A Comparison of the Proteins of Rat Skeletal Muscle and Liver Ribosomes by Two-Dimensional Polyacrylamide Gel Electrophoresis

OBSERVATIONS ON THE PARTITION OF PROTEINS BETWEEN RIBOSOMAL SUBUNITS AND A DESCRIPTION OF TWO ACIDIC PROTEINS IN THE LARGE SUBUNIT*

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SUMMARY

We have determined the number of proteins in rat skeletal muscle 80 S ribosomes and ribosomal subparticles by two-dimensional polyacrylamide gel electrophoresis. The proteins were compared with those from liver ribosomes. The small subunit of muscle ribosomes contained 31 proteins: it had two proteins (S1 and S2) which were not found in liver 40 S particles; one protein (S3*) was always found in the small subunit of muscle ribosomes but was present in the 40 S subparticle of liver ribosomes only if they were prepared in buffer containing 500 mM potassium and 3 mM magnesium (it was absent if the cation concentrations during preparation were 830 and 12.5 mM, respectively); muscle small subparticles lacked S10 and S22; finally, S8 was absent if muscle subunits were prepared in buffer containing lower concentrations of potassium and magnesium (500 mM potassium and 12.5 mM magnesium) whereas it was present if the cation concentration was higher (830 mM potassium and 12.5 mM magnesium). The large subunit of muscle ribosomes had 38 proteins: it lacked two (L1 and L41*) that always were present in liver 60 S subunits; and lacked one (L20) found only in the large subunit of liver ribosomes prepared in buffer containing 830 mM potassium and 12.5 mM magnesium; L20 is also absent from the large subunit of liver ribosomes if they are prepared in 500 mM magnesium and 3 mM potassium. We have detected two acidic proteins (L40* and L41*) in the large subunit of ribosomes that may be homologous with Escherichia coli L7 and L12. Preparations of muscle 80 S monomers contained five proteins (1, 2, 3, L1, M4) not present in either subunit; one of the five proteins (M4) was absent from liver ribosomes. We estimate muscle ribosomes have 67 to 74 different proteins; liver ribosomes have 69 to 74.

Eukaryotic ribosomes are complex structures containing 3 molecules of RNA and approximately 70 different proteins (1-8). Some of the proteins are peculiar to a species; but it is not certain whether the proteins of ribosomes in separate organs of a single species differ (6, 8-24). In general, one-dimensional polyacrylamide gel electrophoresis of eukaryotic ribosomal proteins from diverse tissues or organs (9-12, 14, 17, 22), and from various stages of development (10, 13, 18, 23), has failed to show any qualitative differences, although quantitative alterations have been observed (9, 14, 17, 22, 23). An exception is the report of differences in the ribosomal proteins of membrane bound and free liver ribosomes (10), a difference which could have been due to contamination of the bound ribosomes with membrane protein.

Two-dimensional polyacrylamide gel electrophoresis is extremely useful in comparing eukaryotic ribosomes since each protein is displayed as a single zone (4-8, 15, 19-21, 25-28). The technique was used by Huynh-Van-Tan et al. (5) and Delaunay et al. (20) to compare the proteins of 80 S ribosomes from rabbit reticulocytes, liver, kidney, and cecal appendix; a number of differences (albeit, not precisely defined) were found in the proteins of the ribosomes from the four tissues, particularly between the proteins of reticulocyte ribosomes and those of ribosomes from the other tissues (20). Rodgers (8) has compared the proteins of 80 S ribosomes from several mouse tumors, and from liver, kidney, intestine, and brain by two-dimensional gel electrophoresis. He found differences in the apparent quantity of ribosomal proteins of the normal tissues, as well as an additional...
protein in brain ribosomes. The tumor ribosomes had a number of unique proteins and lacked some present in the ribosomes from normal tissues (8). It should be pointed out that Wikman-Coffelt et al. (29) have used immunochemical techniques to provide convincing evidence for two tumor-specific proteins in the 60 S subunit of ribosomes from Novikoff hepatoma ascites cells. On the other hand, no differences were found by electrophoresis in the proteins of ribosomes from normal liver and hepatomas of rats (15, 19).

