Stoichiometry and composition of an aminoacyl-tRNA synthetase complex from rat liver
(subunit structure/heterotypic multi-enzyme complex/active site titration)

DEBORAH L. JOHNSON AND DAVID C. H. YANG*

Department of Chemistry, Georgetown University, Washington, D. C. 20057

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ABSTRACT The particulate aminoacyl-tRNA synthetases of rat liver were copurified about 1000-fold with more than 20% yields for individual synthetase activities. Measurements of aminoacylation activities showed that lysyl-, arginyl-, leucyl-, isoleucyl-, and methionyl-tRNA synthetases in the purified complex co-sedimented at 18 S. The molecular weight of the synthetase complex is about one million, as estimated by gel filtration. The stoichiometry of the synthetase in the complex was determined by active site titration with aminoacyl adenylates. Results indicate that the 18S synthetase complex contains one subunit of methionyl-tRNA synthetase and two subunits of lysyl-tRNA synthetase. Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate showed that the 18S synthetase complex contains eight major protein bands. Proteins with subunit molecular weights of 104,000, 92,000, 69,000, and 67,000 are present in molar ratios of 1:1:2:2, while proteins with subunit molecular weights of 160,000, 150,000, and 135,000 are present in stoichiometric amounts. These results suggest that the particulate aminoacyl-tRNA synthetases exist as a heterotypic multienzyme complex with defined structure.

Aminoacyl-tRNA synthetases play the essential role of aminoacylating specific tRNAs during protein biosynthesis (1). Several aminoacyl-tRNA synthetases from higher organisms have been found consistently to occur in high molecular weight (2-5) and multiple forms (5, 6). We have only a sketchy knowledge of the structure of these particulate synthetases. A physical association of these synthetases has been suggested by their copurification during conventional (7-9) as well as affinity chromatographic procedures (9, 10), and by their sequential dissociation (5). The structure of these synthetases is of interest not only from the viewpoint of understanding the mammalian protein biosynthesis machinery but also as a model system to study protein assembly beyond quaternary structure. The association of enzymes that catalyze parallel reactions, such as aminoacyl-tRNA synthetases, may have a unique yet unknown functional significance—comparable to that of the multienzyme complexes in metabolic pathways (11). However, the association of the particulate synthetases as heterotypic multienzyme complexes has not been demonstrated by direct chemical methods. After dissociation of the particulate synthetases, the subunit structure of free lysyl-tRNA synthetase from rat liver has been determined (12). Recent advances in the purification of the particulate synthetases (9) further facilitated the structural analysis. In this communication, we report the chemical composition and the stoichiometry of an 18S synthetase complex from rat liver.

MATERIALS AND METHODS

Aminoacyl-tRNA synthetases were prepared from 300- to 400-g fresh livers of 6- to 9-month-old Sprague-Dawley fed male rats (Holtzman Albino, Madison, WI) by the procedure of Kellermann et al. (9). tRNA-Sepharose was synthesized according to Remy et al. (13) with unfractionated Escherichia coli tRNA. Analytical methods and the assay of the synthetase activities were carried out as described (5, 12). The purified synthetases had specific activities for lysyl-, arginyl-, methionyl-, leucyl-, isoleucyl-, and glutaminyl-tRNA synthetases of 82, 93, 35, 19, 15, and 7 units/mg, respectively.

NaDodSO4/polyacrylamide gel electrophoresis was carried out according to Laemmli (14). Polyacrylamide gel electrophoresis under non-denaturating conditions was carried out on 2-16% gradient gels for 48 hr at 400 V and 4°C (15). Protein bands were stained with Coomassie brilliant blue R-250 (16) or fast green FCF (17) for at least 10 hr at 25°C before destaining. For quantitative densitometry, the stained gels were scanned at 595 nm for Coomassie brilliant blue or at 610 nm for fast green, using a Gilford 2520 gel scanner. Carbohydrates were stained by incubating gels with 1% sodium metaperiodate, followed by staining with basic fuchsin (18).

Determinations of enzyme-bound aminoacyl adenylate was carried out according to Yarus and Berg (19) with modifications. The incubation mixtures contained, in a total volume of 50 μl, 144 mM Tris-HCl (pH 7.8 at 25°C), 10 mM MgCl2, 10 mM 2-mercaptoethanol, 2.5 mM or no ATP, 14C-labeled amino acid, and 10-20 pmol of the purified synthetase. After incubation at 25°C for 2.5 min, a 40-μl aliquot of the mixture was immediately filtered through a prewashed nitrocellulose filter (Schleicher & Schuell BA85) and washed with 3 ml of buffer containing 130 mM [bis(2-hydroxyethyl)amino]tris(hydroxy-methyl)methane-HCl (pH 5.87 at 25°C). The filter was dried and radioactivity was determined. All assays, including controls, were done in triplicate.

