ELONGATION FACTORS FROM HUMAN LYMPHATIC TISSUE: ISOLATION AND SOME PROPERTIES

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1. Introduction

Two factors complementary in poly Phe synthesis were isolated from human tonsils and separated by chromatography on Sephadex G-200. These factors are apparently analogous to TF I and TF II from rabbit reticulocytes [1] and aminoacyl transferases I and II from rat liver [2]. One of them, TF I, stimulated Phe-tRNA binding to ribosomes at low Mg^{2+} concentrations. Maximum binding of Phe-tRNA required both ribosomal subunits, TF I and GTP; however, some stimulation by TF I and GTP was observed also with 40 S particles alone. At 5 mM Mg^{2+}, non-enzymatic binding hardly occurred with either 40 S or 60 S subunits, but was significant in the presence of both particles. TF II, on the other hand, facilitated a transfer of Phe to puromycin, presumably via translocation of bound Phe-tRNA to the donor site. Data presented suggest that substrate complexes of TF II (and/or TF I) may be able to migrate from one to another ribosome.

2. Methods and materials

Poly Phe synthesis was assayed under the optimized conditions described [3,4]; the reaction mixtures contained 150 pmoles/ml* ribosomes, purified from elongation factors by 3-times washing with 0.5 M NH_4Cl [4]; the amounts of elongation factors were as indicated in the legends. AA-tRNA binding to ribosomes was assayed in 5 mM $MgCl_2$, 50 mM tris-HCl pH 7.4, 60 mM KCl, 100 μg/ml poly U (Boehringer), and 0.6 mg/ml ^{3}H-Phe 2.2% charged with tRNA (*E. coli*), TF I, GTP, and ribosomes, as indicated in legends; ribosomes were purified as above. The amount of Phe-tRNA bound was determined by the cellulose nitrate filter technique [5]. Transfer of Phe to puromycin was assayed as described [6]. Ribosomes and postribosomal supernatant were prepared from human tonsils [3,4]; ribosomal subunits were obtained from NH_4Cl washed ribosomes according to the method of Martin and Wool [7]. tRNA (*E. coli*) was purchased from Schwarz BioResearch and charged with ^{3}H-Phe from the Radiochemical Centre, Amersham (specific activity = 1000 Ci/mole) [8].

* Calculation based upon the approximation: 1 nmole human ribosomes = 4 mg = 52.5 A_{260}-units.

3. Results and discussion

The human elongation factors were enriched from the postmitochondrial supernatant (Fraction I), by removal of ribosomes in 2 hr at 130,000 *g* (Fraction II), and by precipitation between 35 and 70% saturation with $(NH_4)_2SO_4$ (Fraction III); they were separated (fig. 1) by gel filtration on Sephadex G-200 (Fraction IV). TF I was further purified by a procedure similar to that of McKeehan and Hardesty [9]: fractions 14–45 from the G-200 column, containing 60–100 mg protein in 100 ml, depending upon the amount of starting tissue, were pooled and concentrated in Aquacide II (Calbiochem.) into 20–30 ml. Thereafter, Fraction IV proteins of TF 1 were put on Sepharose 4 B column (5 X 20 cm), equilibrated with elution buffer used for G-200 and eluted with the same buffer at 1 ml per min. The second A_{280} peak, containing 30–50 mg Fraction V proteins of TF I in

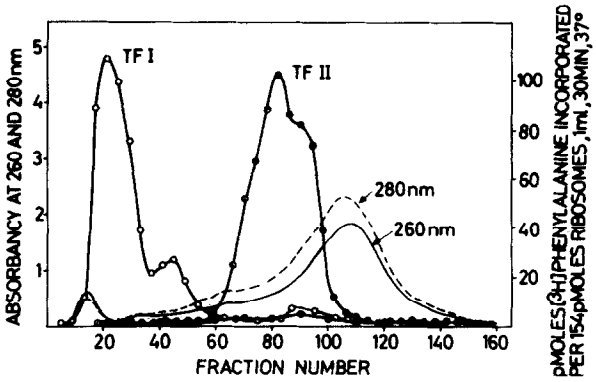

Fig. 1. Separation of elongation factors on a Sephadex G-200 column (5 X 41 cm), equilibrated with elution buffer (containing 50 mM tris-HCl pH 7.4, 100 mM KCl, 0.1 mM K-EDTA, 7 mM 2-mercaptoethanol, and 250 mM sucrose). 320 mg Fraction III proteins in 8 ml dialysis buffer (50 mM tris-HCl pH 7.4, 0.1 mM K-EDTA, 7 mM 2-mercaptoethanol, 250 mM sucrose) were eluted with elution buffer at a flow rate of 0.5 ml per min. After discarding the first 175 ml, 3.5 ml fractions were collected. 10 µl from fractions indicated were assayed for poly Phe synthesis •—• individually; ⊙—⊙ in presence of 10 µl of fraction 18; and ○—○ in presence of 10 µl of fraction 81.