We have used two-dimensional polyacrylamide gel electrophoresis to determine the number of proteins in rat liver ribosomes and ribosomal subunits (6). We now report the results of experiments designed to determine the number of proteins in rat skeletal muscle ribosomes and ribosomal subunits; the proteins contained in ribosomal particles from muscle and liver were compared to determine whether there were tissue-specific differences. During the course of these experiments we have made observations on the partition of selected proteins between ribosomal subunits and detected for the first time two acidic proteins in the large subunit that may be homologous with Escherichia coli L7 and L12.

### Experimental Procedure

**Preparation of Ribosomes—Liver** (30) and skeletal muscle (31) ribosomes were isolated from male Sprague-Dawley rats that weighed 100 to 120 g. The methods usually used to prepare ribosomes from the two tissues differed in two important respects: minced skeletal muscle was stirred in medium containing 4 mm EGTA (32) before homogenization. EGTA chelates calcium ions and causes muscle to relax, making the tissue easier to homogenize, and thereby doubling the yield of ribosomes. The preliminary steps in the preparation of muscle ribosomes were in a medium containing 250 mM KCl, rather than in 80 mM KCl as was used for liver ribosomes. The higher concentration of potassium chloride is to solubilize myosin and prevent it from sedimenting in the supernatant. Preparation of ribosomes from skeletal muscle involved the use of a commercial detergent, Nonidet P-40, as well as of a slower sedimentation procedure (33). The ribosome suspensions were centrifuged at 12,000 X g for 10 min to remove aggregated particles; the ribosomes in the supernatant were sedimented by centrifugation (133,000 X g for 16 hours at 13,500 rpm). The liver and skeletal muscle subunit fractions (prepared in either Medium A or B) were shown to be free of contamination by zonal centrifugation (6, 34). However, preparations of 60 S subunits from skeletal muscle, made in Medium A, had a significant amount of degraded particles that sedimented between 40 and 60 S; the degraded particles were not present if the subunits were made in Medium B. The degraded particles were also present in preparations of liver 60 S subunits if the subparticles were prepared from liver ribosomes that had been produced in the same way as muscle ribosomes; the degraded particles occurred even though the subunits were formed in Medium B.

**Extraction of Ribosomal Proteins**—Proteins were extracted with 67% acetic acid from ribosomes and from ribosomal subunits made in 10 mM Tris-Cl (pH 7.7) and 100 mM MgCl₂ (6, 29). The ribosomal proteins were dialyzed (in acetylated dialysis tubing (40)) against 100 volumes of 1 N acetic acid for 48 hours with three changes of acid. The dialyzed proteins were lyophilized.

**Two-Dimensional Polyacrylamide Gel Electrophoresis**—The ribosomal proteins were separated by two-dimensional polyacrylamide gel electrophoresis, modified from the procedure of Kaltschmidt and Wittmann (41). The only additional change for the present experiments was continuous diffusion destaining of the gels in 17 liters of 7.5% acetic acid (42). A model 425 “Dynaflo” aquarium pump was used to circulate the destaining solution through a filter of activated charcoal (31).

The “standard conditions” of electrophoresis were: 8% acrylamide gel, pH 6.6, for 44 hours at 75 volts in the first dimension; and 18% acrylamide gel, pH 4.2, for 40 hours at 105 volts in the second dimension. The concentrations of gels and the pH of the buffers were selected to maximize separation by charge in the first dimension, and by molecular size in the second. For some experiments the following changes were made: the time of electrophoresis in the first dimension was reduced to 20 hours and in the second dimension to 19 hours; the acrylamide concentration in the first dimension was varied from 5 to 10% and the pH of the electrophoresis buffer from 7.6 to 10.6. The concentration of ribosomal proteins in the sample gel was 6 to 8 mg per ml, and the amount analyzed was 0.4 to 2.4 mg (in 50 to 300 μl). A large portion (perhaps, in some cases, as much as half) of the ribosomal protein remains at the origin. Electrophoresis was at room temperature.