RESULTS

Purification of the Particulate Synthetases. The particulate synthetases, including lysyl-, arginyl-, leucyl-, isoleucyl-, methionyl-, and glutaminyl-tRNA synthetases, were purified from rat liver about 1000-fold with more than 20% yields for the individual aminoacylation activities. During the final column chromatography on tRNA-Sepharose, the synthetases were coeluted as two partially resolved peaks. The relationship between the two activity peaks is at present not understood (9). The second activity peak had higher specific activities for all six synthetases. Fractions in the second peak, which contained eight major protein bands, as analyzed by NaDodSO4/polyacrylamide gel electrophoresis, were used for examination of the molecular properties.

Composition of the Synthetase Complex. As shown in Fig. 1, the aminoacyl-tRNA synthetases for lysine, arginine, leucine, isoleucine, and methionine cosedimented at 18 S in sucrose gradients. A small amount (about 3% of that of lysyl-tRNA

* To whom reprint requests should be addressed.
synthetase) of glutaminyl-tRNA synthetase also cosedimented at 18 S. All other synthetase activities were below 1% of that of lysyl-tRNA synthetase. The rather broad activity peaks probably resulted from partial dissociation of the synthetase complex during sucrose gradient centrifugation (5). These results suggested that in the purified preparation, synthetases were present as an 18S complex with aminoacylation activities for lysine, arginine, leucine, isoleucine, methionine, and possibly glutamine.

Direct chemical analysis showed that the synthetase complex contained less than 1.5% RNA (4) and less than 0.5% cholesterol esters (20). No carbohydrate could be detected by NaDodSO₄ gel electrophoresis in any of the protein bands of the purified synthetase complex under conditions that detected the carbohydrates in ovalbumin (3%) (21).

**Molecular Weight of the Synthetase Complex.** Gel filtration of the 18S synthetase complex on a column of Ultrogel AcA 22 (LKB) with thyroglobulin, β-galactosidase, catalase, and free lysyl-tRNA synthetase as standards, resulted in a molecular weight estimate of about one million. Addition of bovine serum albumin (1 mg/ml) to the buffer was necessary to eliminate nonspecific interaction of the synthetase complex with agarose (22). The synthetase complex did not elute symmetrically from Ultrogel. The number-average molecular weight was estimated by numerical integration to be 970,000 ± 70,000, whereas polyacrylamide gel electrophoresis under nondenaturing conditions gave an estimate of 800,000–1,000,000. These estimates should be considered tentative, because standards with higher molecular weight than the complex were not available.

**Active Site Titration of the Synthetase Complex.** Specific binding of aminoacyl adenylate to the cognate synthetase (23, 24) (with saturating amounts of 14C-labeled amino acid and ATP) was used to determine the molar amounts of individual synthetases (Table 1). On the basis of an average molecular weight of 970,000 for the synthetase complex, 1 mol of the complex bound 2 mol of lysyl adenylate. This amount remained the same when the concentration of lysine was doubled, indicating that the binding of lysyl adenylate was saturated (Table 1). The same results were consistently obtained for different preparations of the synthetase complex. Similarly, 1 mol of synthetase complex bound approximately 1 mol of methionyl adenylate (Table 1).

The binding of isoleucyl adenylate was 0.5–1 mol per mol of complex, whereas that of leucyl adenylate was weaker and did not saturate under the assay conditions. At various concentrations of leucine, the saturating level of bound leucyl adenylate corresponded to 0.58–0.68 mol per mol of the synthetase complex (Table 1).

**Subunit Structure of the Synthetase Complex.** Fig. 2 shows that the purified synthetase complex consists of eight major polypeptides with subunit molecular weight of 160,000, 150,000, 135,000, 104,000, 92,000, 69,000, 67,000, and 48,000 (bands I through VIII). The protein of band VI has previously been identified as lysyl-tRNA synthetase with α₂-type subunit structure (12). The molar ratios of the eight proteins were determined by quantitative densitometry and standardization with band VI (Table 2).

For every 2 mol of lysyl-tRNA synthetase subunits, there

![Figure 1](image)

**FIG. 1.** Sucrose gradient centrifugation of the purified synthetase complex. Purified synthetase complex (0.2 mg) from tRNA-Sepharose chromatography was sedimented in a 20–40% linear gradient of sucrose in 50 mM Tris-HCl (pH 7.5 at 25°C)/5 mM Mg(OAc)₂/2 mM dithioerythritol/25 mM KCl for 6 hr in a Beckman SW 60 rotor at 60,000 rpm and 4°C. Fractions (0.3 ml) were collected and assayed for lysyl- ( ), arginyl- ( ), and methionyl- ( ) tRNA synthetase activities (left scale) and leucyl- ( ) and isoleucyl- ( ) tRNA synthetase activities (right scale). Recovery of lysyl-tRNA synthetase activity was greater than 90%. Standards included β-galactosidase (16S) and catalase (11.3 S).

