Determination of the Number of Proteins in Liver Ribosomes and Ribosomal Subunits by Two-Dimensional Polyacrylamide Gel Electrophoresis*

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SUMMARY

The number of proteins in rat liver ribosomes was determined by two-dimensional polyacrylamide gel electrophoresis. There were 30 proteins in the 40 S subunit, 39 in the 60 S subunit. The proteins of the two subunits, with one possible exception, are unique. Preparations of 80 S ribosomes contained three proteins not present in either subunit; thus, we estimate that eukaryotic ribosomes contain between 68 and 72 different proteins.

The isolation, purification, and characterization of ribosomal proteins is a prerequisite for analysis of the structure and function of the organelle. Ribosomal proteins are heterogeneous (1). The 30 S subunit of Escherichia coli ribosomes contains 21 proteins, which have been shown to be unique by amino acid composition, tryptic peptides, and molecular weights (2-15); the 50 S subunit has approximately 34 proteins (8, 9, 15-17). Thus, the ribosomes from E. coli have approximately 55 different proteins, whose molecular weights range from 10,000 to 65,000 (7, 10). Since the proteins yield unique tryptic peptides (3, 4, 7, 14), and since antibodies to the proteins (with one exception) show no cross-reactivity (18), one must conclude (with one exception) that there are no homologies amongst the proteins. The exception is two of the proteins of the large subunit which differ only in that the amino-terminal serine of one of the two is acetylated (19, 20).

The proteins contained in eukaryotic ribosomes have not been isolated and purified, although a start has been made on their characterization (21-30). The results would indicate that ribosomal proteins from eukaryotic cells have the same general characteristics as those from prokaryotic organisms.

We undertook to determine the number of proteins contained in the subunits of eukaryotic ribosomes, and especially whether the proteins in the subunits were unique. For the analysis we used two-dimensional polyacrylamide gel electrophoresis, a technique developed by Kaltschmidt and Wittmann (40) and shown by them to be of great utility in resolving E. coli ribosomal proteins (9, 10, 40). Two-dimensional gel electrophoresis has been used to separate eukaryotic ribosomal proteins also (32-34, 38, 41, 42).

EXPERIMENTAL PROCEDURE

Preparation of Ribosomes--Liver ribosomes were isolated (43) from male Sprague-Dawley rats that weighed 100 to 120 g. The particles used to prepare 80 S ribosomal proteins for electrophoresis were incubated (43) to remove nascent peptide and endogenous messenger RNA, we refer to the particles as stripped ribosomes. Ribosomes were suspended (7 mg per ml) in Medium A (50 mM Tris-HCl, pH 7.8-12.5 mM MgCl₂, 80 mM KCl) containing 0.1 mM puromycin, 5 mM ATP, 0.5 mM GTP, 465 μg per ml of phosphoenolpyruvate, 10 mM 2-mercaptoethanol, 3.6 mg per ml of liver supernatant protein (G-25 fraction), prepared as described by Leader et al. (44). The ribosome suspension was incubated for 30 min at 37°. The mixture was centrifuged at 12,000 × g for 10 min to remove ribosomal aggregates; the ribosomes in the supernatant were sedimented by centrifugation (133,000 × g for 5 hours at 4° in a Spinco Ti 50 rotor) through 4 ml of 0.5 mg sucrose in Medium B (50 mM Tris-HCl, pH 7.8-12.5 mM MgCl₂, 880 mM KCl).

