Thermostability of Mammalian Brain Ribosomes and the Effects of Nucleoside Triphosphates on Their Heat-Sensitivity

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Mammalian brain ribosomes were found to be heat-labile. On preincubation of the ribosomes at 37°C, their ability to participate in polypeptide-synthesis reactions was substantially diminished. Despite the sensitivity of ribosomal protein synthesis to heat-inactivation, preincubation resulted in no significant alterations in ribosomal sedimentation profiles or changes in the integrity of the ribosomal RNA. The thermolability of brain ribosomes was shown to be associated with their inability to bind both template RNA and aminoacyl-tRNA. Similar experiments with brain ribosomal subunits demonstrated that the small (40S) subunit was more sensitive to heat-inactivation than the large (60S) subunit. The presence of ATP (1 mM) protected ribosomes from thermal inactivation, although this protection was shown to be temporary. The protection appeared to be specific to nucleoside triphosphates, since GTP and UTP also stabilized ribosomes to thermal denaturation whereas nucleoside diphosphates (ADP) and nucleoside monophosphates (AMP and cyclic AMP) did not alter ribosomal sensitivity to heat. Although 1 mM concentrations of nucleoside triphosphates protected ribosomes from heat-inactivation, the presence of higher concentrations resulted in complete inactivation of ribosomal activity.

Although a considerable amount of information about the synthesis and assembly of ribosomes is accumulating (Kurland *et al.*, 1969; Nomura, 1970), less data are available on the stability and maintenance of the ribosomal particle. These latter considerations are particularly critical to our understanding of the differential activity of ribosomes under various metabolic conditions (Wool & Cavicchi, 1967; Lerner & Johnson, 1970; Andrews & Tata, 1971) and the recycling of ribosomes and ribosomal subunits in eukaryotic cells (Baglioni *et al.*, 1969; Kaempfer, 1969). Several investigators have shown that protein synthesis can be modulated by chemical modification of ribosomal proteins without causing a major change in the structural integrity of the ribosome (Kabat, 1970; Majumder & Turkington, 1972; Liew & Gornall, 1973). In mammalian cells protein synthesis has been shown to be sensitive to both increases in temperature (McCormick & Penman, 1969) and the presence of nucleoside triphosphates (Pronczuk *et al.*, 1968). Since ribosomes are ribonucleoprotein complexes, one might suspect that ribosomal activity may be sensitive to heat-denaturation. However, there is little information about the thermal stability of ribosomes with regard to their structural integrity and their ability to participate in polypeptide synthesis.

In the present study, we sought to examine the mechanism of thermal inactivation of mouse brain ribosomes and the possible consequences of their

interactions with small molecules known to be essential for peptide synthesis (nucleoside triphosphates). In order to measure ribosomal thermostability, we measured the activity of mouse brain ribosomes in protein synthesis after a prior incubation period at 37°C. We were also able to measure the effects of preincubation on the individual events required for polypeptide synthesis and to analyse the effects of thermal inactivation on the physical properties of the ribonucleoprotein particles. In addition, various concentrations of nucleoside triphosphates were tested for their effects on thermal inactivation of ribosomes as well as for their more direct effects on ribosome stability and function.

Materials and Methods

Isolation of mouse brain ribosomes and post-microsomal supernatant fraction

Ribosomes were isolated as previously described (Lerner & Johnson, 1970). Whole brains from 100–200 Ha/ICR albino mice were removed and immediately placed in ice-cold RSB buffer [10 mM-Tris-HCl (pH 7.4) – 10 mM-KCl – 1.5 mM-MgCl₂]. Brains were homogenized with six to eight strokes of a motor-driven glass-Teflon homogenizer in the presence of 1.5 mg of bentonite per ml of homogenate. Nuclei, mitochondria and synaptosomes were removed from the homogenate by centrifugation at

