SUBRIBOSOMAL PARTICULATE FORMS OF ELONGATION FACTORS AND AMINOACYL-tRNA SYNTHETASES IN HUMAN CELLS

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Summary

Several enzymes required for translation in eucaryotic cells have been found in a subribosomal particulate form especially during maximal protein synthesis of exponentially growing HeLa cells. In sucrose gradients containing post-ribosomal cell extracts, elongation factors 1 and 2 as well as aminoacyl-tRNA synthetases were found in multiple forms with sedimentation values from 5-28 S. The heavier forms of the enzymes associated upon Sepharose chromatography to high molecular weight complexes of greater than 1 X 10^6 daltons.

We have recently studied the subcellular distribution of elongation factor - 2 (EF-2, aminoacyl transferase II, T2) in HeLa cells under asynchronous and synchronous growth conditions (1). During this investigation it was observed that the enzyme was associated with a subribosomal particulate fraction ("soluble pellet") with a sedimentation coefficient smaller than approximately 30S. The specific activity of EF-2 in this subcellular fraction was high during logarithmic cell growth and rapid protein synthesis and decreased considerably when the cells entered stationary growth phase and the rate of protein synthesis declined. These results suggested that the EF-2 containing particulate fraction might be a complex of biological significance from which the enzyme could be donated to the polyribosomes during protein synthesis.

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Materials and Methods

Aminoacyl-tRNA synthetase activity was measured by two independent assays: amino acid dependent ATP - $^{32}PP_i$ exchange was measured essentially as described by Fangman and Neidhardt (15) in the presence of 13 L-amino acids. The assay of aminoacyl-tRNA formation was carried out according to standard procedures (16, 17) utilizing E. coli tRNA (stripped) and a mixture of twenty ^{14}C-L-amino acids. EF-1 activity was assayed as described by Raeburn et al. (18). EF-2 activity was assayed by the procedure employed for measuring EF-1 activity, but the incubation mixture contained a saturating amount of partially purified EF-1 (rat liver) instead of EF-2. The diphtheria toxin catalyzed transfer of ^{3}H-adenosine diphosphoribose from ^{3}H-adenosine-NAD to EF-2 was described earlier (1, 20). Sepharose 6B gel filtration - The gel column was prepared according to the manufacturer (Pharmacia). The void volume (V_o=60.7 ml) was determined with a 0.5% solution of blue dextran (Pharmacia), and T_2O was used to measure the total bed volume (V_t=166 ml). The proteins (thyroglobulin, ferritin, catalase, BSA) used as molecular weight markers (22) were applied to the column separately in 5 ml samples (10 mg protein in reticulocyte standard buffer (pH 7.4), containing 1mM DTT and 0.3M sucrose).

Results and Discussion

The significance of the complex is emphasized by the observation that the "soluble pellet" in addition to EF-2 contained EF-1 (elongation factor-1, aminoacyl transferase I, T1) and aminoacyl-tRNA synthetases (Table 1). Preliminary results further indicated that initiation factor activity also was present.

Because of the apparent relationship between cell growth, rate of protein synthesis and EF-2 association with the "soluble pellet", we studied the postribosomal distribution of the enzymes indicated in

Table I. Demonstration of particle - character of enzymes involved in protein synthesis

Enzyme Source	Aminoacyl-tRNA synthetases ATP-PP exch. (cpm/10 min.)	Aminoacyl-tRNA synthetases t-RNA charging (dpm/10 min.)	EF-1 Leucine incorp. (dpm/10 min.)	EF-2 Leucine incorp. (dpm/10 min.)	EF-2 Diphtheria toxin assay (dpm/60 min.)
None	61	40	79	102	20
"Soluble pellet"					
10 µg protein	585	197	--	--	143
50 µg protein	--	--	752	978	--

The "soluble pellet" was obtained as described earlier (1): postmitochondrial supernatant was layered on 28 ml 15-30% sucrose gradients and after centrifugation for 18 hours at 18,000 rpm (for preparation of ribosomal subunits) in a SW 25.1 rotor, the portion of the gradients containing material with s values less than approximately 30 were collected. After dilution with reticulocyte standard buffer (1) and centrifugation at 100,000 x g for 15 hours a gelatinous pellet ("soluble pellet") was obtained.

