AN *ARTEMIA SALINA* FACTOR WHICH STIMULATES THE ACTIVITY OF HIGHLY PURIFIED INITIATION FACTOR eIF-2 FROM *A. SALINA* AND RETICULOCYTES

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1. Introduction

In a variety of eukaryotic cells, there is a protein synthesis initiation factor which makes a ternary complex with GTP (or non-hydrolyzable GTP analogs) and eukaryotic initiator Met-TRNA$_i$ [1–10]. This factor is referred to as eIF-2 according to the nomenclature in [11]. A protein factor from reticulocytes which stimulates the GTP-dependent binding of Met-tRNA$_i$ to highly purified reticulocyte eIF-2 was isolated [12]. This stimulatory factor is referred to as CO-eIF-2 here. The discovery of CO-eIF-2 in reticulocytes is significant since about 90% of the input eIF-2 was bound to Met-tRNA$_i$ in a GTP-dependent manner in the presence of CO-eIF-2 [12]. In contrast, it has been reported that only a relatively small fraction of highly purified or homogeneous reticulocyte eIF-2 makes a ternary complex with GTP and Met-tRNA$_i$ in the absence of added reticulocyte CO-eIF-2 [12,13].

eIF-2 is a key initiation factor which is involved in one of the early steps of chain initiation, namely the formation of 40 S subunit · Met-tRNA$_i$ preinitiation complexes [11]. Any factor (like CO-eIF-2) which modulates the activity of eIF-2 is also likely to modulate the overall rate of polypeptide chain initiation. It is, therefore, of considerable interest to establish the presence of CO-eIF-2 in a system other than reticulocytes and to study its mechanism of action. Evidence is provided here which shows that a factor, functionally analogous to reticulocyte CO-eIF-2, is also present in developing *Artemia salina* embryos. Furthermore, we have observed that *A. salina* and reticulocyte CO-eIF-2 preparations are interchangeable, i.e., CO-eIF-2 isolated from either source can stimulate the activity of highly purified eIF-2 from *A. salina* and reticulocytes. The action of CO-eIF-2 apparently does not require GTP hydrolysis since our results show that CO-eIF-2 also stimulates the amount of ternary complex formed in the presence of the non-hydrolyzable analog, GMP-P(NH)P (5′-guanylyl-imidodiphosphate).

2. Materials and methods

A. salina CO-eIF-2 was prepared by the following procedure. Development of *A. salina* embryos and preparation of 35–80% $(NH_4)_2SO_4$ fraction from high-salt ribosomal washes of developing embryos were carried out as in [10]. The $(NH_4)_2SO_4$ fraction was dialyzed against buffer (buffer A) containing 20 mM Tris–HCl, pH 7.4, 70 mM KCl, 0.2 mM dithiothreitol and 10% (v/v) glycerol for 6 h with change of buffer after every hour. Dialyzed $(NH_4)_2SO_4$ fraction (1.5 ml; 16.6 mg/ml) was loaded onto a DEAE-cellulose column (Whatman DE-52; 0.5 × 4 cm) pre-equilibrated with buffer A. The column was washed stepwise with buffer A and then with buffer A containing 100 mM KCl. Fractions (0.7 ml) were collected. The bulk of the eIF-2 activity was present in fractions eluted with buffer A containing 100 mM KCl. These fractions were pooled and kept frozen at −70°C. The pooled solution (3 ml; 0.5 mg/ml) was thawed and put on a column of CM–Sephadex (Pharmacia C-50; 0.4 × 0.8 cm) pre-equilibrated with buffer (buffer B) containing 20 mM Tris–HCl, pH 7.1, 100 mM KCl, 0.2 mM dithiothreitol and 10% glycerol. The column was then washed with a small volume of buffer B. The flow-through material, not retained by the CM–

Sephadex column, contains CO-eIF-2 activity and is essentially free of eIF-2.

Reticulocyte CO-eIF-2, highly purified reticulocyte eIF-2 and reticulocyte [^{35}S]Met-tRNA$_i$ [12] were generously provided by N. K. Gupta, University of Nebraska, Lincoln. Highly purified eIF-2 was prepared from ribosomal salt washes of developing *A. salina* embryos by gel filtration on Sephadex G-200 followed by chromatography on DEAE-cellulose and CM–Sephadex [10]. eIF-2 activity was assayed by retention of ternary complexes on millipore filters as in [14]. Crystalline bovine serum albumin was a product of Armour. Other materials and methods were the same as described [14].

3. Results

The effect of reticulocyte CO-eIF-2 on the activity of highly purified reticulocyte and *A. salina* eIF-2 is shown in table 1. We have confirmed the results [12] that reticulocyte CO-eIF-2 stimulates the activity of highly purified reticulocyte eIF-2 in ternary complex formation with GTP (exp. 1, lines 2, 4), and that reticulocyte CO-eIF-2 by itself cannot form a GTP-dependent ternary complex (exp. 3, lines 5, 6). In addition, we report for the first time that:

(i) Reticulocyte CO-eIF-2 stimulates the amount of ternary complex formed with reticulocyte eIF-2 and GMP-P(NH)P (exp. 1, lines 3, 5).

