Macromolecular Complexes from Sheep and Rabbit Containing Seven Aminoacyl-tRNA Synthetases

I. SPECIES SPECIFICITY OF THE POLYPEPTIDE COMPOSITION*

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Using a three-step procedure designed to minimize the risks of proteolysis, high molecular weight complexes containing the seven aminoacyl-tRNA synthetases specific for isoleucine, leucine, methionine, lysine, arginine, glutamic acid, and glutamine were purified from sheep liver and spleen, as well as from rabbit reticulocytes and liver.

The polypeptide composition of these complexes, as revealed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, is characteristic of the animal species from which they are derived. The complexes from sheep liver and spleen display indistinguishable polypeptide patterns composed of 11 major components. Of the 10 common components which characterize the complexes of rabbit reticulocytes and liver, 4 are also shared by the complexes from sheep, while 6 have distinctly different electrophoretic mobilities. Furthermore, in the case of the complex from rabbit reticulocytes, it is shown that the enzyme and polypeptide composition of the complex is independent of the purification method employed.

The isolation of high molecular weight complexes of identical aminoacyl-tRNA synthetase and polypeptide compositions from two cell types as radically different as rabbit reticulocytes and hepatocytes suggests that these multienzyme complexes do not arise as artifacts of preparation and supports the view that they reflect a structural organization existing within the cell.

A large body of data pertaining to the existence of high molecular weight complexes of aminoacyl-tRNA synthetases in mammalian cells has been accumulated. The complexes described range from fragile supramolecular assemblies containing all or most aminoacyl-tRNA synthetases (1-4) to those containing only a limited yet variable number of these enzymes, in sufficiently stable association to withstand purification by conventional procedures (5-11). In most of these cases variable proportions of the aminoacyl-tRNA synthetases encountered as high molecular weight complexes were also present in extracts as low molecular weight entities. A common feature of the aminoacyl-tRNA synthetase composition of the complexes purified so far is the invariable presence of methionyl-, lysyl-, leucyl-, and isoleucyl-tRNA synthetases, and the frequent occurrence of arginyl-, glutaminyl-, and glutamyl-tRNA synthetases. With rare exceptions (10, 11), the polypeptide composition of the purified complexes has not been examined. Owing to the widely different purification procedures employed, it has been impossible to discern if the observed variations in size and enzyme composition reflect a cell type specificity or result from methodological differences. That differences in purification techniques can be responsible for the variations observed is exemplified by the multienzyme complex from rat liver, which was successively purified as an entity comprising five (5), eight (9), or six (11) aminoacyl-tRNA synthetases.

Our earlier (12, 13) and more recent (14) studies have demonstrated that high molecular weight complexes containing methionyl-tRNA synthetase were highly susceptible to proteolysis, which generated fully active lower molecular weight forms of this enzyme. These results suggest that uncontrolled proteolysis may be a major factor responsible for the markedly heterogenous elution profiles encountered in the early chromatographic steps of most of the published purifications procedures (5-9), as well as for the variations in size and enzyme composition of the purified complexes. Indeed, a survey of publications concerning complexes of aminoacyl-tRNA synthetases clearly indicates that, with very few exceptions, this problem has not received the attention it deserves.

Using a three-step procedure which laid major emphasis on minimizing the risks of proteolysis, we described the extensive purification, from sheep liver, of a high molecular weight complex of defined polypeptide composition, comprising seven aminoacyl-tRNA synthetases specific for Met, Leu, Ile, Lys, Arg, Glu, and Gin (10). Throughout the purification steps, each of the seven aminoacyl-tRNA synthetases was present exclusively as a high molecular weight entity.