Preparation of Ribosomal Subunits--Liver ribosomes (1 to 1.4 g) that had not been stripped were dissociated by incubation for 15 min at 37° in Medium B containing 0.1 mM puromycin and 20 mM 2-mercaptoethanol. The ribosomal subunits were separated by centrifugation at 13,500 rpm for 17 hours at 25° in a Spinco Ti 15 zonal rotor with the use of a hyperbolic sucrose density gradient (45). The gradient was formed over a cushion of 45% sucrose; the concentration of sucrose in Medium B was 35% at 3.5 cm and 37.2% at 8.6 cm from the rotor core. The sample, in a linear sucrose gradient of 0 to 45% sucrose in Medium B (50 mM Tris-HCl, pH 7.8-12.5 mM MgCl₂, 880 mM KCl), was layered on the hyperbolic gradient. After centrifugation the gradient was displaced with 60% sucrose, and 10-ml fractions were collected. The fractions containing the 40 S subunits and those containing the 60 S subunit (Fig. 1) were pooled and dialyzed against 8 liters of Medium C (10 mM Tris-HCl, pH 7.6-0.1 mM MgCl₂, 80 mM KCl) for at least 36 hours with four to five changes of buffer. (Sucrose and high concentrations of potassium interfere with the subsequent precipitation of ribosomes by ethanol (46).) The concentration of magnesium in the subunit suspensions was made 12 mM by addition of suffi-
Fig. 1. Sedimentation of liver ribosome subunits in a zonal rotor. Liver ribosome subunits (1.38 g) were separated by centrifugation for 17 hours at 13,500 rpm in a Spinco Ti15 zonal rotor using a hyperbolic sucrose density gradient. Fractions (10 ml) were collected, and those indicated by the interrupted lines were pooled; the other fractions were discarded. One hundred seventeen milligrams of 40 S and 243 mg of 60 S subunits were recovered.

Fig. 2. Sedimentation of 60 S subunits of liver ribosomes in a zonal rotor. Fractions containing 730 mg of 60 S subunits from three previous centrifugations were pooled and centrifuged again for 17 hours at 13,500 rpm in a Spinco Ti15 zonal rotor using a hyperbolic sucrose density gradient. The fractions indicated by the interrupted lines were pooled; 321 mg of 60 S subunits were recovered.

Fig. 3. Sedimentation of subunit fractions in sucrose gradients. The purity of subunit fractions collected after centrifugation in a zonal rotor (Figs. 1 and 2) was determined after dialyzing the samples against Medium C. The samples were analyzed on 10 to 30% sucrose gradients in Medium D; centrifugation was at 60,000 rpm for 40 min at 25°C in a Spinco SW 65 rotor. a, 40 S fraction from Fig. 1, 18 µg; b, 60 S fraction from Fig. 1, 36 µg; c, 60 S fraction from Fig. 2, 36 µg.

The purity of the subunit fractions was determined by zonal centrifugation (Fig. 3). The samples were dialyzed against Medium C and layered on 10 to 30% linear sucrose gradients in Medium D (10 mM Tris-HCl, pH 7.6-7.9; MgCl₂-500 mM KCl); centrifugation was at 60,000 rpm for 40 min at 25°C in a Spinco SW 65 rotor. The distribution of the particles in the gradient was determined with an ISCO density gradient analyzer (47, 48). The purified 60 S subunits prepared as described above were free of contamination with 40 S subunits (Fig. 3c).

Extraction of Ribosomal Proteins—Proteins were extracted from ribosomes and from ribosomal subunits with acetic acid by a modification of a procedure described by Hardy et al. (6). Ribosomes were suspended (50 to 60 mg per ml) in 10 mM Tris-HCl (pH 7.7) containing 100 mM magnesium acetate; 2 volumes of glacial acetic acid were added, and the mixture was stirred for 1 hour at 0°C. The ribosomal RNA was removed by centrifugation at 15,000 × g for 10 min; the pellet was washed with 2 volumes of the same mixture of acetic acid-Tris-HCl-magnesium acetate and recentrifuged. The combined supernatant, which contained the ribosomal proteins, was dialyzed (in acetylated dialysis tubing (49)) against about 50 volumes of 10% acetic acid for 48 hours with five to six changes of acid. The dialyzed proteins were lyophilized.

