Vol. 85, No. 3, 1978 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
December 14, 1978 Pages 999-1006

OCCURRENCE OF AMINOACYL-tRNA SYNTHETASE COMPLEXES IN QUIESCENT WHEAT GERM

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Received October 2, 1978

SUMMARY

The cellular distribution of arginyl-tRNA, leucyl-tRNA, methionyl-tRNA and phenylalanyl-tRNA synthetases from quiescent wheat germ shows that these enzymes participate to various high molecular weight complexes from 3.10^6 to 3.10^5. Two types of interactions were discriminated by salt washing. The greater part of these complexes are weakly bound and destroyed by conventionnal enzymatic extraction procedures which release the free enzymes. Sedimentation constants of complexes show that they have a common size at 18-20S, but higher sedimentation constants were also observed (≈ 40-60 S). Investigations by gel filtration lead to evidence that the complexes contain a little amount of conjugates.

INTRODUCTION

It is well established that the aminoacyl-tRNA synthetases of animal cells are included in complexes of various sizes. In addition to the free forms of these enzymes, the constant presence of a complex with a sedimentation coefficient of 18 to 25 S has also been demonstrated (1-4).

Other high molecular weight structures have also been found, either in the form of enzymatic complexes or in the form of ribosome-bound enzymatic activities (3-7). Certain have been isolated and it has been shown that they are composed of either a limited number (6-8) or the majority of synthetases (1,7). Regardless of the type of macrostructure with which these enzymes are associated, they are always found with other molecules of the genetic translation system, such as tRNA (1,2,6,9), rRNA (1,3,6,9), elongation factors (10,11) or even lipids (12).

The present report is an initial study of the distribution and properties of the complexes in which arginyl-tRNA, leucyl-tRNA, methionyl-tRNA and phenylalanyl-tRNA synthetases are found in the cells of wheat germ embryos. We have concentrated on the sub-cellular distribution of

Abbreviations: PMSF : phenylmethylsulfonyl fluoride. Synthetases : arginyl-tRNA- (E.C.6.1.1.19), leucyl-tRNA- (E.C.6.1.1.4), methionyl-tRNA- (E.C.6.1.1.10), phenylalanyl-tRNA- (E.C.6.1.1.20).

0006-291X/78/0853-0999$01.00/0

these enzymes and the conditions governing the maintenance and the dissociation of these complexes.

MATERIALS AND METHODS

Biological material. Fresh, quiescent wheat germ embryos were obtained by mechanical sorting and were used as the source of different tRNAs, ribosomes, synthetases.

Preparation of post-microsomal fractions. Wheat germ embryos were homogenized for 2 minutes at 4°C in 5 volumes of buffer A : 50 mM Tris-HCl, pH 8.05, 5 mM magnesium acetate, 50 mM KCl, 0.1 mM PMSF and 6 mM 2-mercaptoethanol. Cell debris, nuclei and mitochondria were eliminated by two centrifugations for 15 minutes each at 21,000 g. After readjusting the pH of the supernatant to 7.7 (crude extract), it was centrifuged for 3 hours at 105,000 g. The lipid layer of the supernatant was discarded and the upper 2/3 of the transparent underlying phase was removed and used as fraction S 105 (post-microsomal supernatant). The clear microsomal pellet, fraction C 1 (0.5 ml), was the source of crude ribosomes. Fraction S 105 was then centrifuged for 8 hours at 105,000 g. The pellet was rinsed before being "dissolved" in the above Tris buffer and was used as fraction C 2 (0.5 ml). The supernatant was then centrifuged at the same speed for 32 hours. The pellet was treated as was C 2 and constituted fraction C 3 (0.5 ml) ; the supernatant was termed fraction S 3 (30 ml).

Washing of crude ribosomes. Crude ribosomes pellets were divided into four aliquots and were suspended in buffer A adjusted with 20 % (v/v) glycerol and 0.125, 0.250, 0.500 or 1 M KCl. The ribosome concentration was equivalent to 100 A_{260} units/ml. Incubation was for 1 or 16 hours at 4°C, after which ribosomes were recovered by centrifugation, resuspended in the above buffer containing 50 mM KCl and dialyzed against the same buffer. Ribosomes were stored at - 20°C at a concentration equivalent to 200 to 300 A_{260} units/ml.