The objective of the present study was to extend the application of this purification procedure, to determine if a multienzyme complex of defined aminoacyl-tRNA synthetase and polypeptide composition such as was previously found in sheep liver could be found in other cell types from the same or other species. It is shown that complexes containing the same seven enzymes can be isolated from sheep liver and spleen, as well as from rabbit liver and reticulocytes. The polypeptide composition of these complexes is characteristic of the animal species from which they are derived. Furthermore, in the case of rabbit reticulocytes, it was verified that the polypeptide composition of the purified complex is independent of the purification method employed. The results suggest that these multicomponent systems represent discrete physical entities of defined protein composition, which are physiologically relevant.
**Materials**

Reticulocytes were prepared by acetylphenylhydrazine treatment of 3-month-old New Zealand rabbits (10). Fresh livers from untreated rabbits of the same origin, as well as livers and spleen from 3- or 4-month-old sheep were excised and homogenized in extraction buffer containing DFP, within 10 min of death of the animals (10). Sheep liver tRNA containing 70-75 A260 units of unfraccionated Escherichia coli RNA or brewers' yeast tRNA/ml of gel bed, was prepared as described previously (10). Ultragel A4R-heparin (4-6 mg of heparin/ml of gel bed) was purchased from Pharmindustrie (France).

**Methods**

**Standard Aminoacyl-tRNA Synthetase Assay**

The enzymes were assayed by the aminoacylation of tRNA as reported previously (10). Assay conditions were optimized for methionyl-tRNA synthetase. The reaction mixture contained, in a total volume of 0.1 ml: 20 mM imidazole-HCl at pH 7.5, 150 mM KCl, 5 mM MgCl₂, 3 mM ATP, 60 μM 14C-labeled aminoacid (specific activity, 50 Ci/mol), 0.5 mM dithioerythritol, and saturating amounts of either unfraccionated brewers' yeast RNA (Boehringer) or partially purified tRNAs recovered after benzoyl-DEAE-chromatography of beef liver tRNA (10). Yeast and beef liver tRNAs were equally effective substrates for methionyl-tRNA synthetase. The other aminoacyl-tRNA synthetases were assayed using appropriate fractions of the partially purified beef liver tRNA. The reaction was started by addition of limiting amounts of enzyme (0.025 ml), after appropriate dilution in 10 mM Tris-HCl at pH 7.5 containing 10 mM 2-mercaptoethanol, and 10% glycerol. A unit of enzyme activity is defined as the amount of enzyme required to form 1 nmol of aminoacyl-tRNA/min at 25 °C.

**Analytical Procedures**

Protein concentration was determined as reported previously (10). SDS-polyacrylamide gel electrophoresis was conducted as described in the following paper (14).

**Buffers**

Where indicated, specified amounts of DFP (Serva) were added to the buffers immediately before use from an 0.2 mM stock solution in anhydrous isopropl alcohol. Buffer A contained 25 mM potassium phosphate at pH 7.5, 10 mM 2-mercaptoethanol, 0.1 mM EDTA, and 10% glycerol. Buffer B was composed of 50 mM Tris-HCl at pH 7.5, 55 mM KCl, 10 mM 2-mercaptoethanol, 5 mM MgCl₂, 0.1 mM EDTA, and 10% glycerol. A 50% (w/v) stock solution of PEG 6000 (Merck, Darmstadt) was made up in 50 mM Tris-HCl buffer at pH 7.5, containing 5 mM MgCl₂, 0.1 mM EDTA, and 1 mM dithioerythritol.

**Purification of the Aminoacyl-tRNA Synthetase Complexes from Sheep Liver and Spleen**

The purification and partial characterization of a high molecular weight complex from sheep liver, containing aminoacyl-tRNA synthetases specific for isoleucine, leucine, methionine, lysine, arginine, glutamic acid, and glutamine, was described earlier (10, 17). The method, as described previously, involved preparation of the crude extract by Waring Blender homogenization of fresh tissue in the presence of 1 mM DFP, followed by fractional precipitation with PEG, gel filtration through 6% agarose, and chromatography on Sepharose-bound tRNA. All purification steps were carried out at 0-4 °C.

