THE INVOLVEMENT OF 5S RNA IN THE BINDING OF tRNA TO RIBOSOMES[+]

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Summary: The tRNA fragment TpΨpCpGp was found to bind to 5S RNA. This binding is ten times increased when a specific 5S RNA-protein complex is used. The ability of TpΨpCpGp to bind to the complex could be abolished by selective chemical modification of two adenines in 5S RNA. Such 5S RNA, when incorporated into 50S ribosomal subunits, yielded particles with greatly reduced biological activities. From the results presented we conclude that 5S RNA is most likely part of a site with which the TΨC-loop of tRNA interacts on the ribosome.

Attempts to elucidate the precise function of 5S RNA in the ribosome have led to the following observations: 1. 5S RNA is essential for ribosomal activity, since 50S ribosomal subunits lacking 5S RNA were inactive when tested in a series of different biological assays (1). 2. One of the functions of 5S RNA is structural since -5S RNA ribosomal particles are lacking several ribosomal proteins (1). 3. The 5S RNA binding proteins were subsequently identified for Bacillus stearothermophilus (2) and Escherichia coli (2, 3) by reconstituting specific 5S RNA-protein complexes from 5S RNA and 50S ribosomal protein components. 4. Modification of the 3'-terminal end of 5S RNA followed by 50S reconstitution experiments have established that the 3'-terminal nucleoside of the 5S RNA is not essential for its biological function (4, 5). 5. Reconstitution experiments have shown that different prokaryotic 5S RNAs can be incorporated in B. stearothermophilus 50S ribosomal subunits to

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[+] Paper No. 6 on "Structure and Function of 5S RNA". Preceding paper is reference 8.

yield biologically active particles, whilst eukaryotic 5S RNAs cannot (6). 6. Recently both, GTPase and ATPase activities have been found in association with specific 5S RNA-protein complexes isolated from B. stearothermophilus (7, 8) and with rat liver systems (9). This enzymatic activity may implicate, that 5S RNA is either at or near the peptidyl transferase center of the ribosome.

It has been proposed that 5S RNA interacts on the ribosome with tRNA during protein synthesis (10). Such proposed interaction would take place between the TΨC-loop of tRNA and part of the CGAAC sequence of 5S RNA. Indeed, it has been reported that TpΨpCpGp inhibits non-enzymatic binding of aminoacyl tRNA to ribosomes (11, 12). Recently it has also been observed that TpΨpCpGp inhibits enzymatic binding of aminoacyl tRNA to ribosomes and additionally that it prevents magic spot (pppGpp and ppGpp) formation (13). The binding site of TpΨpCpGp still remains to be identified. We decided to further investigate this problem by reducing the ribosome-tRNA system to smaller components, such as 5S RNA or 5S RNA-protein complexes and the tRNA fragment TpΨpCpGp. The results reported in this communication indicate that TpΨpCpGp weakly binds to free 5S RNA and strongly to specific 5S RNA-protein complexes. Selective modification of two adenines in 5S RNA prevents this interaction.

Materials and Methods

Isolation of B. stearothermophilus and E. coli 50S and 30S ribosomal subunits, reconstitution of B. stearothermophilus 50S subunits and poly(U) directed polyphenylalanine incorporating assays were carried out as previously published (1).

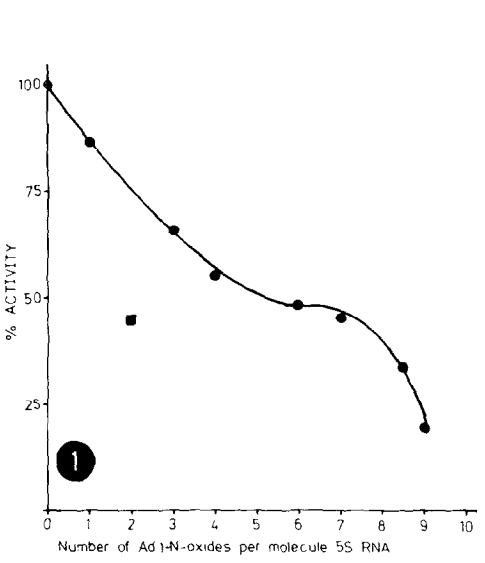
Chemical modification of 5S RNA with monoperphthalic acid was performed as described (14-16). For details of 5S RNA modification on the ribosome see legends to Figure 2. The number of adenine 1-N-oxides per RNA molecule was determined as previously reported (14-16). 5S RNA (120 nucleotides) contains 23 adenines per molecule (17) and 23S RNA (3000 nucleotides) was estimated to contain 800 adenines per molecule (14).

