AMINOACYL-tRNA SPECIFICITY OF A 40S RIBOSOMAL SUBUNIT BINDING FACTOR FROM RABBIT RETICULOCYTES

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A factor, isolated from rabbit reticulocytes, catalytically promotes the codon directed, GTP independent binding of aminoacyl-tRNA to 40S ribosomal subunits. High specificity is observed for binding of Met-tRNA$_f$ in the presence of ApUpG or ApUpG(U)$_n$, but much of this apparent specificity is lost when poly(A,G,U) is used as the template. The factor promotes poly(A,G,U) directed binding of at least Met-tRNA$_m$ and Val-tRNA in addition of Met-tRNA$_f$ to 40S ribosomal subunits. However, Met-tRNA$_f$ is almost exclusively bound when both Met-tRNA species are present in a ratio similar to that observed in reticulocytes.

We have described an isolation procedure for a factor present in the rabbit reticulocyte postribosomal supernatant and ribosomal wash that promotes the codon directed, GTP independent binding of aminoacyl-tRNA (1) and deacylated tRNA (2) to 40S ribosomal subunits. The factor was shown to be a heat sensitive protein that is inactivated by N-ethylmaleimide. It has a molecular weight of approximately 50,000 and is composed of two nonidentical subunits of about 30,000 and 20,000. Formation of a factor mediated 40S·poly(U)·Phe-tRNA initiation complex lowers the Mg^{++} concentration optimum for subsequent polyphenylalanine synthesis from about 8 mM to 4 mM (1).

A functionally similar factor has been isolated from the brine shrimp, Artemia salina, by Zasloff and Ochoa (3). This factor, named EIF-1, is also a sulfhydryl reagent sensitive protein, but its molecular weight was estimated to be 145,000. It appeared to contain two identical subunits of approximately 74,000. The A. salina EIF-1 has been shown to promote the ApUpG directed binding of both fMet-tRNA$_f$ and Met-tRNA$_f$ to 40S ribosomal subunits (4). It was shown to be interchangeable with preparations of IF-M$_1$ in lowering the Mg^{++} concentration optimum for polyphenylalanine

synthesis in conjunction with IF-M_2 (5). Rabbit reticulocyte IF-M_1 has a molecular weight of 65,000 (6).

MATERIALS AND METHODS

Poly(U), poly(A,G,U), ApUpG and UDP were obtained from Miles Laboratories, Inc., Elkhart, Ind.; ^{35}S methionine (2 Ci/mmole) and [^{3}H] methionine (9 Ci/mmole) were obtained from Amersham/Searle, Arlington Heights, Ill.; [^{14}C] amino acids and BD-cellulose were obtained from Schwarz/Mann, Orangeburg, N.Y.; and Micrococcus lysodeikticus polynucleotide phosphorylase (12 U/mg) was obtained from Worthington Biochemical Corp., Freehold, N.J.

Preparation of unfractionated tRNA from rabbit liver, ribosomes and soluble enzymes used with the reticulocyte cell-free system have been described before (7,8). Reticulocyte ribosomal subunits were prepared by the procedure of Falvey and Staehelin (9) with minor modifications (10). ApUpG$(U)_n$ was prepared from ApUpG and UDP in the presence of polynucleotide phosphorylase from Micrococcus lysodeikticus (11). Aminoacyl-tRNAs were formed from deacylated rabbit liver tRNA (12). Met-$tRNA_f$ was separated from Met-$tRNA_m$ by chromatography on BD-cellulose (13). N-acetylMet-$tRNA_f$ was formed by the treatment of Met-$tRNA_f$ with acetic anhydride (14).

A detailed description of the isolation procedure of the rabbit reticulocyte tRNA binding factor from the postribosomal supernatant and ribosomal wash fractions has been presented (1).

Standard Binding Assay System

The incubation mixture of the standard Met-$tRNA_f$ binding assay system contained the following in a final volume of 0.10 ml: 50 mM Tris·HCl (pH 7.5), 125 mM KCl, 5 mM $MgCl_2$, 10 mM β-mercaptoethanol, 1.0 A_{260} units of 40S ribosomal subunits, 0.1 A_{260} units of ApUpG, 20 pmoles [^{35}S] Met-$tRNA_f$, and 0.3 μg of rabbit reticulocyte tRNA binding factor. After incubation for 20 min. at 0°, Met-$tRNA_f$ bound to 40S ribosomal subunits was determined as material retained on nitrocellulose filters (15). The

reaction mixture was diluted by addition of 5 ml of a cold solution containing 20 mM Tris·HCl (pH 7.5), 100 mM KCl, 5 mM $MgCl_2$ and filtered immediately. Each filter was washed 3 times with the same cold solution, then dried at 120° for 5 min. and counted by liquid scintillation.