Precisely the same procedure was used to purify the corresponding complex from sheep spleen. Only methionyl-tRNA synthetase activity was assayed to monitor the purification.

**Purification of the Aminoacyl-tRNA Synthetase Complex from Rabbit Reticulocytes**

**Method I**

Each of the seven relevant aminoacyl-tRNA synthetases were assayed at every step of the purification.

**Preparation of the Crude Extract—Rabbit reticulocytes** were lysed by the addition of 3 volumes of 5 mM Tris-HCl at pH 7.5 containing 5 mM MgCl₂ and 2 mM DFP to 1 volume (130 ml) of pelleted washed cells, with gentle stirring. After 2 min, the lysate was diluted with 1 volume of 250 mM Tris-HCl buffer at pH 7.5 containing 1.5 mM KCl, 10 mM MgCl₂, 5 mM dithioerythritol, 5 mM DFP, 0.5 mM EDTA, and 50% glycerol, and the cell debris and unlysed cells were removed by centrifugation at 10,000 x g for 30 min. The resulting supernatant (540 ml) was centrifuged at 140,000 x g for 90 min to remove the ribosomal fraction which was recovered and assayed for residual aminoacyl-tRNA synthetase activities.

**Fractional Precipitation with Poly(ethylene Glycol) 6000**—To the high speed supernatant (530 ml), a 50% stock solution of PEG was slowly added, with stirring, to raise the concentration to 10% (w/v). The suspension was stirred for 30 min and the precipitate was recovered by centrifugation for 30 min.

**Gel Filtration on 6% Agarose**—The precipitate from the preceding step was dissolved in 30 ml of Buffer A and applied on a 1700-ml column of Bio-Gel A-5m equilibrated in the same buffer. Ascending chromatography was performed as described for Fig. 2. Fractions 34-48, which contained the seven relevant aminoacyl-tRNA synthetase activities, were combined (306 ml).

**Chromatography on Sepharose-bound tRNA**—The pooled fractions from the preceding step were directly applied on a 218-ml column of Sepharose-bound tRNA equilibrated in Buffer A. Ascending chromatography was performed as described for Fig. 3. Fractions 31-42 (peak B) were pooled, concentrated by ultrafiltration (TCP2 system from Amicon equipped with a PM30 membrane), and dialyzed against 100 volumes of 50 mM potassium phosphate (pH 7.5) containing 1 mM dithioerythritol, 0.1 mM EDTA, and 50% glycerol, and finally stored at -18 °C.

**Method II**

Only methionyl-tRNA synthetase was assayed in the course of this purification.

**Preparation of the Crude Extract—Washed rabbit reticulocytes** (20 ml of pelleted cells) were lysed exactly as described in Method I, except that the lysate was diluted with 1 volume of the Tris-HCl buffer from Method I containing 250 mM KCl instead of 1.5 mM KCl. Cell debris was removed by centrifugation at 10,000 x g for 30 min.

**Chromatography on Ultragel A4R-Heparin**—The superna-
tant (84 ml) was directly applied on 25 ml of Ultragel A4R-heparin equilibrated in Buffer B. Ascending chromatography was performed as described for Fig. 5. Fractions 50-53 (44 ml) were pooled and immediately diluted by slow addition, with stirring, of 2.5 volumes of dilution buffer composed of 10 mM potassium phosphate at pH 7.5 containing 10 mM 2-mercaptoethanol, 0.1 mM EDTA, and 10% glycerol.

**Chromatography on Sepharose-bound tRNA**—The diluted solution (154 ml) was applied on a 40-ml column of Sepharose-bound yeast tRNA equilibrated in Buffer A. Ascending chromatography was performed as described for Fig. 6. Fractions 42-62 (110 ml), which contained the seven relevant aminoacyl-tRNA synthetase activities, were combined, concentrated by vacuum dialysis in U10/25 and slow dialysis against 100 volumes of 50 mM potassium phosphate at pH 7.5, and finally stored at -18 °C.