The protein spots from each ribosomal subunit were numbered separately along horizontal lines beginning at the upper left (6). The exceptions are S81*, L40*, and L41*, which were identified after the original numbering system was adopted. The 80 S subunit proteins are designated by an L (for large subunit), the 40 S subunit proteins by an S (for small subunit). Proteins found only in the small subunit of muscle ribosomes have been designated Sm. Proteins found in the 80 S ribosome, but not in either subunit, have no letter prefix. The protein found only in muscle 80 S ribosomes is designated by the prefix M.

Some of the spots on the gels were clearly visible to the eye, but are barely discernible or cannot be seen in the photographs. Those proteins are included in the schematic drawings of the gels. The schematics do not represent with fidelity the intensity of the staining of the individual spots; moreover, they are compositions from a large number of electrophoretograms, and as such may differ in detail from individual gels.
RESULTS

Proteins of 40 S Subunit of Skeletal Muscle Ribosomes—Two-dimensional polyacrylamide gel electrophoresis in standard conditions of the proteins of the 40 S subunit of rat skeletal muscle revealed 28 to 31 spots (Fig. 1, a and b; the pattern is portrayed schematically in Fig. 3a). The 40 S subunit of rat liver ribosomes when prepared in Medium A contained 30 proteins (6); when prepared in Medium B, 31 (Fig. 2a; a schematic of the pattern is given in Fig. 4a).

The variability in the number of proteins (28 to 31) in the 40 S subunit of muscle ribosomes was due in part to a group of one to three spots which, after electrophoresis in the second dimension, appeared directly under S27. The intensity of S27 varied inversely with the number and intensity of the three satellite spots. It would seem that the satellite spots are altered forms of protein S27. The satellite spots are not due to extraction of proteins with acetic acid since they occur when other procedures for the preparation of ribosomal proteins are used (results not shown). The satellite zones were not seen in electrophoretograms of 80 S ribosomal proteins. The liver ribosomal protein S27 behaves in the same manner. Protein S21 of the small subunit of muscle ribosomes migrated in either direction to the boundary between the spacer and separation gels, as does S21 from liver ribosomes. Finally, one muscle small subunit protein, S8, was present if the subunits had been prepared in Medium A (containing 830 mM KCl and 12.5 mM MgCl₂; Fig. 1a) but was absent if the subunits had been prepared in Medium B (containing 500 mM KCl and 3 mM MgCl₂; Fig. 1b).

With the exceptions already noted (S8, S21, and S27) the position and intensity of the spots were precisely reproducible. When electrophoresis was shortened from 44 hours to 20 in the first dimension and from 40 hours to 19 in the second, two more spots were seen, S29 and S30 (see Fig. 3a, inset). Varying the acrylamide concentration of the first dimension from 5 to 10% and the pH of the electrophoresis buffer from 7.6 to 10.6 revealed no additional spots. It is not to be expected that proteins with different sizes or charges (or both) would migrate together in all the conditions that we used; therefore, we assume that each spot contains a single protein.