We did not extract ribosomal proteins by other methods, as, for example with lithium chloride-urea (4), or guanidine-HCl (50). Ford has reported (51) that acetic acid (52) is not as efficient as the other methods for the extraction of proteins from Xenopus ribosomes. However, the method he used (52) did not embody the modification (high concentrations of magnesium) introduced by Hardy et al. (6) which considerably increases the efficiency of the procedure. Nonetheless, we do not know that we have extracted all the proteins of liver ribosomes.

Two-dimensional Polyacrylamide Gel Electrophoresis—The procedure we used was that of Kaltschmidt and Wittmann (40). The “standard conditions” for two-dimensional electrophoresis...
of ribosomal proteins, to which we shall refer frequently, were 8% acrylamide gel, pH 8.6, for 40 hours at 90 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 size in the second. The concentration of ribosomal proteins in the sample gel was 6 to 8 mg per ml; the amount analyzed was 0.4 to 3 mg (in 50 to 400 μl). Electrophoresis was at room temperature.

Several minor changes in the Kaltschmidt-Wittmann procedure (40) were made. The 8 M urea solution used in the preparation of separation or sample gels for electrophoresis in the first dimension was passed through a column of Bio-Rad AG501-X8(D) resin to remove cyanate. Cyanate can lead to carbamylation of lysine residues (53), and hence adventitiously increase the number of protein bands or spots obtained on electrophoresis of ribosomal proteins. Urea was omitted from the buffer solution, used for electrophoresis in the first dimension; instead 55 ml of 2 N NaOH was added to maintain the pH at 8.6. A 150-μl aliquot of sample gel was polymerized on either side of the gel that actually contained the sample to serve as spacer gels.

The gels were stained for 20 min in 1% Amido black in 7.5% acetic acid. The dye had been purified by electrophoresis through 10% acrylamide gel at 100 ma for 40 hours; the dye remaining on the anode side of the gel was used. The gels were destained for 1 hour in running distilled water and for 3 to 4 days in eight changes of 17 liters of 7.5% acetic acid. The protein spots from each subunit were numbered separately along horizontal lines beginning at the upper left. The 60 S subunit proteins are designated by an L (for large subunit), the 40 S subunit proteins by an S (for small subunit).

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 composites from a large number of electropherograms, and as such they may differ in detail from individual gels. We did not include on the schematics the material that remained at the origin in the first dimension and migrated directly toward the anode in the second dimension.

RESULTS

When ribosomal proteins were analyzed by two-dimensional electrophoresis there were shadowed streaks in the upper portion of the gel plates on the cationic side in a region where one would expect to find the proteins of greatest molecular weight (Fig. 4). The streaks are probably caused by protein aggregates linked by disulfide bonds. Kaltschmidt and Wittmann (9) have shown that similar streaks, which occur when two-dimensional electrophoresis of E. coli ribosomal proteins is carried out, can be eliminated if the proteins are oxidized with performic acid or reduced and alkylated with iodoacetamide. We have not repeated that procedure. On occasion proteins migrated directly down from the origin in the second dimension, sometimes forming bands rather than spots (Fig. 4). The formation of the bands was concentration dependent. 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 pH in the first dimension). The proteins would be expected to be soluble at the pH (4.2) used in the second dimension. The bands do not contain species of proteins in addition to those that do migrate in the first dimension (see “Discussion”).

