 23S r-RNA as well as toward single stranded RNA from the MS2 phage. The final products of extensive degradation do not contain mononucleotides and cleavage does not require metal ions. These facts, as well as the source of the preparation, appear to exclude RNAses I, II and III and suggest the involvement of an endonuclease of the RNAse IV type found in the RNAse I^- E. coli strain MRE 600 (5). Ribosomal subunits with their respective RNA's "halved" have been found fairly active in the in vitro polyphenylalanine synthesis system.

Materials and Methods. Ribosomes were prepared from E. coli Q13 (harvested in the early exponential phase) according to Iwasaki et al. (ref. 3). Final purification included overnight washing in 0.01M Tris-Ac, pH 7.8, 0.01M MgAc, 1M NH_4Cl, 0.005M mercaptoethanol (two times) and fractionation on a DEAE-SH cellulose column. Fractionation of ribosomes into subunits was obtained by centrifugation through a linear sucrose gradient (5 to 30%, w/v) in a Spinco SW 25.1 rotor run at 23,500 rpm for 12 hrs at 4°. 150 A_{260} units of ribosomes suspended in 0.5 ml of 0.02M Tris-Ac, 0.002M MgAc, 0.5M NH_4Cl, 0.005M mercaptoethanol were layered on 27 ml of gradient in the same buffer. About 15 A_{260} units of the 30S ribosome and about 20 A_{260} units of the 50S ribosome were routinely collected from each tube thereby reducing cross-contamination to 3-5%. Ribosomal core particles and split protein were prepared according to Staehelin and Meselsohn (4). Ribosomal RNA was extracted from 70S, 50S and 30S ribosomes and from core particles by the phenol method in the presence of Macaloid (6). ^{14}C 23S and ^{14}C 16S RNA was purchased from Miles Lab., and was purified prior to use in a sucrose gradient (5 to 25%, w/v, SW 25.1 rotor, 23,500 rpm, 13 hrs at 3°). ^{32}P MS2 RNA ($s_{20,w}$-29.5S) was a gift from Dr. K. Iwasaki. The poly-U- dependent binding of Phe-tRNA to ribosomes and polyphenylalanine synthesis were assayed according to published procedures (7, 8).

Results and Discussion. When purified 70S or 50S ribosomes were kept at 0° in 10mM Mg^{2+} for 3-4 weeks before RNA extraction it was found that the 23S RNA peak disappeared almost completely from the sucrose density gradient profile and a homogenous peak sedimenting at 16S appeared instead (Fig. 1A and B). Crude 70S ribosomes, i.e., not washed with NH_4Cl, behaved similarly with respect to the disappearance of 23S RNA, the bulk of their RNA, however, sedimented broadly around the 12-16S region indicating more extensive cleavage. 16S RNA from the 30S subunit remained unchanged under these conditions if the subunit was kept in association with the 50S; after subunit fractionation the RNA in the 30S subunit becomes also accessible to cleavage and after a month at 0° the bulk of the RNA sedimented at 8-10S (Fig. 1C). Separated subunits were immediately centrifuged in 5M CsCl and resolved into core particles (42S cores from the 50S subunit and 23S core from the 30S subunit, ref. 4) and the corresponding 30S and 50S split protein fractions. No RNA cleavage has

