IN VIVO INACTIVATION OF SOME RIBOSOMAL FUNCTIONS IN A POTASSIUM DEPLETED MUTANT OF *E. COLI*

Yael KAUFMANN, Ruth MISKIN and Ada ZAMIR

Department of Biochemistry,
The Weizmann Institute of Science, Rehovot, Israel

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1. Introduction

Isolated *E. coli* ribosomes require K^+ ions (NH_4^+, Rb^+ and Cs^+ are also effective) to maintain a number of specific functions, e.g. peptidyl transferase activity of 50 S subunits [1], aminoacyl-tRNA binding to 30 S subunits [2] and the ability of both subunits to bind certain antibiotics [3–6]. Removal of the specific monovalent cation inactivates these functions, but they can be restored by heating the inactive ribosomes in the presence of K^+, NH_4^+ or Rb^+ [1, 2]. It was suggested that these interconversions may reflect a physiological behaviour of ribosomes [1].

To test this hypothesis we have studied some ribosomal activities in a mutant of *E. coli* B that fails to accumulate potassium [7]. When mutant cells are depleted of potassium, cell division and protein synthesis stop, but RNA synthesis and its degradation continue [7, 8].

We have found that the maintenance of peptidyl transferase activity *in vivo* depends on the intracellular concentration of potassium ions. Inactivation of this function as well as of the related ability to bind chloramphenicol and erythromycin took place when the bacterial growth medium was depleted of potassium. Moreover, these properties could be restored to the mutant ribosomes by activating them *in vitro*. These results may also explain the inhibition of protein synthesis in potassium depleted cells.

2. Experimental

2.1. *Preparation of bacterial cells and ribosomes*

E. coli B-207 [7] was a gift from Dr. M. Lubin. Growth conditions were as described by Lubin and Ennis [8] except that the medium contained 10 mM KCl and 90 mM NaCl instead of 100 mM KCl. The culture was chilled at 6×10^8 cells/ml and harvested in a Sharples centrifuge. Half of the cells were frozen at $-15°$. The rest were resuspended to the above density in a medium that lacks potassium (Na^+–A) [8], and aerated vigorously for 1 hr at 37°. This depletes the cells of potassium and stops their growth [8]. The cells were then harvested and kept at $-15°$.

Preparation of 30 S fractions from both types of cell was essentially as described by Friedman et al. [9] but the standard buffer employed contained: 7 mM $Mg(OAc)_2$, 50 mM NH_4Cl, 50 mM Tris-HCl pH 7.5 and 1 mM 2-mercaptoethanol. In this buffer active and inactive ribosomes each maintained their initial state of activity in the cold and no interconversion from one form into the other took place.

Ribosomes were pelleted from the 30 S fractions by centrifugation at 150,000 *g* for 3 hr at 2°, resuspended in the above buffer and stored in liquid air.

2.2. *In vitro reactivation of ribosomes*

The reactivation conditions were as follows: 200 μg of ribosomes were incubated in 50 μl of 5 mM $Mg(OAc)_2$, 15 mM Tris-HCl pH 7.5, 0.2 mM mercaptoethanol and 0.1 M of the monovalent cation tested, for 10 min at 26°.

2.3. *Assays*

Peptidyl transferase activity was assayed with the alcohol reaction essentially as described by Monro et al. [10, 11] and terminated according to Miskin et al. [1]. The aqueous component (0.15 ml) was 26 mM $Mg(OAc)_2$, 0.5 M KCl, 75 mM Tris-HCl pH 7.5, 0.1 mM

Table 1
Effect of *in vivo* potassium depletion on a number of ribosomal activities and its activation *in vitro*.

Assay	Not activated			Activated		
	Na^+-ribosomes	K^+-ribosomes	Ratio $\frac{Na^+\text{-ribosomes}}{K^+\text{-ribosomes}}$	Na^+-ribosomes	K^+-ribosomes	Ratio $\frac{Na^+\text{-ribosomes}}{K^+\text{-ribosomes}}$
Peptidyl transferase (pmoles fMet-puromycin formed)	0.9	7.8	0.11	9.0	10.0	0.90
Chloramphenicol binding (pmoles)	4.5	14.1	0.32	22.1	24.1	0.92
Erythromycin binding (pmoles)	5.0	27.0	0.18	50.0	53.8	0.94

Ribosomes were thawed and immediately assayed (not activated) or reactivated in the presence of NH_4^+ as described in Experimental, chilled to 0° and then assayed.

neutralized puromycin, 9000 cpm of ^{14}C-labelled fMet-tRNA 220 mCi/mM (prepared essentially as in [12]) and 200 μg ribosomes. The reaction was started by addition of 50 μl methanol and carried out for 25 min at 0°. A background of 0.03 pmole obtained without ribosomes was subtracted.

Bindings of ^{14}C-chloramphenicol and ^{14}C-erythromycin were performed at 0°, for 20 or 30 min, respectively. The reaction mixture (0.1 ml) contained 200 μg ribosomes (77 pmoles), 10 mM $Mg(OAc)_2$, 100 mM NH_4Cl, 50 mM Tris-HCl pH 7.5, 1 mM dithiothreitol, and 18,000 cpm (1×10^{-5} M) of ^{14}C-chloramphenicol (10.2 mCi/mM, Radiochemical Centre, Amersham) or 6400 cpm (5×10^{-6} M) of ^{14}C-erythromycin (7.9 mCi/mM, kindly supplied by Dr. J.C.H. Mao). Under these conditions maximal antibiotic binding to ribosomes was obtained. The mixtures were further treated as described by Vogel et al. [5]. Zero time controls of 11 pmoles and 4 pmoles were subtracted from chloramphenicol and erythromycin binding values, respectively.

