# RNase CATALYZED HYDROLYSIS OF RIBOSOMES IN SEVERAL FUNCTIONAL STATES.*

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Summary. The RNase A catalyzed hydrolysis of rRNA in ribosomes has been studied for nonwashed 50S and 70S ribosomes, for washed 50S and 70S ribosomes, for runoff 50S ribosomes and for 70S ribosomes in polysomes. The regions available to hydrolysis in the 50S ribosome remain available when the 50S ribosomes become a part of a 70S ribosome or a polysome. The regions available to hydrolysis in the 30S ribosome become unavailable when the 30S ribosome becomes part of a 70S ribosome or a polysome. Removal of tRNA, mRNA and factors from the 50S and 70S ribosome lowers the rate of hydrolysis of one site in the 23S rRNA. This shows that the conformation of one region of the 23S RNA changes for ribosomes in different functional states.

Introduction. RNA makes up about 60% of 50 and 30S ribosomes and their 70S association product. Several studies have appeared in which details of the intra-ribosomal structure of the ribosomal RNA (rRNA) have been deduced by hydrolyzing the ribosomes with various RNases.[1] The details of the role of rRNA in the steps of protein synthesis are still, however, largely unknown.

We have studied the RNase catalyzed hydrolysis reactions for ribosomes in various states of association and function. Rate constants have been measured and the number of RNA fragments have been determined. The data suggest that the configuration of one particular region of 23S rRNA in the 50S ribosome differs for ribosomes with or without bound tRNA, mRNA and factors. Regions of 16S RNA in the 30S ribosome may be implicated in holding the 30 unit to the 50S unit to form the 70S unit.

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Experimental. In this work ribosomes were prepared in several different ways to obtain particles in different stages of protein synthesis. E. coli Q13 (-RNase I) were grown in tryptone-NaCl medium, collected by centrifugation, and washed in 10 mM $MgAc_2$, 5 mM tris pH 7.2 ($TM^{-2}$) by resuspension and repelleting.

Nonwashed 70S ribosomes in $TM^{-2}$ were prepared by standard methods.[2] Nonwashed 50S and 30S subunits were obtained by suspending a 70S pellet in 1 mM $MgAc_2$, 50 mM $NH_4Cl$, 5 mM tris, pH 7.2 ($TAM^{-3}$) and were separated by zonal centrifugation.[3]

Washed 70S ribosomes were obtained by pelleting 70S from $TM^{-2}$ plus 0.5M $NH_4Cl$. The pellet was resuspended in $TM^{-2}$ to obtain washed 70S ribosomes or in $TAM^{-3}$ to obtain washed 50S and 30S ribosomes which were separated as above.

Runoff ribosomes and polysomes were prepared as described previously.[4, 5]

Hydrolysis reactions were carried out in 50 µl of solution containing 0.5 or 0.6 absorbance units of ribosomes in 5 mM $MgAc_2$, 5 mM tris pH 7.2 (S-buffer), or in S-buffer plus 25 mM $NH_4Cl$. Concentrations of pancreatic RNase A (Worthington) were either 0.01 µg/ml or 0.8 µg/ml. Hydrolysis reactions took place at 0°C and were stopped by the addition of sodium dodecylsulfate (SDS) to 0.2% which also dissociates rRNA from ribosomal protein. One-tenth volume of glycerin was added and the solution was pipetted on top of a 3% polyacrylamide gel. Electrophoresis took place for 2.5 hr at 5 milliamps per gel. Gels were then removed and scanned on a Gilford gel scanning attachment and recorded on a Sargent potentiometric recorder.[6]

Results and Conclusions. We will now compare the RNase catalyzed hydrolysis

reactions of ribosomal particles in a number of functional and structural states obtained by various methods of preparation.

a) Comparison of 50S ribosomes from various sources.

When nonwashed 50S ribosomes are hydrolyzed at 0.01 μg/ml RNase, two fragments called 50A and 50B are obtained by electrophoresis (Fig. 1, a, b).[6]

