Perturbation of the Aminoacyl-tRNA Synthetase Complex by Salts and Detergents

IMPORTANCE OF HYDROPHOBIC INTERACTIONS AND POSSIBLE INVOLVEMENT OF LIPIDS*

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Ram K. Sihag and Murray P. Deutscher
From the Department of Biochemistry, University of Connecticut Health Center, Farmington, Connecticut 06032

In order to gain some insight into the structural parameters important for aminoacyl-tRNA synthetase complex formation, we have examined the effect of various salts and detergents on the stability and structure of the synthetase complex. Certain neutral salts were found to inactivate aminoacyl-tRNA synthetase activities in the complex, and the order of effectiveness in this process followed a classical Hofmeister series. In addition, one of these salts, NaSCN, was also effective in partially dissociating the complex. Detergents varied in their ability to inactivate synthetases, with ionic detergents being most effective and nonionic detergents being much less destructive. Detergents, by themselves, could partially disrupt the complex; however, in the presence of 1 M NaCl, nonionic detergents did lead to considerable dissociation of synthetases and generation of low molecular weight forms of these enzymes. Removal of lipids from the complex with the nonionic detergent, Triton X-114, rendered arginyl-tRNA synthetase sensitive to the addition of NaCl. However, this salt sensitivity was abolished by read-}

dition of a lipid extract isolated from the complex. These results implicate hydrophobic interactions in the stability of the synthetase complex, and suggest the possible involvement of lipids in maintaining its structural integrity.

Aminoacyl-tRNA synthetases isolated from eukaryotic cells generally are obtained as high molecular weight complexes containing multiple synthetase activities (reviewed in Refs. 1 and 2). Although these findings presumably are important for our understanding of the structural organization of the protein synthetic machinery in vivo, there has been relatively little increase in our knowledge of these complexes since their initial description about ten years ago (3–6). Particularly, almost nothing is known about how the individual synthetases are associated with each other, and whether there might be some "glue" which holds them together. Various molecules, such as lipids (7, 8), carbohydrates (9, 10), and RNAs (3, 8, 11) have been suggested as playing a role in aminoacyl-tRNA synthetase structure, but it is not known whether any of these components actually are involved in the associations among synthetase molecules.

As a first step toward gaining some insight into the factors responsible for aminoacyl-tRNA synthetase complex formation, we have examined the effect of various known pertur-bative agents on the activity and structure of these high molecular weight complexes. The agents used included different neutral salts with varying degrees of effectiveness in disrupting protein structure (12), various types of detergents, or combinations of the two. Our results indicate that even though the isolated aminoacyl-tRNA synthetase complex is a relatively stable entity, it can be partially or totally dissociated by certain treatments. Furthermore, evidence is presented which implicates lipids in the stability and structure of this complex. These studies help to define the types of interactions that are important for maintaining the functional and structural integrity of this complicated structure. In addition, this information should be useful for devising methods to efficiently dissociate the complex as a prerequisite to purifying and studying its individual components.

EXPERIMENTAL PROCEDURES

Materials—Hydroxylapatite (Bio-Gel HT) was obtained from Bio-Rad Laboratories and Sephacryl S-300 from Pharmacia. Amino acids, DTT, 1 PMSF, protein standards, NaDodSO4, Triton X-114, Tween 80, Lubrol PX, and polyoxyethylene W1 were purchased from Sigma Chemical Co. Radioactive amino acids were obtained from either New England Nuclear or ICN. Sodium cholate and sodium deoxycholate were from Calbiochem. CHAPS was obtained from Polyscience Corp. and NP-40 from Particle Data Laboratories. Rabbit liver tRNA was prepared as described previously (13). Bio-Beads SM-2 were purchased from Bio-Rad.

Animals—Female Sprague-Dawley rats (150–200 g each) were obtained from Charles River Laboratories.

Enzyme Assays—Aminoacyl-tRNA synthetases were assayed as described previously (14) except that 1.5 mg/ml of tRNA and 0.1 mM C-labeled amino acid were generally used. Recoveries of synthetase activity after chromatography were compared to samples assayed immediately prior to loading on the column.

