INTERCONVERSIONS BETWEEN INACTIVE AND ACTIVE FORMS OF RIBOSOMAL SUBUNITS

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Received 3 March 1969

1. Introduction

We have previously reported that the peptidyl transferase activity of the 50 S subunit of *E. coli* ribosomes is lost if the ribosomes are exposed to media lacking NH_4^+ and K^+. Activity can be restored, but this requires both (a) the readdition of one of these ions, and (b) heat. The heat requirement is discerned only if the ribosomes are assayed at 0°, where they are active only if they were previously heated in the presence of NH_4^+ or K^+ [1].

We have now studied a specific function of the 30 S subunit, the non-enzymatic binding of phenylalanyl-tRNA directed by poly U and assayed at 0°, and have found a similar inactivation and reactivation, with similar effects of specific monovalent cations and heat. In addition, the 30 S subunit is inactivated if the Mg^{++} concentration is lowered to 1 mM (as is commonly done to dissociate 70 S ribosomes), even if NH_4^+ is present. It is of particular interest, however, that ribosomes that have been inactivated toward non-enzymatic binding are active at 0° in the enzymatic binding reactions mediated by initiation factors or transfer factor T, even if they have not been previously heated.

2. Experimental

All buffers contained 1 mM dithiothreitol. Bacterial extracts were prepared from *E. coli* MRE-600 [2]. 70 S ribosomes were purified according to Kurland [3]. Each preparation was divided into 2 parts before the dialysis step. One part was dialyzed against and suspended in TSM buffer (10 mM $MgCl_2$, 10 mM Tris-succinate, pH 8.0) and the other treated with the same buffer to which was added 0.1 M NH_4Cl. Isolated 30 S and 50 S subunits were obtained by differential centrifugation [4]. Preparation of initiation factors and the binding of f-Met-tRNA to ribosomes were according to Vogel et al. [5]. Transfer factor T (probably Tu+Ts) was obtained by DEAE-Sephadex chromatography [6]. All assays were performed at 0°, using either isolated subunits or 70 S ribosomes. The standard assay for peptidyl transferase activity was that already described [1], slightly modified. The standard assay for non-enzymatic binding was the poly U directed binding of phe-tRNA [7]. Each reaction mixture in 50 μl, contained: 50 mM Tris-Cl, pH 7.2; 0.1 M NH_4Cl; 25 mM Mg acetate; 5 μg poly U; 5000 cpm of Phe-tRNA (specific activity 298 μc/μmole) and 150 μg of 70 S ribosomes or 25 μg of 30 S subunits. Incubation was for 10 min at 0°.

3. Results

Table 1 shows that NH_4^+, K^+ and Cs^+ ions (in that order of effectiveness) preserve the non-enzymatic binding activity of ribosomes, a function that involves the 30 S subunit. Na^+ and Li^+ are not effective. Identical results were seen with the peptidyl transferase activity of 50 S subunits [1] (Cs^+, not reported earlier, is also effective with 50 S subunits). It is also seen that 30 S subunits, when prepared from 70 S ribosomes dissociated in 1 mM Mg^{++} and 0.1 M NH_4Cl, are inactive. (Isolated 50 S subunits prepared in the

Table 1
Non-enzymatic binding activity of ribosomes after dialysis against salts of different monovalent cations.

	Experiment	1						2
In dialysis medium	Mg^{++} conc. (mM)	10	10	10	10	10	10	1
	Salt (0.1 M)	–	NaCl	LiCl	CsCl	KCl	NH_4Cl	NH_4Cl
	Phe-tRNA bound (cpm)	164	112	180	560	602	772	17

Experiment 1: 70 S ribosomes; 10 mM Tris-succinate, pH 8.0, in all solutions.
Experiment 2: 30 S subunits; 10 mM Tris-HCl, pH 7.2, in all solutions. (The thermal reactivation of this preparation is shown in fig. 1b.)

Table 2
Non-enzymatic binding: effectiveness of different monovalent cations in the thermal reactivation of 30 S subunits.