3. Results

Three activities of the 50 S ribosomal subunit, namely, peptidyl transferase, chloramphenicol binding and erythromycin binding, were assayed in "K-ribosomes" and "Na-ribosomes" (ribosomes isolated from K^+-containing or K^+-depleted *E. coli* B-207 cells, respectively). It was found that the peptidyl transferase activity of "Na-ribosomes" was one tenth the activity of "K-ribosomes" (table 1). Moreover, the ability to bind the antibiotics chloramphenicol and erythromycin, previously shown to be related to peptidyl transferase activity [5], was also markedly lower in "Na-ribosomes".

Next, we tried to activate the "Na-ribosomes" by incubating them under conditions known to restore the lost activities to ribosomes inactivated *in vitro* [1]. Table 1 shows that following this treatment the *in vivo* inactivated ribosomes regained their activity. Although this treatment raised somewhat the activity level of "K-ribosomes", its effect on "Na-ribosomes" was by far more pronounced and the two types of ribosomes became nearly equally active.

The resemblance of "Na-ribosomes" to ribosomes inactivated *in vitro* by removal of essential monovalent cation is further emphasized by the experiment summarized in table 2.

Activation of "Na-ribosomes" in the presence of different monovalent cations was measured. The order of cation effectiveness observed was the same as that previously found in the reaction of *in vitro* inactivated ribosomes [1], i.e. $NH_4^+ = Rb^+ > K^+ > Cs^+$. Na^+ and Li^+ not only failed to reactivate but rather

Table 2
Reactivation of Na-ribosomes in the presence of various monovalent cations.

Reactivation salt	Peptidyl transferase (pmoles fMet-puromycin formed)	Chloramphenicol binding (pmoles)	Erythromycin binding (pmoles)
NH_4Cl	8.5	20.2	43.0
RbCl	8.5	not tested	not tested
KCl	8.3	18.3	38.0
CsCl	6.4	17.4	35.0
NaCl	0.8	3.7	4.0
LiCl	0.5	4.1	3.0
not activated	2.1 (0.9)	11.0 (4.5)	12.0 (5.0)

Reactivation and assays were as described in Experimental section. To achieve identical salt conditions the activated ribosomes were tested in the standard reaction mixtures containing also the chloride salts of all the 6 cations (33 mM each in peptidyl transferase assay medium and 25 mM each in the antibiotics binding medium). The addition of these salts caused reduction of 15% in peptidyl transferase activity, 15% of chloramphenicol binding and 20% of erythromycin binding in ribosomes activated with NH_4^+. On the other hand these assay conditions activated somewhat Na-ribosomes which were not activated prior to the assays (values obtained when the salt mixture was omitted from the assay are written in parentheses). Thus the results reported here, especially for Cs^+, could be slightly affected by an activation which took place during the assay.

reduced the residual activity of "Na-ribosomes" as they also did to fully active ribosomes (unpublished). Activation of the ability to bind chloramphenicol and erythromycin parallelled the activation of peptidyl transferase in all cases.

4. Discussion

The results show that depletion of intracellular potassium in *E. coli* B-207 causes a significant loss of ribosomal activities associated with the 50 S ribosomal subunit. This inactivation is similar to the inactivation of isolated ribosomes from *E. coli* MRE 600 [1]. The similarity rests on the following: 1) The inactivation is brought about by potassium deprivation. 2) In both cases the same 50 S subunit activities are affected. 3) Normal levels of activity are restored by the same heat treatment in the presence of Mg^{2+} and of K^+ [4]. Other monovalent cations may replace potassium in the reactivation procedure of both kinds of inactive ribosomes with similar effectiveness. It is possible, therefore, that these two forms of ribosomes are indeed identical.

Potassium seems to be the only monovalent cation involved in the physiological interconversions of ribosomes in *E. coli* B-207 cells. Though NH_4^+ is a good activator *in vitro*, it can not stimulate growth of K^+-deprived B-207 cells. This result may be explained by the low intracellular concentration of NH_4^+ [8]. Sodium, on the other hand, does penetrate the cells; its concentration in cells growing under K^+-deprivation is 80–90 mM [8]. Still, these cells contained inactive ribosomes. Thus Na^+ could not reactivate the ribosomes *in vivo*, as is also the case *in vitro*.

The studies of Lubin and Ennis of the *E. coli* B-207 mutant have long established that potassium depletion does not interfere considerably with RNA synthesis and RNA degradation but inhibits protein synthesis at the ribosomal stage [8, 13]. Attempts by these authors to locate the direct effect of K^+ in protein synthesis indicated that neither tRNA aminoacylation [8] nor polysome stability [14] were affected. Our results indicate that 50 S ribosomal subunit functions are impaired and perhaps other ribosomal functions. The inactivation of peptidyl transferase function is sufficient to stop completely the process of protein synthesis.

Potassium ions thus may control protein synthesis by influencing the state of activity of ribosomes.

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