7000g (r_{av} , 11 cm) for 10 min at 4°C. Microsomes were isolated from the post-mitochondrial fraction by centrifugation at 200000g (r_{av} , 5.5 cm) for 45 min at 4°C. The post-microsomal supernatant was dialysed against 10 mM-Tris - HCl (pH 7.8) - 10 mM-MgCl₂ - 6 mM-2-mercaptoethanol overnight at 4°C. Protein concentrations were determined by the method of Lowry *et al.* (1951) and the dialysed post-microsomal enzyme fraction was stored at -70°C. Ribosomes were prepared by resuspending the microsomal pellet in 5% (w/v) sucrose-RSB, sodium deoxycholate being added to a final concentration of 0.5% (w/v), and pelleting the ribosomes by centrifugation at 200000g for 45 min at 4°C. The purified ribosomes were resuspended in 5% (w/v) sucrose-RSB, repelleted by centrifugation and stored at -70°C.

Aminoacylation of brain tRNA and Escherichia coli tRNA

Aminoacyl-tRNA synthetases from mouse brain and *E. coli* K12 were prepared by the method of Gilbert & Johnson (1972). Brain tRNA was isolated by the procedure of Chou *et al.* (1971) and brain tRNA and *E. coli* tRNA were aminoacylated with [³H]- or [¹⁴C]-phenylalanine by using homologous synthetases (Gilbert & Johnson, 1972).

Preincubation of ribosomes

Brain ribosomes (10 E_{260} units/ml) were preincubated at 37°C in 0.1 or 0.2 ml of 5 mM-Tris-HCl (pH 7.4) - 5 mM-KCl - 0.75 mM-MgCl₂. After preincubation for various lengths of time, samples were used to test various functional and physical properties of the ribosomes. Any additions to the preincubation mixtures are described in the legends to the Figures.

Polyphenylalanine synthesis with ribosomes

Polyphenylalanine synthesis was measured by the technique reported by Gilbert *et al.* (1972) by using [¹⁴C]phenylalanyl-tRNA from *E. coli* K12 and a brain post-microsomal supernatant fraction as the source of transferase enzymes. After incubation (see legend to Fig. 1) at 37°C for 10 min, the reaction was terminated by the addition of 1 ml of cold buffer [10 mM - Tris - HCl (pH 7.4) - 50 mM - KCl - 10 mM - MgCl₂ - 6 mM-2-mercaptoethanol]. The products were collected on Millipore filters (0.45 μ m), washed with 15 ml of the above buffer solution and the radioactivity was determined.

Binding of [³H]poly(U) and [³H]phenylalanyl-tRNA (brain) to ribosomes

The binding of template RNA to mouse brain ribosomes was performed by the procedure of Lerner & Johnson (1971).

The [³H]phenylalanyl-tRNA binding reaction has been described by Chou *et al.* (1971). Binding was done in the presence of post-microsomal enzymes and 10 mM-MgCl₂, which are the optimal conditions for poly(U)-dependent phenylalanyl-tRNA binding (Chou *et al.*, 1971).

Polyphenylalanine synthesis with ribosomal subunits

Subunits were prepared from ribosomes isolated as described above. Ribosomal suspensions were treated with 0.5 mM-puromycin, layered on a 10-35% (w/v) linear sucrose gradient, and the small and large subunits were purified and concentrated from the gradient (Gilbert & Johnson, 1973). The subunits at a molar ratio of 1:1.2 (40S:60S) were added to a polyphenylalanine-synthesizing system using phenylalanyl-tRNA as previously described (Gilbert & Johnson, 1973).