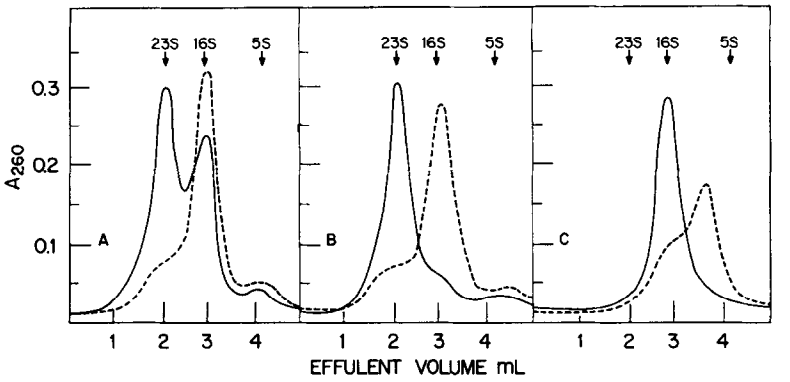

Fig. 1. Sucrose density gradient centrifugation profile of ribosomal RNA. 3 to 5 A_{260} units in 0.15 ml buffer (see below) were layered on 5 ml of linear gradient (5-25% sucrose, w/v) and centrifuged at 50, 000 rpm at 3° for 4 hrs.
——, RNA extracted within less than 72 hrs after cell harvest.
---, RNA extracted 1 month later; respective ribosomal preparations were kept at 0° in 0.02M Tris, pH 7.8, 0.5M NH_4Cl, 0.01M MgAc, 0.005M mercaptoethanol.
A- RNA from the 70S ribosome; B- RNA from the 50S subunit; C- RNA from the 30S subunit.

been observed in either core particle and RNA extracted after storage at 0° (up to 3 months) gave rise to homogenous 23S and 16S peaks when analyzed by sucrose gradient centrifugation. It was apparent that nucleolytic activity-if any-is confined to the split protein fractions.

Incubation of several single-stranded RNA's (synthetic, ribosomal and viral) with large excesses of split protein from either subunit did not produce acid-soluble products and it was necessary to analyze each reaction mixture by gradient centrifugation in order to follow the degradation. The results of extensive degradation of both r-RNA's and viral MS2 RNA are presented in Fig. 2. Incubation was carried out at low salt concentration to prevent excessive reconstitution of ribosomal components (4). Split protein from either subunit

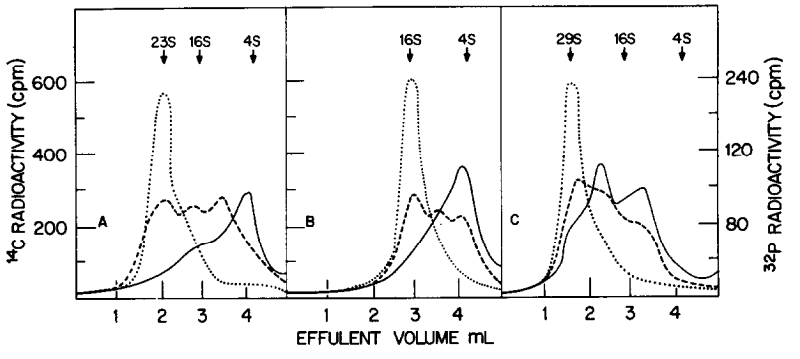

Fig. 2. Degradation of ^{14}C r-RNA and ^{32}P MS2 RNA by split proteins. 25 μg of split protein was incubated with 0.1 A_{260} unit of 23S or 16S r-RNA (about 3000 cpm), or with 0.1 A_{260} unit of MS2 RNA (about 1500 cpm) for 120 min at 30° in 0.1 ml buffer (see below). The sample was diluted to 0.5 ml with 0.1 M NaAc, pH 5.3, 5 A_{260} unit of unfractionated r-RNA was added and protein was extracted with 80% phenol in the presence of 0.5% sodium dodecyl sulphonate.* The RNA was precipitated with 2 volumes of ethanol and centrifuged in a gradient as described in Fig. 1. Gradient fractions were counted in a Packard Tri-Carb scintillation spectrometer. A- 23S RNA and 30S split protein; B- 16S RNA and 50S split protein; C- MS2 RNA and 50S split protein.
——, incubation in 0.02 M Tris, pH 7.8, 0.005 M EDTA.
---, incubation in 0.02 M Tris, pH 7.8, 0.05 M NH_4Cl, 0.01 M MgAc.
. . ., control without split protein.
* Incubation mixtures were not layered directly on the gradients since split protein "sticks" easily to tube walls and part of the RNA was found bound to the protein and could not be recovered.