There were several differences between the ribosomal proteins of liver and muscle 40 S subunits. Muscle 40 S subunits contained two proteins not present in the small subunit of liver ribosomes, Sₘ₁ and Sₘ₂. Both the small and large subunits of muscle contained protein Sₘ₁; it appeared in a position that corresponded to L31 of liver. Sₘ₂, however, was unique to the 40 S subunit of muscle ribosomes. This protein did not occur in the large subunit of muscle ribosomes or in either subunit of liver ribosomes. Sₘ₁* was found with the proteins of the small subunit of muscle ribosomes (and with the proteins of liver 40 S subunits prepared in Medium B) and migrated to the position occupied by L20 of the large subunit of liver ribosomes made in Medium A. (L20 is not present in the large subunit of muscle ribosomes.) Finally, the 40 S subunit of muscle ribosomes lacked two of the proteins of liver 40 S subunits, S₁₀ and S₂₂. The separation of S₂₂ requires a change in conditions during electrophoresis of liver ribosomal proteins (6); however, S₁₀ and S₂₂ never appeared on electrophoretograms of muscle 40 S or 80 S ribosomal proteins.

We conclude that the 40 S subunit of muscle ribosomes contains 31 proteins; only 29 are the same as those of liver 40 S ribosomal subunits.

Proteins of 60 S Subunit of Skeletal Muscle Ribosomes—The 60 S subunits of muscle ribosomes, unlike large subunits from liver ribosomes, were not entirely stable in Medium A (830 mM KCl and 12.5 mM MgCl₂). Analysis of the subunits on sucrose gradients revealed that a portion of the 60 S particles prepared in Medium A was degraded. Proteins extracted from preparations containing degraded particles were poorly resolved and had many satellite spots. Therefore all our analyses were of proteins extracted from muscle 60 S subunits prepared in Medium B (500 mM KCl and 3 mM MgCl₂), in which conditions the large subunit remained intact.

The proteins extracted from purified muscle 60 S ribosomal subunits were analyzed by two-dimensional gel electrophoresis in standard conditions; 38 spots were detected on the electrophoretograms (Fig. 1C; portrayed schematically in Fig. 3b). The 60 S subunit of liver ribosomes contained 41 proteins when prepared in Medium A, and 40 when prepared in Medium B (L20 was missing) (Fig. 2b; the pattern is given schematically in Fig. 4b).

The intensity of three spots, L₁₉*, L₂₅, and L₃₇, in the electrophoretograms of muscle 60 S ribosomal proteins varied inversely with the intensity of L₁₉, L₂₅, and L₃₇, respectively. The former are likely to be derivatives (or satellites) of the latter. The satellite spots also occurred when other procedures were used to prepare the ribosomal proteins. A similar phenomenon has been observed by Kaltschmidt and Wittmann with E. coli ribosomal proteins (43, 44). L₂₉ and L₃₄ stained lightly and were usually not visible when ordinary amounts (1.0 mg) of protein were used. L₂ and L₂₄ from liver 60 S ribosomal subunits on occasion stain lightly, also. Again, the concentration of acrylamide in the first dimension and the pH of the electrophoresis buffer were varied. When the conditions were different from the standard ones there was an additional spot, L₃₃, which apparently had migrated during electrophoresis with L₃₂. The same occurs with the proteins of the large subunit of liver ribosomes (6). Two other spots, L₃₉ and L₄₀* (Fig. 3b, inset), were seen when the duration of electrophoresis in the first dimension was reduced to 20 hours. Thus, the large subunits of muscle ribosomes contained no proteins not present in the 60 S subunit of liver ribosomes. However, muscle 60 S subunits lacked three proteins, L₁, L₂₀, and L₄₁*. There was a muscle small subunit protein, S₃₁*, that migrated to the position occupied by L₂₀. L₂₀ was found among the proteins of the large subunit of liver ribosomes if they were prepared in Medium A. However, if the liver subparticles were made in Medium B, S₃₁* was found with the small subunit and L₂₀ was absent from the large subunit, exactly as happened with muscle. We conclude then that the 60 S subunit of skeletal muscle ribosomes contains 38 proteins.