Results

Thermostability of preincubated ribosomes in a protein-synthesizing system

To study ribosome inactivation quantitatively, a poly(U)-directed protein-synthesizing system *in vitro* was used to evaluate ribosomal activity and to permit separation of the individual steps involved in peptide-chain synthesis. In order to assay the effects of temperature on ribosomal activity, purified brain ribosomes were preincubated at 37°C. Subsequently the preincubated ribosomes were added to a protein-synthesizing system which was terminated after 10 min. These conditions allowed the measurement of ribosomal activity while the incorporation of [¹⁴C]phenylalanine was still linear (Gilbert & Johnson, 1972). Ribosomal activity proved to be extremely sensitive to prior incubation of the ribosomes at 37°C, the temperature normally used in cell-free protein-synthesizing reactions (Fig. 1). When ribosomes were preincubated for different lengths of time and tested for their ability to incorporate phenylalanine into polypeptides, they exhibited a dramatic decrease in protein-synthetic activity. Within 10 min only 50% of the initial activity remained and by 30 min incorporation was decreased to 33%. In further experiments preincubation of brain ribosomes at 22°C resulted in a considerably slower rate of inactivation in that 75% of the ribosomal activity remained after 30 min, and ribosomes could be maintained at 0°C for several hours without an appreciable loss of their ability to incorporate amino acids into proteins (Grove & Johnson, 1973).

Fig. 1. Effect of preincubation on the ability of ribosomes to synthesize polypeptides

Each point represents the length of time that a sample of ribosomes ($10 E_{260}$ units/ml) was preincubated at 37°C before ribosomal protein-synthetic activity was tested. The protein-synthesis reaction mixture (0.2 ml) contained 10 mM-Tris-HCl, pH 7.4, 50 mM-KCl, 10 mM-MgCl₂, 6 mM-2-mercaptoethanol, 0.8 mM-GTP, 10 mM-phosphoenolpyruvate, 8 mg of pyruvate kinase, $40 \mu\text{g}$ of poly(U), 0.3 mg of dialysed post-microsomal supernatant fraction, $0.4 E_{260}$ unit of ribosomes and 8 pmol (5500 d.p.m. of [¹⁴C]phenylalanyl-tRNA (*E. coli*)). Reactions were incubated at 37°C for 10 min.

Effect of preincubation on ribosome sedimentation properties and rRNA integrity

In order to understand better the events occurring during the preincubation of ribosomes, we tested thermally inactivated ribosomes for alterations in their physical and chemical properties. Analysis of ribosome-sedimentation profiles after sucrose-gradient centrifugation showed that even after 30 min, by which time a dramatic loss in protein-synthetic activity was observed, the sedimentation properties of ribosomes were not significantly altered (Fig. 2).

Although the sedimentation profiles of preincubated ribosomes appeared normal, the possibility still existed that the rRNA may have been enzymically degraded. This possibility was ruled out by preincubating ribosomes for 30 min at 37°C and examining the rRNA by sodium dodecyl sulphate-polyacrylamide-gel electrophoresis (Fig. 3). The RNA of preincubated ribosomes showed no evidence of hydrolysis when compared with the RNA isolated from control (unheated) ribosomes. Thus, no major change in ribosomal sedimentation properties or RNA integrity could be detected as a result of thermal inactivation.

Effect of ribosomal preincubation on poly(U) binding and aminoacyl-tRNA binding to ribosomes

Since thermal inactivation was originally detected by a decreased capacity of the ribosomes to synthesize peptides, functional changes in the individual

Fig. 2. Sucrose-gradient profiles of control ribosomes (not preincubated) (a), ribosomes preincubated at 37°C for 10 min (b) and ribosomes preincubated at 37°C for 30 min (c)

Approx. $5 E_{260}$ units of brain ribosomes were layered on an 11 ml (5–20%) linear sucrose-RSB gradient. The samples were centrifuged at $180000g$ (r_{av} , 10.25 cm) for 75 min at 4°C . After centrifugation, E_{254} was monitored. The gradient profiles are similar to those previously reported for brain ribosomes prepared in this manner (Lerner & Johnson, 1970). The major peak is composed primarily of 80S monoribosomes.

events required for protein synthesis were studied. Preincubated ribosomes were first tested for their ability to bind the synthetic mRNA, [^3H]poly(U). Preincubated ribosomes did indeed bind less mRNA (Fig. 4). After 10 min of preincubation, ribosomes retained only 66% of their initial binding capacity.