**Purification of the Aminoacyl-tRNA Synthetase Complex from Rabbit Liver**—Purification was achieved by following precisely the standard procedure described for sheep liver (10), and only methionyl-tRNA synthetase activity was assayed in the course of purification. As in the case of rabbit reticulocytes (Fig. 3), elution of the enzyme from Sepharose-bound tRNA gave rise to peaks A and B, in the relative proportions of about 1 to 4, respectively. The material from peak B was either directly processed for characterization of its aminoacyl-tRNA synthetase and polypeptide compositions, or was subjected to an additional fractionation step on hydroxyapatite.

**Chromatography on Hydroxyapatite**—The combined fractions corresponding to peak B (25 mg of protein) were diluted 2.5-fold by slow addition, with stirring, of 10 mM potassium phosphate buffer at pH 7.5 containing 10 mM 2-mercaptoethanol, 0.1 mM EDTA, and 10% glycerol. This solution was applied on a column of hydroxyapatite (2.6 x 20 cm) equilibrated in 100 mM phosphate buffer at pH 7.5 containing the same additives as above. The column was washed with 2 volumes of the same bu.cor. at a flow rate of 3 column volumes/hr. Elution was achieved by a linear gradient (800 ml) of potassium phosphate at pH 7.5 (100 to 400 mM) containing the same additives. Methionyl-tRNA synthetase activity emerged as a single, symmetrical peak at a phosphate concentration of about 350 mM. Fractions containing activity were pooled and processed as described for the complex from rabbit reticulocytes.

1 The abbreviations used are: DFP, diisopropyl fluorophosphate; PEG, poly(ethylene glycol) 6000; SDS, sodium dodecyl sulfate.
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Immunotitation of the Methionyl-tRNA Synthetase Component of the Purified Complexes—The preparation of an antiserum raised against trypsin-modified monomeric methionyl-tRNA synthetase from sheep liver was described earlier (12). Purified IgG was prepared according to the method of Livingston (18). Immunoassays were conducted by incubating 1 volume of the purified complex (10 μg of protein/ml in 10 mM potassium phosphate buffer at pH 7.5 containing 5 mM 2-mercaptoethanol, 10% glycerol, and 5% Dextran T-500 (19) from Pharmacia) with 1 volume of appropriate dilutions of IgG in the same buffer, as indicated in Fig. 5. After 2 h at 0 °C, residual methionyl-tRNA synthetase activity was assayed.

RESULTS

Aminoacyl-tRNA Synthetase Complexes from Sheep Liver and Spleen—The purification and partial characterization of the high molecular weight complex from sheep liver, containing seven aminoacyl-tRNA synthetases specific for Ile, Leu, Met, Lys, Arg, Glu, and Gln, was described earlier (10, 17). A typical SDS-polyacrylamide electrophoretic pattern of its polypeptide composition is shown in Fig. 1, lane A.

To search for the existence of a similar complex in another cell type from the same species, methionyl-tRNA synthetase was purified from sheep spleen by following precisely the procedure described for sheep liver. The behavior of this enzyme at each step of the procedure was essentially identical with that reported for sheep liver, except that in the first step, precipitation occurred between 5 and 9% PEG, instead of 2–5%. As in the case of sheep liver (10), gradient elution of methionyl-tRNA synthetase bound to Sepharose-tRNA gave rise to two, partially overlapping peaks (A and B) of nearly equal size, emerging at phosphate concentrations of 0.13 and 0.21 M, respectively. The specific activity of the enzyme in peak A was 3-fold lower than in peak B (19 and 59 units/mg, respectively). The material from the latter peak was recovered as described earlier (10), for comparison with the corresponding fraction from sheep liver. This fraction contained only the same seven aminoacyl-tRNA synthetases activities previously reported in sheep liver, and the methionyl-tRNA synthetase activity was eluted as a single, symmetrical peak of apparent molecular weight of 107 when subjected to analytical gel filtration on Bio-Gel A-50-m under the conditions described previously (10) (results not shown). Moreover, the polypeptide composition of this fraction, as revealed by SDS-polyacrylamide gel electrophoresis, was virtually indistinguishable from that of sheep liver (Fig. 1, lane B).