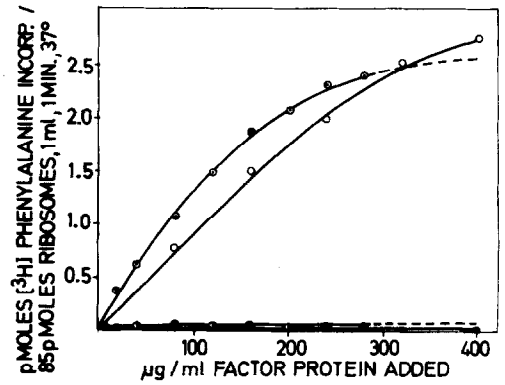

Fig. 2. Effect of TF I (Fraction VI) and TF II (Fraction V) concentrations, respectively, tested individually or, in presence of the complementary factor, on the maximum rate of poly Phe synthesis. Standard conditions were used; ribosomes were kept limiting. ⊙—⊙ TF I alone; •—• TF II alone; ⊙—⊙ TF I in presence of 320 µg/ml TF II; ○—○ TF II in presence of 200 µg/ml TF I.

120–140 ml, was pooled. 6–10 ml hydroxyapatite suspension (Serva) were added slowly under continuous stirring during 45 min. The hydroxyapatite was regained by 5 min centrifugation at 10,000 *g*. It was eluted twice with 20 ml 140 mM phosphate buffer, pH 7.0, 7 mM 2-mercaptoethanol and regained. Thereafter, TF I activity was eluted with 10 ml 300 mM phosphate buffer pH 7.0, 7 mM 2-mercaptoethanol as Fraction VI which was dialysed overnight against dialysis buffer. For TF II, G-200 fractions 55–100 with 200–400 mg Fraction IV proteins of TF II in 150 ml were dialysed against dialysis buffer and, thereafter, put on a DE 32 (Whatman) column (2 X 15 cm), equilibrated with dialysis buffer. TF II was eluted at 0.5–0.7 ml per min by a linear salt gradient from 0 to 100 mM KCl in dialysis buffer as Fraction V. Alternatively, TF II was eluted stepwise with 100 mM KCl in the same buffer. The factors purified in this way were fully complementary in poly Phe synthesis (fig. 2). The similar chromatographic and functional properties of the two human elongation factors suggest their analogy with TF I and TF II from rabbit reticulocytes and with aminoacyl transferases I and II from rat liver. TF I together with GTP

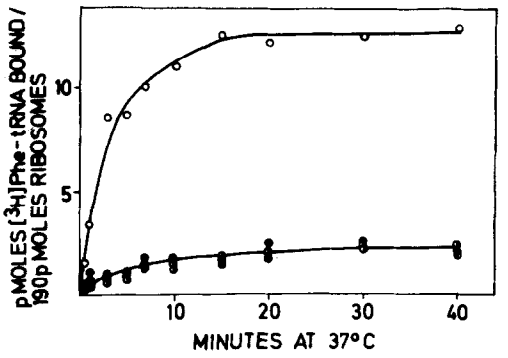

Fig. 3. Kinetics of ^{3}H-Phe-tRNA binding to ribosomes. Standard conditions were used (5 mM Mg^{2+}). •—• – TF I, – GTP; •—• – TF I, + 0.2 mM GTP; ⊙—⊙ + 80 µg/ml TF I (Fraction IV proteins), – GTP; ○—○ + 80 µg/ml TF I (Fraction IV proteins) + 0.2 mM GTP.

markedly stimulated Phe-tRNA binding to ribosomes (fig. 3). Most of this stimulation occurred at lower (4–8 mM) Mg^{2+} concentrations only. Chromatographic analysis of the product showed that only 1–3% of the enzymatically bound Phe-tRNA had been converted to diPhe-tRNA [10].

Table 1 demonstrates involvements of ribosomal subunits in coded Phe-tRNA binding at 5 mM Mg^{2+}.

Table 1
Enzymatic and non-enzymatic ^{3}H Phe-tRNA binding to ribosomal subunits, 30 min at 37°

Additions	^{3}H-Phe-tRNA bound (pmoles)	
	Experiment I	Experiment II
40 S	1.3	1.2
60 S	1.1	1.0
40 S and 60 S	3.9	2.9
40 S, TF I and GTP	3.1	1.7
60 S, TF I and GTP	1.4	0.9
40 S, 60 S, TF I and GTP	8.2	7.9
Filter blank	0.8	–

The binding reaction was assayed at 5 mM Mg^{2+}. 38 pmoles/ml 40 S, 115 pmoles/ml 60 S subunits and 150 μg/ml Fraction IV proteins of TF I were added as indicated. GTP concentration, if present, was 0.2 mM.

