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Altered Aminoacyl-tRNA Synthetase Complexes in G_1-Arrested Chinese Hamster Ovary Cells†

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ABSTRACT: Aminoacyl-tRNA synthetase complexes existing in Chinese hamster ovary (CHO) cells were shown to undergo alterations as a function of the growth state of the cell. The distribution pattern for 13 particulate postribosomal aminoacyl-tRNA synthetases in 10–30% (w/v) exponential sucrose gradients was determined for the enzymes from CHO cells as they exist under three different culture conditions: exponential growth, G_1 arrest induced by isoleucine deficiency, and G_1 arrest induced by leucine deficiency. The synthetases specific for the amino acids Arg, Asp, Cys, Gln, His, Lys, Met, Thr, and Val have indistinguishable distribution patterns in all three cell types. However, the synthetases specific for Glu, Pro, Leu, and Ile have a unique distribution of synthetase forms in the G_1-arrested cultures and this distribution is independent of whether G_1 arrest was induced by isoleucine or leucine deficiency. The distribution of synthetase forms in G_1-arrested cells differs in a definite, reproducible manner from the profiles obtained with the exponentially growing cells, and this fact is strong evidence for an in vivo role for the synthetase complexes.

The existence of aminoacyl-tRNA synthetase complexes in mammalian systems is well documented and numerous references are cited in recent reviews (Kisselev and Favorova, 1974; Söll and Schimmel, 1974). However, no direct evidence has been available that suggests a possible functional role for these complexes.

Recent work from our own laboratory has identified the existence of aminoacyl-tRNA synthetase complexes in Chinese hamster ovary (CHO)[1] cells (Hampel and Enger, 1973; Ritter et al., 1976). In this paper we have studied the synthetase complexes existing in CHO cells as a function of the growth state of the cell.

CHO cells can be very clearly arrested in the G_1 phase of the cell cycle by a condition of isoleucine deficiency (Tobey, 1973). It was previously shown that DNA synthesis immediately ceases with isoleucine deficiency while protein synthesis continues for some time, being 57% of control when complete G_1 arrest is obtained after 30 h (Enger and Tobey, 1972).

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[1] Abbreviations used are: CHO, Chinese hamster ovary; Ile^- cells, cells grown in isoleucine-deficient medium; Leu^- cells, cells grown in leucine-deficient medium; buffer A, 100 mM KCl–10 mM Tris-HCl (pH 7.5 at 25 °C)–1.5 mM $MgCl_2$–0.1 mM dithiothreitol; buffer B, 10 mM KCl–10 mM Tris-HCl (pH 7.5 at 25 °C)–1 mM $MgCl_2$–0.1 mM dithiothreitol; Tris-HCl, 2-amino-2-hydroxymethyl-1,3-propanediol hydrochloride.

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Isoleucine-deficient G_1-arrested cells appear to have enough protein synthetic capacity to allow cells already in the S phase to complete the S phase but not enough to allow entry into S from G_1. The synthesis of some unknown essential component appears to be prevented in G_1-arrested cells (Tobey, 1973). This argues that isoleucine deficiency induced G_1 arrest may be due to some unique alteration in the basic protein synthetic machinery. In the present study we analyzed the aminoacyl-tRNA synthetase complexes for 13 particulate synthetases in isoleucine-deficient G_1-arrested cells and found unique form changes in the enzyme complexes for four specific synthetases. An independent G_1 arrest induced by leucine deficiency (Everhart and Prescott, 1972) produced identical changes. The four altered aminoacyl-tRNA synthetases are specific for the amino acids Glu, Pro, Leu, and Ile. The nine unchanged particulate synthetases are specific for the amino acids Arg, Asp, Cys, Gln, His, Lys, Met, Thr, and Val. This is the first alteration in the basic protein synthetic machinery that has been reported in amino acid deficient G_1-arrested cells.

Experimental Procedures

CHO cells were grown exponentially in regular F-10 medium at 37 °C in spinner culture as previously described (Enger et al., 1973). These cells are referred to as exponential cells.

