A SOLUBLE FACTOR FROM YEAST WHICH PROMOTES A GTP-DEPENDENT BINDING OF *N*-ACETYLPHENYLALANYL-tRNA TO THE RIBOSOMES*

A. TORAÑO, A. SANDOVAL, C. SANJOSÉ and C.F. HEREDIA

Instituto de Enzimología del Consejo Superior de Investigaciones Científicas, Facultad de Medicina de la Universidad Autónoma, Madrid-34, Spain

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1. Introduction

Yeast possess two different protein synthesizing systems: a bacterial-like one in the mitochondria [1, 2] and other in the cytoplasm with properties similar to those from mammalians [3]. Soluble protein elongation factors have been isolated from both the mitochondria [2, 4] and the cytoplasm [5, 6] of yeast cells. These factors are functionally equivalent to the elongation factors T and G from bacteria [7].

Purified yeast ribosomes bind phenylalanyl-tRNA at low Mg^{2+} concentrations in response to one of the yeast elongation factors in a reaction which requires GTP [8]. This binding reaction does not occur when *N*-acetylphenylalanyl-tRNA is substituted for the phenylalanyl-tRNA [8]. In this communication we present evidence indicating that in the soluble fraction of yeast extracts there is an activity, different from the elongation factors, which catalyzes a GTP-dependent binding of *N*-acetylphenylalanyl-tRNA to purified yeast ribosomes.

2. Methods and materials

The strain of yeast, conditions of growth, composition of the growth medium and preparation of crude extracts have been previously described [9]. Ribosomes were obtained and purified as shown elsewhere [8]. A partially purified preparation of soluble proteins was obtained by treating the 105,000 *g* supernatant with Alumina Cγ at pH 6.5 and a ratio of mg gel/mg protein of 0.16. The gel was separated by centrifugation and washed with 0.1 M phosphate–10 mM mercaptoethanol pH 6.5.The proteins adsorbed in the gel were eluted with 0.5 M phosphate–10 mM mercaptoethanol pH 6.5 (1/15 of the initial volume of the 105,000 *g* supernatant fraction) for 5 min at 4°. The eluate was dialyzed overnight against a buffer containing 10 mM Tris-HCl pH 7.5, 10 mM mercaptoethanol, 5 mM magnesium acetate. The resulting preparation is referred to as "soluble protein fraction". Partially purified elongation factors were obtained as described [6].

The reaction mixture (0.1 ml) for the binding of aminoacyl-tRNA to the ribosomes contained the following components: 50 mM Tris-acetate pH 6.5, 100 mM ammonium acetate, 50 μg poly U, 1 mM GTP, 10 mM magnesium acetate, 2 A_{260} units of purified ribosomes, aminoacyl-tRNA, soluble protein fractions and other components as indicated in each case. The mixtures were incubated at 30° usually for 20 min, diluted with 3 ml of a cold buffer containing 50 mM Tris-acetate pH 6.5, 100 mM ammonium acetate and 10 mM magnesium acetate and filtered through Millipore filters [10]. The filters were washed 3 times with 3 ml each time of the same buffer, dried, and counted in a liquid scintillation spectrometer. The polymerization of phenylalanine from phenylalanyl-tRNA was followed as described [8].

Uniformly labeled L-^{14}C-phenylalanyl-tRNA was obtained from the Radiochemical Centre, England. Soluble yeast ribonucleic acid and GTP were from Sigma Chemical Co. The preparation of ^{14}C-phenylal-

*A preliminary report of this work was presented elsewhere [15].

Table 1
Requirements for the enzymic binding of *N*-acetylphenylalanyl-tRNA to yeast ribosomes.

Conditions	*N*-acetylphenylalanyl-tRNA bound pmoles
Complete system	4.5
– Soluble protein fraction	0.6
– GTP	0.5
– Ammonium	1.2
GTP replaced by GMP-PCP (1 mM)	0.5

Conditions for the binding reaction as indicated in Methods and materials, using 25 pmoles of *N*-acetylphenylalanyl-tRNA and 50 μg of the soluble protein fraction.

anyl-tRNA and *N*-acetyl-^{14}C-phenylalanyl-tRNA was as previously described [8]. Poly U was from Boehringer, and GMP-PCP (5′-guanylyl-β-γ-methylene diphosphonate) was from Miles Chemical Co.