Proteins of Skeletal Muscle 80 S Ribosomes—Ribosome monomers were prepared by a method expected to yield particles free of contamination with extraneous proteins. Electrophoresis, in standard conditions, of the proteins extracted from the particles revealed 66 zones (Figs. 1d and 3d); 57 migrated to a position occupied by a single protein of either the large or small subunit. The 58 included S₂₁, which migrated to the boundary between the spacer and separation gel in either direction; S₂₇, which formed a single spot, Sₘ₂, which appeared in the same position as when muscle 40 S subunit proteins were analyzed; L₂ and L₂₄, which stained more intensely than in preparations of proteins extracted from the muscle 60 S subunit; and L₁₉, L₂₅, and L₃₇, which showed single, sharply defined spots of constant intensity.

Of the nine remaining spots, four resulted from pairs of proteins migrating together during electrophoresis: L₃₂ and L₃₃, S₁₈ and L₂₃, S₈ and L₁₃, and Sₘ₁ and L₃₁. Of these pairs,
FIG. 1. Two-dimensional electrophoretograms of skeletal muscle ribosomal proteins. Electrophoresis was in standard conditions. The anode was at the left in the first dimension, at the top in the second. (a) 1 mg of protein from 40 S subunits prepared in Medium A; (b) 1 mg of protein from 40 S subunits prepared in Medium B; (c) 1 mg of protein from 60 S subunits; (d) 1.6 mg of protein from 80 S ribosomes. The shadowed streaks in the upper portion of some of the gels (see especially (c)) on the cationic side where one would expect to find the proteins of the greatest molecular weight, are probably protein aggregates linked by disulfide bonds (43). Proteins migrated directly down from the origin in the second dimension, generally forming bands rather than spots. We suspect the bands to be proteins that did not migrate in the first dimension because of the reduced solubility of ribosomal proteins at pH 8.6; the proteins would be expected to be soluble in the pH 4.2 buffer used in the second dimension (6). Some of the spots on the gels were clearly visible to the eye, but are barely discernible (S1) or cannot be seen (S12, L1, L2) in the photograph; these proteins are included in the schematic (Fig. 3).
L32 and L33 and S18 and L23 separated when the concentration of acrylamide in the first dimension was increased from 8 to 10% or the pH was varied. S8 and L13 and S4,1 and L31 were never separated. Recall, however, that if 40 S subunits were prepared in Medium B, S8 was missing. Four additional spots (S29, S30, L39, and L40*) were seen when the duration of electrophoresis was decreased (Fig. 3c, inset). Thus, all 69 of the proteins of the two subunits were accounted for; 57 were identified immediately; four pairs, or eight proteins, migrated together when electrophoresis was in the standard conditions, and four were seen when the duration of electrophoresis was decreased. There were five proteins (1, 2, 3, L1, M4) in extracts of muscle 80 S ribosomal monomers that did not appear in either muscle subunit. Of the five, 1, 2, and 3 were found also in extracts of liver 80 S monomers, but, once again, not in subunits. L1 is contained in the 60 S subunit of liver ribosomes as well as in the 80 S monomer,
whereas in muscle L1 occurs only in the 80 S ribosome. M4 is not present at all in liver ribosomes. Protein S10, which is a component of liver 40 S and 80 S particles, is missing from muscle ribosomes.

Muscle 80 S ribosomes, therefore, have from 67 to 74 ribosomal proteins. If S8 and L13 and S,n1 and L31 are different proteins, the total is 69; if the five proteins of the 80 S monomer not found in either subunit are included, the number is 74. Liver ribosomes have between 70 and 74 proteins (Fig. 4c).

Proteins of Liver Ribosomes Prepared with EGTA and 250 mM KCl—Rat liver ribosomes were isolated by the method usually used to prepare skeletal muscle ribosomes, e.g. with EGTA and 250 mM KCl. Subunits from these EGTA-liver ribosomes were made and purified in Medium B (containing 500 mM KCl and 3 mM MgCl2).