Table 1
Effect of reticulocyte CO-eIF-2 on the activity of highly purified eIF-2 from *A. salina* and reticulocytes

Exp. no.	Incubation	[^{35}S]Met-tRNA$_i$ bound (cpm)	Reticulocyte CO-eIF-2 stimulation (fold)
1	Reticulocyte eIF-2	1167 –	–
	+ GTP	8133 (6966)	–
	+ GMP-P(NH)P	20 535 (19 368)	–
	+ GTP + CO-eIF-2	15 894 (14 727)	2.1
	+ GMP-P(NH)P + CO-eIF-2	35 261 (34 094)	1.7
2	*A. salina* eIF-2	458 –	–
	+ GMP-P(NH)P	3880 (3422)	–
	+ CO-eIF-2	1289 –	–
	+ GMP-P(NH)P + CO-eIF-2	13 798 (12 509)	3.6
3	*A. salina* eIF-2	222 –	–
	+ GTP	3347 (3125)	–
	+ CO-eIF-2	622 –	–
	+ GTP + CO-eIF-2	8616 (7994)	2.6
	CO-eIF-2 alone	258 –	–
	CO-eIF-2 + GTP	212 (0)	–

Exp. 1: Binding of Met-tRNA$_i$ to eIF-2 was assayed by the Millipore filtration method [12] using 6 pmol reticulocyte [^{35}S]Met-tRNA$_i$ (9610 cpm/pmol), 3 μg highly purified reticulocyte eIF-2 and 15 μg reticulocyte CO-eIF-2 (where indicated). Albumin was not added. Exp. 2, 3: Binding of Met-tRNA$_i$ to to eIF-2 was assayed by the Millipore filtration method [14] using 2.4 μg highly purified *A. salina* eIF-2, 30 μg reticulocyte CO-eIF-2 (where indicated) and either 4 pmol reticulocyte [^{35}S]Met-tRNA$_i$ (exp. 2; 8200 cpm/pmol) or 2 pmol calf liver [^{35}S]Met-tRNA$_i$ (exp. 3; 13 240 cpm/pmol). Albumin (18 μg) was added to all reaction mixtures. The concentration of albumin solution was measured spectrophotometrically [20]. Values within parentheses represent net binding promoted by GTP or GMP-P(NH)P (0.27 mM in exp. 1 and 0.20 mM in exp. 2, 3).

(ii) Reticulocyte CO-eIF-2 stimulates the activity of highly purified *A. salina* CM–Sephadex eIF-2 as assayed by GTP- (or GMP-P(NH)P-)dependent ternary complex formation (exp. 2, 3).

(iii) This stimulatory effect of reticulocyte CO-eIF-2 is seen using either reticulocyte [^{35}S]Met-tRNA$_i$ (exp. 2) or calf-liver [^{35}S]Met-tRNA$_i$ (exp. 3).

In contrast, the activity of less purified *A. salina* eIF-2 preparations was not stimulated by reticulocyte CO-eIF-2 (data not shown). These results suggested that less purified preparations of *A. salina* eIF-2 contain a factor analogous to reticulocyte CO-eIF-2.

When partially purified *A. salina* DEAE-cellulose eIF-2 is chromatographed on CM–Sephadex (see section 2), CO-eIF-2 activity is easily detectable in the flow-through fraction not retained by the column. The results are summarized in table 2. Like reticulocyte CO-eIF-2, *A. salina* CO-eIF-2 also cannot make a ternary complex with [^{35}S]Met-tRNA$_i$ and GTP or GMP-P(NH)P in the absence of eIF-2 (exp. 3, lines 5–7). However, *A. salina* CO-eIF-2 significantly stimulates the amount of ternary complex formed with highly purified *A. salina* eIF-2, [^{35}S]Met-tRNA$_i$ and either GMP-P(NH)P (exp. 1, 2) or GTP (data not shown). *A. salina* CO-eIF-2 also significantly stimulates the activity of highly purified reticulocyte eIF-2 as assayed by ternary complex formation using either GMP-P(NH)P (exp. 3) or GTP (exp. 4). The stimulatory effect of *A. salina* CO-eIF-2 is also seen in the presence of high concentrations of albumin using eIF-2 from either *A. salina* (exp. 2) or reticulocytes (exp. 4). It should also be pointed out that, relative to GMP-P(NH)P, less ternary complex is formed with GTP using eIF-2 from either reticulocytes (table 1, exp. 1) or *A. salina* [14].