Linear sucrose gradient (5 to 20 %) were made in buffer A. Samples (0.5 ml) and standards (ribosomal subunits : 60 S and 40 S, rRNA : 18 S and tRNA : 5 S) were loaded on identical gradients and centrifuged simultaneously for 8 hrs at 4°C and 27,000 rpm in an SW 27 rotor.

Molecular filtration. Columns of Sephadex G 200 and Sepharose 6B (90 x 1.5 cm) were equilibrated with buffer A with 10 % (v/v) glycerol. Void volumes (V_o) were determined with dextran blue. Columns were calibrated with cytochrome C, ovalbumin, bovine serum albumine, aldolase, catalase and ferritin. Samples (300 µl) were applied on the gels in the same conditions.

Aminoacyl-tRNA assays were performed according to Carias et al. (13,19) and Chazal et al. (14).

RESULTS AND DISCUSSION

I - CELLULAR DISTRIBUTION OF AMINOACYL-tRNA SYNTHETASES - A heterogeneity of aminoacyl-tRNA synthetase distribution was observed after centrifugation (Fig. 1). There is approximatively 17 % of total activity of the three enzymes in fraction C 1 (> 80 S) ; but maximal activities were found to be in fraction C 2 (10 - 80 S) with 30 % and S 3 with 46,5 % of total activity. Thus, the synthetases from post-microsomal supernatant containing no 80 S ribosomes or larger subcellular structures, are present as either heavy aggregates or are associated with multimolecular complexes and represent

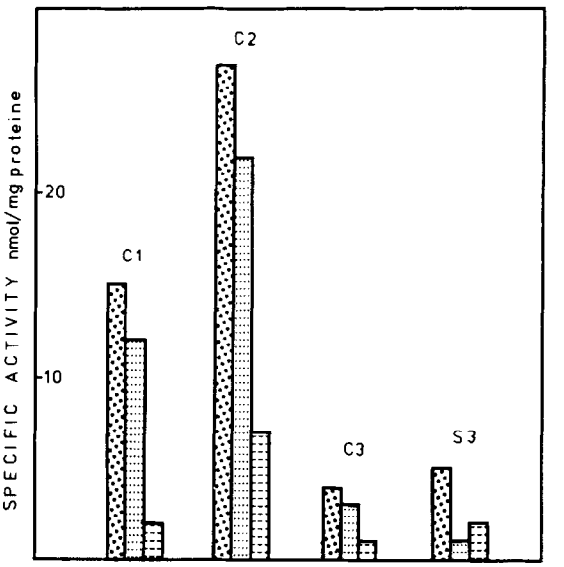

Fig. 1 - Distribution of leucyl-tRNA, arginyl-tRNA and phenylalanyl-tRNA synthetases in fractions C1, C2, C3 and S3 obtained by centrifugation (see materials and methods). Specific activity, determined in aliquots (2-4 µg) is expressed as nmol of [^{14}C] aminoacid charged per mg of protein in 3 min. The amount of proteins was estimated by Lowry method to 700 µg for C1, 651 µg for C2, 1044 µg for C3 and 6866 µg for S3.

●●● leucyl-tRNA synthetase ; - - - phenylalanyl-tRNA synthetase ; ••• arginyl-tRNA synthetase.

more than 30 % of total synthetase activity. Fraction C 3, whose sedimentation coefficient of about 7 S corresponds to that of the heaviest purified synthetases (15,16), exhibits enzymatic activities of 7 % of the total activity. Since this is the opposite of that which is observed in bacterial systems (17,18), it thus appears that plant cells, as animal cells, (1-11), contain aminoacyl-tRNA synthetases which are associated with high molecular weight subcellular structures.

The subcellular synthetase distribution data furnished by centrifugation do not exclude the possibility that these structures result from non-specific interactions. It should be noted that although these complexes are found in various types of animal cells, virtually nothing is known concerning the bonds which might assure the structural integrity of these macro-complexes. Invoking the hypothesis that electrostatic attractions are concerned, a series of experiments involving variations of the ionic strength of the medium were undertaken.

