A ROLE FOR PROTEIN–PROTEIN INTERACTIONS IN THE MAINTENANCE OF ACTIVE FORMS OF AMINOACYL-tRNA SYNTHETASES

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1. Introduction

Reports have appeared which indicate that in mammalian cells aminoacyl-tRNA synthetases may exist in the form of high molecular weight aggregates. These aggregates were isolated and shown to contain either all the aminoacyl-tRNA synthetases [1] or only some of them [2–10]. The synthetases have also been reported to be associated with ribosomes [11-13].

The intermolecular aggregates of aminoacyl-tRNA synthetases contain tRNA [1] and lipids in the form of cholesterol esters [14]. Cholesterol-14-methyl hexadecanoate appears to be associated with aminoacyl-tRNA synthetases and to stimulate their activity [15].

Although one may speculate that supramolecular organization of aminoacyl-tRNA synthetases and other components of protein biosynthesis apparatus might be essential for its adequate functioning in vivo, almost no data exist which indicate the actual role of these aggregates. However, some kind of functional interactions between ribosomes and aminoacyl-tRNA synthetases have been found in rabbit reticulocytes [16] and wheat germ [17] systems. The changes in the aminoacyl-tRNA synthetase complexes in G_1-arrested Chinese Hamster ovary cells were considered as strong evidence for their in vivo role [10]. It has recently been reported that multimeric aminoacyl-tRNA synthetases from wheat germ [17,18] and human placenta [19] are inactivated upon dilution. The inactivation was overcome by ribosomes, and by high concentrations of bovine serum albumin [17].

Here, data are presented which indicate that, in addition to aminoacyl-tRNA synthetase–ribosome interactions, there are functional interactions between yellow lupin aminoacyl-tRNA synthetases themselves. Highly purified yellow lupin aminoacyl-tRNA synthetases are partially or totally inactive when assayed in the absence of other proteins. Activity is regained when the enzymes are assayed in the presence of ribosomes or some proteins. Not all proteins tested are able to reactivate lupin aminoacyl-tRNA synthetases. The screening of over 20 different homo- and heterologous proteins indicates that some degree of specificity exists in the interactions of ribosome with synthetase and synthetase with synthetase. It is suggested that the interactions between synthetases themselves and between ribosomes and synthetases are essential for the maintenance of their active structures. This function provides a rationale for the existence of intermolecular aggregates of aminoacyl-tRNA synthetases within the cell.

2. Materials and methods

Valyl-, seryl- and tryptophanyl-tRNA synthetases from yellow lupin seeds were purified to apparent homogeneity as in [20,21]. Highly purified phenylalanyl-, tyrosyl- and arginyl-tRNA synthetases were prepared from yellow lupin seeds by methods to be described elsewhere.

Bovine serum albumin, ovalbumin, myoglobin (horse), cytochrome *c*, catalase (cow), ferritin (horse), chymotrypsinogen A, soybean trypsin inhibitor were from Serva. α-Amylase, creatine phosphokinase (rabbit), DNase I (bovine pancreas), hemoglobin (cow), RNA polymerase (*E. coli*) were from Sigma.

Lysozyme was purchased from BDH Chemicals Ltd and concanavalin A from Pharmacia.

Homogenous *S*-adenosylhomocysteinase (EC 3.3.1.1.) [22] and adenosine kinase (EC 2.7.1.20.) [23] both from yellow lupin seeds, were kindly donated by Dr Andrzej Guranowski.

Ribosomes from wheat germ and from yellow lupin seeds were prepared according to [24]. The concentration of ribosomes was determined spectroscopically. 14.5 A_{260} units correspond to 1 mg/ml. The M_r of ribosomes was taken as 5×10^6 [17]. The sources of other reagents were described in [20,21].