RESULTS AND DISCUSSION

As shown in Table I, the rabbit reticulocyte tRNA binding factor efficiently promotes binding of Met-$tRNA_f$ and N-acetylMet-$tRNA_f$ to 40S ribosomal subunits in the presence of either ApUpG of $ApUpG(U)_n$. Also, these tRNAs are bound enzymatically to ApUpG·ribosomal subunit complexes

TABLE I

ApUpG and $ApUpG(U)_n$ Directed Met-tRNA Binding to 40S Ribosomal Subunits

tRNA	mRNA	Mg^{++} Concentration mM	Factor Addition	Binding pmoles
Met-$tRNA_f$	ApUpG	5	-	0.94
		5	+	10.84
		40	-	18.29
	$ApUpG(U)_n$	5	-	0.46
		5	+	4.92
Met-$tRNA_m$	ApUpG	5	-	0.30
		5	+	0.88
		40	-	1.13
	$ApUpG(U)_n$	5	-	0.18
		5	+	0.44
N-AcMet-$tRNA_f$*	ApUpG	5	-	0.87
		5	+	11.05
		40	-	17.89
	$ApUpG(U)_n$	5	-	0.52
		5	+	5.62

*N-acetylMet-$tRNA_f$.

The standard assay procedure was modified by substitution of the indicated type of tRNA and 0.8 A_{260} units of $ApUpG(U)_n$ where indicated.

TABLE II

Aminoacyl-tRNA Binding to 40S Ribosomal Subunits in the Presence of poly(A,G,U)

tRNA	Binding Factor µg	Total Binding pmoles
Met-$tRNA_f$	-	0.09
	+	2.77
Met-$tRNA_m$	-	0.08
	+	1.76
Val-tRNA	-	0.45
	+	1.07
Phe-tRNA	-	0.37
	+	0.57
Lys-tRNA	-	0.35
	+	0.41
Ser-tRNA	-	0.35
	+	0.37

The standard assay procedure was modified by substitution of the indicated type of tRNA and of 10 µg of poly(A,G,U). Where indicated, 0.3 µg of tRNA binding factor was added.

in reaction mixtures containing 40 mM Mg^{++}. Very little Met-$tRNA_m$ is bound under either of these sets of conditions.

In contrast, Met-$tRNA_m$ was observed to bind to 40S ribosomal subunits at 40 mM Mg^{++} if poly(A,G,U) was used as the template (16). Thus, poly (A,G,U) was used to test the activity of the binding factor with other tRNAs. The results shown in Table II indicate that the binding factor can promote

binding of at least Met-tRNA$_m$ and Val-tRNA in addition to Met-tRNA$_f$. Also, Phe-tRNA is bound very efficiently in the presence of poly(U) (1). On this basis we conclude that the binding factor is not specific for tRNA$_f^{Met}$. However, poly(A,G,U) should contain codons for Phe-tRNA, Lys-tRNA and Ser-tRNA. Factor-dependent binding of these species is very low. The significance of this low level of binding is not clear. It may be important that the three aminoacyl-tRNAs efficiently bound with poly(A,G,U), Met-tRNA$_f$, Met-tRNA$_m$ and Val-tRNA, are coded by the triplets AUG and/or GUG.

Even though the binding factor can promote binding of several species of tRNA with poly(A,G,U), the data of Table II indicate that binding of Met-tRNA$_f$ is relatively efficient compared with binding of Met-tRNA$_m$ or Val-tRNA. The difference in binding of Met-tRNA$_f$ and Met-tRNA$_m$ was investigated further by thermal decay studies of the Met-tRNA binding complexes and by competition experiments with the two Met-tRNA species. Figure 1 shows the thermal decay curves for the Met-tRNA$_f$ and Met-tRNA$_m$