	Phe-tRNA bound (cpm)					
Salt	–	NaCl	LiCl	CsCl	KCl	NH_4Cl *
Not heated	17	66	60	28	22	40
Heated	100	19	16	220	352	440

30 S subunits were inactivated by dialysis against 1 mM Mg acetate, 10 mM Tris-HCl, pH 7.2. They were reactivated by being heated 5 min at 40° at a concentration of 4 mg/ml in 10 mM Mg acetate, 10 mM Tris, and the indicated salts at a concentration of 0.1 M.

* As shown in fig. 1b, reactivation can be much higher if Mg^{++} or NH_4^+ concentrations are raised.

same medium remain active.)

Once lost, activity can be restored by heating the ribosomes in an appropriate medium. This was confirmed with both Phe-tRNA and *N*-acetyl-Phe-tRNA and with both poly U and the trinucleotide UUU as messengers. Table 2 shows the effect of different monovalent cations in promoting the thermal reactivation of the non-enzymatic binding activity of 30 S subunits. The order of effectiveness is the same as in preventing inactivation (see table 1). Similar results have already been reported for 50 S subunits [1] as assayed with the ethanol-requiring fragment reaction with puromycin [8]. The inactivation and reactivation of 50 S subunits have now been confirmed with a different assay, namely, the direct puromycin reaction of ribosome-bound f-Met-tRNA. Although the substrate can be bound enzymatically to inactive 70 S ribosomes (see below), it does not react with puromycin.

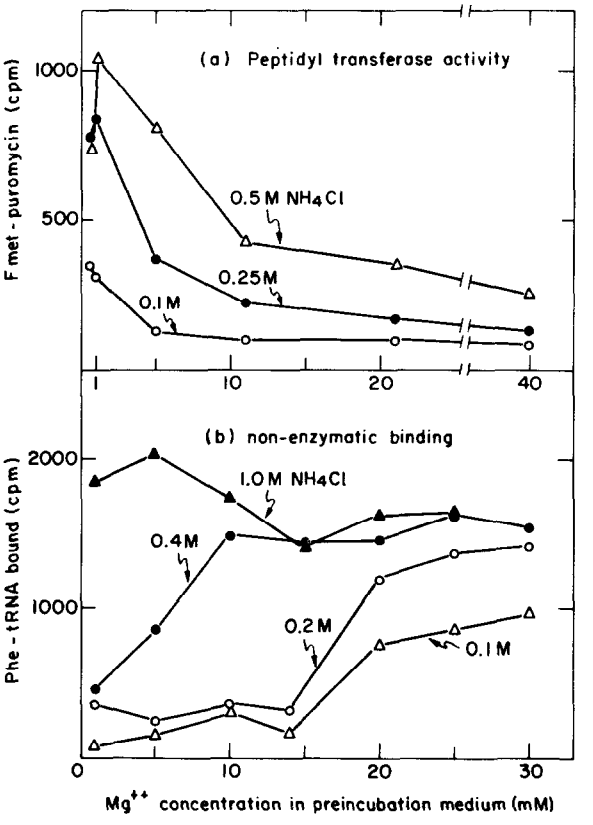

Fig. 1. Effect of Mg^{++} and NH_4^+ concentration on the rate of reactivation of ribosomes. (a) Inactive 70 S ribosomes were heated in the indicated media for 6 min at 30°, immediately chilled to 0°, and subsequently assayed for peptidyl transferase activity in the fragment reaction (10 min). Essentially the same results are obtained with isolated 50 S ribosomes.
(b) Inactive 30 S subunits were heated in the indicated media for 5 min at 40°, immediately chilled to 0°, and then assayed for non-enzymatic binding activity (experimental). In both cases the assay media were adjusted to correct for the salts introduced with the ribosome samples.