Fig. 3. Polyacrylamide-gel-electrophoresis analysis of RNA from non-heated control ribosomes (a) and ribosomes preincubated at 37°C for 30 min (b)

Ribosomal RNA was analysed by polyacrylamide-gel electrophoresis essentially as described by Bishop *et al.* (1967). The preparation of ribosome samples and polyacrylamide gels [3.1% (w/v); 0.4 cm $\times$ 6.5 cm] has been described previously (Johnson, 1973). The electrophoresis unit was operated for 90 min at 5 mA/gel and E_{260} was recorded with a Gilford model 2400 spectrophotometer adapted with a linear transport.

To ensure that the measured loss in poly(U) binding was a reflexion of ribosomal protein synthesis, the ability of the ribosome-template RNA complexes to bind aminoacyl-tRNA was measured. Ribosomes were preincubated for different lengths of time and then added to a [^3H]phenylalanyl-tRNA binding reaction. Preincubated ribosomes were deficient in their ability to bind aminoacyl-tRNA (Fig. 5). After 10 min of preincubation at 37°C, the preheated ribosomes formed 60% as many complexes with phenylalanyl-tRNA as did control ribosomes. This decrease in aminoacyl-tRNA-binding capacity paralleled the impairment of protein synthesis by preincubated ribosomes (Fig. 5).

Effect of preincubation of ribosomal subunits

The results of poly(U)- and phenylalanyl-tRNA-binding experiments substantiated the idea that initiation of protein synthesis was particularly sensitive to thermal inactivation. Since both mRNA and aminoacyl-tRNA binding are primarily associated with the small ribosomal subunit (Gasior & Moldave, 1972), the effects of preincubation on the protein-synthetic capacity of purified subunits was measured. Neither the small nor the large subunits

Fig. 4. Binding of template RNA to preincubated ribosomes

Ribosomes, preincubated at 37°C for different lengths of time, were tested for their ability to bind template RNA. The reaction was performed at room temperature on a membrane filter (Chou *et al.*, 1971). Samples of 2.0 E_{260} units of control or preincubated ribosomes and 0.05 μCi of [^3H]poly(U) (1.25 mCi/mmol) were allowed to react for 3 min. The reaction was stopped by the addition of buffer [10 mM-Tris-HCl (pH 7.4)–50 mM-KCl–10 mM-MgCl₂], the filters were washed six times with 2 ml of the buffer solution and the bound radioactivity was determined.

Fig. 5. Binding of [³H]phenylalanyl-tRNA (brain) to ribosomes after preincubation (○) compared with [¹⁴C]phenylalanine incorporation into peptides (●)

The [³H]phenylalanyl-tRNA (brain) binding mixture (0.2 ml) contained 10 mM-Tris-HCl, pH 7.4, 50 mM-KCl, 10 mM-MgCl₂, 6 mM-2-mercaptoethanol, 0.8 mM-GTP, 40 μg of poly(U), 0.4 *E*₂₆₀ unit of ribosomes, 0.3 mg of dialysed post-microsomal supernatant fraction and 1.6 pmol (4450 c.p.m.) of [³H]phenylalanyl-tRNA (brain). Samples were incubated at room temperature for 10 min as described for Fig. 1.

alone could sustain protein synthesis, although active incorporation of phenylalanine into polypeptides was obtained in the presence of both native (unheated) subunits (Table 1). When the 60S subunits were preincubated at 37°C for 20 min, ribosomal protein synthesis remained unaltered. However, whenever the 40S ribosomal subunits were preheated in a similar manner, the incorporation of amino acids was significantly decreased. Thus the small ribosomal subunit appeared to be preferentially susceptible to thermal inactivation.

Effect of nucleoside triphosphates on thermal inactivation of ribosomes

From preliminary experiments we found that ATP appeared to protect ribosomes from thermal inactivation. To investigate further the interaction of ATP with ribosomes, we preincubated ribosomes in the presence of various concentrations of ATP and subsequently tested their protein-synthetic activity. Ribosomes preincubated for 10 min in the presence of 1 mM-ATP were as active as non-preincubated control ribosomes (Table 2). However, the concentration of ATP that stabilized ribosomes to heat-inactivation was shown to be quite stringent. Exposure of ribosomes to concentrations of ATP of 2 mM and greater resulted in a rapid and complete inactivation of protein-synthetic activity. Thus, it appeared that ATP had two distinct effects on ribosomes; one concentration (1 mM) fully protected ribosomes from thermal inactivation whereas higher concentrations essentially destroyed ribosomal activity.