**Table 1. Stoichiometry of aminoacyl-tRNA synthetases based on active site titration**

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Amino acid present, nmol</th>
<th>Aminoacyl adenylate bound, mol/mol of complex</th>
<th>Stoichiometry</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lys</td>
<td>0.8</td>
<td>1.92 ± 0.25</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>1.6</td>
<td>1.72 ± 0.08</td>
<td>1</td>
</tr>
<tr>
<td>Met</td>
<td>5.0</td>
<td>1.29 ± 0.15</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>0.97 ± 0.15</td>
<td>1</td>
</tr>
<tr>
<td>Ile</td>
<td>0.8</td>
<td>1.05 ± 0.02</td>
<td>0.5–1</td>
</tr>
<tr>
<td></td>
<td>1.6</td>
<td>0.89 ± 0.05</td>
<td>1</td>
</tr>
<tr>
<td>Leu</td>
<td>0.8</td>
<td>0.12</td>
<td>0.26</td>
</tr>
<tr>
<td></td>
<td>1.1</td>
<td>0.18</td>
<td>0.29</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>0.21</td>
<td>0.40</td>
</tr>
<tr>
<td></td>
<td>2.3</td>
<td>0.21</td>
<td>0.41</td>
</tr>
<tr>
<td></td>
<td>4.6</td>
<td>0.27</td>
<td>0.43</td>
</tr>
<tr>
<td></td>
<td>5.6</td>
<td>0.33</td>
<td>0.44</td>
</tr>
<tr>
<td>Saturating</td>
<td></td>
<td>0.58</td>
<td>0.68</td>
</tr>
</tbody>
</table>

* Two different preparations of the synthetase complex were assayed for the binding of aminoacyl adenylate; 9.67 and 15 μg of protein were used for preparations I and II, respectively. Errors are SD.

† The saturating level was obtained from a double reciprocal plot fitted by linear regression.
were 2 mol of band VII protein and 1 mol each of band IV and V proteins in the synthetase complex (Table 2). The molar ratios of bands IV through VIII proteins did not vary significantly with different preparations of the synthetase complex, staining with Coomassie brilliant blue or fast green, or with various amounts of proteins loaded onto the gel (data not shown). Conversely, band I, II, and III proteins showed variable and substoichiometric amounts (0.3–0.7 mol of each subunit for every 2 mol of lysyl-tRNA synthetase subunit). The synthetase complex preparation was apparently heterogeneous with respect to these proteins.

Table 2. Subunit structure of the 18S synthetase complex based on NaDodSO₄ gel electrophorograms

<table>
<thead>
<tr>
<th>Protein band</th>
<th>Molecular weight (kDa)</th>
<th>Molar ratio*</th>
<th>Stoichiometry</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>160,000</td>
<td>0.73 0.38 0.44 0.28 0.17</td>
<td>&lt;1</td>
</tr>
<tr>
<td>II</td>
<td>150,000</td>
<td>0.57 0.74 0.53 0.40 0.39</td>
<td>&lt;1</td>
</tr>
<tr>
<td>III</td>
<td>135,000</td>
<td>0.45 0.58 0.35 0.25 0.30</td>
<td>&lt;1</td>
</tr>
<tr>
<td>IV</td>
<td>104,000</td>
<td>0.91 1.06 1.1 1.1 1.1</td>
<td>1</td>
</tr>
<tr>
<td>V</td>
<td>92,000</td>
<td>1.1 1.18 1.2 0.86 1.1</td>
<td>1</td>
</tr>
<tr>
<td>VI</td>
<td>69,000</td>
<td>2.0 2.0 2.0 2.0 2.0</td>
<td>2</td>
</tr>
<tr>
<td>VII</td>
<td>67,000</td>
<td>2.0 2.11 1.9 2.7 2.0</td>
<td>2</td>
</tr>
<tr>
<td>VIII</td>
<td>48,000</td>
<td>2.2 2.03 1.3 0.77 1.0</td>
<td>1–2</td>
</tr>
</tbody>
</table>

* The amounts of the protein bands were determined from the area under each peak of the intensity profiles of the NaDodSO₄ gel electrophorograms stained by Coomassie brilliant blue. Molar ratios were obtained after correction for the molecular weight of each protein and standardization with lysyl-tRNA synthetase (band VI).

† Columns A and B give values for two typical preparations.
‡ Columns C, D, and E give values for fractions 6, 7, and 8, respectively, from the sucrose gradient shown in Fig. 1.