Aminoacyl-tRNA Synthetase Complex from Rabbit Reticulocytes—Two procedures were used for the purification of the aminoacyl-tRNA synthetase complex from rabbit reticulocytes. Procedure I is essentially that described for the purification of the corresponding complex from sheep liver (10), with the following modifications: cells were lysed by osmotic shock and the ionic strength of the ensuing lysate was raised to 0.3 M KCl to prevent interaction of aminoacyl-tRNA synthetases with ribosomes (20, 21). The relevant aminoacyl-tRNA synthetases from the postribosomal supernatant were found exclusively as high molecular weight complexes by analytical gel filtration on Bio-Gel A-50m (result not shown). Fractional precipitation with 10% PEG resulted in extensive co-purification of the relevant aminoacyl-tRNA synthetases, which were recovered in the precipitate together with only 3% of the cellular proteins. Upon preparative gel filtration on Bio-Gel A-50m, the seven relevant enzymes from the preceding step were co-eluted as a fairly broad peak close to the void volume of the column (Fig. 2). It is noteworthy that valyl-tRNA synthetase activity, 30% of which was recovered in the

Fig. 1. Polypeptide compositions of the purified aminoacyl-tRNA synthetase complexes. The complexes purified from sheep liver (lane A) and sheep spleen (lane B) were subjected to SDS-polyacrylamide gel electrophoresis as described under "Methods".

Fig. 2. Preparative gel filtration of the protein fraction from rabbit reticulocytes precipitated by 10% PEG. The precipitate was dissolved in 39 ml of Buffer A and applied on a column (6 x 87 cm) (Pharmacia, type B50/50) of Bio-Gel A-50m (200–400 mesh) equilibrated in Buffer A. Ascending chromatography was carried out at a flow rate of 1 ml/min. Fractions of 18 ml were collected and assayed for enzyme activities and absorbance at 280 nm.
10% PEG precipitate, was also eluted as a high molecular weight entity as expected (21), yet clearly lagging behind the other activities assayed.

Extensive further purification of the seven relevant aminoacyl-tRNA synthetases was achieved by chromatography on Sepharose-bound tRNA. As shown in Fig. 3, the enzymes were co-eluted in two distinct peaks, A and B, emerging at phosphate concentrations of about 0.12 and 0.2 M, respectively. However, whereas the enzymes from sheep liver (10) and spleen were distributed in two peaks of nearly equal size, the enzymes from reticulocytes were consistently found preferentially in peak B. The specific activities of the aminoacyl-tRNA synthetases from peak A were approximately 4-fold lower than those from peak B, probably due to contamination by other proteins eluted in the early portion of the gradient, as exemplified by the behavior of the residual valyl-tRNA synthetase activity (Fig. 3). The basis for this bimodal distribution of the enzymes is not understood (10).

The purification of the complex from reticulocytes by the standard procedure is summarized in Table I. Only seven aminoacyl-tRNA synthetases were present in the purified complex, and their specific activities were substantially higher than those reported earlier (6). Methionyl-tRNA synthetase, the only enzyme assayed under optimal conditions, displayed the same specific activity as that reported for the enzyme from sheep liver (10). Since the seven activities were co-eluted at each chromatographic step of the purification, the variations observed in the relative recoveries of activities are likely to be due to differences in the relative stabilities of the aminoacyl-tRNA synthetases.

The apparent molecular weight of the purified complex was
estimated by gel filtration on 6% agarose. As shown in Fig. 4, the seven relevant enzymes co-eluted as a major peak corresponding to a molecular weight of about 10^6. The distinct shoulder afforded by several aminocyl-tRNA synthetases may reflect partial dissociation of the purified complex due to the high salt concentration (0.2 M phosphate buffer) used to prevent adsorption to the matrix (17). No free enzymes were detected in the region between β-galactosidase and the inclusion volume.

