

The Eucaryotic Aminoacyl-tRNA Synthetase Complex: Suggestions for its Structure and Function

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ABSTRACT Aminoacyl-tRNA synthetases from eucaryotic cells generally are isolated as high molecular weight complexes comprised of multiple synthetase activities, and often containing other components as well. A model is proposed for the synthetase complex in which hydrophobic extensions on the proteins serve to maintain them in their high molecular weight form, but are not needed for catalytic activity. The structural similarity of these enzymes to certain membrane-bound proteins, and its implications for synthetase localization and function *in vivo*, are discussed.

Aminoacyl-tRNA synthetases are a group of enzymes present in all cells which catalyze the first step in protein biosynthesis, the attachment of an amino acid to its respective tRNA. Since the fate of an amino acid in protein synthesis is determined once it has been linked to tRNA, these enzymes play an important role in the overall specificity of amino acid incorporation into protein. Extensive study of aminoacyl-tRNA synthetases from various organisms has revealed that those isolated from higher eucaryotes differ in that they generally are found as high molecular weight complexes comprised of multiple synthetase activities, and often containing other components as well (1-3). These complexes are of interest not only because of what they may reveal about the associative properties of aminoacyl-tRNA synthetases *in vitro*, but more importantly because they may be representative of a supra-molecular organization for the whole protein synthetic machinery *in vivo*.

Considerable effort over the past decade has gone into studies that demonstrate the existence of synthetase complexes in extracts of many types of eucaryotic cells, and into attempts at purification and analysis of their components (1-3). However, relatively little is yet understood about the structure of these entities, and what their *in vivo* function might be. Moreover, studies from various laboratories have revealed substantial differences among the synthetase complexes isolated by different workers.

The purpose of this article is not to present an extensive review of the field, but to provide some suggestions as to the structure of the aminoacyl-tRNA synthetase complex, and to what might be the forces holding it together. These ideas lead to certain possibilities for the localization and functioning of synthetases *in vivo*. It is hoped that these thoughts might

provide a further stimulus to experiments to help clarify what is a potentially important area of research.

WHAT IS THE OVERALL STRUCTURE OF THE SYNTHETASE COMPLEX?

Since high molecular weight forms of aminoacyl-tRNA synthetases are the general rule in extracts of higher eucaryotes, there is no doubt that these enzymes have the capacity for either self-aggregation or heterotypic associations. Considerable evidence obtained from studies such as co-purification of synthetases (4-7), affinity chromatography (8), and immunoprecipitation (9) now support the view that the complexes contain multiple synthetase activities. The widespread occurrence of these multienzyme complexes raises questions of why synthetases have such a high degree of affinity for each other, what kinds of forces are involved in maintaining the complexes, and whether they serve any function or simply are *in vitro* artifacts. The latter possibility seems unattractive since there does not yet appear to be any reasonable explanation for why specific associations would occur among synthetase molecules in the presence of the many other proteins present in cell extracts. Rather, it is more likely that the specific interactions observed *in vitro* reflect some type of structural organization within the cell.

Clarification of the structure of the aminoacyl-tRNA synthetase complex has been a particularly vexing problem which has led to considerable confusion. In some laboratories it has been observed that essentially all of the aminoacyl-tRNA synthetases are found in high molecular weight forms (10-15). At a minimum, these findings indicate that most of the enzymes have the capacity to participate in complex forma-

tion, although as yet there is no direct evidence that all the synthetases are necessarily present in a single structure rather than in a spectrum of assemblies with overlapping activities.

In contrast, other workers have identified, and in some cases extensively purified, complexes containing only eight (16), seven (6, 17), five (4, 18, 19), or even two (5) aminoacyl-tRNA synthetases. The enzyme composition of these smaller, defined complexes (Table I) indicates a common relationship among them even though they were obtained from different sources. It is clear from the existence of these small, stable complexes that certain of the synthetases are very tightly associated with each other and can withstand extensive purification. Nevertheless, conditions have been found under which even these stable structures can break down further (16, 20), and Dang and Yang (16) have demonstrated a progressive breakdown of one of these complexes, originally containing eight synthetases, to generate the various smaller forms identified by others (4, 6, 17-19). These latter findings clearly demonstrate how complexes of seven, five, or two synthetases can be obtained from one containing eight of these enzymes, and they raise the important question of whether the complexes of seven or eight synthetases are, themselves, breakdown products of even larger structures. If that were so, it could reconcile very simply the disparate observations of many laboratories. However, it would also suggest that some synthetases are very easily dissociated from the larger structures, whereas others remain tightly associated. The explanations for such differences are not yet clear, but they would indicate that not all the synthetases in the complex are structurally equivalent.