3. Results and discussion

It was reported previously that *N*-acetylphenylalanyl-tRNA is bound to purified yeast ribosomes at high Mg^{2+} concentration (20 mM) in the absence of soluble protein factors and GTP [8]. This binding reaction does not occur when the Mg^{2+} concentration is lowered to 10 mM or below [8]. The results presented in table 1 show that at 10 mM Mg^{2+} concentration the binding of *N*-acetylphenylalanyl-tRNA to purified yeast ribosomes is dependent on a partially purified soluble protein fraction and GTP. Removal of either of these two components from the incubation mixtures results in a decrease in the amount of *N*-acetylphenylalanyl-tRNA bound. When spermidine (1 mM) is present in the reaction mixtures the Mg^{2+} concentration required for this binding reaction decreases to 5 mM. As in the case of phenylalanyl-tRNA [8], GTP cannot be replaced by its structural analogue GMP-PCP. The binding of *N*-acetylphenylalanyl-tRNA is greatly reduced when NH_4^+ is omitted in the reaction mixtures; K^+ is much less efficient than NH_4^+ for the binding reaction. The optimal NH_4^+ concentration is around 50 mM. The NH_4^+ requirement is not observed for the non-enzymic binding of *N*-acetylphenylalanyl-tRNA which

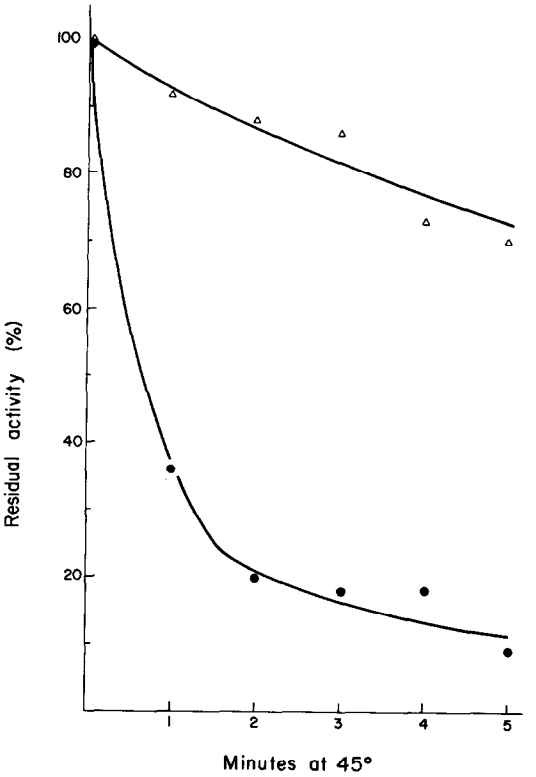

Fig. 1. Differential inactivation of the soluble protein factors. Aliquots of the soluble protein fraction (0.2 ml) in 0.5 M phosphate buffer pH 6.5 were maintained at 45° for the times indicated. After cooling in an ice bath, the binding of *N*-acetylphenylalanyl-tRNA (●–●–●) or the polymerization of phenylalanine from phenylalanyl-tRNA (Δ–Δ–Δ) was followed in a final volume of 0.4 ml as described in Methods and materials, using 0.02 ml of the soluble protein fraction, 15 pmoles of *N*-acetylphenylalanyl-tRNA and 35 pmoles of phenylalanyl-tRNA.

takes place at high Mg^{2+} concentrations (not shown here).

The partially purified protein preparations used in these experiments also contain elongation factors, and it is known that one of these factors catalyzes the binding of phenylalanyl-tRNA to purified yeast ribosomes [8]. The different nature of this aminoacyl-tRNA binding factor and the one involved in the binding of *N*-acetylphenylalanyl-tRNA is suggested by the following facts. Preparations practically devoid of the activity responsible for the binding of *N*-acetylphenylalanyl-tRNA can be obtained by heating the

Table 2
Factor dependent binding of aminoacyl-tRNA to purified yeast ribosomes.
Effect of *N*-ethylmaleimide (NEM).

Exp. No.	Additions	Aminoacyl-tRNA bound	
		N-(Ac)-Phe-tRNA (pmoles)	Phe-tRNA (pmoles)
1.	None	0.3	0.4
	Soluble protein fraction	2.3	–
	NEM-treated soluble protein fraction	0.3	4.3
2.	Elongation factor T	–	3.1
	NEM-treated factor T	–	2.7
3.	None	0.5	0.4
	Soluble protein fraction	2.5	–
	Elongation factor T	0.4	2.5
	Elongation factor G	0.4	–

Conditions for the binding of aminoacyl-tRNA were those described in Methods and materials using *N*-acetylphenylalanyl-tRNA (15 pmoles) or phenylalanyl-tRNA, 35 pmoles (exps. 1 and 2) or 20 pmoles (exp. 3). Approx. 50 μg of protein of each of the protein fractions were used. When indicated protein fractions were preincubated with *N*-ethylmaleimide (20 mM) at 30° for 5 min.