Cells were arrested in the G_1 phase of the cell cycle by using the isoleucine deficiency method of Tobey (1973). In these instances, exponentially growing cells at a cell density of near 4×10^5 cells/mL were collected by centrifugation at 500*g* for 2 min, washed once with the isoleucine-deficient medium (Ile^- medium; see next paragraph), and then grown in Ile^- medium at 2×10^5 cells/mL until G_1 arrest was complete (30 h). These arrested cells are referred to as Ile^- cells.

Ile^- medium is regular F-10 medium from which isoleucine has been omitted and glutamine concentration has been doubled. Serum for Ile^- medium is prepared by filtering untreated serum through glass wool and then dialyzing 50-mL aliquots against a 10X volume of Earle's balanced salt solution at pH 7.2 for 3 days at 4 °C with daily salt solution changes. After dialysis, serum was stored at −20 °C.

Other cells were arrested in G_1 using a modification of the leucine deficiency method of Everhart and Prescott (1972). Cells were grown exponentially in regular F-10 medium and then transferred to a leucine-deficient medium (Leu^- medium) until G_1 arrest occured in about 40 h. These arrested cells are termed Leu^- cells. Leu^- medium was prepared similarly to Ile^- medium, except no leucine was added to the regular F-10 formulation containing 2× glutamine.

G_1 arrest was verified in both Ile^- and Leu^- cells by cell staining and flow microfluorometric analysis of DNA content (Kraemer et al., 1972).

All cells were collected with centrifugation, washed with 0.25 M sucrose, suspended in buffer [100 mM KCl–10 mM Tris-HCl (pH 7.5 at 25 °C)–1.5 mM $MgCl_2$–0.1 mM dithiothreitol] (18 mL of buffer/10^9 cells), and stored at −90 °C. Cells (10^9) were broken in buffer A (20 mL) containing 1% Nonidet P-40 (Particle Data Labs), and nuclei and cell debris were removed by low-speed centrifugation. The supernatant was centrifuged at high speed to remove ribosomes. This postribosomal supernatant was centrifuged further to give a 6–40S subcellular pellet which was resuspended in buffer B [10 mM KCl–10 mM Tris-HCl (pH 7.4 at 25 °C)–1 mM $MgCl_2$–0.1 mM dithiothreitol], layered on 10–30% (w/v) exponential sucrose gradients in buffer B, and centrifuged 22 h at 25 000 rpm in a Spinco SW-25.2 rotor at 4 °C. Further details of this procedure and gradient analysis were as previously published (Enger et al., 1974).

TABLE I: Distribution of Aminoacyl-tRNA Synthetases Specific for Glu, Pro, Leu, and Ile in CHO Cells Growing Exponentially or in G_1 Arrest Induced by Leu^- or Ile^- Medium.[a]

		6–10 S	10–22 S	22–35 S[b]
Glu	Exp	1.5	9.0	89.5
	Ile^-	5.2	25.4	69.4
	Leu^-	4.6	24.9	70.5
Pro	Exp	0	0.6	99.4
	Ile^-	11.3	15.7	73.0
	Leu^-	13.2	16.4	70.4
Leu	Exp	17.2	15.4	67.4
	Ile^-	25	28.1	46.9
	Leu^-	21.2	29.2	49.6
Ile	Exp	0.7	11.3	88
	Ile^-	1	24.9	74.1
	Leu^-	1	27.9	71.1

[a] Values are given as percent of total enzyme units for each specific synthetase found on sucrose gradients. [b] The range of sedimentation coefficients ($s_{20,w}$) are calculated from calibrated sucrose gradients.

The sucrose gradients were assayed for aminoacyl-tRNA synthetase activity specific for each of the 13 amino acids Arg, Asp, Cys, Gln, Glu, His, Ile, Leu, Lys, Met, Pro, Thr, and Val. Assays were performed by linking ^{14}C-labeled amino acids to cognate rat liver tRNA and determining products formed ([^{14}C]aminoacyl-tRNAs) as described elsewhere (Hampel et al., 1978).