Assays of aminoacyl-tRNA synthetase activities in the tRNA aminoacylation reaction were carried out at 25°C in 75 μl total vol. in a medium containing 100 mM Hepes (pH 8.0), 10 mM $MgCl_2$, 8 mM 2-mercaptoethanol, 1 mM ATP, 0.2 mM EDTA, 4 mg/ml unfractionated lupin tRNA, 10–40 μM ^{14}C-labelled amino acid and the components specified in the legend to figure and tables. The reactions were initiated by the addition of a portion of appropriately diluted aminoacyl-tRNA synthetase and 15 μl samples were periodically taken for [^{14}C]aminoacyl-tRNA determination [25]. No activity was found in the absence of some exogenous proteins or ribosomes. It has been excluded that this is brought about by an inhibitor present in the solutions. No evidence for such an inhibitor has been found by running experiments in which the concentrations of the components of the aminoacylation mixture were changed over a wide range.

Aminoacyl-tRNA synthetases, stored at −20°C as 1–6 μM stock solutions in 30 mM potassium phosphate buffer (pH 6.8), 5 mM 2-mercaptoethanol, 40% glycerol, were diluted 250–333-times in 20 mM Hepes (pH 8.0), 40 mM 2-mercaptoethanol at 0°C and 15 μl aliquots of this solution were used to initiate the reactions.

The molar concentrations of the aminoacyl-tRNA synthetases were determined by titration with either [^{14}C]aminoacyl adenylate or [^{14}C]aminoacyl-tRNA using the nitrocellulose filter assay [26,27].

3. Results and discussion

Aminoacyl-tRNA synthetases are assayed routinely in the presence of bovine serum albumin. Under these conditions ribosomes stimulate most (but not all) yellow lupin aminoacyl-tRNA synthetases by 10–70%. This has also been shown to be the case with wheat germ leucyl- and arginyl-tRNA synthetases [17]. Several rabbit reticulocyte aminoacyl-tRNA synthetases and *E. coli* phenylalanyl-tRNA synthetases were stimulated by ribosomes to a much greater extent [16]. Omission of bovine serum albumin from the reaction mixture leads to complete loss of the activity of most of lupin aminoacyl-tRNA synthetases. The activity can be restored by the addition of either ribosomes or bovine serum albumin (fig.1). The interpretation that this reflects interactions of either ribosomes or bovine serum albumin with the synthetases is strengthened by the observation that ribosomes and bovine serum albumin protect valyl-tRNA synthetase against thermal inactivation (not shown). It should be noted that bovine serum albumin at 4×10^{-4} M produces almost the same effect as ribosomes at 1.6×10^{-7} M. Although these data point to some degree of specificity in the ribosome–synthetase interaction [17] in the lupin system, it was interesting to find out if these interactions are the inherent property of ribosomes and bovine serum albumin and whether other

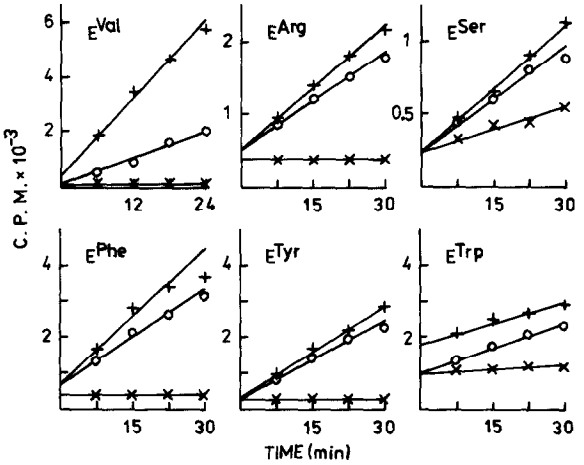

Fig.1. Reactivation of lupin aminoacyl-tRNA synthetases by ribosomes and bovine serum albumin. Stock solutions (1–6 μM) of the enzymes were diluted 250 times and assayed as in section 2. The reactions were carried out in the presence of: (+) ribosomes (0.8 mg/ml); (○) bovine serum albumin (26.7 mg/ml); and (×) in their absence. The ribosomes exhibited only negligible activities of endogenous synthetases which amounted to max. 100 cpm increase in appropriate [^{14}C]aminoacyl-tRNA during 30 min incubation at 25°C.

proteins can interact with lupin aminoacyl-tRNA synthetases or not. Of particular interest was to find out if one synthetase can restore the activity of another synthetase, since this interaction might occur in vivo. Many proteins were therefore screened and the results are presented in tables 1,2.