Table 2
Phe-puromycin-formation from ^{3}H-Phe-tRNA-carrying ribosomes.

System	^{3}H-Phe-puromycin (pmoles formed per 250 pmoles (1 mg) ribosomes)
Complete	3.57
– puromycin	0.14
– GTP	0.23
– TF II	0.29
– TF II, + TF I	0.55
+ TF I	3.80
– TF II, + 16 pmoles ribosomes with complexed TF II	0.78
– TF II, + 97 pmoles ribosomes with complexed TF II	2.14

^{3}H-Phe-tRNA-carrying ribosomes were formed under the conditions for enzymatic AA-tRNA binding. After incubation, 6 ml of reaction mixture were layered over 5.5 ml homogenization buffer (50 mM tris-HCl pH 7.4, 25 mM KCl, 5 mM $MgCl_2$, 7 mM 2-mercaptoethanol, 250 mM sucrose), centrifuged for 4 hr at 100,000 *g*, and resuspended in homogenization buffer. 250 pmoles ribosomes carried 27.0 pmoles ^{3}H-Phe-tRNA before, and 19.7 pmoles ^{3}H-Phe-tRNA after centrifugation. 100 μl complete reaction mixtures contained 10 mM $MgCl_2$, 50 mM tris-HCl pH 7.4, 60 mM KCl, 8 mM 2-mercaptoethanol, 2 mM GTP, 42 μg TF II Fraction IV proteins, 1 mM puromycin and 105 pmoles of ^{3}H-Phe-tRNA-carrying ribosomes. 12.5 μg/100 μl TF I (G-200 fraction) proteins were added as specified. After incubation for 30 min at 37°, Phe-puromycin was extracted according to Heintz et al. [7]. Ribosome-TF II complexes had been formed by incubating in a 2 ml reaction mixture (containing 10 mM $MgCl_2$, 60 mM KCl, 50 mM tris-HCl pH 7.4, 7 mM 2-mercaptoethanol and 2 mM GTP) 328 pmoles ribosomes and 800 μg TF II Fraction IV proteins for 10 min at 37°. The complexes formed were subsequently collected as described above and were redissolved overnight by standing at 2° under a small volume of homogenization buffer.

Without TF I and added GTP, each subunit alone was virtually inactive. As was shown recently by Castles and Wool [11], at a higher Mg^{2+} concentration, 40 S ribosomes would bind Phe-tRNA, just as demonstrated originally for the smaller bacterial subunits [12]. The considerable binding seen in the presence of both 40 S and 60 S particles indicates how very little cross contamination both of these subunits contained. Binding to these apparently pure 40 S particles was stimulated to varying degrees by TF I and GTP. This observation extends the recent finding by Rao and Moldave who demonstrated binding of aminoacyl transferase I to 40 S subunits of rat liver ribosomes [13,14-. 60 S particles, on the other hand, are not stimulated by TF I and GTP. 60 S subunits apparently do, however, stimulate enzymatic but also non-enzymatic, The-tRNA binding to 40 S particles. Both Mg^{2+} and 60 S particles have an apparently similar stimulating effect upon coded Phe-tRNA binding to the small subunit. In *E. coli*, it seems as if T factor and GTP would stimulate Phe-tRNA binding only in the presence of both 30 S and 50 S particles [15].

TF II, on the other hand, was required for the formation of Phe-puromycin (table 2). This reaction required Phe-tRNA bound to ribosomes, TF II, GTP, and puromycin (Serva). TF I could not replace TF II, and its addition to the complete system only slightly enhanced the extent of Phe-puromycin formation. Ribosomes, preincubated with TF II and GTP, and afterwards collected by centrifugation, could replace TF II when these were added to an equivalent amount of Phe-tRNA carrying ribosomes; they led to 59% of the Phe-puromycin formation seen in the presence of directly added TF II. This experiment may be demonstrating that TF II bound in the presence of GTP can leave one ribosome and function on another ribosome. Different evidence led Moldave and co-workers to a similar conclusion [14,16]. TF II is generally believed to be engaged in translocation. However, the observed Phe-puromycin synthesis from the two complex sub-

strates being primarily bound to different ribosomes may involve as well or instead a migration of TF I (and Phe-tRNA). This phenomenon deserves further investigation.

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