Results

Aminoacyl-tRNA synthetases from cultured Chinese hamster ovary cells were found in complexes of defined size. Sedimentation distribution profiles on calibrated 10–30% (w/v) exponential sucrose gradients showed that the number and nature of the aminoacyl-tRNA synthetase forms varied in a defined manner. Synthetases from exponentially growing cells sediment in three general size classes: AlaRS, AsnRS, SerRS, TrpRS, and TyrRS have a single activity peak that sediments at 6–10S; CysRS, HisRS, PheRS and ValRS have a 15–21S form plus an 8–10S form; and AspRS, ArgRS, GlnRS, GluRS, IleRS, LeuRS, LysRS, MetRS, ProRS, and ThrRS have a 30S form plus, in some cases, one or more slower sedimenting forms.

The size distribution of 13 particulate postribosomal aminoacyl-tRNA synthetases from exponentially growing and G_1-arrested CHO cells were compared on calibrated logarithmic sucrose gradients. Cells were arrested in G_1 at 37 °C in F-10 medium by isoleucine deficiency and, separately, by leucine deficiency. Nine of the synthetases (ArgRS, AspRS, CysRS, GlnRS, HisRS, LysRS, MetRS, ThrRS, and ValRS) have, within experimental error, the same identical profiles in exponential, Ile^- and Leu^- cells.

Four synthetases (GluRS, ProRS, LeuRS, and IleRS) show characteristic, reproducible shifts in the particulate forms of the enzymes following G_1 arrest. GluRS in exponentially growing cells exists primarily in a large 30S particle (peak 3); however, in Ile^- and Leu^- cells a characteristic increase in 20S synthetase activity appears (peak 2).The change is the same in both Ile^- and Leu^- cells (Figure 1, Table I).

The shifts in the synthetase forms for the remaining three synthetase activities (Pro, Leu, and Ile) on sucrose gradients are similar and the results are summarized in Table I. ProRS from exponentially growing cells also exists primarily as a

single, large 30S particle (peak 3). When cells are arrested in G_1, two distinct, slower sedimenting forms of this synthetase (peaks 1 and 2) appear in the 8S and 20S region, respectively (Table I). The same change again occurs in both Ile^- and Leu^- cells.

LeuRS from exponentially growing cells exists in three separable particulate forms. Two predominate forms exist; one being a very large particulate complex at 30S and the other a smaller form at 8S. In addition, low but reproducible LeuRS activity is found at 20 S (peak 2). This synthetase profile changes, however, when cells are arrested in G_1. The relative amount of 20 S LeuRs increases to the point that it is present in amounts nearly equivalent to the 8S and 30S forms of the enzyme. The same reproducible change in the profile is again seen in both Ile^- and Leu^- cells (Table I).

Reproducible changes in the particulate forms of IleRS were found as a function of G_1 arrest induced by isoleucine or leucine deficiency. Typically, exponential cells show a large predominant form of IleRS at 30 S (peak 3). Minor but generally reproducible forms of the enzyme are found at 15 (peak 2a), 28 (peak 3), and 35 S. Cells arrested in G_1 by isoleucine or leucine deficiency show a large reproducible increase in the relative amounts of IleRS in peak 2a at 15 S. Again, the same type of particulate enzyme change was seen independently of the method used to achieve G_1 arrest (Table 1).

IleRS activity is sensitive to extraction and/or isolation conditions. The total units of the IleRS detected on the sucrose gradients occassionally varied from one experiment to the next with some gradients being totally void of detectable activity. Those gradients containing little or no IleRS contained normal levels of other synthetases. Although the total activity of IleRS was variable, the sedimentation profile was generally reproducible in those gradients containing detectable levels of the enzyme. The factors responsible for the loss of IleRS activity have not been identified.