Equilibrium dialysis and synthesis of ^{3}H labeled UpUpCpG was carried out as described (18). TpΨpCpGp was isolated from ^{32}P labeled E. coli tRNA using essentially the method reported (12). Two-dimensional polyacrylamide gel electrophoresis was according to (19).

Results and Discussion

Reaction of monoperphthalic acid with RNA modifies non base-paired adenines to their 1-N-oxides (14-16). Such adenine 1-N-oxides are unable to participate in normal Watson-Crick type base pairing (16) and it was this reason why N-oxidized 5S RNA was used in parts of this investigation. To check the importance of non base-paired adenines in 5S RNA, E. coli 5S RNA was N-oxidized to various extents and then checked for biological activity in B. stearothermophilus reconstituted 50S subunits. Figure 1 shows that with increasing number[+] of adenine 1-N-oxides per 5S RNA molecule an increasing amount of biological activity was lost. The inhibition curve reached an intermediary plateau at 45 % residual activity when an average of seven adenines were modified per 5S RNA molecule. Further N-oxidation of 5S RNA yielded another decrease in poly(U) directed polyphenylalanine synthesis. Analysis of B. stearothermophilus reconstituted 50S ribosomes for their RNA content showed, that only when more than eight adenines per 5S RNA had been modified, the 5S RNA was incorporated in less than equimolar amounts. Parallel with this reduced incorporation of 5S RNA the binding of the 5S RNA proteins decreased, such as B-L5 and B-L22, which have previously been identified as 5S RNA binding proteins (2). These data indicates that when less than eight adenines were modified per 5S RNA, its biological function was affected, but not its structural function. This suggests that some non base-paired adenines of 5S RNA are essential for ribosomal activity.

[+] The numbers of adenine-1-N-oxide per RNA molecule were determined spectrophotometrically and by base analysis (14-16) and refer to the average number of adenines modified.



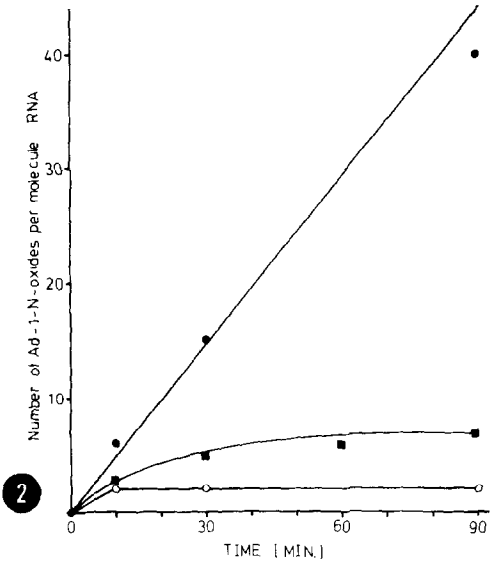


Fig. 1. Poly(U) directed polyphenylalanine synthesis activity of B. stearothermophilus 50S ribosomes reconstituted with N-oxidized E. coli 5S RNA modified free in solution and then incorporated in 50S subunit (●——●). The solid square (■) in the graph represents the activities obtained with E. coli 5S RNAs which had been N-oxidized on the E. coli 50S ribosomal subunit prior to incorporation into B. stearothermophilus 50S subunits (see also Figure 2). 100 % activity (11 moles phenylalanine polymerized per mole 50S ribosomes) corresponds to reconstituted B. stearothermophilus ribosomes with unmodified E. coli 5S RNA.