Electrophoresis of the proteins from EGTA-liver 40 S subunits revealed 31 zones. The electrophoreograms were the same as those of liver small subunit ribosomal proteins prepared in the normal way, except that the amount of S10 was greatly reduced; the spot was barely visible. S,n1 and S,n2, which are found among the proteins of muscle 40 S subunits, were not present in preparations from EGTA-liver 40 S subunits.

Electrophoreograms of EGTA-liver 60 S subunit proteins showed no L1 (or L20), and reduced amounts of L24. L41* was present. There were many satellite spots, including L19', L30', and L37', that had been prominent in electrophoreograms of proteins from the partially degraded skeletal muscle 60 S subunits prepared in Medium B. The presence of the satellite spots we presume to be due to partial degradation of a portion of the EGTA-liver large subunits during preparation of the ribosomes or isolation of the subunits.

Electrophoreograms of the EGTA-liver 80 S proteins showed
DISCUSSION

There are a number of differences in the proteins contained in rat skeletal muscle ribosomes and ribosomal subunits and those in liver particles. We wished to sort out which of the discrepancies might be due to differences in the expression of the genome for ribosomal proteins in the two tissues, and which might be the result of trivial changes, i.e. artifacts produced during the preparation of ribosomes or ribosomal subunits, during the extraction of the proteins, or during electrophoresis. Finally, we were led to consider the possibility, raised by our results, that certain ribosomal proteins are shared by the subunits and may be partitioned between one or the other or both subparticles depending on the conditions of their preparation. The following pairs of proteins lie in that category: S31* and L20, S_m1 and L31, S8 and L13 (Table 1).

Before we undertake a discussion of the differences between the proteins of muscle and liver ribosomes, a limitation of gel electrophoresis needs to be made explicit. Two proteins having different amino acid sequences might migrate together on two-dimensional polyacrylamide gel electrophoresis provided they had the same net charge and the same molecular size.

The position on two-dimensional electrophoretograms of the muscle small subunit protein S31*, corresponded exactly to that occupied by the liver large subunit protein L20. Whether S31* occurred with the small subunit or L20 with the large subparticle was dependent upon the method used to prepare the particles. If liver ribosomal subunits were prepared in Medium A (which contained 830 mM KCl and 12.5 mM MgCl₂) L20 was present in the large subunit; if the liver subunits were made in Medium B...
Potassium (830 mM) and magnesium (12.5 mM) but not if the latter was extracted from the small subunit because of the interaction between the protein and the RNA of the other subunit. On the other hand, the interaction may be different for the preparation of the ribosomes and ribosomal subunits (Table I). King et al. (2) did find that the treatment of rabbit reticulocytes with EDTA caused the transfer of a protein from the large to the small subunit.

Differences in the conditions of preparation of muscle and liver ribosomes might account for some of the variances in the proteins of the subunits. For example, protein S10 is absent from skeletal muscle ribosomes and ribosomal subunits. When liver ribosomes are prepared in the same manner as muscle particles, the 80 S monomers and the 40 S subunits contain greatly reduced quantities of S10. Thus, it is likely that S10 is extracted from ribosomes during treatment with EGTA or 250 mM KCl or both.

Preparation of ribosomes in EGTA and 250 mM KCl seems to make protein L1 susceptible to extraction during dissociation of the monomer. The protein is always absent from muscle 60 S subunits: it is absent from liver large subunits as well if the ribosomes were made in the same way as muscle particles. L1 is, however, present in both liver and muscle 80 S monomers no matter how they are prepared.

S2 is found with the proteins of the small subunit of skeletal muscle but not liver ribosomes. However, muscle ribosomes lack protein S22 which in standard conditions migrates with S24. The latter (S24) is located very close to S2 in the electrophoretogram. Thus, S2 and S22 may be proteins unique to muscle and liver ribosomes, respectively, or, perhaps what is more likely, muscle S2 is an altered form of liver S24 and muscle S24 is an altered form of liver S22. Since the apparent differences in the behavior of muscle and liver S22 and S24 cannot be accounted for by the conditions of preparation of the ribosomes and subunits they may reflect tissue-specific variations (Table I).