Discussion

The synthetase complexes for the aminoacyl-tRNA synthetase activities specific for Glu, Pro, Leu, and Ile are altered during the arrest of CHO cells in the G_1 phase. No changes occurred in the particulate synthetase complexes for another nine particulate synthetases. The reasons some synthetase complexes are changed while others are not is presently unknown. However, the fact that in vivo changes in CHO cells can uniquely and reproducibly affect specific synthetase complexes in vitro strongly supports the suggestion that the synthetase complexes we have analyzed do in fact exist in vivo and are not artifacts. The same identical alterations in synthetase complexes were observed during amino acid deficiency induced G_1 arrest independently of whether it was caused by isoleucine deficiency or leucine deficiency. This argues for a general in vivo role for the complexes of these four synthetases.

The regulation of cell growth is little understood and is a critical area of biochemistry (for recent reviews, see Leffert and Koch, 1977; Holley, 1975). Limiting isoleucine in cultured CHO cells shuts down DNA synthesis very quickly while protein synthesis continues at a surprising rate even up to time of complete G_1 arrest (Enger and Tobey, 1972). The polysome profiles during G_1 arrest remain very similar to those in exponential cells, supporting the fact that there is enough protein synthesis occurring for cells to complete late interphase and enter G_1 but not enough to allow cells to enter S. We have now detected altered aminoacyl-tRNA synthetase complexes in such G_1-arrested cells. We have not yet determined whether the synthetase complex alterations are directly involved in maintaining the G_1 state or whether the complex alterations are a consequence of the G_1-arrested state. However, in either case, a role for the various synthetase complexes in metabolic changes related to G_1 arrest would definitely be indicated. What this role may be is largely speculative, but based on our results and observations from other laboratories one can suggest possible functional mechanisms.

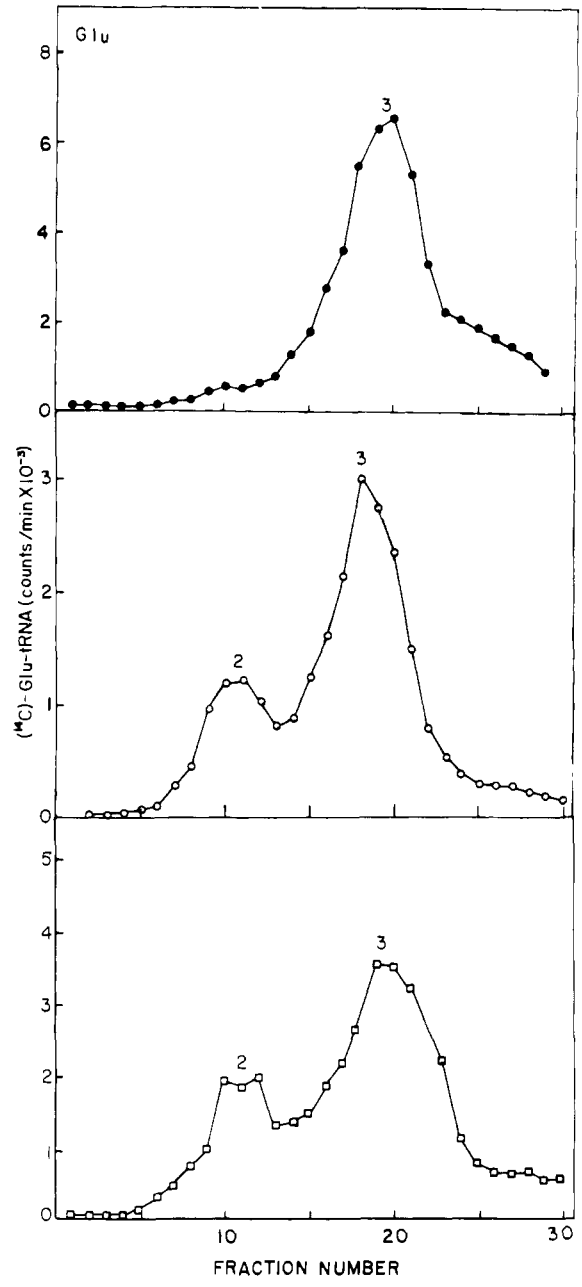

FIGURE 1: Size distribution of glutamyl-tRNA synthetase activity. Enzyme activity in the particulate postribosomal subcellular fraction was determined on 10-30% (w/v) logarithmic sucrose gradients for GluRS from exponentially growing cells (●), cells in G_1 arrest due to isoleucine deficiency (○), and cells in G_1 arrest due to leucine deficiency (□).