Table 2
Effect of *A. salina* CO-eIF-2 on the activity of highly purified eIF-2 from *A. salina* and reticulocytes

Exp. no.	Incubation	[^{35}S]Met-tRNA$_i$ bound (cpm)	*A. salina* CO-eIF-2 stimulation (fold)
1	*A. salina* eIF-2	197 –	–
	+ GMP-P(NH)P	3319 (3122)	–
	+ GMP-P(NH)P + CO-eIF-2	7166 (6969)	2.2
2	*A. salina* eIF-2	172 –	–
	+ GMP-P(NH)P	4781 (4609)	–
	+ GMP-P(NH)P + CO-eIF-2	10 663 (10 491)	2.3
3	Reticulocyte eIF-2	568 –	–
	+ GMP-P(NH)P	9813 (9245)	–
	+ CO-eIF-2	846 –	–
	+ GMP-P(NH)P + CO-eIF-2	19 149 (18 303)	2.0
	CO-eIF-2 alone	109 –	–
	CO-eIF-2 + GTP	100 (0)	–
	CO-eIF-2 + GMP-P(NH)P	125 (16)	–
4	Reticulocyte eIF-2	363 –	–
	+ GTP	4261 (3898)	–
	+ GTP + CO-eIF-2	8169 (7806)	2.0

Binding of Met-tRNA$_i$ to eIF-2 was assayed by the Millipore filtration method [14], using 2 pmol rabbit liver [^{35}S]Met-tRNA$_i$ (25 220–27 300 cpm/pmol), 16.4 μg *A. salina* CO-eIF-2 (where indicated) and either highly purified *A. salina* eIF-2 (2.4 μg exp. 1; 1.8 μg exp. 2) or highly purified reticulocyte eIF-2 (3 μg exp. 3; 1.8 μg exp. 4). Albumin (18 μg) was added to reaction mixtures in exp. 2, 4. Values within parentheses represent net binding promoted by 0.2 mM GTP or GMP-P(NH)P.

4. Discussion

The present study demonstrates that a factor can be isolated from ribosomal salt washes of developing *A. salina* cysts which stimulates the GMP-P(NH)P- or GTP-dependent binding of initiator [^{35}S]Met-tRNA$_i$ to eIF-2 (table 2). This *A. salina* factor (CO-eIF-2) appears to be analogous to the factor isolated [12] from rabbit reticulocytes. These two factors are interchangeable. As assayed by ternary complex formation, both *A. salina* and reticulocyte CO-eIF-2 can significantly stimulate the activity of highly purified eIF-2 prepared from *A. salina* or reticulocytes (tables 1,2). Our results suggest that, like eIF-2, CO-eIF-2 may also be present in a wide variety of eukaryotic cells.

The ability of *A. salina* or reticulocyte CO-eIF-2 to stimulate the activity of eIF-2 does not appear to be due to any non-specific stabilizing effect resulting from addition of extra protein. There is some stimulation of ternary complex formation when reaction mixtures containing eIF-2 (*A. salina* or reticulocyte), [^{35}S]Met-tRNA$_i$ and GTP or GMP-P(NH)P are supplemented with serum albumin (data not shown). However, it is important to note that the stimulatory effect of CO-eIF-2 is still observed in the presence of high saturating concentrations of albumin (table 1, exp. 2, 3 and table 2, exp. 2, 4).

Treatment of reticulocyte eIF-2 with hemin-controlled translation inhibitor (called HCR) results in the phosphorylation of the smallest subunit of eIF-2 [15–17]. GTP-dependent ternary complex formation promoted by *A. salina* DEAE-cellulose eIF-2 was partially inhibited by prior incubation of the factor with ATP and HCR [18]. A relation between phosphorylation and inactivation of eIF-2 was indicated by the lack of inhibition when ATP was omitted [18]. In contrast to these results obtained with partially purified *A. salina* eIF-2, prior incubation of highly purified *A. salina* eIF-2 with ATP and HCR did not result in any inhibition of ternary complex formation [18]. Our results indicate that while CO-eIF-2 is present in apparently saturating levels in partially purified preparations of *A. salina* DEAE-cellulose eIF-2, it may be present in only limiting amounts (if at all) in highly purified CM–Sephadex eIF-2. The possibility exists, therefore, that the partial inhibition of ternary complex formation observed [18] is somehow related to the presence of CO-eIF-2 in the *A. salina* DEAE-cellulose eIF-2 preparations used by these investigators. It may be speculated that the interaction of CO-eIF-2 with eIF-2 (which results in an increased level of ternary complex formation) is prevented when eIF-2 is phosphorylated by prior incubation with ATP and HCR. An apparent inhibition of ternary complex formation is thus observed under these conditions. However, when highly purified eIF-2 (essentially free of CO-eIF-2) was treated with ATP and HCR, no inhibition was observed since the ability of eIF-2 per se to form a ternary complex appears not to be affected by phosphorylation [17,19].

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