During the course of the present experiments we discovered zones L40* and L41* on electrophoreograms of the proteins of the large subunit of liver ribosomes: we had failed to detect them before (6). L40* and L41* are by far the most acidic proteins of eukaryotic ribosomes, which account in part for our failure (and that of others as well) to notice them earlier: since they are so

<table>
<thead>
<tr>
<th>Ribosomal proteins</th>
<th>Classification</th>
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<tr>
<td>S31* and L20</td>
<td>Possible identity; partition.</td>
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<tr>
<td>S1 and L31</td>
<td>Possible identity; partition.</td>
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<tr>
<td>S8 and L13</td>
<td>Possible identity; partition.</td>
</tr>
<tr>
<td>S10</td>
<td>Absent from muscle small subunit; possible preparation artifact.</td>
</tr>
<tr>
<td>S2</td>
<td>Present in muscle may correspond to liver S24.</td>
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<tr>
<td>S24</td>
<td>Muscle S24 may correspond to liver S22, which is absent from muscle.</td>
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<tr>
<td>L40* and L41*</td>
<td>Acidic proteins of large subunit of liver ribosomes (muscle had only L40*); may be homologous to Escherichia coli L7/L12.</td>
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a Possible identity is used to designate pairs of proteins that might be the same. b Partition indicates that the protein occurs with one subunit or the other, or both subunits (e.g. partitions) depending on the manner in which the ribosomes or the subunits are prepared. c Protein S10 is absent from the small subunit of skeletal muscle ribosomes and is found in reduced amounts in liver particles if they are prepared in the same way as muscle ribosomes. d Muscle S2 and S24 may be altered forms of liver S22 and muscle S24 of liver S22. Muscle has no normal S22. The differences may be tissue-specific.
Conversely, reticulocyte ribosomes had four proteins missing proteins of which 12 were not to be found in reticulocyte particles. Huynh-Van-Tan et al. (5) found a considerable number of differences in the proteins of rabbit reticulocyte ribosomes and rabbit liver (liver, kidney, caecal appendix, and reticulocytes) identically. They found no qualitative differences between the proteins of reticulocyte ribosomes and the ribosomal proteins from the other tissues (20). It may be important that they did not analyze the proteins of ribosomal subunits but depended entirely on an examination of ribosome monomer proteins. The analysis and comparison is considerably simplified if the number of proteins is reduced by using ribosomal subunits rather than the intact particle. Moreover, special pains need to be taken to ensure that the ribosomes are free of contaminants. A number of proteins, probably fortuitous contaminants and protein synthesis factors, are removed from ribosomes during the preparation of subunits (6).

Most, if not all, of the variations which we found between the proteins of rat skeletal muscle and liver ribosomes can be accounted for by differences in the preparation of the ribosomes, in the preparation of the subunits, or a differential apportionment of certain proteins between the two subunits during dissociation of the ribosome. We can not be certain that the ribosomal proteins of the two tissues are identical (the method of analysis does not permit certainty); nonetheless, it is unlikely that there are more than minor variances, hardly sufficient to account for marked differences in the character of protein synthesis in muscle and liver.

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REFERENCES


acidic they are lost in the anode buffer during electrophoresis in the first dimension unless the duration of electrophoresis is reduced to 20 hours; moreover, the proteins always stain very lightly with Amido black. L40* and L41* migrate in a virtually identical manner to E. coli ribosomal proteins L7 and L12. The only difference in the structure of E. coli L7 and L12 is that the N11-terminal serine of L7 is acetylated (47-49). The two proteins have been shown to be essential for the reactions which require GTP and the translation factors EF-G and EF-Tu (49-52). Liver L40* and L41* may be homologous with E. coli L7 and L12. Antisera to E. coli L7 and L12 cross-react with the proteins of the large subunit of rabbit liver ribosomes and antiserum to liver 60 S ribosomal proteins cross-react with E. coli L7 and L12 and no other E. coli ribosomal protein.7

Whereas liver 60 S ribosomal subunits have L40* and L41*, muscle large subunits have only L40*. Moreover, L41* is absent from both liver and muscle 80 S monomers. Since the proteins stain lightly at best we can not be sure of the significance of the absence of L41* (which may be homologous with E. coli L12, the nonacylated from of the protein).