Table 1
Effect of heterologous proteins and ribosomes on reactivation of lupin valyl-tRNA synthetase

Additions	Conc. (μM)	Reactivation effect (cpm/min incubation)
1. Wheat ribosomes	0.08	236
	0.04	164
	0.02	36
	0.01	0
2. RNA polymerase (*E. coli*)	0.6	73
	0.2	13
	0.07	0
3. Ferritin	5.6	35
4. Cytochrome *c*	57	155
	23	150
	11.5	75
	5.8	10
5. Hemoglobin	87	143
	29	62
	10	0
6. Bovine serum albumin	262	114
	87	40
	29	10
	10	0
7. Myoglobin	150	40
	75	6
	38	0
8. Lysozyme	83	17
	41.5	10
	21	3
9. Soybean trypsin inhibitor	1200	20
10. Concanavalin A	24	0
11. Chymotrypsinogen A	107	0
12. Catalase	22.4	0
13. DNase I	108	0
14. Creatine phosphokinase	82	0
15. α-Amylase	69	0
16. Ovalbumin	148	0

Activity of lupin valyl-tRNA synthetase (1 nM) was determined in the presence of specified additions as in section 2. The enzyme exhibits no activity when assayed in the absence of either ribosomes or bovine serum albumin (see fig.1). This is due to severe dilution since the enzyme exhibits 15%, 5% and 1% activity relative to that determined in the presence of ribosomes when assayed at 32 nM, 16 nM and 8 nM, respectively. Where the concentration dependence of the reactivation effect is given, the highest activity represents the greatest reactivation attainable with a given protein or ribosomes. The proteins and ribosomes neither contain endogenous valyl-tRNA synthetase nor inhibit its catalytic activity with the exception of hemoglobin which inhibited the aminoacylation at > 0.1 mM. The order of listing reflects the potency of proteins in reactivation of valyl-tRNA synthetase

Table 2
Effect of homologous lupin proteins and ribosomes on reactivation of lupin valyl-tRNA synthetase

Additions	Conc. (μM)	Reactivation effect (cpm/min)	Endogenous Val-tRNA synthetase activity (cpm/min)
1. Ribosomes	0.032	265	15
	0.016	260	7
	0.008	150	0
	0.004	9	–
2. Phe-tRNA synthetase	0.29	100	0
	0.14	32	0
	0.07	0	–
3. Tyr-tRNA synthetase	0.2	50	–
	0.1	20	0
	0.05	10	–
4. Arg-tRNA synthetase	0.03	60	25
	0.015	50	13
5. Ser-tRNA synthetase	0.7	70	20
6. Trp-tRNA synthetase	0.29	80	240
7. Adenosine kinase	15	0	0
8. *S*-Adenosylhomocysteinase	3	0	0

The reactivation effect of the additions listed in the table was measured as in section 2. The concentration of lupin valyl-tRNA synthetase was 1 nM and of [^{14}C]valine (175 Ci/mol) was 22.5 μM (~0.5 K_m). The rate of 180 cpm/min incubation at 25°C corresponds to rate constant of 50 min^{-1}. The magnitude of the effect is not necessarily the highest due to limitations in availability of adequately concentrated preparations of the enzymes used for reactivation of valyl-tRNA synthetase. The endogenous valyl-tRNA synthetase activity of the enzymes and ribosomes, given in the last column of the table, is subtracted where needed. The ribosomes and the enzymes do not inhibit the enzymatic activity of valyl-tRNA synthetase