Table 2. Postribosomal distributions of EF-1 and EF-2 in a 5-20% sucrose gradient

S Range	EF-1 (^{14}C-leucine incorp.)		EF-2 (diphtheria toxin assay)	
	Total incorp. (dpm/100µl)	Spec. activity (dpm/µg prot.)	Total incorp. (dpm/200µl)	Spec. activity (dpm/µg prot.)
5-7	1,556	6.9	4,500	14.8
8-10	1,547	8.6	2,000	8.2
15-17	780	36.4	1,000	12.3

2 ml. of postmitochondrial extract from 3 x 10^8 HeLa cells was layered on a 5-20% sucrose gradient (36 ml.) and centrifuged for 16 hours at 27,000 rpm (SW27 rotor). Fractions of 10 drops (approximately 0.6 ml.) were collected, and pooled fractions covering the S range indicated above were assayed for EF-1 and EF-2 as described in Materials and Methods. Purified ^{14}C-labeled 28 S, 18 S and 4-5 S RNA from HeLa cells were centrifuged on a separate gradient without sample and served as sedimentation markers.

Table 1 by sucrose gradient centrifugation, in order to characterize their possible association. A detailed account of this investigation will be reported elsewhere (2). Purified radioactively labeled 5 S, 18 S and 28 S RNA from HeLa cells were used as markers of sedimentation rates. Table 2 shows that both EF-1 and EF-2 were present in the sucrose gradients at positions corresponding to the sedimentation coefficients (5-7 S) reported for the purified enzymes from rabbit reticulocytes (3) and rat liver (4), respectively. It should be noted, however, that significant amounts of the enzymes, of high specific activity, could be demonstrated in a 15-17 S region of the gradient. In addition, a high concentration of morphologically

distinct particles were observed by electron microscopy at 15-17 S. A description of these subribosomal particles will appear elsewhere (2). It is of great interest that other investigators also have reported that EF-1 exists in multiple forms in extracts of cells of animal origin (5-8), similarly, EF-2 released from polyribosomes of rabbit reticulocytes by EDTA treatment was heterogeneously distributed in a 5-15 S region (9).

High molecular weight forms of aminoacyl-tRNA synthetases were apparent in both the 14-21 S and the 25-30 S regions of DTT-sucrose gradients of postribosomal extracts. Recently, high molecular weight forms of various aminoacyl-tRNA synthetases have been demonstrated in other eucaryotic cell types (10-14); the sedimentation coefficients of these complexes correspond very well with the range observed in this study. Our demonstration of the presence of EF-1 as well as EF-2 in combination with aminoacyl-tRNA synthetases suggests that this subribosomal cell fraction may play an important role as an intermediate in protein synthesis.

In order to obtain further evidence for a biologically significant association between the amino acid activating enzymes and elongation factors, a high molecular weight region (10-12 S) of a sucrose gradient containing postribosomal cell extract was subjected to gel filtration on a Sepharose 6B column. These experiments will be reported in detail elsewhere (2). As a control, a 5 S region of the same gradient, which presumably contained the enzymes in their free forms (3, 4), was chromatographed on the same Sepharose column. An approximate size distribution was calculated for the eluted material based on a calibration of the gel with proteins of known molecular weights. Table 3 shows that EF-2 and aminoacyl-tRNA synthetases were eluted mainly as high molecular weight complexes during gel filtration of 10-12 S material (>1 million dalton), whereas the

Table 3. Gel filtration of postribosomal cell extract

Approx., mol. wt. (dalton)	Distribution of eluted enzyme activities (% of total)			
	EF-2		Aminoacyl-tRNA synthetases	
	10-12 S material	5 S material	10-12 S material	5 S material
Void volume	53.7	0.8	7.1	4.0
1-3 million	22.2	3.5	55.2	11.1
2000,000-1 million	0.0	14.0	10.2	3.3
90,000-200,000	5.5	45.8	25.6	81.6
90,000	18.6	35.9	1.9	0.0