One additional problem related to the structure of synthetases is that in many systems some of these enzymes may also be found in low molecular weight forms (21). A possible explanation that has been suggested by Waller and co-workers (7, 22) is that the smaller forms arise by proteolytic action. The possibility of uncontrolled proteolysis during preparation of extracts may be a serious problem, not only for generation of the low molecular weight forms of the synthetases but also for the apparent fragility of complexes containing a complete complement of synthetases. This may occur owing to direct action on the synthetases, or on a component necessary for maintaining the structural integrity of the complex. It is also possible that the presence of both high and low molecular weight forms of synthetases does have some physiological significance (21, 23).

TABLE I
Composition of Complexes Containing Less than a Full
Complement of Synthetases

Source	Synthetases present	Number	Reference
Rat liver	Arg, Glu, Gln, Ile, Leu, Lys, Met, Pro	8	16
Sheep liver	Arg, Glu, Gln, Ile, Leu, Lys, Met	7	6
Human placenta	Arg, Glu, Gln, Ile, Leu, Lys, Met	7	17
Rat liver	Gln, Ile, Leu, Lys, Met	5	18
Rabbit reticulo-cytes	Arg, Ile, Leu, Lys, Met	5	19
Rat liver	Arg, Ile, Leu, Lys, Met	5	4
Rat liver	Arg, Lys	2	5

HYDROPHOBIC DOMAINS ARE PRESENT ON SYNTHETASES AND PLAY A ROLE IN COMPLEX FORMATION

Although synthetases can associate with each other in stable assemblies, the forces involved in maintaining these structures are not well understood. However, several pieces of evidence have implicated hydrophobic interactions in the structural integrity of the complexes. For example, one procedure that has proven effective in dissociating the synthetase complex (16) and in separating free from complexed synthetases (5) is hydrophobic interaction chromatography. Since free synthetases are retained on these columns, whereas high molecular weight forms pass through (5), it is likely that dissociation exposes hydrophobic domains that normally serve to hold the complex together. Further evidence comes from studies testing the effects of various perturbing agents (20). Whereas high ionic strength has little effect on the complex, the presence of chaotropic salts or detergents affects both the structure and stability of aminoacyl-tRNA synthetases (20).

It has also been shown that certain purified synthetases have the ability to self-aggregate or to associate with other synthetases. Thus, tryptophanyl-tRNA synthetase polymerizes into rod-like particles which can be visualized by electron microscopy (24), and glycyl-tRNA synthetase tends to bind to other synthetases (25). Aspartyl-tRNA synthetase also self-aggregates, but this can be prevented by ammonium sulfate or the nonionic detergent octylglucoside (26). The conclusion from the latter study was that the enzyme contains hydrophobic domains that are involved in its self-aggregation properties. Lysyl-tRNA synthetase has been shown to exist in two forms, one native and one proteolyzed, but only the native form has a tendency to aggregate, suggesting that the protein fragment removed by proteolysis may be responsible for the aggregation phenomenon (27).

One other possible feature of aminoacyl-tRNA synthetase complexes that deserves mention is that they contain lipids. A number of reports have appeared showing that lipids co-elute with the synthetase complex (11, 28, 29), but it has not been proven that the lipids actually are associated with synthetases rather than with contaminating proteins. More convincing is the suggestion that a homogeneous Lys-Arg complex contains glycolipid (30). Of most significance are the few functional studies that show that delipidation of individual synthetases (27, 31) or the synthetase complex (20) leads to loss of activity or decreased stability of the enzymes, which can be reversed or prevented by the re-addition of lipids. The implication of these findings is that lipids play a role in the structure and/or stability of this group of enzymes. However, further work is required to substantiate this point.

