STUDIES ON FREE AND 5 S RNA-BOUND RIBOSOMAL GTPASE AND ATPASE

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1. Introduction

A complex consisting of one molecule of 5 S RNA and two molecules of ribosomal protein was recently reconstituted in bacterial systems [1]. This complex was shown to be associated with both a GTP and an ATP hydrolyzing activity [2,3]. A 5 S RNA–protein complex was isolated previously by Blobel [4] by treatment of rat liver ribosomes with EDTA. Using a similar procedure to isolate this mammalian 5 S RNA–protein complex, we looked for GTPase and ATPase activities like these in the reconstituted bacterial 5 S RNA–protein complex. As reported previously [5], we found the proteins associated with 5 S RNA active both in GTP and ATP hydrolysis. In this paper we describe the purification and the chemical composition of this complex. Furthermore, the kinetic behaviour and the thermal stability of the GTPase and ATPase associated with 5 S RNA are compared with those of GTPase and ATPase not bound to RNA.

2. Material and methods

60 S subunits of rat liver ribosomes were isolated as described previously [5,6]. The pellets of the 60 S subunits were suspended in 10 mM Tris–HCl, pH 7.8, 6 mM 2-mercaptoethanol at a concentration of 150–200 A_{260} units/ml. One μmole of EDTA (pH 7.0) was added per 10 A_{260} units of ribosomal subunits. Two ml of this solution were then layered on the top of a 5–20% sucrose gradient containing 10 mM Tris–HCl, pH 7.8, 10 mM KCl, 6 mM 2-mercaptoethanol, and centrifuged for 22 hr at 27 000 rpm and 2°C in a Spinco rotor SW 27. Gradients were fractionated into 20 fractions each and the optical density was monitored at 260 nm. Twenthy five μl of each fraction were assayed for both GTPase and ATPase activity. The last 3 fractions were previously dialyzed against 10 mM Tris–HCl, pH 7.8, 1 mM NH_4Cl, 1 mM $MgCl_2$, 6 mM 2-mercaptoethanol to remove EDTA. The assay conditions for the GTPase and ATPase activities have been described elsewhere [5]. Ribosomal proteins were isolated by extraction of ribonucleoprotein fractions with 0.25 N HCl and precipitation of the proteins with 5 vol of acetone. SDS gel electrophoresis was carried out according to Laemmli [7], in 3 mm thick slab gels as described previously [5]. Gradient gels with 10–20% acrylamide were run at 150 V and stained with 0.2% Coomassie blue.

3. Results and discussion

When rat liver 60 S ribosomal subunits are treated with EDTA several proteins as well as 5 S RNA are released. After sucrose centrifugation of this 5 S RNA and protein mixture, two optical density peaks are revealed (fig. 1A). The UV-absorbing material sedimenting at 4 S represents small amounts of tRNA still present in the 60 S subunits, as checked by coelectrophoresis in 10% acrylamide gels with rat liver tRNA which could be charged with amino acids. From the second peak fraction (sedimentation coefficient about 7 S) a RNA can be isolated by phenol extraction which sediments at 5 S (fig. 1B). Thus, this fraction comprises a 5 S RNA–protein complex.

When aliquots of each gradient fraction were assayed for GTPase and ATPase activity two maxima of activity were found for both hydrolytic activities (fig. 1A). One maximum of GTPase and ATPase activity is coincident with the 7 S absorbancy peak. The

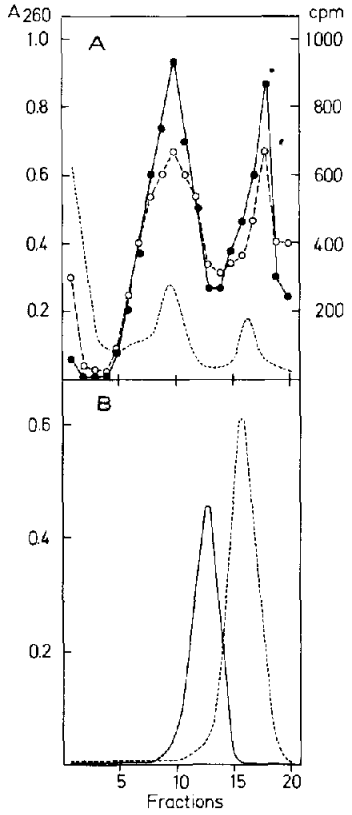

Fig. 1. Sucrose gradient centrifugation of EDTA-treated ribosomal 60 S subunits. Sedimentation from right to left, details in Material and methods. (A) . . . . . , UV absorbance at 260 nm, ○ - - - ○, GTPase activity, ●——●, ATPase activity. (B) . . . . . , tRNA measured at 260 nm, – – –, 5 S RNA isolated from the 7 S peak of (A).