Preparation of the Aminoacyl-tRNA Synthetase Complex—Fifteen rats were used in a typical preparation. The high speed supernatant fraction and the hydroxylapatite peak II were prepared as described earlier (14), except that the concentrations of DTT, EDTA, and PMSF were increased to 0.2 mM. The hydroxylapatite peak II, which contained the synthetase complex, was pooled and concentrated with an Amicon PM-30 membrane, and then dialyzed against a solution containing 10 mM sodium phosphate, pH 7.4, 0.2 mM DTT, 0.2 mM EDTA, 0.5 mM PMSF, 10% glycerol, 0.1 M NaCl (Buffer l). The concentrated material was applied in 8 ml fractions to a column of Sephacryl S-300 (2.5 x 96 cm) equilibrated with the same solution used for dialysis. Arginyl-, lysyl-, and glutamyl-tRNA synthetase activities were generally measured and eluted in the void volume. This region was pooled and concentrated with an Amicon YM-10 or YM-10 membrane, and the concentrated material stored frozen in aliquots at –20 °C. These samples, containing 1.0 to 1.2 mg of protein/ml, were used as the starting material for the experiments described

1 The abbreviations used are: DTT, dithiothreitol; PMSF, phenyl-methylsulfonyl fluoride; NaDodSO4, sodium dodecyl sulfate; EDTA, ethylenediaminetetraacetic acid; CHAPS, 3(3-cholamidopropyl)-dimethylammonio]-propane sulfonate; NP-40, PEG (polyethylene glycol) 6000-10.

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here. Samples stored in this manner retained full activity for at least 2 months. Protein was determined by the method of Bradford (15).

Delipidation of the Aminoacyl-tRNA Synthetase Complex—The concentrated synthetase complex, purified on Sephacryl S-300, was made 1% in Triton X-114 by addition of a 10% solution of the detergent. After incubating in ice for 30 min, the temperature was raised to 37 °C for 1 min to allow clouding of the Triton (16). The detergent was removed by centrifugation for 2 min at 20 °C in an Eppendorf Zentrifuge 3200. The upper aqueous layer was removed and the detergent treatment repeated with 0.5% Triton X-114. After incubating in ice for 30 min, the temperature was raised to 37 °C for 1 min to allow clouding of the Triton (16). The detergent phase with the aqueous layer. Ultraviolet absorption measurements at 276 nm of the delipidated sample indicated that, at most, the detergent treatment repeated with 0.5% Triton X-114. After incubating in ice for 30 min, the temperature was raised to 37 °C for 1 min to allow clouding of the Triton (16). The detergent phase with the aqueous layer. Ultraviolet absorption measurements at 276 nm of the delipidated sample indicated that, at most, about 0.05% Triton X-114 remained with the sample. Thin layer chromatography of a lipid extract of the delipitated sample revealed that all the detectable lipid had been removed. Delipidation of the low molecular weight form of arginyl-tRNA synthetase was carried out by the same procedure. For the experiment presented in Table II, residual detergent was removed from the delipidated sample by passage through a column of Bio-Beads SM-2 (0.6 × 1.5 cm) equilibrated in Buffer 1 lacking NaCl.

Preparation of a Lipid Extract of the Aminoacyl-tRNA Synthetase Complex—The concentrated aminoacyl-tRNA synthetase complex was extracted at room temperature with 5 volumes of CHCl₃:CH₃OH (2:1). The CHCl₃ phase was washed 2 times with an equal volume of 50% aqueous CH₃OH containing 0.1 M NaCl. After drying, the sample was redissolved and stored in a known volume of CHCl₃. Prior to use for reconstitution experiments, an aliquot of the lipid extract was dried and dispersed by sonication for 2 min in one-fifth the original volume of 10 mM NaK phosphate, pH 7.2-0.15 M NaCl.

RESULTS

Properties of the Complex—The aminoacyl-tRNA synthetase complex used in these studies, prepared as described under “Experimental Procedures,” was purified 20- to 30-fold from the high speed supernatant fraction based on the increase in specific activity of several of the synthetase activities. The apparent molecular weight of this preparation was 1.0-1.1 × 10⁶ determined from its elution position on Sephacryl S-300 (Fig. 1). Activity for 14 amino acids was present in the high molecular weight fraction eluted from Sephacryl S-300, and for at least 10 amino acids, synthetase activities coeluted from the column (activities for 4 amino acid are shown in Fig. 1). These included the activities for arginine, lysine, leucine, isoleucine, glutamic acid, glutamine, methionine, tyrosine, valine, and glycine. Thus, this complex contained the synthetases generally found to be associated, and presumably most stably bound (2). In the experiments described here, perturbation of the complex was evaluated by determination of synthetase activity for arginine, leucine, glutamic acid, and in some cases, lysine. Structural changes were evaluated by chromatography on Sephacryl S-300, and assay of synthetase activities.