PROTEOLYSIS OF THE SYNTHETASE COMPLEX RELEASES ACTIVE ENZYMES

Studies of purified bacterial (32, 33), yeast (34), or mammalian (35, 36) aminoacyl-tRNA synthetases have shown that limited proteolysis can yield active enzymes from which a significant fraction of the protein has been removed. These data indicate that part of the structure of aminoacyl-tRNA synthetases may be involved in functions other than catalytic activity. This point has recently been clearly demonstrated in work with the *Escherichia coli* alanyl-tRNA synthetase (37) which showed that the C-terminal half of the protein was not

needed for catalytic activity but for maintaining the protein in its tetrameric form for its function in gene regulation (38).

A similar situation seems to be operative for the mammalian aminoacyl-tRNA synthetase complex. Waller and co-workers (39, 40) have demonstrated that controlled proteolysis of the sheep liver complex with trypsin or elastase leads to the release of low molecular weight forms of methionyl- and lysyl-tRNA synthetase, respectively. The molecular weights of the released enzymes are substantially lower than when these enzymes are present in the complex, indicating that proteolysis removes a significant portion of the proteins. Likewise, in our laboratory we have shown that papain treatment of the rat liver synthetase complex leads to the formation of low molecular weight forms of arginyl- and leucyl-tRNA synthetase (R. K. Sihag and M. P. Deutscher, unpublished observations). Of most interest is the fact that in all cases the released enzymes are fully active, which indicates that the fragment removed by proteolysis is essential not for catalytic activity but only for maintaining the synthetase in a high molecular weight structure.

A MODEL FOR THE AMINOACYL-tRNA SYNTHETASE COMPLEX IN VITRO

On the basis of the differences in molecular weights between eucaryotic and procaryotic synthetases, it has been suggested that in addition to their catalytic domain, mammalian synthetases have another domain that participates in complex formation (40). A reasonable extension of this idea is that this additional region, which can be removed by proteolysis, represents the hydrophobic domain present on synthetases. Thus, the aminoacyl-tRNA synthetase complex, as isolated in vitro, would contain multiple synthetases whose catalytic centers are exposed to the environment, but which are held together by a hydrophobic core (Fig. 1). This core would be made up of the hydrophobic extensions present on the individual proteins, and may also contain lipids or other components, acting as a "glue." However, if lipids are present in the core, they are not absolutely essential to the structure of the complex; delipidation with detergents, although it destabilizes synthetase activities, does not necessarily lead to breakdown of the complex (20). Most likely, interactions among the hydrophobic regions of the proteins themselves are sufficient to maintain the synthetases in a high molecular weight form, although the increased lability of the enzymes suggests some structural change has occurred.

One attractive feature of this model is that it is easily testable. Structural comparisons of aminoacyl-tRNA synthetases isolated directly from the complex with those released by proteolysis would reveal whether the former contain an additional hydrophobic extension. We have already observed that arginyl-tRNA synthetase isolated from the complex by detergents and chaotropic salts has a tendency to aggregate and is quite unstable (R. K. Sihag, G. J. Vellekamp, and M. P. Deutscher, unpublished observations), whereas the low molecular weight form previously purified in our laboratory (21) displays none of these properties. Analogous results were obtained by Dimitrijevic and Godefroy-Colburn (27) in their studies of a native and proteolyzed form of yeast lysyl-tRNA synthetase.

The proposed model for the complex also is of interest because the attachment of synthetases to this assembly would

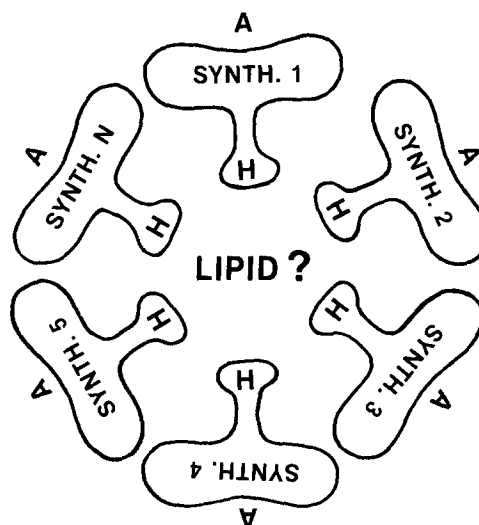


FIGURE 1 Schematic representation of the aminoacyl-tRNA synthetase complex isolated in vitro. Depicted is a cross section through the synthetase complex showing a hydrophobic interior made up of the hydrophobic extensions (*H*) of the individual synthetases, and possibly containing lipids. The hydrophilic portions of the individual synthetases containing the active site (*A*) are exposed to the environment.