The polypeptide composition of the complex was analyzed by SDS-polyacrylamide gel electrophoresis. As shown in Fig. 5 (lane C), the complex from reticulocytes displayed a distinctive pattern composed of ten major components.

A multicomponent complex of essentially similar polypeptide composition was obtained when methionyl-tRNA synthetase from rabbit reticulocytes was purified by a two-step procedure (Method II) involving chromatography on Ultrogel-heparin as a substitute for fractionation by PEG and gel filtration, followed by chromatography on Sepharose-tRNA. The crude lysate obtained by hypotonic shock was adjusted to 50 mM KCl instead of 300 mM, since heparin was expected to displace ribosome-bound aminocyl-tRNA synthetases, as was previously found for ribosome-bound initiation factors (22). A typical elution profile of a reticulocyte lysate on Ultrogel-heparin is presented in Fig. 6. As shown in Table II, a 126-fold purification was thus achieved in a single step. Subsequent chromatography on Sepharose-bound yeast tRNA lead to the separation of methionyl-tRNA synthetase activity into two peaks, A and B, in the approximate proportions of 1 to 10 (Fig. 7). Methionyl-tRNA synthetase, which was recovered from fraction B with a global yield of 92% and a specific activity of 73 units/mg, displayed an apparent molecular weight of about 10^6 by gel filtration on 6% agarose (result not shown). The polypeptide composition of this material, as revealed by SDS-polyacrylamide gel electrophoresis (Fig. 5, lane D) was essentially the same as that of the reticulocyte complex purified by the standard procedure. The only significant difference resided in the presence of a contaminant which comigrated with the seventh component from the top, accounting for the intense staining of this band.

**Fig. 5.** Polypeptide compositions of the purified aminocyl-tRNA synthetase complexes. SDS-polyacrylamide gel electrophoretic patterns of the complexes originating from sheep liver (lane A) and rabbit liver (lane B) purified by the standard procedure; rabbit reticulocytes prepared according to Method I (lane C) or Method II (lane D); rabbit liver prepared by the standard procedure (lane E) or further purified by hydroxylapatite chromatography (lane F).

**Fig. 6.** Chromatography on Ultrogel 4AR-heparin of the 10,000 × g supernatant from a reticulocyte lysate. The supernatant (84 ml) was applied on a column (2.5 × 5 cm) of Ultrogel-heparin equilibrated in Buffer B. Ascending chromatography was performed at a flow rate of 33 ml/h and fractions of 11 ml were collected. After washing with 4 column volumes of Buffer B, elution was achieved with a linear gradient (300 ml) of KCl (35 to 400 mM) in Buffer B. Fractions 50–53, which contained methionyl-tRNA synthetase activity, were pooled for further purification.

**Aminoacyl-tRNA Synthetase Complex from Rabbit Liver—** The complex from rabbit liver was purified exactly according to the standard procedure described for sheep liver (10). Only methionyl-tRNA synthetase was assayed to monitor the purification. The behavior of this enzyme at each step of the procedure was the same as that reported for sheep liver, with one notable exception. Elution of methionyl-tRNA synthetase
Aminoacyl-tRNA Synthetase Complexes from Sheep and Rabbit

TABLE II

Purification of the aminoacyl-tRNA synthetase complex from rabbit reticulocytes by Method II

The purification was monitored by following methionyl-tRNA synthetase activity.