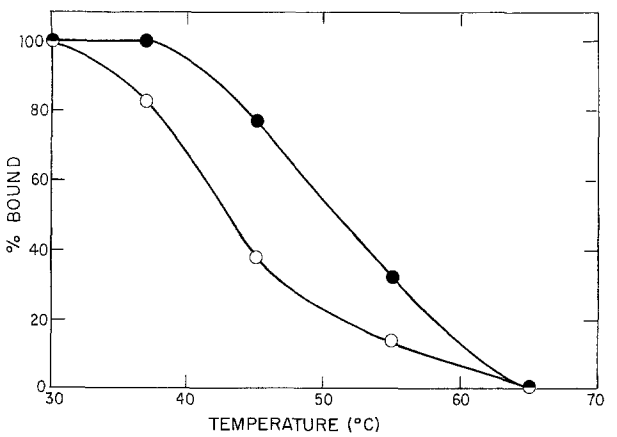

Figure 1. Thermal stability of factor promoted Met-tRNA·40S complexes formed in the presence of poly(A,G,U). Factor dependent binding of Met-tRNA$_f$ and Met-tRNA$_m$ to 40S ribosomal subunits was carried out as described in the legend to Table II. The preformed complexes were incubated an additional 5 min. at the indicated temperatures; then the bound Met-tRNA was determined as described under Methods. The percent bound is calculated from the amount of Met-tRNA bound before and after the second incubation.

●——● Met-tRNA$_f$

○——○ Met-tRNA$_m$

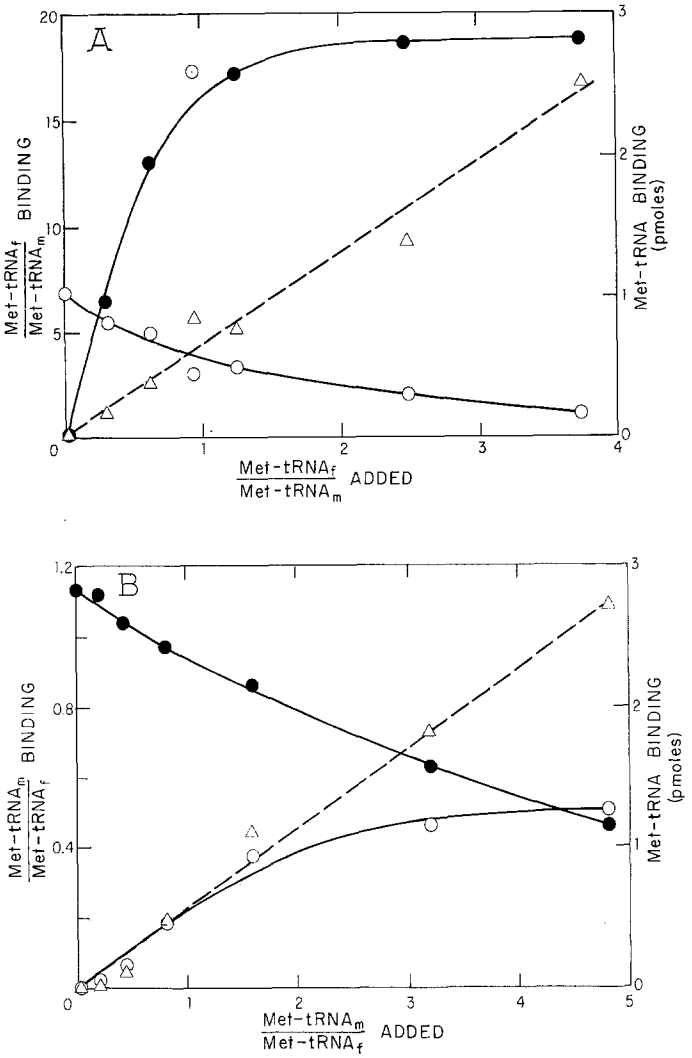

Figure 2. Competition of Met-tRNA$_f$ and Met-tRNA$_m$ for binding in the presence of poly(A,G,U).

A. Factor dependent Met-tRNA binding was determined as described in the legend to Table II. Each incubation mixture contained 8 pmoles of [^{3}H] Met-tRNA$_m$ and [^{35}S] Met-tRNA$_f$ to give the indicated ratio.

B. Each reaction mixture contained 8 pmoles of [^{3}H] Met-tRNA$_f$ and [^{35}S] Met-tRNA$_m$ to give the indicated ratio.

40S ribosomal subunit complexes. In these experiments Met-tRNA was bound to 40S ribosomal subunits in the presence of poly(A,G,U) and the tRNA

binding factor. Then the reaction mixture was incubated for an additional 5 min. at the indicated temperatures. The Met-tRNA$_f$ complex appears to be reasonably stable. Its T_m value is 51°. The Met-tRNA$_m$ complex has a T_m value of only 43°.