soluble protein fraction at 45° for 2 min. The heated preparations contain however the two elongation factors, as shown by the ability to polymerize phenylalanine from phenylalanyl-tRNA (fig. 1). Additional evidence is presented in table 2. The binding of *N*-acetylphenylalanyl-tRNA is abolished by treatment of the soluble protein fraction with 20 mM *N*-ethylmaleimide (exp. 1). This inactivation is prevented by dithioerythritol (50 mM), although this compound cannot reactivate the inactive preparations. The *N*-ethylmaleimide treated preparations retain, however, the capacity to promote a GTP-dependent binding of phenylalanyl-tRNA to the ribosomes, as corresponds to the insensitivity of elongation factor T to inactivation by *N*-ethylmaleimide (exp. 2). Finally, experiments in which isolated elongation factors were used have shown that neither of these two factors are able to catalyze binding of *N*-acetylphenylalanyl-tRNA to purified yeast ribosomes while under the same experimental conditions the elongation factor T promotes binding of phenylalanyl-tRNA (exp. 3).

The binding of *N*-acetylphenylalanyl-tRNA to purified *E. coli* ribosomes at low Mg^{2+} concentration requires initiation factors and GTP [11]. Incubation of *E. coli* ribosomes with *N*-acetylphenylalanyl-tRNA and initiation factors results in a shift in the Mg^{2+} requirements for the poly U dependent polymerization of phenylalanine [11]. The results presented in fig. 2 show that yeast ribosomes bearing enzymically bound *N*-acetylphenylalanyl-tRNA show a very high rate of phenylalanine polymerization at low Mg^{2+} concentration (2.5 mM) as compared with the rate observed when uncharged ribosomes are used. No difference in the rates of polyphenylalanine synthesis is observed if the Mg^{2+} concentration is increased to 10 mM or above. In separated experiments we have found that part of the *N*-acetylphenylalanyl-tRNA enzymically bound to the ribosomes is incorporated as amino terminal residue into the polyphenylalanine chain.

The activity implicated in the binding of *N*-acetylphenylalanyl-tRNA to yeast ribosomes shares some of the properties found for soluble protein factors present in rat liver [12, 13] and *Artemia salina* [14] which has been suggested to be implicated in peptide chain initiation. A major difference is the fact that while in these two systems the binding reaction occurs only with 40 S ribosomal subunits and does not require GTP, in yeast the binding of *N*-acetylphenylalanyl-tRNA takes place with 80 S ribosomes and requires GTP.

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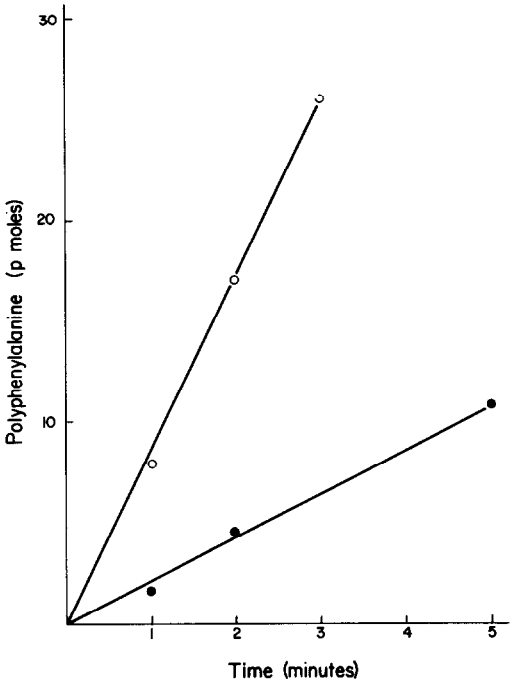

Fig. 2. Polymerization of phenylalanine at 2.5 mM Mg^{2+} by ribosomes bearing enzymically bound *N*-acetylphenylalanyl-tRNA. Purified ribosomes (1.8 A_{260} units) were preincubated at 30° for 5 min with (o–o–o) or without (•–•–•) *N*-acetylphenylalanyl-tRNA (10 pmoles) in a reaction mixture (0.1 ml) containing 100 mM Tris-HCl pH 6.5, 50 mM ammonium chloride, 50 μg poly U, 1 mM GTP, 10 mM magnesium acetate and 55 μg of soluble protein fraction. After incubation, the reaction mixtures were supplemented with ^{14}C-phenylalanyl-tRNA (35 pmoles), 100 μg of soluble protein fraction and GTP, ammonium and Tris-HCl pH 6.5 to give the final concentrations shown above, and the volume was adjusted to 0.4 ml. After incubation at 30° for the times indicated, the amount of polyphenylalanine was estimated as described [8].

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