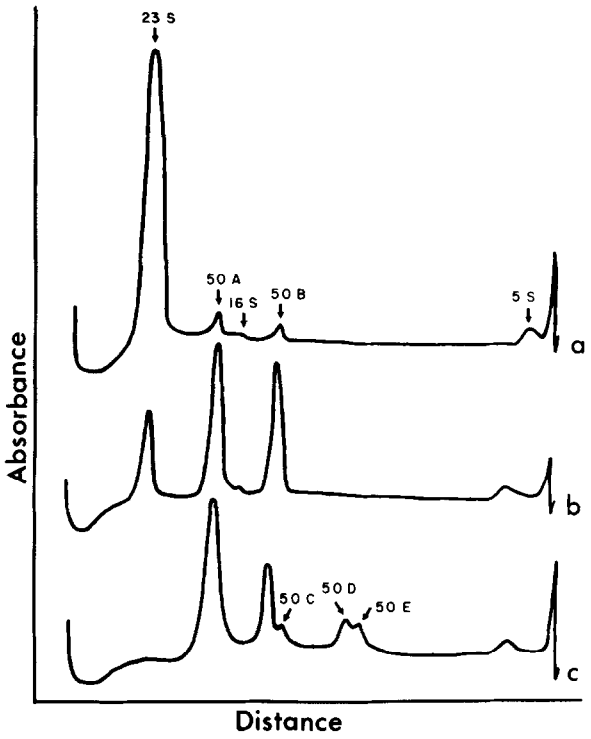


Figure 1. Electrophoresis profiles for the RNA from 50S and 70S ribosomes.
(a) 50S ribosome, no hydrolysis.
(b) 50S ribosome hydrolyzed with 0.01 μg/ml of RNase A at 0°C.
(c) 50S ribosomes hydrolyzed with 0.8 μg/ml RNase A at 0°C.
(d) 70S ribosomes, no hydrolysis
(e) 70S ribosomes hydrolyzed with 0.01 μg/ml of RNase A at 0°C.
(f) 70S ribosomes hydrolyzed with 0.8 μg/ml RNase A at 0°C.

This suggests one site of rapid hydrolysis called 50-1 in the 23S RNA in the 50S subunit. The pseudo first order rate constant (k') for the hydrolysis of site 50-1 is 12 x $10^{-2}$ $min^{-1}$ (Table 1). Site 50-1, which may be a region of RNA rather than a single nucleotide residue, appears to be in a strained configuration which has a greatly increased rate of hydrolysis compared with a nonstrained sample such as uridylyl (3'-5') uridine.[7]

For nonwashed 50S ribosomes hydrolyzed at 0.8 μg/ml RNase, we observe three new RNA bands (fig 1, c) denoted as 50C, 50D and 50E, which implies at least two new sites of hydrolysis called 50-2 and 50-3.

Hydrolysis of washed 50S subunits at both 0.01 and 0.80 μg/ml RNase gave the same electrophoresis profiles as found for nonwashed 50S subunits (fig 1b, c). The k' value for hydrolysis of the washed 50S subunit is about 6 x $10^{-2}$ (Table 1) or about one half the rate of the nonwashed 50S subunit. This shows that the nonwashed 50S ribosome, which contains some factors and tRNA, still has the same sites of hydrolysis as the washed 50S ribosome which should be partially free of tRNA, factors or fragments of mRNA. Since k' is significantly lower for washed ribosomes, the RNA of site 1 may be in a less strained configuration or may have more steric shielding than in the nonwashed ribosome.

Runoff 50S ribosomes which contain little or no tRNA, mRNA, or proteins were hydrolyzed at 0.01 μg/ml RNase and also gave fragments 50A and 50B, but k' for site 50-1 is one-sixth (2 x $10^{-2}$ $min^{-1}$) of the value found for nonwashed 50S, and is one-third of the value of washed 50S subunits (Table 1). The difference between k' for runoff and washed ribosomes may be the degree to whic tRNA, mRNA and factors have been removed from the ribosome. If we assume that the fraction of runoff ribosomes with bound tRNA, etc., is much lower than the fraction of washed ribosomes with bound tRNA, etc, the decrease in

Table 1. Hydrolysis of Ribosomes in Several Functional States.

| Substrate | 0.50 $NH_4Cl$ Wash | RNase μg/ml | RNA Fragments | Mol. Wht. X $10^{-3}$ | Sites of Hydrolysis | Rate of Hydrolysis of 50-1 |
|---|---|---|---|---|---|---|
| 50S | No | 0.01 | 50A | 660 | 50-1 | 12 x $10^{-2}$ $min^{-1}$ |
| | | | 50B | 440 | | |
| 50S | Yes | 0.01 | 50A | 660 | 50-1 | 6.5 x $10^{-2}$ $min^{-1}$ |
| | | | 50B | 440 | | |
| | | | 50A | 660 | | |
| | | | 50B | 440 | 50-1 | |
| 50S | No | 0.80 | 50C | 374 | 50-2 | —— |
| | | | 50D | 229 | 50-3 | |
| | | | 50E | 199 | | |
| 'Runoff' 50S | No | 0.01 | 50A | 660 | 50-1 | 20 x $10^{-2}$ $min^{-1}$ |
| | | | 50B | 440 | | |
| 70S | No | 0.01 | 50A | 660 | 50-1 | 12.7 x $10^{-2}$ $min^{-1}$ |
| | | | 50B | 440 | | |
| 70S | Yes | 0.01 | 50A | 660 | 50-1 | 8.0 x $10^{-2}$ $min^{-1}$ |
| | | | 50B | 440 | | |
| | | | 50A | 660 | | |
| | | | 50B | 440 | 50-1 | |
| 70S | No | 0.80 | 50C | 374 | 50-2 | —— |
| | | | 50D | 229 | 50-3 | |
| | | | 50E | 199 | | |
| Polysome | No | 0.01 | 50A | 660 | 50-1 | 8.0 x $10^{-2}$ $min^{-1}$ |
| | | | 50B | 440 | | |
| | | | 50A | 660 | | |
| | | | 50B | 440 | 50-1 | |
| Polysome | No | 0.80 | 50C | 374 | 50-2 | —— |
| | | | 50D | 229 | 50-3 | |
| | | | 50E | 199 | | |

k' is explained. We conclude that there is a region of 23S RNA (site 50-1) which is open to hydrolysis in the presence or absence of bound tRNA, mRNA and factors. The configuration of this region of RNA appears to change as tRNA, mRNA, etc, are removed.

b) Comparison of 70S ribosomes from various sources.