Figure 2A shows the relationship between the ratio of Met-tRNA$_f$ to Met-tRNA$_m$ added to the binding reaction and the ratio of Met-tRNA$_f$ to Met-tRNA$_m$ bound to the 40S ribosomal subunits. In this experiment the amount of [^{3}H] Met-tRNA$_m$ was held constant while increasing amounts of [^{35}S] Met-tRNA$_f$ were added to the incubation mixture. Approximately four times more Met-tRNA$_f$ than Met-tRNA$_m$ is bound when the two Met-tRNA species are present in the incubation mixture at a ratio of one to one. A similar relationship is expected if the concentration of [^{3}H] Met-tRNA$_f$ if held constant and increasing amounts of [^{35}S] Met-tRNA$_m$ are added to the reaction mixture. This anticipated result is observed, as shown in Figure 2B. In this case, the ratio of Met-tRNA$_m$ to Met-tRNA$_f$ bound to ribosomes is approximately 0.25 when the ratio of the two Met-tRNA species present in the reaction mixture is one.

Smith and McNamara (17) have reported that in rabbit reticulocytes tRNA$_f^{Met}$ and tRNA$_m^{Met}$ account for 85.9% and 14.1% respectively, of the total tRNA^{Met} present in these cells. Similar results have been reported by Hardesty et al. (18). At this ratio of Met-tRNA$_f$ to Met-tRNA$_m$, in vitro, the factor promotes binding almost exclusively of Met-tRNA$_f$. This may reflect the specificity for Met-tRNA$_f$ during peptide initiation in vivo.

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REFERENCES

1. Cimadevilla, J.M. and Hardesty, B. (1974). J. Biol. Chem., submitted.
2. Hardesty, B., Som, K., Cimadevilla, M. and Irvin, J. (1974). In Modern Trends in Human Leukemia, (Neth, R., ed.), 314-326, J.F. Lehmanns Verlag, Munich.

3. Zasloff, M. and Ochoa, S. (1973). J. Mol. Biol. 73, 65-76.
4. McCroskey, R.P., Zasloff, M. and Ochoa, S. (1972). Proc. Nat. Acad. Sci. U.S.A. 69, 2451-2455.
5. Picciano, D.J., Prichard, P.M., Merrick, W.C., Shafritz, D.A., Graf, H., Crystal, R.G., and Anderson, W.C. (1973). J. Biol. Chem. 248, 204-214.
6. Merrick, W.C., Safer, B., Adams, S. and Kemper, W. (1974). Fed. Proc. 33, 1262.
7. Hardesty, B., McKeehan, W. and Culp, W. (1971). In Methods in Enzymology (Grossman, L. and Moldave, K., eds.), vol.12, part C, 316-330, Academic Press, New York.
8. Hardesty, B. and McKeehan, W. (1971). In Methods in Enzymology (Grossman, L. and Moldave, K., eds.), vol.12, part C, 330-337, Academic Press, New York.
9. Falvey, A.K. and Staehelin, T. (1970). J. Mol. Biol. 53, 1-19.
10. Obrig, T., Irvin, J., Culp, W. and Hardesty, B. (1971). Eur. J. Biochem. 21, 31-41.
11. Thach, R.E. (1966). In Procedures in Nucleic Acid Research (Cantoni, G.L. and Davies, D.R., eds.), vol.1, 520-534, Harper and Row, New York.
12. Culp, W., Morrisey, J. and Hardesty, B. (1970). Biochem. Biophys. Res. Commun. 40, 777-785.
13. Kerwar, S., Spears, C. and Weissbach, H. (1970). Biochem. Biophys. Res. Commun. 41, 78-84.
14. Haenni, A. and Chapeville, F. (1966). Biochim. Biophys. Acta, 114, 135-148.
15. Nirenberg, M. and Leder, P. (1964). Science 145, 1399-1407.
16. Morrisey, J. and Hardesty, B. (1972). Arch. Biochem. Biophys. 152, 385-397.
17. Smith, D.W.E. and McNamara, A.L. (1972). Biochim. Biophys. Acta, 269, 67-77.
18. Hardesty, B., Obrig, T., Irvin, J. and Culp, W. (1973). In Gene Expression and its Regulation (Kenney, F.F., Hamkalo, B.A., Favelukes, G. and August, J.T., eds.), 377-392, Plenum Press, New York.