Proteins of 40 S Subunit—The 40 S subunit fraction obtained by zonal centrifugation (Fig. 1) was free of contamination with 60 S subunits (Fig. 3a). Two-dimensional acrylamide gel elec-

![Fig. 4. Two-dimensional electrophoretograms of liver ribosomal proteins. Standard conditions were used for electrophoresis. The anode was at the left in the first dimension, at the top in the second. A, 1 mg of protein from 40 S ribosomal subunits; B, 1 mg of protein from 60 S ribosomal subunits; C, 1.6 mg of protein from 80 S ribosomes. Some of the spots on the gels were clearly visible to the eye, but are barely discernible or cannot be seen in the photograph. Those proteins are included in the schematics (Figs. 5 to 8).](http://www.jbc.org/content/244/17/4462/F4.large.jpg)
Fro. 5. Schematic of the two-dimensional electrophoretogram of the proteins of the 40 S subunit of liver ribosomes. The solid spots were always seen; the cross-hatched spots either varied in location (S21) or in intensity of staining (S27 and its three satellite spots); the open spot (S22) was only seen when the conditions of electrophoresis were changed. The diagram includes spots that are difficult to see in the photographs (Fig. 4). The intensity of the staining of the spots is not represented with fidelity.

Electrophoresis of the proteins of the 40 S subunit in standard conditions revealed 28 to 30 spots (the pattern is portrayed schematically in Fig. 5). The variability is in a group of one to three spots which, after electrophoresis in the second dimension, appear directly under S27. The intensity of S27 varies inversely with the number and intensity of the three satellite spots. It would seem that the latter are altered forms of protein S27 (they were not seen in electrophoretograms of 80 S ribosomes where S27 stains intensely; see below). Protein S21 usually migrates toward the anode in the first dimension, to the boundary between the spacer and separation gels; however, on occasion it moves toward the cathode. In either case, it migrates the same distance in the second dimension.

With the exceptions we have already noted (S27 and S21), the position of the spots and the intensity with which they were stained could be reproduced precisely. To verify that no spot contained more than one protein 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. In any but the standard conditions of electrophoresis, there was an additional spot, S22, which apparently had undergone co-electrophoresis with S24. Two more spots, S29 and S30 (see Fig. 5, inset), were discovered when electrophoresis was shortened from 40 hours to 18 in the first dimension and from 40 hours to 19 in the second. In standard conditions, protein S29 and S30 were lost into the cathode buffer vessel during electrophoresis in the first dimension.

It is not to be expected that proteins with different sizes or charges (or both) would migrate together in all the conditions we have tried; therefore, we assume that each spot contains a single protein and that the number of proteins in the 40 S subunit is 30.

Proteins of 60 S Subunit—Even the purest fraction containing 60 S subunits obtained from a single centrifugation in a zonal rotor was contaminated with a small number of 40 S subunits (Fig. 3b). When the proteins extracted from that preparation of 60 S subunits were analyzed by two-dimensional electrophoresis, almost all of the proteins of the 80 S ribosome were present (results not shown). Clearly, to determine the number of proteins in the 60 S subunit, and whether they differ from the 40 S proteins, it is necessary that the large subunits be scrupulously free of contamination with small subunits (Fig. 3c).

Proteins extracted from purified 60 S subunits were analyzed by two-dimensional acrylamide gel electrophoresis in standard conditions (Fig. 4B); 37 spots could be detected on electrophoretograms (the pattern is portrayed schematically in Fig. 6). L1 and L2 stained lightly and were only seen when large amounts (1.6 rather than 0.8 mg) of protein were used. Once again the concentration of acrylamide in the first dimension was varied from 5 to 10% and the pH of the electrophoresis buffer from 7.6 to 10.6. In all but standard conditions of electrophoresis there was an additional spot, L33, which apparently had undergone co-electrophoresis with L32. One more spot, L39 (see Fig. 6, inset) was seen when electrophoresis in the first dimension was decreased to 18 hours and in the second to 19 hours. We conclude that the 60 S subunit contains at least 39 separate proteins.

Proteins of 80 S Ribosome—Ribosome monomers were prepared by a method expected to yield particles free of contamination with extraneous proteins. Electrophoresis, using standard conditions, of the proteins extracted from the particles revealed...
FIG. 7. Schematic of the two-dimensional electrophorogram of the proteins of rat liver ribosome monomers. The solid spots were always seen; the cross-hatched spot (S21) varied in location; the open spots (1, 2, 3) were seen when ribosome monomer proteins were analyzed but not when subunit protein was subjected to electrophoresis.