Fig. 2. Number of adenines N-oxidized per molecule RNA with respect to time. 5S RNA modified free in solution (■——■). 23S RNA (●——●) and 5S RNA (○——○) modified with monoperphthalic acid in E. coli 50S ribosomal subunits were N-oxidized with slight modifications as previously described (14). The reaction was carried out at room temperature in a total volume of 12.0 ml, which consisted of 6.0 ml 50S subunits (10 mg per ml) in TMA I buffer (0.01 M Tris-HCl, pH 7.8, 0.01 M $MgCl_2$, 0.03 M NH_4Cl, 0.006 M β-mercaptoethanol), 0.120 ml 1.0 M phosphate buffer, pH 7.0, 0.06 ml 1.0 M $MgCl_2$, 1.0 ml monoperphthalic acid (1.0 M) and 5.82 ml water. After 10, 30 and 90 minutes 4.0 ml of the reaction mixture was withdrawn and diluted with 20 ml TMA I buffer. Each sample was dialyzed against 5 liters of TMA I at 0°C. The rRNAs were then extracted with buffer saturated phenol and separated by Sephadex G-100 chromatography (4). Extent of N-oxidation was determined as previously described (14-16).

Next, we explored the possibility to selectively modify those adenines in 5S RNA, which are essential for its biological activity. Therefore, E. coli 50S subunits were reacted with monoperphthalic acid. It was expected that adenines of 5S RNA important for biological function, would be exposed and, thus, readily available for the chemical reaction. Figure 2 shows the

average number of adenines per 5S RNA and 23S RNA modified in the 50S ribosome with respect to time. The results demonstrate that only two adenines of 5S RNA were modified after 10, 30 and 90 minutes of reaction, whilst 23S RNA was increasingly modified over this time period. If free 5S RNA was modified under similar conditions seven adenine 1-N-oxides were found per molecule RNA after 90 minutes of reaction (Figure 2).

Employing 5S RNA, which had been modified on the ribosomal subunit, in 50S reconstitution experiments showed that it was normally incorporated into the particles, but that those particles had only 45 % activity in a poly(U) directed polyphenylalanine synthesizing system (Figure 1). Each of the 5S RNA samples modified on the ribosome for 10, 30 and 90 minutes behaved identically. Two-dimensional polyacrylamide gel electrophoresis analysis of such 50S reconstituted subunits showed no loss in protein content, i.e. the 5S RNA binding proteins were present in normal amounts (data not shown). From these results we conclude that 5S RNA contains two non base-paired adenines which are on the surface of the ribosome and which are essential for the biological activity of the ribosome.

It is worth noting that all tRNAs involved in chain elongation contain the sequence GpTpΨpCpPup (20-21) and that all prokaryotic 5S RNAs so far sequenced contain the sequence CpGpApApCp (17, 22). This observation led Forget and Weissman (10) to propose that tRNA could possibly interact with 5S RNA on the ribosome. To investigate this possibility the following experiments were carried out. The binding of the synthetic oligonucleotide UpUpCpG, which should be an analogue of TpΨpCpGp, to 5S RNA was measured by equilibrium dialysis. Table 1 shows that UpUpCpG weakly binds to E. coli 5S RNA. This binding is significantly increased with specific 5S RNA-protein complexes. Selective modification of two adenines per 5S RNA drastically reduces binding (Table 1). Similar results, but with significantly higher binding constants, were obtained when UpUpCpG was replaced by TpΨpCpGp (Table 1). Again the 5S RNA-protein complex showed nearly a ten fold higher

Table 1

Molar association constants of oligonucleotides with *E. coli* 5S RNA and 5S RNA-protein complexes at -2°C in 0.35 M KCl, 20 mM $MgCl_2$, 30 mM Tris-HCl buffer pH 7.4.