There were differences between muscle and liver 80 S ribosomal proteins in the region of the gel occupied by high molecular weight proteins which migrate toward the anode in the first dimension (pH 8.6) and which stain lightly. There are five proteins in that group in liver 80 S ribosomal monomers (1, 2, 3, L1, L2); the latter two are in extracts of the proteins of the 60 S subunit as well. There are six proteins from 80 S monomers of muscle ribosomes (1, 2, 3, L1, L2, M4) in the same region of the gel, one only of which (L2) is to be seen with 60 S subunit proteins. M4 is among the proteins of skeletal muscle but not liver 80 S ribosomes. However, M4 does not occur with the proteins of either subunit of muscle ribosomes. There must, of course, be some question as to whether the proteins which occur in 80 S monomers but not in either subunit are properly to be considered as ribosomal.

On some of our gels proteins formed bands directly below the origin after electrophoresis in the second dimension (Figs. 1 and 2), a phenomenon also observed by Welfle et al. (23). We have argued (6) that those bands contain no additional species of protein, but rather are a sample of all the proteins, a sample that did not migrate in the first dimension because of reduced solubility of ribosomal proteins at pH 8.6. Conceivably, proteins which were absent in certain preparations might merely have been retarded at the origin during electrophoresis in the first dimension. We do not consider that to be likely. The distance the bands migrate directly below the origin is the same as the distance the protein spots migrate in the second dimension. However, there are no bands consistently found directly below the origin at the level at which these sometimes absent proteins (S8, S10, S22, S31*, S41, S5, S6, S7, S8, L1, L20, M4) would normally appear in the second dimension. Therefore, the evidence favors the conclusion that these proteins are lacking in the particles from which the preparations were made.

The ribosomal proteins from several normal mouse tissues (liver, kidney, intestines) were compared by two-dimensional polyacrylamide gel electrophoresis and found to be the same except that brain seemed to have one additional protein (8). Huynh-Van-Tan et al. (5) found a considerable number of differences in the proteins of rabbit reticulocyte ribosomes and rabbit liver ribosomes. Rabbit liver ribosomes were said to have 7 proteins of which 12 were not to be found in reticulocyte particles. Conversely, reticulocyte ribosomes had four proteins missing from liver ribosomes; thus reticulocyte ribosomes had only 67 proteins. Obviously, they find far more differences between the proteins of rabbit reticulocyte and liver ribosomes than we discern between rat liver and skeletal muscle ribosomes. There were significant differences in the methods by which they prepared rabbit liver and reticulocyte ribosomes, which certainly could account for at least some of the differences they observed. Delaunay et al. (20) attempted to meet that objection by preparing ribosomes from several rabbit tissues (liver, kidney, caecal appendix) and reticulocytes) identically. They found no qualitative differences between the proteins of ribosomes from liver, kidney, or caecal appendix. However, there were still many differences between the proteins of reticulocyte ribosomes and the ribosomal proteins from the other tissues (20). It may be important that they did not analyze the proteins of ribosomal subunits but depended entirely on an examination of ribosome monomer proteins. The analysis and comparison is considerably simplified if the number of proteins is reduced by using ribosomal subunits rather than the intact particle. Moreover, special pains need to be taken to ensure that the ribosomes are free of contaminants. A number of proteins, probably fortuitous contaminants and protein synthesis factors, are removed from ribosomes during the preparation of subunits (6).

1 G. Stöfler and I. G. Wool, unpublished observation.
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