II - EFFECTS OF SALT CONCENTRATION ON THE BINDING OF AMINOACYL-tRNA SYNTHETASES. Particle-synthetase interactions of variable strengths could be demonstrated by washing the particles from the fraction C 1 (17 % of total enzymatic activity) with increasing concentrations of KCl. Although the four enzymes studied remain bound to particles after a control wash of crude ribosomes in KCl-free buffer, it can be observed that leucyl-tRNA and phenylanalyl-tRNA synthetases are easily released by 0.125 M KCl ; residual activity of washed ribosomes is only 2 to 3 %. In the case of arginyl-tRNA and methionyl-tRNA synthetases, however, even a long wash of 16 hours in 1 M KCl does not remove more than 50 % of the activity. Synthetase activities released from the particles and found in the wash complement the residual activities of washed ribosomes. Losses of methionyl-tRNA and leucyl-tRNA synthetases activities are only between 3 and 10 %, depending on the length of the wash and the ionic strength. We may thus, at least in this case, eliminate the possibility of KCl inhibition of particle-associated synthetases. The loss of phenylalanyl-tRNA synthetase activity in the wash is higher, reaching a mean value of 30 %. KCl-released arginyl-tRNA synthetase activity on the other hand, is three times higher than that of non-washed ribosomes, although a KCl concentration upper than 75 mM inhibits the aminoacylation of $tRNA^{Arg}$ carried out in the assays with 30 mM KCl. This has already been reported for other enzymes by Smulson (11) and confirms research currently in progress (unpublished), which shows that arginyl-tRNA synthetase has a highly stable functionnal structure, unlike the other three synthetases presently studied. It can be noticed that the free form in the wash solution has a higher activity than that complexed to the particle. Considering the very strong interactions apparently responsible for the cohesion of the complex, it appears improbable that the distribution of free and bound forms of this enzyme obeys a simple association-dissociation model.

Phenylalanyl-tRNA and leucyl-tRNA synthetases are easily detached from the complexes with which they are normally associated. This is in agreement, especially for the former, with the results of Roberts (3). The bonds involved in this association are most likely weak, ionic-type bonds. In the cases of methionyl-tRNA and arginyl-tRNA synthetases, however, the bonds involved between enzyme and particle are apparently relatively strong. Although the exact nature of these bonds cannot yet be determined, an occasional or non-specific interaction can be eliminated.

Results about subcellular distribution and nature of bonds involved in the constitution of the enzymatic complexes are summarized (table 1). When enzyme extractions were accomplished by conventionnal methods (13,14,19) 95 % of enzymatic complexes are easily dissociated and release the well-known

TABLE 1 - SUBCELLULAR DISTRIBUTION OF WEAKLY- AND STRONGLY-BOUND ENZYMATIC ACTIVITIES

Sub-cellular fractions	Enzymatic forms				
	High molecular weight complexes		Low molecular weight complexes		Free enzyme
	WB	SB	WB	SB	
C 1	16	1	3	-	-
C 2	-	-	25	5	-
C 3 + S 3	-	-	-	-	50

WB = Weakly-bound : enzymatic activity released from particles by 0,5 M KCl treatment. SB = strongly-bound : enzymatic activity resistant to 16 hrs 0,5 M KCl treatment.
Sub-cellular activities were expressed as % of total four enzymatic activities.

molecular weight four enzymes. Only a little enzymatic fraction (about 6 %) was resistant to dissociating treatment and could be assigned to strongly bound enzymatic forms.

III - ANALYSIS OF AMINOACYL-tRNA SYNTHETASE ACTIVITIES BY DENSITY GRADIENT CENTRIFUGATION of fraction C 2 (Fig. 2) demonstrate that these enzymes are associated with complexes of varying size. Four forms of leucyl-tRNA synthetase can be separated : one sedimenting at 4-5 S corresponds to the free form and the others are associated with complexes, one sedimenting at 15-20 S and the remaining two having sedimentation coefficients close to 40 and 60 S. The distributions of methionyl-tRNA and arginyl-tRNA synthetases are superimposed on that of the leucyl enzyme in the 15-20 S region, with a macrocomplex including the methionyl-tRNA enzyme at 40 S, and the free form of arginyl-tRNA synthetase at 5 S. Since the fraction studied was obtained from a pellet and thus containing no free forms ($\simeq$5 S), the appearance of activity peaks at 5 S demonstrates that the complexed enzyme was partially dissociated during the experiment. This is most pronounced for leucyl-tRNA and arginyl-tRNA synthetases. The only form commun to the three synthetases sediments in the region of 15-20 S.