ATP appeared to interact directly with the ribosomes, since neither preincubation of the post-microsomal supernatant fraction with 2 mM-ATP nor

Table 1. *Effect of preincubation on polyphenylalanine synthesis by mouse brain ribosomal subunits*

Purified ribosomal subunits (0.5 *E*₂₆₀ unit of 40S and/or 1.3 *E*₂₆₀ units of 60S) and 2.2 pmol of phenylalanyl-tRNA (8000 d.p.m.) were incubated at optimal Mg²⁺ concn. (18 mM) for 20 min at 37°C in the reaction mixtures previously described (Gilbert & Johnson, 1973). Heated ribosomal subunits were preincubated at 37°C for 20 min before their addition to the protein-synthetic reaction mixtures. When small subunits were present in the reaction mixture, phenylalanine incorporation was calculated per *E*₂₆₀ unit of 40S subunits, since this was the rate-limiting component (Gilbert & Johnson, 1973).

Ribosomal subunit		10 ² × Phenylalanine incorporated (pmol)	10 ² × Specific activity	
40S	60S		(pmol/ <i>E</i> ₂₆₀ unit)	(% of native)
Native	Native	15.4	30.8	100
Native	—	2.3	4.6	15
—	Native	4.8	3.7	12
Native	Heated	15.1	30.2	98
Heated	Native	10.0	20.0	65
Heated	Heated	10.0	20.0	65

Table 2. *Effect of ATP on heat-inactivation of mouse brain ribosomal activity*

Ribosomes were preincubated in the concentrations of ATP indicated for 10 min at 37°C. Subsequently ribosomes were tested for protein-synthetic activity. Protein synthesis with [¹⁴C]phenylalanyl-tRNA (*E. coli*) was carried out as described in Fig. 1. [³H]Aminoacyl-tRNA (brain) binding was measured as described for Fig. 5.

ATP concn. (mM)	[¹⁴ C]Phenylalanine incorporated		[³ H]Phenylalanyl-tRNA bound	
	(pmol/ <i>E</i> ₂₆₀ unit)	(% of control)	(pmol/ <i>E</i> ₂₆₀ unit)	(% of control)
Control	5.0	100	0.27	100
0	2.6	52	0.11	41
0.5	3.2	63	—	—
1.0	5.0	100	0.22	81
2.0	0.1	2	0	0
4.0	0.05	1	0	0

Table 3. *Effect of Mg²⁺ on the thermostability of mouse brain ribosomal activity*

Ribosomes were preincubated for 10 min at 37°C. After preincubation, ribosome-directed protein synthesis was measured as described for Fig. 1.

Mg ²⁺ concn. (mM)	ATP concn. (mM)	[¹⁴ C]Phenylalanine incorporated	
		(pmol/ <i>E</i> ₂₆₀ unit)	(% of control)
0.75	—	6.0	100
(non-heated)			(control)
0.75	—	3.5	58
1.75	—	3.0	50
5.00	—	1.3	22
10.00	—	1.1	18
0.75	2.0	0.1	2
1.75	2.0	4.0	67
5.00	2.0	5.7	95
10.00	2.0	6.0	100
20.00	2.0	6.1	102

direct addition of up to 2 mM-ATP to protein-synthesizing reactions significantly affected ribosomal activity. Also the interaction of ATP with ribosomes was found to be temperature-dependent, for ribosomes maintained at 0°C for 10 min in the presence of 2 mM-ATP were not inactivated.