Previous work has shown that mild hydrolysis of nonwashed 30S ribosomes gives four RNA fragments (30A, 30B, 30C and 30D).[6] Experiments were therefore carried out to determine if the sites of hydrolysis in the separated 30S and 50S ribosomes remained available in the 70S ribosome. First it was determined that 70S ribosomes do not dissociate into subunits when hydrolyzed for 10 min with either 0.01 or 0.80 μg/ml RNase. Sucrose gradient sedimentation showed that no major differences exist in the sedimentation profiles of 70S ribosomes with or without hydrolysis. In particular no increase in absorbance was seen in the 30S and 50S regions.

Hydrolysis of nonwashed 70S ribosomes at 0.01 μg/ml RNase gives fragments 50A and 50B with k' equal to $12.7 \times 10^{-2}$ (fig. 1, e and Table 1). Since this value of k' is the same as that for a nonwashed 50S subunit, we may conclude that the configuration of site 50-1 remains nearly the same for 50S subunits in both states.

Hydrolysis of nonwashed 70S ribosomes at 0.8 μg/ml RNase gives the same five RNA fragments observed with 50S subunits alone. We conclude that sites 50-1, 50-2 and 50-3 are not covered by the 30S subunit in the 70S ribosome. These sites are therefore not implicated in the binding of subunits.

Hydrolysis of washed 70S ribosomes gave the same fragments as nonwashed 70S. The rate constant for the hydrolysis of site 50-1 was however reduced to $8 \times 10^{-2}$ (Table 1). Again we obtain a diminution of k' upon washing.

This may mean that as tRNA and factors are removed the RNA of site 50-1 relaxes from a strained configuration which increases the energy barrier for hydrolysis.

We next compare the hydrolysis of free 30S subunits with hydrolysis of the 30S subunit as part of 70S ribosomes. The treatment with 0.80 μg/ml RNase is sufficient to hydrolyze the 16S RNA in a nonwashed 30S subunit. The hydrolysis of 70S ribosomes with 0.8 μg/ml produces no fragments which correspond to 30A, 30B, 30C or 30D (fig. 1 f).[6] We conclude that sites of hydrolysis on free 30S subunits do not exist in the 70S ribosome. These sites are either covered by the 50S subunit or the structure of 30S subunit changes when 30S is absorbed to 50S to form the 70S ribosome.

c) Hydrolysis of polyribosomes.

When 70S ribosomes in the form of polysomes are hydrolyzed at 0.01 μg/ml RNase, fragments 50A and 50B again appear with $k' = 8 \times 10^{-2}$ $min^{-1}$. At 0.80 μg/ml RNase all five fragments from 50S again appear, and no fragments of RNA from the 30S subunit were observed. This means that the conclusions for single 70S ribosomes also apply to 70S ribosomes in polysomes.

The significance of the lower value of k' for hydrolysis at site 50-1 in polysomes compared with that of nonwashed 50S or 70S is obscured due to the fact that the hydrolysis of polysomes took place in S-buffer plus 25 mM $NH_4Cl$ which causes a decrease in the rate constant when compared with S-buffer alone.[8] Past work has shown that k' value for nonwashed 50S ribosomes also goes from $12 \times 10^{-2}$ to $8 \times 10^{-2}$ $min^{-1}$ when $NH_4Cl$ is made up to 25 mM in S-buffer.[8]

References

1. J. C. Pinder and W. B. Gratzer, Eur. J. Biochem. 26, 73 (1972).

2. A. Tissieres, J. D. Watson, D. Schlessinger and B. Hollingsworth, J. Mol. Biol. 1, 221 (1959).

3. E. F. Eikenberry, T. A. Bickle, R. R. Traut, and C. A. Price, Eur. J. Biochem. 12, 113 (1970).

4. H. Friedman, P. Lu and A. Rich, Nature 223, 909 (1969).

5. C. P. Flessel, P. Ralph and A. Rich, Science 158, 658 (1967).

6. K. A. Hartman, J. Amaya and E. M. Schachter, Science 170, 171 (1970).

7. K. A. Hartman and N. W. Clayton, in preparation.

8. K. A. Hartman, J. C. Nolan and J. Amaya, Biochem. Biophys. Res. Commun. 45, 1307 (1971).