be similar to the association of a number of proteins with membranes. Proteins such as cytochrome *b*₅ (41), cytochrome *b*₅ reductase (42), γ -glutamyltranspeptidase (43), and cytochrome P-450 reductase (44), as well as others (45, 46), contain terminal hydrophobic regions that anchor these proteins in membranes, but have catalytic centers present within a hydrophilic domain that can be released from the membrane as a soluble protein by proteolytic treatment. Treatment of the membranes with detergents releases amphipathic molecules displaying the aggregative properties of typical membrane proteins.

IMPLICATIONS FOR THE IN VIVO LOCALIZATION AND FUNCTION OF AMINOACYL-tRNA SYNTHETASES

The apparent similarity between aminoacyl-tRNA synthetases and certain membrane-bound proteins raises a number of interesting points regarding the disposition of aminoacyl-tRNA synthetases in vivo. The presence of hydrophobic domains was an unexpected development, since these enzymes must interact with highly charged substrates, and implies that these regions may serve a function other than catalysis in vivo. One intriguing possibility is that at least a portion of the aminoacyl-tRNA synthetase population may be membrane associated within the cell. This suggestion is not unreasonable in light of the large body of evidence that extracellular amino acids can be direct precursors for protein synthesis without mixing with the intracellular amino acid pool (47-52). One way this could occur is if aminoacylation of tRNA can take place on or near the plasma membrane (48-53). In addition, it has been shown that aminoacyl-tRNA synthetases can influence amino acid transport in mammalian cells (54, 55).

It is also clear that some fraction of aminoacyl-tRNA derives from the intracellular amino acid pool (47-52), and presumably would not depend on plasma membrane-associated synthetases. A model has been proposed by Pahuski et

al. (23) in which the high molecular weight forms of aminoacyl-tRNA synthetases would use amino acids from the extracellular pool while the low molecular weight forms would derive amino acids from the intracellular pool. An alternative possibility is that amino acids from the intracellular pool are activated by synthetases associated with the endoplasmic reticulum membrane. This suggestion is compatible with the recent observation of Dang et al. (56) that methionyl-tRNA synthetase is present on the endoplasmic reticulum membrane. It would also agree with the findings of Dimitrijevic (57) that yeast lysyl-tRNA synthetase fractionates with cytoplasmic and endoplasmic reticulum membranes. Another possibility that should also be considered is that the aminoacyl-tRNA synthetases, as well as the whole protein-synthesizing apparatus, is associated with the cytoskeletal framework of the cell (58, 59). Nevertheless, whatever the disposition of aminoacyl-tRNA synthetases within the cell might be, the available evidence on amino acid precursor pools for protein synthesis suggests the likelihood of two populations of synthetases, one for exogenous amino acids and one for the intracellular pool. In fact, a recent paper by Tanaka and Ichihara (60) also demonstrates that the two amino acid compartments are used for the synthesis of different proteins.

A final point that needs to be considered is the relation of the synthetase complex as isolated in vitro to synthetases in vivo. If, as suggested above, synthetases are associated with membranes in vivo, this association must be relatively weak since these enzymes do not fractionate as typical membrane proteins. Therefore, it is probable that the complex obtained in vitro represents at least a partially rearranged structure compared with the in vivo entity. If the synthetase complex functions in vivo to increase the efficiency of protein synthesis, it might also be expected that synthetases would be associated with other components of the protein-synthesizing machinery to enhance the transfer of aminoacyl-tRNA from synthetase to elongation factor to ribosome, and back again. These ideas would suggest the possibility of a much larger protein-synthesizing structure within the cell, which does not survive homogenization and fractionation. It is now well known that many enzymes and pathways previously regarded as "soluble" within the cell are in fact organized into multi-enzyme aggregates or associated with subcellular structures, although with various degrees of stability (61). It is not unreasonable to consider the protein-synthesizing apparatus in the same light.

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