<table>
<thead>
<tr>
<th>Purification step</th>
<th>Total protein</th>
<th>Specific activity</th>
<th>Yield</th>
<th>Purification factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>10,000 × g supernatant</td>
<td>4,250</td>
<td>0.07</td>
<td>100</td>
<td>1</td>
</tr>
<tr>
<td>Ultrogel-heparin eluate</td>
<td>20</td>
<td>8.7</td>
<td>59</td>
<td>126</td>
</tr>
<tr>
<td>Sepharose-tRNA eluate (peak B)</td>
<td>1.3</td>
<td>73</td>
<td>32</td>
<td>1,055</td>
</tr>
</tbody>
</table>

* The lysate was prepared from 20 ml of washed, pelleted cells.

b Units of activity are as defined in Table I.

Fig. 7. Chromatography on Sepharose-bound tRNA on methionyl-tRNA synthetase from reticulocytes purified according to Method II. The diluted pool from the Ultrogel-heparin step was applied on a column (2.5 × 8 cm) of Sepharose-bound yeast tRNA equilibrated in Buffer A, at a flow rate of 55 ml/h. The column was washed with 5 column volumes of the same buffer, after which elution was achieved using a linear gradient (400 ml) of potassium phosphate at pH 7.5 (25 to 400 mM) containing 10 mM 2-mercaptoethanol, 0.1 mM EDTA, and 10% glycerol, at a flow rate of 20 ml/h. Fractions of 5.4 ml were collected and assayed for methionyl-tRNA synthetase activity. Fractions 42 to 62 were pooled and processed as described under "Methods". The arrow indicates the beginning of the gradient.

from Sepharose-bound tRNA by the linear phosphate gradient gave rise to an activity profile which was similar to that obtained from rabbit reticulocytes: the relative proportion of peaks A and B were close to 1 to 4, and the specific activity of the enzyme was 3-fold higher in peak B (Table III). The latter fraction contained the same seven aminoacyl-tRNA synthetases found in rabbit reticulocytes. Its polypeptide composition, as visualized by SDS-polyacrylamide gel electrophoresis, is presented in Fig. 5, lanes B and E. Further fractionation by hydroxylapatite chromatography of peak B yielded an indistinguishable band pattern (lane F). Comparison of the polypeptide compositions of rabbit liver and reticulocytes showed a remarkable similarity, the only notable difference being the virtual absence of the 4 minor contaminants present in the complex from reticulocytes.

Antigenic Properties of Methionyl-tRNA Synthetase—As will be shown (14, 23), the polypeptide components from rabbit and sheep liver which correspond to methionyl-tRNA synthetase have distinct electrophoretic mobilities on SDS-polyacrylamide gels, corresponding to molecular weights of 108,000 and 103,000, respectively (14). In spite of this difference, the two enzymes share common antigenic properties, as revealed by immunotitration experiments presented in Fig. 8. Antibodies raised against homogeneous, trypsin-modified monomeric methionyl-tRNA synthetase from sheep liver, specifically inactivated methionyl-tRNA synthetase from complexes of sheep and rabbit liver with equal efficiency. The same result was obtained with the purified complex from rabbit reticulocytes (result not shown). Furthermore, controlled trypsin treatment of the purified complex from rabbit reticulocytes generated a fully active fragment of methionyl-tRNA synthetase with a molecular weight of close to 70,000, as previously found with the complex from sheep liver (12). This fragment was also inactivated by the antibodies raised against the monomeric enzyme from sheep liver (results not shown).

TABLE III

Purification of the aminoacyl-tRNA synthetase complex from rabbit liver

The purification was monitored by following methionyl-tRNA synthetase activity.

<table>
<thead>
<tr>
<th>Purification step</th>
<th>Total protein</th>
<th>Specific activity</th>
<th>Yield</th>
<th>Purification factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>10,000 × g supernatant</td>
<td>107,000</td>
<td>0.11</td>
<td>100</td>
<td>1</td>
</tr>
<tr>
<td>PEG precipitate</td>
<td>3,920</td>
<td>1.79</td>
<td>38</td>
<td>113</td>
</tr>
<tr>
<td>Bio-Gel A-5m eluate</td>
<td>342</td>
<td>12.5</td>
<td>618</td>
<td>1</td>
</tr>
<tr>
<td>Sepharose-tRNA eluate</td>
<td>24</td>
<td>23</td>
<td>40</td>
<td>1</td>
</tr>
<tr>
<td>Peak A</td>
<td>29</td>
<td>68</td>
<td>17</td>
<td>1</td>
</tr>
<tr>
<td>Peak B</td>
<td>17</td>
<td>76</td>
<td>12</td>
<td>1</td>
</tr>
</tbody>
</table>

a The amount of sheep liver used was 885 g.