Gel filtration on Sepharose 6B was performed on pooled fractions of a 5-20% sucrose gradient containing postribosomal HeLa cell extract. The sedimentation coefficients of the pooled material are indicated in the table. 3 mg protein (10-12 S material) and 6 mg protein (5 S material) in 5 ml volumes were chromatographed separately. Figure 1 describes the calibration of the gel with proteins of known molecular weights. EF-2 concentrations were measured by the diphtheria toxin assay, and aminoacyl-tRNA synthetase activity was assessed by the t-RNA charging reaction.

enzymes eluted from 5 S postribosomal extract have molecular weights corresponding to those reported for the free aminoacyl-tRNA synthetases and EF-2 from other mammalian cell types (90,000-200,000 dalton). Probably due to the low sensitivity of the assay and the very small amount of protein eluted, it was not possible to detect EF-1 in the eluate. A recording of the optical density of the fractions eluted from 10-12 S material showed that the large majority of proteins were eluted corresponding to molecular weights between 200,000-400,000 dalton, thus demonstrating that protein aggregation was not a general phenomenon during gel filtration. It seems conceivable that the complexes containing synthetases and EF-2 of very high molecular weight were assembled during gel filtration from preformed smaller segments. The data might suggest that there are other, perhaps highly specific, macromolecules (RNA, protein?) in the 10-30 S region of the postribosomal cell extract, which hold certain enzymes together. The size of the enzyme-aggregates eluted from the Sepharose column were extremely large. It should be realized, however, that the particle weight of a complex, composed of all protein factors necessary for translation of mRNA, should amount to several million dalton.

In summary, our studies suggest that several, if not all, of the enzymes involved in translation may occur as macromolecular complexes in the intact eucaryotic cell. Earlier results (1) further suggest that the relative amounts of the various enzymes within the complexes may be regulated in order to correspond to the require ments of cellular protein synthesis.

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References

1. Henriksen, O., and Smulson, M., Arch. Biochem. Biophys., 150, 175 (1972).
2. Henriksen, O., and Smulson, M., Manuscript in preparation.
3. McKeehan, W.L., and Hardesty, B., J. Biol. Chem., 244, 4330 (1969).
4. Collins, J.F., Raeburn, S., and Maxwell, E.S., J. Biol. Chem., 246, 1049 (1971).
5. Klink, F., Kloppstech, K., Kramer, G., and Dimigen, J., Biochim. Biophys. Acta, 134, 373 (1967).
6. Richter, D., and Klink, F., Biochemistry, 6, 3569 (1967).
7. Schneir, M., and Moldave, K., Biochim. Biophys. Acta, 166, 58 (1968).
8. Collins, J.F., Moon, H.M., and Maxwell, E.S., Personal communication.
9. Collier, R.J., and Traugh, J.A., Cold Spring Harbor Symp. Quant. Biol., 34, 589 (1969).
10. Bandyopadhya, A.K., and Deutscher, M.P., J. Mol. Biol., 60, 113 (1971).
11. Roberts, W.K., and Coleman, W.H., Biochem. Biophys. Res. Commun., 46, 206 (1972).
12. Vennegoor, C.J.G.M., Stols, A.L.H., and Bloemendal, H, J. Mol. Biol., 65, 375 (1972).
13. Vennegoor, C., and Bloemendal, H., Eur. J. Biochem., 26, 462 (1972).
14. Irvin, J.D., and Hardesty, B., Biochemistry, 11, 1915 (1972).
15. Fangman, W.L., and Neidhardt, F.C., J. Biol. Chem., 239, 1839 (1964).
16. Smulson, M.E., and Rabinovitz, M., Arch. Biochem. Biophys., 124, 306 (1968).
17. Bandyopadhya, A.K., and Deutscher, M.P., J. Mol. Biol. 60, 113 (1971).
18. Raeburn, S., Collins, J.F., Moon, H.M., and Maxwell, E.S., J. Biol. Chem., 246, 1041 (1971).

19. Skogerson, L., and Moldave, K., Arch. Biochem. Biophys. 125, 497 (1968).
20. Smulson, M.E., and Rideau, C., J. Biol. Chem., 245, 5350 (1970).
21. Andrews, P., Biochem. J., 91, 222 (1964).
22. Giorgio, N.A., Jr., Yip, A.T., Fleming, J., and Plaut, G.W.E., J. Biol. Chem., 245, 5469 (1970).