Out of 15 heterologous proteins tested, 7 were totally inactive in the restoration of lupin valyl-tRNA synthetase activity, whereas 8 were active to different extents (table 1). No single protein was as effective as ribosomes. Lupin valyl-tRNA synthetase was most effectively stimulated by ribosomes when assayed in the presence of bovine serum albumin. Among the proteins tested, RNA polymerase, ferroproteins and bovine serum albumin are the most effective. However, there is a great difference in the effective concentrations of ribosomes and proteins: the former are active at 10^{-8}–10^{-7} M, whereas the latter are active at 10^{-5}–10^{-4} M.

Out of 7 homologous proteins tested, 5 were active in the restoration of lupin valyl-tRNA synthetase activity; all these are other lupin aminoacyl-tRNA synthetases. Two homologous proteins, adenosine kinase and *S*-adenosylhomocysteinase, which may be regarded as functionally unrelated to aminoacyl-tRNA synthetases, were inactive. Again, homologous ribosomes were the most active.

Inspection of the concentration dependence of the reactivating effects indicate that in most cases the effect does not vary proportionally with the concentration of the reactivating protein, e.g., in the case of ribosomes and phenylalanyl-tRNA synthetase shown in table 2, and of ribosomes, RNA polymerase, cytochrome *c*, hemoglobin and myoglobin shown in table 1. This indicates that ribosomes and the reactivating proteins themselves may undergo unfolding upon dilution, resuming an inactive conformation.

If this is the case, the mixtures of 2, 3 or more reactivating proteins would be more effective than is expected from simple addition of their reactivating effects. In other words, the reactivating effects would be synergistic. This point is illustrated in table 3. Indeed, lupin valyl-tRNA synthetase is reactivated more effectively by mixtures of 2 or 3 other amino-

Table 3
Synergistic interactions between lupin aminoacyl-tRNA synthetases

aa-tRNA synthetase (E^{aa}) added	Reactivation effect cpm/min incubation
1. E^{Arg} (4 nM)	7
2. E^{Phe} (70 nM)	2
3. E^{Tyr} (50 nM)	6
4. E^{Tyr} (50 nM) + E^{Phe} (70 nM)	34
5. E^{Tyr} (50 nM) + E^{Arg} (4 nM)	45
6. E^{Arg} (4 nM) + E^{Phe} (70 nM)	17
7. E^{Arg} (4 nM) + E^{Phe} (70 nM) + E^{Tyr} (50 nM)	59

The reactivation of valyl-tRNA synthetase (1 nM) was measured in the presence of various combinations of homologous aminoacyl-tRNA synthetases as in section 2. Reactivation effect is defined as the rate of aminoacylation of $tRNA^{Val}$ in terms of cpm/min incubation at 25°C. The assays no. 1,5,6 and 7 are corrected for endogenous valyl-tRNA synthetase present in E^{Arg} preparation. The correction is 3 cpm

acyl-tRNA synthetases than is expected on the basis of the reactivating action of each one of them.

The data presented above indicate that, in addition to ribosomes, some proteins can interact with lupin aminoacyl-tRNA synthetases and that these interactions are essential for the maintenance of active conformation of the synthetases. In particular, it is likely that the interactions between ribosomes and aminoacyl-tRNA synthetases, and between the synthetases themselves do occur in vivo. These interactions are detectable at 10^{-8} M ribosomes and 10^{-8}–10^{-7} M synthetases. No other protein tested affects the activity of valyl-tRNA synthetase at $< 10^{-6}$ M.

The ribosome–synthetase and synthetase–synthetase interactions provide the functional rationale for the existence of high molecular weight complexes of aminoacyl-tRNA synthetases within the cell. One of the functions of these complexes may be the stabilization of aminoacyl-tRNA synthetases in their active conformations.

Recently the intermolecular complexes of aminoacyl-tRNA synthetases have also been isolated from a plant (wheat germ) system [28].

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