65 spots (Figs. 4C and 7); 58 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, generally toward the cathode, S27, which formed a single spot without the three satellite spots, and L1 and L2, which stained darker than in preparations of proteins extracted from the 60 S subunit. Of the seven remaining spots, four were the result of co-electrophoresis of pairs of proteins: S22 and S24; L32 and L33; L32 and L33, separated when the proteins of the small and large subunits, respectively, underwent electrophoresis alone in any conditions other than standard (Figs. 5 and 6). L2 and S18 were separated when the concentration of acrylamide in the first dimension was increased from 8% to 10%. However, L13 and S8 migrated together even when the acrylamide concentration in the first dimension was varied from 8% to 10%. However, L13 and S8 migrated together even when 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. L13 and S8 are the only proteins of the large and small subunit which were not separated, and hence the only subunit proteins which have not been demonstrated to have a unique size or charge, or both. Of course, they may well have unique sequences of amino acids.

Three additional spots (S29, S30, and L39) were seen when electrophoresis was done under standard conditions; the number accounted for can be increased to 63 if the conditions of electrophoresis are altered. Four proteins (S22 and S24, and L32 and L33) formed separate spots only when the subunit proteins underwent electrophoresis alone in any condition other than standard. L13 and S8 were not separated.

The three remaining spots, which were seen when the proteins from 60 S ribosomes underwent electrophoresis, migrate toward the anode (1, 2, 3 in Fig. 7); they were not present when either 40 S or 60 S ribosomal protein underwent electrophoresis.

Equal amounts by weight of 40 S and 60 S ribosomal proteins were subjected to electrophoresis at the same time (Fig. 8). The pattern was like that of ribosome monomer proteins, except that the three S27 satellite spots were present, and the three anionic spots (1, 2, 3) were absent. S21 migrated toward the cathode.

**FIG. 8.** Schematic of the two-dimensional electrophorogram of an equal mixture by weight of rat liver 40 S and 60 S ribosomal proteins. The solid spots were always seen; the cross-hatched spots either varied in intensity (L1, L2, S27 and its three satellites) or in location (S21).

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**DISCUSSION**

We believe it unlikely that proteins with different size or charge (or both) would migrate together during two-dimensional electrophoresis despite variations in gel concentration, pH of the buffers, and time of electrophoresis. We assume then that each spot contains a single protein. There is one reservation; two different proteins might fortuitously have the same size and charge. Assuming each spot to be a single protein, it need be pointed out that the intensity with which the spots are stained with Amido black varies a great deal. Moreover, the differences in the intensity of the staining (with the exception of S27 and its three satellites) are reproducible. The variation might be due to an intrinsic difference in the intensity with which proteins stain, or it might be the result of a difference in the number of copies of individual proteins, and hence reflect ribosome heterogeneity as some have assumed (9, 31).
One protein in the 40 S subunit (S12) and two in the 60 S subunit (L1 and L2) migrated toward the anode and thus have isoelectric points lower than pH 8.6. The isoelectric point of S21 is probably very close to pH 8.6 since it did not migrate appreciably from the origin (the variability that occurred in the direction of the migration was probably due to minor variations in the experimental conditions). *E. coli* ribosomes are quite different in that they have 11 proteins with isoelectric points lower than pH 8.6 (12). Our results are not in agreement with those of Huynh-Van-Tan et al. (33) nor Welfle et al. (32) who found no other ribosomal proteins that migrated toward the anode during two-dimensional electrophoresis. More recently Welfle reported (38) that two proteins from liver ribosomes migrate toward the anode.