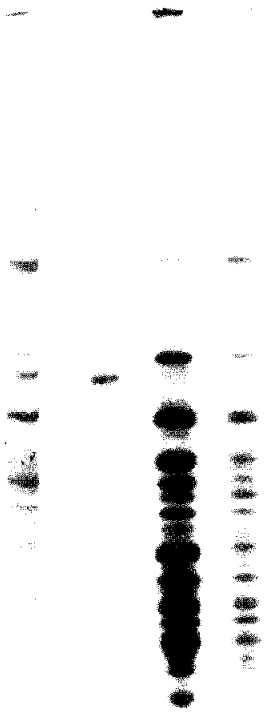

Fig. 2. Electrophoretogram of ribosomal proteins. From right to left. 60 S subunit protein (20 μg), protein from EDTA-treated 60 S subunits (60 μg), protein from the 7 S peak fraction (1 μg), protein from the < 4 S fraction.

second peak of both enzymatic activities has a sedimentation coefficient of < 4 S. This bimodal distribution of GTPase and ATPase probably represents the enzyme(s) bound to 5 S RNA (7 S peak) and in the free form not associated with RNA (< 4 S).

The protein of both the 7 S and the < 4 S fraction was isolated and compared with the protein moiety of the 60 S subunits before and after EDTA-treatment. As can be seen in fig. 2, there is only one single band visible in the protein fraction of the 7 S peak. In the gradient fractions comprising the RNA-free GTPase and ATPase (< 4 S) several bands including that of the 7 S peak can be seen. The protein of the 7 S peak has a molecular weight of 32 000, as determined by SDS-gel electrophoresis with several marker proteins.

To check whether the free and 5 S RNA-bound enzyme(s) has the same enzymatic properties, we have studied the kinetics of both the free and the 5 S RNA-bound GTPase and ATPase. The Lineweaver–Burk plots (fig. 3 and 4) indicated that the V_{max} of both the GTPase and ATPase is slightly but significantly higher in the 7 S peak fraction (7 S:0.94 pmole/min for GTP and 0.8 pmole/min for ATP; < 4 S:0.43 pmole/min for GTP and 0.28 pmole/min for ATP). The bound enzyme(s) also exhibits a higher substrate affinity than the free enzyme(s) for both the GTP and ATP hydrolysis (7 S:K_m 0.9 μM for GTP and

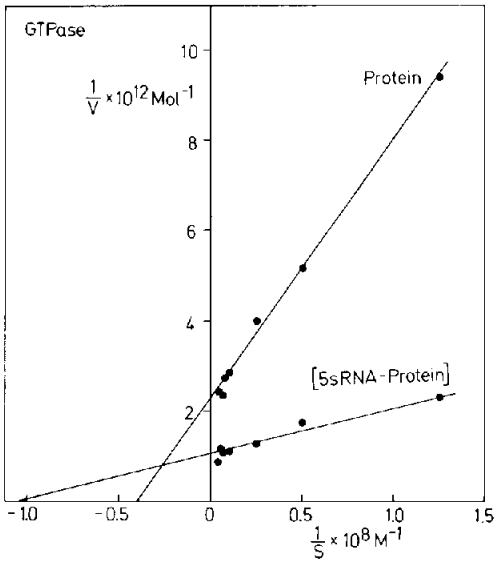

Fig. 3. Determination of K_m and V_{max} for GTP hydrolysis with the 7 S and < 4 S peak fractions. S corresponds to substrate concentration. 25 µl of enzyme fraction were assayed. Other conditions see [5].