The molar ratios of band I through VIII proteins were then determined after sucrose gradient centrifugation of the purified synthetase complex, in an attempt to ascertain that all proteins cosedimented with the synthetases. As shown in Table 2, band IV through VIII proteins maintained essentially the same molar ratios after sucrose gradient centrifugation. Band II through VIII proteins cosedimented with the synthetases at 18 S (see Fig. 1). The decrease of band I, II, and III proteins likely reflects their weak association with the synthetase complex.

DISCUSSION

In the present investigation, eight major proteins were copurified with six aminoacyl-tRNA synthetases. The subunit structure of the highly purified 18S synthetase complex was examined by quantitative densitometry and active site titration. The two independent but complementary methods demonstrated that some of the proteins as well as synthetases are present in stoichiometric amounts. Protein bands IV, V, VI, and VII are present in molar ratios of 1:1:2:2 in the complex. Active site titration of the synthetase complex showed that the complex contains two subunits of lysyl-tRNA synthetase and one subunit of methionyl-tRNA synthetase. For the other synthetases and protein components, stoichiometric amounts were not observed, possibly because of lability of the complex.

Densitometry of the synthetase complex indicated that bands I, II, and III are present in variable and substoichiometric amounts and are significantly decreased after ultracentrifugation. Bands II and III proteins cosediment with the synthetases after sucrose gradient centrifugation. It appears, therefore, that these proteins may be weakly associated with the complex. Their association with the complex is supported by the fact that the protein bands present in stoichiometric amounts are insufficient to account for the estimated molecular weight of the complex.

Band VIII protein decreased from a molar ratio of 2 to approximately 1 after ultracentrifugation. Because band VIII protein moved closer to the dye front and diffused more than the other proteins, its molar amount could be significantly underestimated at low protein concentration. This was verified by using the complex preparation before centrifugation. It is thus likely that the molar ratio of band VIII protein after ultracentrifugation was underestimated.

Active site titration with leucyl and isoleucyl adenylates did not clearly show stoichiometric amounts of these two synthetases. It is difficult at present to discern whether the results are due to the limitation of the method (23, 24), lability of the structure of the synthetase complex (5), or the instability of the synthetase activities (25). Hence, it is possible that these synthetases could actually be present in stoichiometric amounts. The molar amounts of arginyl- and glutamyl-tRNA synthetases in the complex cannot be determined by active site titration with aminoacyl adenylates (23).

The molecular weights of band VI, IV and VIII proteins agreed well with the molecular weight of lysyl-tRNA synthetase previously determined and those suggested for leucyl- and arginyl-tRNA synthetases, respectively (12). The molar amount of lysyl adenylate bound to the synthetase complex also correlated well with the α₂-type subunit structure of free lysyl-tRNA synthetases (12).

Glutamyl- and glutamyl-tRNA synthetases dissociate early in the disassembly scheme of rat liver synthetase complex (5). We consistently failed to detect any glutamyl-tRNA synthetase activity after polyethylene glycol fractionation. Glutamyl-tRNA synthetase, if present in the complex, is in rather low and unstable amounts. However, both enzymes were reported to be present in a synthetase complex from sheep liver purified...
by the same procedure (9). These results suggest that our 18S synthetase complex may be partially dissociated but structurally more stable.

On the basis of our present evidence and that of earlier studies by ultracentrifugation (5) and affinity chromatography (10), the synthetase complex evidently is a heterotypic multienzyme complex with defined structure. Our results provide the basic information necessary for further studies of the structural organization of the synthetase complex. Those proteins present in stoichiometric amounts are likely peripheral proteins, and they are weakly associated with the synthetase complex. Band I, II, and III proteins had molecular weights higher than any known subunit molecular weight of aminoacyl-tRNA synthetases (1, 23) and thus may be nonsynthetase components of the complex. Because of the lability of the synthetase complex, further structural studies by chemical crosslinking or immunochemical techniques are apparently needed to ascertain that all protein components identified are physically associated.

The functional significance of the association of different aminoacyl-tRNA synthetases is unknown. No enzymatic interactions among synthetases have been reported, although the nonsynthetase components may have a regulatory role in the synthetase activities (26). The possible involvement of synthetase complexes in the regulation of protein biosynthesis or the transport (27) and the biosyntheses (28) of amino acids is yet to be explored.

In view of the structural relationship of the multiple forms (5) and the subunit structure of the particulate synthetases, we propose the term ligasome as a collective name for synthetase complexes. The term accounts for the aminoacyl-tRNA ligase activities and their high molecular weight nature. Ligasomes may prove to be an excellent model to advance our knowledge of protein assembly and cellular compartmentation.

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