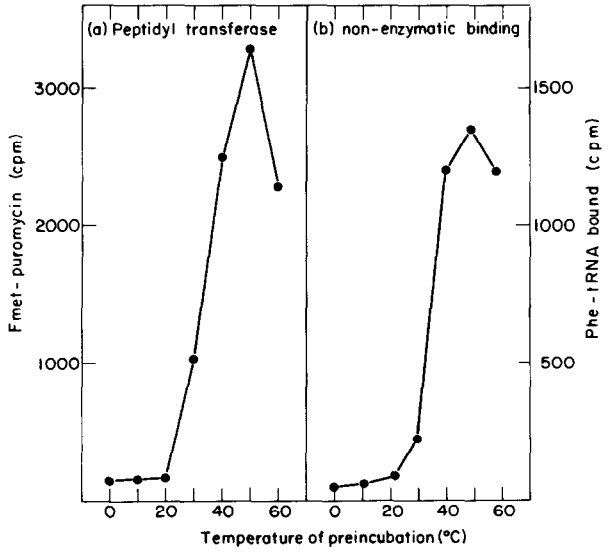

Fig. 2. Effect of preincubation temperature on the rate of reactivation of 50 S and 30 S ribosomal subunits. (a) Inactive 50 S subunits were heated for 2 min at the indicated temperature in 10 mM Tris, pH 7.8, 50 mM NH_4Cl, 5 mM Mg acetate, immediately chilled, and assayed for peptidyl transferase activity (fragment reaction, 4 min). (b) Inactive 30 S subunits were heated for 2 min at the indicated temperatures in 10 mM Tris, pH 7.2, 0.25 M NH_4Cl, 20 mM Mg acetate, immediately chilled, and assayed for non-enzymatic binding activity (experimental).

With reactivated ribosomes, all the bound substrate reacts.

The rate of thermal reactivation of both subunits depends strongly on the concentrations of Mg^{++} and the monovalent cation (e.g., NH_4^+), although the two subunits do not show identical response (fig. 1). Also, different preparations may show somewhat different optima. Fig. 2 shows the temperature dependence of the rate of reactivation to be quite similar for both subunits under the conditions employed. No significant reactivation is seen below 20° at these incubation times, although on prolonged storage it may take place even at 0°.

The binding of aminoacyl-tRNA to ribosomes is known to be mediated by soluble proteins of the initiation and transfer factor sets. Table 3 shows the effect of initiation factors in promoting the enzymatic binding of f-Met-tRNA to ribosomes that were either active or inactive in non-enzymatic binding, as

Table 3
Enzymatic binding: effect of initiation factors on the binding of f-Met-tRNA.

Mg^{++} conc. (mM)	Initiation factors	f-Met-tRNA bound (cpm) ribosomes *	
		"Inactive"	"Active"
5	Absent	92	53
5	Present	954	1033
25	Absent	117	240
25	Present	417	333

* Ribosomes. "Inactive": dialyzed against TSM; low activity in non-enzymatic binding of Phe-tRNA. "Active": dialyzed against TSM + 0.1 M NH_4Cl; high activity in non-enzymatic binding of Phe-tRNA.

described above. Only the "active" ribosomes at high Mg^{++} concentration showed low but significant activity in the absence of factors. Initiation factors stimulated the binding activity markedly, particularly at low Mg^{++} concentration; but most significantly, both kinds of ribosome were now active. (It should be noted, however, that the Mg^{++} dependence of the initiation factor-mediated binding follows a different pattern for each type of ribosome. Although both are active, they are not equally active at all Mg^{++} concentrations.) In other experiments, transfer factor T was found to cause a twofold enhancement of the binding of Phe-tRNA to both kinds of ribosomes at 0°. Thus, ribosomes which are inactive in non-enzymatic binding are active at 0° in enzymatic binding in the presence of initiation or transfer factors.

4. Discussion

It has been shown that functions associated with both the 50 S and 30 S ribosomal subunits may be inactivated by exposure to media often employed in their purification. Such inactivation is difficult to detect; since it is readily reversed under ordinary assay conditions, i.e., at temperatures of 30° or more and in the presence of K^+ or NH_4^+. In many cases the apparent characteristics of a reaction involving ribosomes may actually be those of the thermal reactivation process rather than of the reaction. Our results show how this difficulty may be overcome.

The temperature dependence of the reactivation process suggests that there may be a structural difference between the active and inactive ribosomes. Since ribosomes which are inactive in non-enzymatic binding become active in factor-promoted binding, it is possible that the described thermal reactivation of ribosomes mimics a natural process. Conceivably, ribosomal action may entail a cycle of structural changes mediated by certain of the soluble initiation and transfer factors.

Acknowledgements

We are grateful to Miss Nurith Tolnai for technical assistance. This work was partially supported by the National Science Foundation (GB-6970).

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