On some of our gels proteins formed bands directly below the origin after electrophoresis in the second dimension (Fig. 4). A similar phenomenon has been observed by Welfle (38). It is, of course, important to determine whether those bands contain unique proteins which were not recognized as individual spots on the electrophoretograms. We believe the internal evidence supports the conclusion that the bands contain no additional species of protein, but rather that they are an aliquot of all the proteins, an aliquot that did not migrate in the first dimension because of reduced solubility of ribosomal proteins at pH 8.6. First, it should be noted that no additional spots were observed on those occasions when the bands were absent. The second argument derives from the behavior of protein S21. The isoelectric point of S21 is close to 8.6, the pH of the buffer, but the protein always migrated in the first dimension (albeit the direction was not constant). Thus it must be insolubility in the buffer, rather than an isoelectric point the same as the pH of the buffer, that accounts for protein remaining at the origin. (We assume that only an isoelectric point close to 8.6 or insolubility will cause a protein to remain at the origin during electrophoresis.) After undergoing electrophoresis in the second dimension at pH 4.2, the proteins may be supposed to have been solubilized and migrated from the original sample gel to form bands in the second dimension gel. It is important that the migration distances of the bands were the same as those of the proteins that appeared as spots in the second dimension. If the bands contained unique proteins one would have to make the additional (highly unlikely) presumption that every one of them had a molecular size that corresponded to the size of proteins that were resolved as spots in the second dimension. Therefore, we conclude the bands contained no additional species of ribosomal proteins.

Originally ribosomal proteins were defined as those proteins which were associated with the particle from the time it was formed until it was destroyed, a definition that clearly excluded initiation and translation factors. However, the aptness of that definition has been questioned (54). If ribosomes are heterogeneous with respect to their proteins, and especially if "fractional" proteins can be exchanged by ribosomes, then clearly the definition will not do. There is difficulty in arriving at even an acceptable operational definition, i.e. ribosomal proteins are the set of proteins required for function of the ribosome. That definition is not satisfactory if there are structural proteins which are not essential, but rather amplify ribosome function (54, 55). Given the difficulty of deciding which proteins are ribosomal, it is not surprising that the estimate of the number in the set varies. For example, there is not yet agreement as to the exact number of proteins in *E. coli* ribosomes; the estimate of the total varies from 48 (16) to 55 (9). With those reservations in mind, we conclude that the 40 S subunits of rat liver ribosomes contain 30 proteins and the 60 S subunits 39. The proteins of the two subunits, with one possible exception for each (S8 and L19), are unique; hence the 80 S ribosome monomer contains 68 or 69 different proteins. We found that rat liver ribosome monomers prepared in a manner likely to remove initiation and translation factor, nascent peptide, and proteins bound to the ribosome factually during isolation of the particle, actually contained the 68 or 69 proteins of the subunits and three additional spots (albeit the last three stained lightly). The identity of those three proteins is not known to us, but we have no reason to believe they are ribosomal proteins since they are not present in either subunit and the subunits function normally, at least in the translation of polyuridylic acid (47, 48) and encephalomyocarditis RNA (56).

Others have estimated the number of proteins in eukaryotic ribosomes and in ribosomal subunits. Pickle and Traut (39) determined the molecular weight distribution of proteins from mouse placenta ribosomes by electrophoresis in polyacrylamide gels containing sodium dodecyl sulfate. The gels were scanned with a spectrophotometer and from the quantity of protein in the bands it was possible to calculate the weight average and number average molecular weights of the subunits. The values for the 40 S subunit were 34,000 and 29,000, respectively, and for the 60 S particle, 32,000 and 27,000. An estimate of the number of moles of protein in each subunit was obtained by dividing the daltions of protein in the particle by the average molecular weight. The number they obtained for the 40 S subunit was 18 to 27 (depending on whether they used the weight average or number average molecular weight); that is to be compared with our finding of 30 proteins. They also estimated that the 60 S subunit had 42 to 52 proteins, we found 39. We would suggest their prediction for the small subunit was somewhat low, and for the large subunit high.