Since it was possible that the effects of ATP on ribosomal activity may have been mediated by an interaction with Mg²⁺, the effect of Mg²⁺ on both ribosome heat-denaturation and the apparent inactivation of ribosomal activity by 2 mM-ATP was measured. An increase in Mg²⁺ concentration during preincubation actually resulted in an appreciable decrease in ribosome thermal stability (Table 3). This effect was independent of any influence of the Mg²⁺ subsequently introduced to the protein-synthesis reactions, since addition of comparable amounts had no inhibitory effect of amino acid incorporation. It is clear that the inactivation of ribosomal activity by

2 mM-ATP can be prevented by an increase in the Mg²⁺ concentration (Table 3). However, our results suggest that the thermal stability of brain ribosomes preincubated in the presence of 1 mM-ATP (Table 2) cannot be explained directly by the ATP/Mg²⁺ ratio, since a similar ATP/Mg²⁺ ratio with 2 mM-ATP or 1 mM-ATP resulted in inactivation and complete protection respectively.

Since ATP had such pronounced effects on thermal inactivation when ribosomal protein synthesis was measured, the aminoacyl-tRNA binding capacity of ribosomes which had been caused to interact with ATP during preincubation was measured. The preincubation of ribosomes at 37°C in the presence of various concentrations of ATP affected both aminoacyl-tRNA binding to ribosomes and protein synthesis similarly. Ribosomes did not lose their original binding activity after 10 min of preincubation in the presence of 1 mM-ATP. However, both 2 mM- and 4 mM-ATP essentially destroyed aminoacyl-tRNA-binding capacity within 10 min of preincubation (Table 2).

To examine the specificity of both ATP protection and ATP inactivation, several phosphate-containing compounds were assayed for their effects on the heat-stability of ribosomes. Both 1 mM-GTP and 1 mM-UTP protected the ribosomes as well as 1 mM-ATP (Fig. 6). However, in all cases the protection was not permanent. After 10 min of preincubation in the presence of any of these three nucleoside triphosphates, the ribosomes began losing their ability to incorporate amino acids into polypeptides. The temporary nature of this protection may have been the result of the hydrolysis of the nucleoside triphosphates by enzymes associated with the brain ribosomes. Ribosomal inactivation was not restricted to ATP, since 2 mM concentrations of either GTP or UTP inactivated ribosomal activity. Other compounds tested which did not alter the temperature-mediated decrease in ribosomal protein synthesis at either 1 or 2 mM concentrations were ADP, AMP, cyclic AMP and P_i.

Fig. 6. Effect of ATP, GTP and UTP on heat-inactivation of ribosomal activity with time

Ribosomes were preincubated alone (●) or in the presence of 1 mM-ATP (○), 1 mM-GTP (△), or 1 mM-UTP (▲) for various lengths of time at 37°C before the protein-synthetic activity was determined. Protein-synthesis reaction components were as described for Fig. 1.

Discussion

Several investigations have been directed at delineating the possible regulatory role of ribosomes in cellular growth (Li & Amos, 1971), tissue differentiation (Majumder & Turkington, 1972), and the antibiotic-sensitivity of prokaryotic cells (Weisblum & Davies, 1968). Recently it has become more apparent that ribosomes and protein synthesis can be modulated by the interactions of effector molecules with the ribonucleoprotein particles (Pronczuk *et al.*, 1968). However, in many cases involving mammalian cell ribosomes, the critical events that are responsible for changes in ribosomal activity have not been elucidated. Several observations have suggested that translational events are particularly sensitive to temperature shifts. McCormick & Penman (1969) reported that the incubation of HeLa cells at 42°C, in contrast with the normal 37°C, resulted in a rapid disaggregation of cellular polyribosomes with a concomitant decrease in polypeptide synthesis. These authors concluded that the temperature-sensitive step appeared to involve the inability of ribosomes to bind mRNA and/or initiate polypeptide synthesis. In the present investigation we were able to illustrate that brain ribosomes are very sensitive to thermal denaturation. This loss in synthetic activity was associated with a decreased capacity in the ability of the ribosomes to bind poly(U) and phenylalanyl-tRNA. This observation is similar to that of Roberts

et al. (1973), who reported that ribosomes from rye grain embryos of decreasing viability have a decreased ability to incorporate amino acids and that this decreased activity was due to a diminished capacity to bind aminoacyl-tRNA.