b Units of activity are as defined in Table I.
Aminoacyl-tRNA Synthetase Complexes from Sheep and Rabbit

We have previously described a simple, rapid, and efficient procedure for the extensive purification from sheep liver of a high molecular weight complex containing seven aminoacyl-tRNA synthetases specific for Ile, Leu, Met, Lys, Arg, Glu, and Gln (10). In developing this procedure, major emphasis was laid on minimizing the risks of proteolytic degradation. The strategy employed to this effect consisted of preparing the crude extracts from fresh tissue in the presence of DFP, followed by rapid separation of the high molecular weight complex from the lower molecular weight components more likely to include proteases.

Applying this 3-step procedure to sheep liver, an extensively purified multienzyme complex containing seven aminoacyl-tRNA synthetase was obtained (10), which displayed a characteristic and reproducible band pattern consisting of 11 polypeptide components, after SDS-polyacrylamide gel electrophoresis. The high level of purity attained was attested by the observation that the electrophoretic pattern was not significantly altered when the purified complex was further subjected to molecular sieve chromatography (17) or to chromatography on DEAE-cellulose, hydroxylapatite, or phosphocellulose (results not shown).

The versatility of this purification procedure is illustrated in the present study. Its application to the purification of methionyl-tRNA synthetase from sheep spleen has led to the isolation of a multiunit complex which was indistinguishable from that purified from sheep liver. This result leads to the conclusion that the multiunit complex containing seven aminoacyl-tRNA synthetases originating from sheep is not tissue-specific. It also raises the question as to whether complexes displaying the same aminoacyl-tRNA synthetase and polypeptide compositions could be isolated from other mammalian sources, using the standard procedure.

To elucidate this point, we first chose to reinvestigate the aminoacyl-tRNA synthetase complex previously purified from rabbit reticulocytes by Som and Hardesty (6). Starting from reticulocytes lysed by osmotic shock in the absence of protease inhibitors, these authors used a 7-step procedure to purify a 16 S complex containing five aminoacyl-tRNA synthetases specific for Ile, Leu, Met, Arg, and Lys. The polypeptide composition of this complex was not determined. The markedly asymmetric elution profiles obtained in the first two chromatographic steps, as well as the absence of glutamyl- and glutaminyl-tRNA synthetase activities in the purified product (6), could conceivably reflect destabilization of the complex in the course of purification, as a consequence of uncontrolled proteolysis. Indeed, rabbit reticulocytes are known to contain high levels of at least eight different intracellular endo- and exopeptidases (Ref. 24 and references therein).

As shown in the present study, application of the standard 3-step procedure to reticulocytes which were osmotically lysed in the presence of DFP, led to isolation of a multiunit complex containing the same seven aminoacyl-tRNA synthetases found in complexes from sheep liver or spleen, but displaying a distinctly different polypeptide pattern after SDS-polyacrylamide gel electrophoresis.

A primary question regarding the purified aminoacyl-tRNA synthetase complexes must be whether they reflect a structural organization that occurs within the cell or represent aggregates of enzymes that are generated as artifacts of preparation. Concern with the possibility that PEG 6000, which was used in the first step of the standard purification procedure, may promote protein-protein interactions (25-27), prompted us to develop an alternative procedure for the rapid purification of the complex. Others have shown that chroma-
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Macromolecular complexes from sheep and rabbit containing seven aminoacyl-tRNA synthetases. I. Species specificity of the polypeptide composition.
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