King et al. (31) counted 35 ± 5 proteins for the small subunit of rabbit reticulocyte ribosomes, 49 ± 12 for the large subunit, and 85 ± 17 for the monomer. Their determinations were based on the intensity of staining after electrophoresis in a single dimension in polysacrylamide gels containing sodium dodecyl sulfate, and given the difference in the resolving power of that procedure and two-dimensional gel electrophoresis, their estimates and ours are in reasonable agreement. There is also good correspondence between our tally (i.e. 69 proteins) and that of Huynh-Van-Tan et al. (33) and Welfle et al. (32). The former found 75 proteins in rabbit liver ribosomes and the latter 76 in rat liver ribosomes; both used two-dimensional electrophoresis for the analysis. Finally, Warner (35) has reported that yeast (Saccharomyces cerevisiae) ribosomes have 80 proteins (30 in the small subunit and 50 in the large); the calculation was from a comparison of the specific radioactivity of bands after electrophoresis in sodium dodecyl sulfate gels. The exact number of proteins in eukaryotic ribosomes is not certain, and in fact may vary from species to species (33), but is surely not far from 70. It is clear that eukaryotic ribosomes have more proteins than the 48 to 55 present in *E. coli* ribosomes (38).

Our experiments are very similar to those of Martini and Gould (34) who analyzed the proteins of rabbit reticulocyte ribosomal subunits by two-dimensional gel electrophoresis. The main difference between their procedure and ours is that they used...
polyacrylamide gels containing sodium dodecyl sulfate for electrophoresis in the second dimension. They found the 40 S subunit to contain 26 proteins, whereas we found 30 proteins. The difference between their results and ours can be accounted for in part by their failure to detect two proteins (S29 and S30) which are lost into the cathode buffer unless the time of electrophoresis is shortened from 40 to 18 hours, and one protein (S22) that is only resolved (from S24) when the conditions of electrophoresis are changed. Martini and Gould also report that the 60 S subunit has 36 or 37 proteins; we found 39. Once again the difference can be reconciled by assuming they failed to detect one protein (L39) which is only seen when the duration of electrophoresis is decreased, and a second (L33) which is only resolved (from L32) when the conditions of electrophoresis are changed.

There is a serious disagreement between our results and those of Welfle et al. (32). They report 27 proteins specific to the small subunit of rat liver ribosomes, 34 to the large subunit, and 15 shared by the two. The small subunit then would have a total of 42 proteins and the large subunit 49. The critical disparity in the two studies is in the number of proteins in the small and large subunit reported to have identical electrophoretic mobilities, 15 in the experiments of Welfle et al. (32), only one in ours. We believe the discrepancy can be accounted for by the difference in the methods used to prepare ribosomal subunits, in the purity of the 60 S subunit, and in the conditions used to carry out two-dimensional electrophoresis. Welfle et al. (32) formed subunits by treating ribosomes with EDTA. The subunits prepared in that way are no longer active in protein synthesis (48, 57). What is more important, because of removal of magnesium the ribosomes and subunits unfold to varying degrees (48, 57-59), complicating the isolation of the particles by zonal sedimentation. It is especially important that the large subunit may cosecond with unfolded ribosome monomers and the small subunit may be contaminated with unfolded large subunits. Contamination of this kind accounted for an earlier report (60) almost certainly in error (26) that 60 S subunits prepared with EDTA contained all of the proteins of the ribosome. In addition, King et al. (31) found that EDTA could cause the transfer of a protein from the large to the small subunit.

We dissociated ribosomes by treating them with high concentrations (0.88 M) of KCl (47, 48). Subunits prepared in that way will recombine to form ribosomes active in protein synthesis (47, 48). It is also important then that the subunits be free of contamination as possible. Finally, we have shown that it is important to vary the conditions of electrophoresis, for only in that way were several of the proteins detected. We feel confident then that the proteins of the two subunits, with one possible exception, are unique.

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