Effect of Neutral Salts on the Synthetase Complex—Certain neutral salts are known to alter associations within and among protein molecules over and above their nonspecific effects on electrostatic interactions (12). In order to determine the importance of these other types of interactions in the synthetase complex, we first examined the influence of various salts, known to affect protein structure differentially, on aminoacyl-tRNA synthetase activities in the high molecular weight complex. The data presented in Fig. 2 show that chloride, iodide, and thiocyanate ions have markedly different effects on the stability of the aminoacyl-tRNA synthetases. With all three synthetases tested (arginyl-, leucyl-, and glutamyl-tRNA synthesis activities follow a classical Hofmeister series (12).

In order to assess the effect of neutral salts on the overall structure of the synthetase complex, samples were subjected to gel filtration on Sephacryl S-300 in the presence of the salt under study. Compared to the control sample run in 0.1 M NaCl (Fig. 1), no significant change in the elution position of arginyl-, glutamyl- or leucyl-tRNA synthetase was observed in 1.0 M NaCl (data not shown), although there was a slight broadening of the peak for leucyl-tRNA synthetase. In contrast, chromatography of the synthetase complex in 0.5 M NaSCN led to a pronounced shift of leucyl-tRNA synthetase

FIG. 1. Gel filtration of the aminoacyl-tRNA synthetase complex in low salt. A 2-ml sample of the synthetase complex was loaded on a Sephacryl S-300 column (1.5 × 92 cm) equilibrated with buffer 1. Fractions of 2.0 ml were collected and 10 μl aliquots were assayed for synthetase activities for 5 min at 37 °C. Recovery of synthetase activity was as follows: arginine ( ), lysine ( ), 95%; leucine ( ), 85%; glutamic acid ( ), 78%. The elution positions of the standard proteins, thyroglobulin ( ), apoferritin ( ), and catalase ( ) are noted by arrows in this and subsequent figures.

FIG. 2. Effect of salts on the stability of the aminoacyl-tRNA synthetase complex. The synthetase complex was diluted with buffer 1 containing no salt and reconstituted to give a final NaCl concentration of 30 mM. Aliquots of 40 μl were adjusted to the indicated concentration of the various salts and to a volume of 50 μl. Samples were divided into two and incubated either at 4 °C for 18 h (A) or at 37 °C for 10 min (B). Aliquots of 2 to 5 μl were assayed for arginyl- ( ), glutamyl- ( ), or leucyl-tRNA synthetase ( ) activity. NaCl (--), KI (--), NaSCN (--).
activity, although arginyl- and glutamyl-tRNA synthetase activities were apparently unaffected (Fig. 3). The very low recovery of lysyl-tRNA synthetase in the presence of NaSCN precluded determination of its elution position. The differential effects of the various salts on both the structure and activity of the synthetase complex draw attention to other than electrostatic interactions in maintaining the integrity of the complex.

Effect of Detergents on the Synthetase Complex — The response of the synthetase complex to the chaotropic salts described above, as well as earlier studies indicating the presence of lipids in the complex (7, 8), suggested that hydrophobic interactions might be playing a role in the organization of this structure. In order to explore this possibility further, we have tested a variety of detergents for their effect on synthetase activities in the high molecular weight complex.

Incubation of the synthetase complex with the ionic detergent, deoxycholate, either at 4 °C for 16 h or at 37 °C for 10 min, led to a dramatic loss of synthetase activity (Fig. 4A).

Thus, a concentration of 2.5 mM deoxycholate was sufficient to totally inactivate arginyl-, glutamyl-, and leucyl-tRNA synthetases at 37 °C, as well as two of these enzymes at 4 °C. Likewise, sodium dodecyl sulfate at a concentration of 0.9 mM, completely inactivated arginyl-tRNA synthetase at 4 °C (data not shown). However, in contrast to the latter two detergents, sodium cholate at 4.8 mM concentration had no effect on arginyl-tRNA synthetase activity during an 18-h incubation at 4 °C (data not shown).

The zwitterionic detergent, CHAPS, also affected the stability of aminoacyl-tRNA synthetases, but it was considerably less destructive than deoxycholate (Fig. 4B). Upon incubation at 4 °C, concentrations of CHAPS as high as 20 mM had no effect on synthetase activities; however, upon incubation at 37 °C for 10 min, 20 mM CHAPS led to almost complete inactivation.