was equally effective toward both r-RNA's and toward MS2 RNA, and the respective sedimentation profiles were similar to those shown in Fig. 2. Removal of Mg^{2+} from the incubation mixtures substantially enhanced degradation. In fact, the most extensive cleavage occurred in the absence of any metal ions and the bulk of both r-RNA's sedimented in the 4-6S region (Fig. 2A and B). It will be noted that degradation follows a rather regular pattern, namely, 23S → 16S → → 4-6S. It is tempting to assume that removal of Mg^{2+} opens up some helical regions and makes them accessible to enzymatic hydrolysis. It is not clear at the present time to what extent this pattern reflects enzyme specificity on the one hand and substrate conformation on the other. As seen from Fig. 2C, MS2 RNA was degraded into several distinct fragments, the sucrose gradient profile varying markedly from that obtained with r-RNA's. Again, no soluble products could be detected and degradation was much more pronounced in the absence of Mg^{2+}. The latter point distinguishes the enzyme involved from RNAses II and III (9, 10). The source of the preparation (*E. coli* Q_{13}) and lack of mononucleotides in the degradation products appear to exclude RNAse I and suggest the involvement of an endonuclease of the RNAse IV type found in the RNAse I^- *E. coli* strain MRE600 (5). No changes in the sedimentation profile of both r-RNA's and MS2 RNA were observed after incubation with 42S and 23S core particles. A slight broadening of the respective 16S, 23S and 29S peaks was observed after prolonged incubation of the three RNA's with ribosomal subunits indicating that the enzyme is tightly bound to a site on the ribosomes. To ascertain that none of the described degradative processes take place in the ribosome in the intact cell, RNA was extracted from bacteria harvested 3-4 hrs after the onset of the stationary phase. No degradation products were found and the sedimentation pattern of r-RNA was undistinguishable from that produced by r-RNA from cells harvested in the early exponential phase.

TABLE 1. In vitro activity of "aged" 50S subunit

Poly U-dependent binding of Phe-tRNA (7) μμmole Phe/μμmole ribosome		Poly U-depdendent polyphenylalanine synthesis (8) mμmoles Phe/mg ribosomal protein	
30S alone 0.24	+ 50S fresh 0.45 (100%)	30S + 50S fresh	12.3 (100%)
	+ 50S aged 0.38 (85%)	30S + 50S aged	11.0 (90%)

Intraribosomal "halving" of the respective r-RNA's was not reflected in the sedimentation profile of the ribosomal subunits themselves, and the question arose as to the activity of these subunits in polyphenylalanine synthesis. The "aged" 50S subunits with a protein content not exceeding 15% of the original (Fig. 1B) was used for this purpose. As seen from Table 1 the activity of the "aged" 50S subunit was not affected to an extent corresponding to its RNA degradation.

Acknowledgements. I wish to thank Miss M. Miller for purified E. coli Q_{13} 70S ribosomes. I am indebted to Dr. S. Ochoa for his support and critical reading of the manuscript.

Aided by grants # FR 05399 from the National Institutes of Health and # E-542 from the American Cancer Society.

REFERENCES

1. Kurland, C.G., J. Mol. Biol., 2, 83 (1960).
2. McIlreary, D.J., and J.E.M. Midgley, Biochim. Biophys. Acta, 142, 47 (1967).
3. Iwasaki, K., S. Sabol, A.J. Wahba, and S. Ochoa, Arch. Biochem. Biophys., 125, 542 (1968).
4. Staehelin, T., and M. Meselson, J. Mol. Biol., 16, 245 (1966).
5. Spahr, P.F., and R.F. Gesteland, Proc. Natl. Acad. Sci., U.S., 59, 876 (1968).
6. Stanley, W.M., Jr., and R. Bock, Biochemsitry, 4, 1302 (1965).
7. Pestka, S., and M. Nirenberg, J. Mol. Biol., 21, 145 (1966).
8. Nirenberg, M., and J.H. Matthaei, Proc. Natl. Acad. Sci., U.S., 47, 1588 (1961).
9. Robertson, H.D., R.E. Webster, and N.D. Zinder, J. Biol. Chem., 243, 82 (1968).
10. Spahr, R.F., J. Biol. Chem., 239, 3716 (1964).