Although the higher molecular weight complexes undergo a degree of dissociation, it is not presently possible to speculate on the mechanism of formation of the free forms. Nevertheless it is clear that dissociation involves the distinct complexes of 60, 40 and 15-20 S ; if not, we would observe dis-

low incorporation into or interaction with the particles (cf. 8). Similar effects have been shown in pentane-extracted mitochondria, that are depleted of their endogenous ubiquinone (Fig. 2).

The rate of reduction of UQ-3 is higher using NADH than succinate as electron donor and the reduction is rotenone-sensitive.

It must therefore be concluded that UQ-3 is reduced by NADH through Complex I of the respiratory chain. The K_m for UQ-3 of NADH-UQ reductase in pentane-extracted mitochondria was 43 μM, in accordance with that in isolated Complex I, while the corresponding value for succinate-UQ reductase was 74 μM.

These experiments allow to conclude that the second hypothesis postulated in the introduction is the correct one.

The fact that UQ-3 reduced by NADH cannot be reoxidized by the cytochrome chain, whereas UQ-3 reduced by succinate is reoxidized is not of obvious explanation and may involve a sidedness of UQ reduction and oxidation in the membrane. To test this possibility we have studied electron transfer using exogenous reduced ubiquinones of different chain lengths as substrates of ubiquinol oxidase in both BHM and SMP which have opposite polarity: as is well known, SMP are inside-out and face the medium with the "matrix" side (M-side)(9). The results are shown in Table I. We can observe that ubiquinol-3 is a good substrate in SMP, but is oxidized at lower rates in BHM; on the other hand, ubiquinols-7 and -8, at similar concentrations, are oxidized at increasingly higher rates in BHM but not in SMP, where there is rather a decrease of oxidation rate with the longer quinols. Only at 15°C there was a lower activity for UQ-7 than for UQ-3 in BHM; a decreased solubility of the long chain quinol could be responsible for the observed effect. The table also shows that the oxidation rate relative to UQ-3 taken as 100%, increases with increasing chain length in BHM, but decreases in SMP.

The relatively low rate of oxidation of ubiquinol-7 and -8 in SMP might be due to the higher protein content of the inner face of the mitochondrial membrane (10) in comparison with the

TABLE 2 - SUBCELLULAR AND MOLECULAR WEIGHT DISTRIBUTION OF PHENYLALANYL-tRNA SYNTHETASE ACTIVITIES

Subcellular fractions	Gel filtration	Approx. mol. wt. (daltons)			% total activity
		$>3.10^6$	$>7.10^5$	$>3.10^5$	
Crude extract	G 200	-	60	40	100
microsomal pellet C 1	6B	1	-	19	20
post-ribosomal supernatant C 2 + C 3 + S 3	G 200	-	40	40	80

Run : 7 ml/hr. Fraction volume : 0.5 ml. Assay volume 30 µl.

total activity from C 1 have a molecular weight corresponding to that reported for the post-microsomal supernatant ($4,5.10^5$ daltons) and the crude extract. Probably due to the very small amount of protein eluted from Sepharose 6B it was impossible to detect the heavy complex ($> 3.10^6$ daltons) in the crude extract or in the post-microsomal supernatant. The same proportion (1 %) of high molecular weight complex ($> 3.10^6$) was observed when the microsomal pellet is salt-washed (0.5 M KCl) before gel filtration analysis; identical results were obtained for the three others enzymes. Salt treatment of the crude extract and post-microsomal supernatant releases the greater part of the free enzymes, but $> 7.10^5$ and $> 3.10^5$ residual complexes were still observed ($\simeq$ 10 %).

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