Nonionic detergents were even more innocuous to aminoacyl-tRNA synthetase activities. For example, NP-40, at concentrations as high as 4% (approximately 60 mM), had essentially no effect on glutamyl-tRNA synthetase at either 4 or 37 °C, slightly activated leucyl-tRNA synthetase at both temperatures, and partially inactivated arginyl-tRNA synthetase, but only at 37 °C (Fig. 4C). However, the latter activity also could be completely stabilized at 37 °C if arginine and ATP were present during the incubation. Essentially identical results were obtained with each of these synthetases with a second nonionic detergent, Triton X-114 (data not shown). Likewise, the other nonionic detergents, Tween 80, Lubrol PX, and polyoxyethylene W1 at concentrations of about 1% had no effect on arginyl-tRNA synthetase during an 18-h incubation at 4 °C.

Gel filtration of the aminoacyl-tRNA synthetase complex in the presence of detergents revealed that some dissociation had occurred in certain instances. For example, chromatography in 10 mM CHAPS led to partial breakdown of arginyl- and lysyl-tRNA synthetases to lower molecular weight forms (data not shown).

Fig. 4. Effect of detergents on the stability of the aminoacyl-tRNA synthetase complex. Samples were prepared as described in Fig. 2 with the indicated concentrations of deoxycholate (A), CHAPS (B), or NP-40 (C) and incubated for either 18 h at 4 °C (closed symbols) or 10 min at 37 °C (open symbols). Aliquots were assayed for arginyl- (●), glutamyl- (■), or leucyl-tRNA synthetase (▲, △) activity.
that detergent-sensitive substances may play a role in the structural integrity of the aminoacyl-tRNA synthetase complex. 

**Effect of Detergents Plus Salt on the Synthetase Complex**—
Although 1 M NaCl had no effect on the size or stability of the aminoacyl-tRNA synthetase complex when present alone, inclusion of this concentration of salt together with the nonionic detergent, NP-40, led to a slightly increased inactivation of glutamyl- and leucyl-tRNA synthetases, particularly at 37 °C (data not shown). Arginyl-tRNA synthetase was extremely sensitive to treatment with salt plus detergent, but as with detergent alone, it could be protected with arginine and ATP, completely at 4 °C and partially at 37 °C. Of more interest was the finding that treatment with NP-40 plus 1 M NaCl led to considerable breakdown of the aminoacyl-tRNA synthetase complex (Fig. 6). In the case of leucyl- and lysyl-tRNA synthetases, activity was found in the region expected for the monomeric form of these enzymes (18). For arginyl-tRNA synthetase, a lower molecular weight form also was observed, but this form still was larger than the free enzyme (14). We suspect that for this enzyme, as well as for glutamyl-tRNA synthetase, the free enzymes are quite unstable under these conditions. In fact, storage overnight of the column fractions also led to considerable loss of activity of the free forms of leucyl- and lysyl-tRNA synthetases. Although some stability problems still remain, these studies indicate that nonionic detergents plus salt are effective for releasing certain aminoacyl-tRNA synthetases from the complex in an active form.

**Possible Involvement of Lipids in the Synthetase Complex**—
The foregoing results strongly implicated hydrophobic interactions in the stability and structure of the high molecular weight synthetase complex. In order to determine whether lipids were involved in these interactions, the complex was treated with the easily removable detergent, Triton X-114 (16). Delipidation with Triton X-114 rendered arginyl-tRNA synthetase activity in the complex extremely sensitive to the addition of NaCl, whereas the untreated complex was unaffected (Table I). Furthermore, treatment of the low molecular weight form of arginyl-tRNA synthetase with Triton X-114 was shown to have a smaller effect on the NaCl sensitivity of this form of the enzyme (Table I). These results suggest that

![Fig. 5. Gel filtration of the aminoacyl-tRNA synthetase complex in NP-40. Chromatography and assays were carried out as described in Fig. 1 except that the sample was made 4% in NP-40 and the column buffer also contained 4% NP-40. Recovery of synthetase activity was as follows: arginine (O), 73%; lysine ( ), 60%; leucine ( ), 83%; glutamic acid ( ), 103%.](image)

![Fig. 6. Gel filtration of the aminoacyl-tRNA synthetase complex in NP-40 plus salt. Chromatography and assays were carried out as in Fig. 5 except that 1 M NaCl was also present in the sample and in the column buffer. Arginine (50 μM) and ATP (200 μM) were also added to the column buffer to help stabilize aminoacyl-tRNA synthetase activity. Recovery of synthetase activity was as follows: arginine ( ), 51%; lysine ( ), 43%; leucine ( ), 59%; glutamic acid ( ), 35%. All recoveries have been corrected for the presence of 0.1 M NaCl in the assays.](image)