For some time it has been known that amino acids from the extracellular pool can be utilized directly in protein synthesis (Vidrich et al., 1977) without going through an intracellular pool. In addition, it has been shown that aminoacyl-tRNA synthetases can be directly involved in amino acid transport in bacteria (Quay et al., 1975) and in the regulation of amino acid transport in mammalian cells (Moore et al., 1977). Thus, the synthetases could easily regulate cellular levels of amino acids and aminoacyl-tRNAs whose levels, in turn, have a

dramatic effect on the regulation of translational initiation (Vaughn and Hansen, 1973) plus an effect on translational elongation as well. The availability of multiple forms of the aminoacyl-tRNA synthetases could facilitate regulation of such cellular events. By changing forms, the particulate synthetases could rapidly adapt to changing regulatory roles while continuing to carry out their classical function of aminoacylation of tRNA. Thus, the different forms of the enzyme may have different enzymatic or regulatory capabilities.

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Discrimination of DNA Polymerase and RNase H Activities in Reverse Transcriptase of Avian Myeloblastosis Virus

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ABSTRACT: The active sites in reverse transcriptase of avian myeloblastosis virus have been selectively modified by various chemical reagents. The DNA polymerase activity is very sensitive to hydrophobic sulfhydryl reagents such as 5,5′-dithiobis(2-nitrobenzoic acid) and *p*-hydroxymercuribenzoate but resistant to sulfhydryl reagents with hydrophilic properties. The RNase H activity, on the other hand, is resistant to both hydrophobic and hydrophilic sulfhydryl reagents, indicating the absence of cysteinyl residues essential for RNase H activity. *N*-Ethylmaleimide (NEM), an amino and sulfhydryl group specific reagent, inactivates both DNA polymerase and RNase H, the later activity being fourfold more stable. Polynucleotides, but not nucleotide triphosphates, protect the two enzymatic activities of reverse transcriptase against NEM. Since pretreatment of the enzyme with 5,5′-dithiobis(2-nitrobenzoic acid) does not prevent *N*-ethylmaleimide from reacting with a residue necessary for DNA polymerase activity, two different reactive groups are probably involved with this enzymatic activity. The pH profile of reverse transcriptase inhibition by *N*-ethylmaleimide also suggests the involvement of two reactive groups essential for the DNA polymerase activity with apparent pK_as of 5.5 and 6.5. Only one reactive group with a pK_a of 7.5 is found associated with the RNase H activity.

Reverse transcriptase (RNA-dependent DNA polymerase) of RNA tumor viruses exhibits three distinct measurable activities in vitro, namely, those of a DNA polymerase (Baltimore, 1970; Temin and Mizutani, 1970), of RNase H (Molling, et al., 1971), and of specific binding of tRNA, the primer for DNA synthesis (Panet et al., 1975). Reverse transcriptase of avian myeloblastosis virus (AMV) is a complex of two polypeptides with molecular weights 65 000 (α) and 105 000 (β) (Grandgenett et al., 1973), the α subunit being derived from the β subunit by proteolytic cleavage (Gibson and Verma, 1974). Both DNA polymerase and RNase H activities were shown to reside in the isolated α subunit (Grandgenett et al., 1973). However, the binding of the primer $tRNA^{Trp}$ could not be demonstrated with the purified α subunit (Grandgenett et al., 1976; Haseltine et al., 1977).

Although AMV reverse transcriptase has been extensively studied, only scanty information is available regarding the properties of its active sites. In addition, it is not clear to what extent these sites, which arise from a single gene product, overlap each other. In this study, we demonstrate that the

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