RNA	Oligonucleotide	K_{app} (M^{-1})
5S RNA	UpUpCpG	3 100
5S RNA (N-ox)	UpUpCpG	1 500
5S RNA-protein	UpUpCpG	22 000
5S RNA	TpΨpCpGp	8 300
5S RNA (N-ox)	TpΨpCpGp	5 000
5S RNA-protein	TpΨpCpGp	72 000
5S RNA-protein (N-ox)	TpΨpCpGp	2 000

affinity for the oligonucleotide than 5S RNA itself. This suggests that binding of specific proteins to 5S RNA changes the conformation of the RNA. It seems, therefore, advisable to treat all studies on the secondary or tertiary structure carried out with free 5S RNA with caution, since they may not be relevant to the native structure of 5S RNA in the ribosome. Again modification of two specific adenines in 5S RNA severely decreased the K-value of the tRNA fragment (TpΨpCpGp). That modification of 5S RNA decreases the oligonucleotide binding in the complex shows that it binds to the RNA and not to the protein.

From the results presented here we conclude the following: 1. The structure of 5S RNA free in solution differs from that in specific 5S RNA-protein complexes. 2. 5S RNA contains two adenines which are involved in binding of TpΨpCpGp. 3. These two adenines of 5S RNA are on the surface of the ribosome and are essential for ribosomal activity.

It seems most likely that tRNA interacts with its TΨC-loop with 5S RNA on the ribosome. This would represent for tRNA a third site of binding,

besides the anticodon and CCA-end. We would like to propose that the presence of the TψC-loop in tRNA has been selected for adding specificity to its binding on the ribosome, i.e. that sequences such as UpUpCpGp would be less effective competitors.

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References

1. Erdmann, V.A., Fahnestock, S., Higo, K., and Nomura, M., Proc. Nat. Acad. Sci. U.S.A., 68, 2932-2936 (1971).
2. Horne, J.R., and Erdmann, V.A., Molec. Gen. Genet., 119, 337-344 (1972).
3. Gray, P.N., Garrett, R.A., Stöffler, G., and Monier, R., Eur. J. Biochem., 28, 412-421 (1972).
4. Erdmann, V.A., Doberer, H.G., and Sprinzl, M., Molec. Gen. Genet., 114, 89-94 (1971).
5. Fahnestock, S.R., and Nomura, M., Proc. Nat. Acad. Sci. U.S.A., 69, 363-366 (1972).
6. Wrede, P., and Erdmann, V.A., FEBS-Lett., 33, 315-319 (1973).
7. Horne, J.R., and Erdmann, V.A., Proc. Nat. Acad. Sci. U.S.A., in press (1973).
8. Erdmann, V.A., Horne, J.R., Pongs, O., Zimmermann, J., and Sprinzl, M., First Symposium on Ribosomes and Ribonucleic Acid Metabolism, Slovak Adademy of Sciences, in press (1973).
9. Grummt, F., Grummt, I., and Erdmann, V.A., manuscript submitted for publication (1973).
10. Forget, B.G., and Weissman, S.M., Science, 158, 1695-1699 (1967).
11. Ofengand, J., and Henes, C., Biol. Chem., 244, 6241-6253 (1969).
12. Shimizu, N., Hayashi, H., and Miura, K., J. Biochem., 67, 373-387 (1970).
13. Richter, D., Sprinzl, M., and Erdmann, V.A., manuscript submitted for publication (1973).
14. Erdmann, V.A., Ph.D. Thesis, Technical University Braunschweig, Germany (1968).
15. Cramer, F., and Erdmann, V.A., Nature (Lond.), 218, 92 (1968).
16. von der Haar, F., Schlimme, E., Erdmann, V.A., and Cramer, F., Bioorg. Chem., 1, 282-293 (1971).
17. Brownlee, G.G., Sanger, F., and Barrell, B.G., Nature (Lond.), 215, 735-737 (1967).
18. Pongs, O., Bald, R., and Reinwald, E., Eur. J. Biochem., 32, 117-125 (1973).
19. Kaltschmidt, E., and Wittmann, H.G., Proc. Nat. Acad. Sci. U.S.A., 67, 1276-1882 (1970).
20. Cramer, F., Progress Nucleic Acid Research and Molecular Biology, 11, 391-421 (1971).
21. Zachau, H.G., Frontiers of Biology, Ed. L. Bosch, 173-218, North-Holland Publishing Co., Amsterdam (1972).
22. DuBuy, B., and Weissman, S.M., J. Biol. Chem., 246, 747- (1971).