**Table I**

| Additions | Synthetase complex | Low molecular weight enzyme |
|-----------|--------------------|----------------------------|
| Control   | Delipidated        | Control                    | Delipidated |
| None      | 100 (80)           | 100 (87)                  |
| 0.6 M NaCl| 109                | 62                        | 98          | 78          |
| 1.0 M NaCl| 100                | 12                        | 97          | 56          |

**Table II**

| Additions | Arginyl-tRNA synthetase activity |
|-----------|---------------------------------|
| None      | Untreated                       |
| 1 M NaCl  | Delipidated                     |
| 0.025% Triton X-114 + 1 μl lipid extract | 74 77 |
| 0.025% Triton X-114 + 3 μl lipid extract | 74 57 |
| 0.025% Triton X-114 + 5 μl lipid extract | 84 53 |
| 0.025% Triton X-114 + 1 M NaCl | 85 9 |
| 0.025% Triton X-114 + 1 M NaCl + 1 μl lipid extract | 98 76 |
| 0.025% Triton X-114 + 1 M NaCl + 3 μl lipid extract | 106 96 |
| 0.025% Triton X-114 + 1 M NaCl + 5 μl lipid extract | 103 113 |
the NaCl sensitivity following delipidation is, to some extent, a property of arginyl-tRNA synthetase in the complex rather than of arginyl-tRNA synthetase itself.

That the NaCl sensitivity after detergent treatment was actually due to lipid removal was shown by adding lipid back to the delipidated sample (Table II). Reactidation of increasing amounts of a lipid extract from the synthetase complex to the detergent-treated material almost completely protected delipidated arginyl-tRNA synthetase against its usual inactivation by low concentrations of detergent and 1 M NaCl (Table II). These results demonstrate that the stability of arginyl-tRNA synthetase is influenced by lipids.

DISCUSSION

During the past ten years, considerable effort has gone into studies confirming the existence of aminoacyl-tRNA synthetase complexes in extracts of many different eukaryotic cells, and into attempts at purification and identification of the component synthetases (see Refs. 1 and 2 for references). However, almost no information has been obtained about the structural organization of the complex or of the factors responsible for the association among this group of proteins. It has been suggested that the complex is heterotypic and that it can be disassembled in a specific fashion (19), but the molecular basis for the interactions among synthetases is not yet understood.

Our goal in this initial study was to begin to define the forces involved in holding the synthetases together by determining which agents could affect the stability and structure of the complex. From a purely practical point of view, this information was also necessary for developing procedures to purify individual components from the synthetase complex. The effects of chaotrope salts and of detergents observed in this study strongly implicate hydrophobic interactions in maintaining the structural organization of the complex. In addition, studies with arginyl-tRNA synthetase suggest that lipids are probably involved in these hydrophobic interactions. However, at the present time, we cannot distinguish whether there are also hydrophobic interactions between the synthetase proteins themselves, or whether there is a core of lipids which acts as a glue for holding the various proteins together. Further work is necessary to identify the lipid(s) involved and to prove that the effects are not due to removal of some other detergent-extractable component. It is interesting that the one other procedure that has been found effective in, at least partly, dissociating the aminoacyl-tRNA synthetase complex is hydrophobic interaction chromatography (18, 19). It is likely that in this procedure hydrophobic interactions between the synthetases and the column matrix are sufficient to break other hydrophobic interactions which normally serve to hold the complex together.

Previous studies from this laboratory and others have shown that lipids are associated with the aminoacyl-tRNA synthetase complex (7, 8), and that they can affect the activity of this class of enzymes (20, 21). In addition, lipids have also been implicated in the structure of protein synthesis initiation and elongation factors (22), and in the overall process of protein synthesis itself (23). At this point, one can only guess what may be the role of lipids in the protein synthetic machinery; however, it is clear that this area deserves further attention.

One other feature of these studies that deserves mentioning is the apparent instability of the synthetases once they are isolated from the complex. This problem has complicated the work because it has made it impossible in certain instances to determine whether the lack of a low molecular weight form of a synthetase is due to lack of dissociation of the complex or inactivation of the synthetase after dissociation. Other workers have also noted that synthetases derived from the complex are unstable (19), and that synthetases help to stabilize each other (24). Perhaps, the loss of certain hydrophobic interactions upon disruption of the complex leads to exposure of regions which destabilize the individual proteins in aqueous environments. Studies are in progress to determine whether addition of lipids or low concentrations of detergents might stabilize these proteins.

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