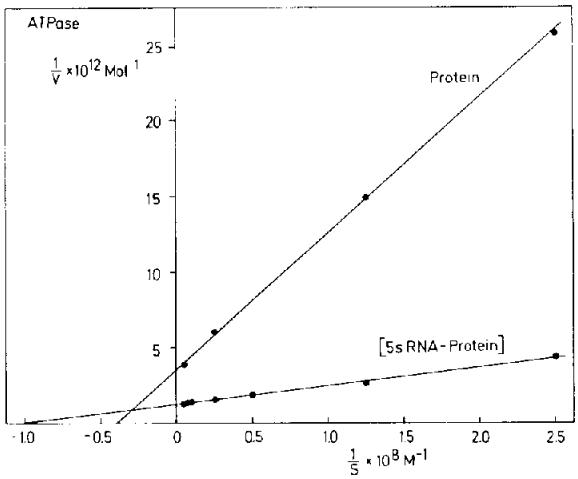

Fig. 4. Determination of K_m and V_{max} for ATP hydrolysis with the 7 S and < 4 S peak fractions. S corresponds to substrate concentration. 25 µl of enzyme fraction were assayed. Other conditions see [5].

K_m 1 µM for ATP; < 4 S:K_m 2.5 µM for GTP and K_m 2.6 µM for ATP).

To ensure that the lower hydrolytic activity of the < 4 S- in comparison to the 7 S-peak enzyme fraction is not due to the presence of the additional proteins in that fraction, we incubated both 25 µl of < 4 S-fraction alone as well as mixed together. As shown in table 1 there was an additive effect on the activities of both the GTPase and ATPase but no inhibition of the activities of the 7 S fraction by the heterogeneous contaminating protein mixture present in the < 4 S fraction. Additionally, we did not find any effect of tRNA when added either to the 7 S- or the < 4 S peak fraction (not shown here).

Table 1
Effect of mixed incubation on the activity of the 7 S and 4 S peak fraction

Enzyme fraction	Hydrolysis of	
	GTP	ATP
	(cpm)	(cpm)
25 µl 7 S	832	1240
25 µl < 4 S	763	1018
25 µl 7 S + 25 µl < 4 S	1587	2341

To check whether the stability of the enzyme(s) is influenced by its binding to RNA we have studied the thermal stability of the GTPase and ATPase activity. As shown in fig. 5, an appreciable increase

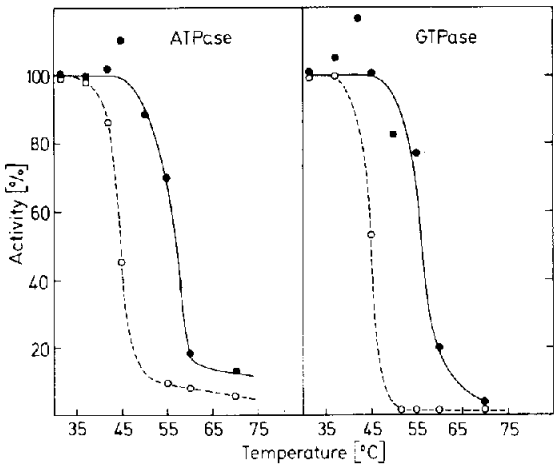

Fig. 5. Thermal stability of GTPase and ATPase activity. ○ - - - ○ enzyme bound to 5 S RNA (7 S peak fraction), ●——● enzyme free of 5 S RNA (< 4 S peak fraction). 25 µl of enzyme fraction were incubated at the temperature indicated on the abscissa for 10 min and then assayed as described in [5].

in the thermal stability of both hydrolytic activities occurs when the enzyme(s) is bound to 5 S RNA. The increase of both the enzymatic activities and the thermal stability of the GTPase and ATPase when bound to 5 S RNA is interpreted as a functionally and structurally important interaction between 5 S RNA and the enzymatic protein(s).

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References

[1] Horne, J. R. and Erdmann, V. A. (1972) Molec. Gen. Genet. 119, 337–344.
[2] Horne, J. R. and Erdmann, V. A. (1973). Proc. Natl. Acad. Sci. U.S. 70, 2870–2873.
[3] Erdmann, V. A., Horne, J. R., Pongs, O., Zimmermann, J. and Sprinzl, M., in: First Symposium on Ribosomes and Ribonucleic Acid Metabolism (Slovak Academy of Sciences, Bratislava), in press.
[4] Blobel, G. (1971) Proc. Natl. Acad. Sci. U.S. 68, 1881–1885.
[5] Grummt, F., Grummt, I. and Erdmann, V. A. (1974) Europ. J. Biochem, in press.
[6] Grummt, F. (1974) Europ. J. Biochem, in press.
[7] Laemmli, V. K. (1970) Nature 227, 680–685.