The finding that the small ribosomal subunit (40S) was preferentially susceptible to heat-inactivation further substantiates the hypothesis that initiation of protein synthesis is particularly sensitive to the effects of temperature. Since the preparation of ribosomal subunits involves the use of 0.5M-KCl, which has been shown to remove ribosome-associated factors (Shafritz & Anderson, 1970), the sensitivity of the ribonucleoprotein particles to thermal denaturation appears to be independent of the function of the ribosome-associated factors.

Although thermal denaturation of mouse brain ribosomes markedly affected their ability to participate in protein synthesis no apparent changes could be detected by sucrose-density-gradient profiles. Analysis of the RNA components of heat-inactivated brain ribosomes clearly showed that the decrease in protein-synthetic activity was not associated with the hydrolysis of rRNA (Fig. 3). This is an important aspect, since Murthy & Rappoport (1965) and Datta (1966) have been able to detect ribonucleases associated with brain ribosomal particles. Datta (1966) has also reported that brain ribosomes possess phosphomonoesterase and phosphodiesterase activities. If such enzymes were activated during preincubation of the ribosomes, it is possible that they could degrade the rRNA, resulting in the observed loss in ribosomal activity. In addition, Johnson (1973) has shown that similar preparations of brain ribosomes are extremely sensitive to exposure to dilute pancreatic ribonucleases. Apparently the alteration in ribosomal activity, as a result of preincubation, was not the result of the activation of a ribonuclease. It is possible that the decrease in ribosomal protein synthesis was the result of a loss or hydrolysis of essential ribosomal proteins, or primarily a reflexion of a conformational change in ribosomal structure.

It is clear from our results that heat-inactivation of brain ribosomal activity was independent of any effects of preincubation on endogenous mRNA. Preincubation did not result in an alteration of sucrose-gradient profiles that would suggest either the release or degradation of mRNA (Fig. 2). In addition, thermal denaturation did not result in an increase in poly(U) binding, but rather a substantial decrease in template-RNA-binding capacity (Fig. 4) and ribosomal subunits, previously 'stripped' of endogenous mRNA, could be used to illustrate that the thermal sensitivity was primarily associated with the small subunit (Table 1).

The presence of 1 mM-ATP during the preincubation resulted in a stabilization of the brain ribosomes to thermal denaturation. However, this protective

effect was temporary, since continual incubation (longer than 10 min) did result in ribosomal inactivation. Although ribosomal activity may be altered as a result of phosphorylation mediated by cyclic AMP-dependent protein kinases and ATP (Kabat, 1970), this mechanism does not appear to be responsible for the stabilization of brain ribosomes to thermal inactivation. Li & Amos (1971) have shown that the protein kinases are removed from ribosomal particles by treatment with sodium dodecyl sulphate. In addition, the stabilization was not restricted to ATP and similar concentrations of UTP and GTP could be substituted. Nucleoside diphosphates (ADP) and monophosphates (AMP and cyclic AMP) did not offer protection to the ribosomes from thermal inactivation.

It was noteworthy that the presence of higher concentrations of those nucleoside triphosphates which offered protection to thermal denaturation at 1 mM actually resulted in a complete inactivation of ribosomal activity. Although the inactivation of brain ribosomal activity by 2 mM-ATP could be inhibited by the presence of increased concentrations of Mg^{2+} (Table 3), for reasons stated previously not all of the ATP-ribosomal interactions appeared to be easily explained by a unifying concept of ATP- Mg^{2+} interaction. The sensitivity of ribosomal activity to high concentrations of nucleoside triphosphates could explain why one must add an energy-regenerating system (phosphoenolpyruvate and pyruvate kinase), instead of an excess of the required nucleoside triphosphates, to cell-free protein-synthesizing reactions. Pronczuk *et al.* (1968) have reported that rat liver polyribosomes were rapidly dissociated in the presence of similar concentrations of nucleoside triphosphates. They suggested that the presence of these nucleoside triphosphates inhibited the hydrolysis of GTP which is a required step in ribosomal peptide elongation.

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