Protein Composition of the Bovine Mitochondrial Ribosome*

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The protein complement of the bovine mitochondrial ribosome has been analyzed by two-dimensional electrophoresis in polyacrylamide gels to determine the number and molecular weights of the ribosomal proteins. Salt-washed ribosomal subunits are found to contain a total of 85 ribosomal proteins, 84 of which are electrophoretically distinct between the two subunits. These proteins are also electrophoretically distinguished from those of cytoplasmic ribosomes. This large number of proteins does not appear to be due to contamination by cytoplasmic ribosomal proteins or by adherent nonribosomal proteins. The molecular weights of these proteins are considerably larger than those of *Escherichia coli* ribosomal proteins, and are similar to those of bovine cytoplasmic ribosomal proteins. The sum of the molecular weights of the 85 proteins agrees well with that predicted by physical-chemical measurements of the total mass of protein in the two subunits. Bovine mitochondrial ribosomes thus contain about twice as much protein as RNA, a highly unusual composition in comparison to the other kinds of ribosomes which have been characterized to date. In addition, it appears that the ribosomal proteins themselves are less basic than the proteins of most other ribosomes.

The mitochondrial ribosomes of higher animals differ considerably in physical and chemical properties from their extramitochondrial counterparts, and from bacterial ribosomes and the mitochondrial ribosomes of other kinds of organisms as well. Animal mitochondrial ribosomes have a comparatively low sedimentation coefficient of 55-60 S (1-5), small rRNA molecules with molecular weights of 0.35 \times 10^6 and 0.54 \times 10^6 (6, 7), and a low buoyant density of 1.40-1.45 g/ml (2, 7-10). Although their slow sedimentation rate and small rRNA might suggest that these ribosomes are unusually small particles, this is not the case. Bovine mitochondrial ribosomes have about the same molecular weight as the ribosomes of *Escherichia coli*, which contain nearly twice as much rRNA and sediment as 70 S particles (11). This observation implies that bovine mitochondrial ribosomes must contain an unusually high proportion of protein, a conclusion which is supported by the low buoyant density of these particles (8). As a basis for more detailed studies into the structure and function of mammalian mitochondrial ribosomes, we have analyzed this large ribosomal protein complement by two-dimensional polyacrylamide gel electrophoresis.

**EXPERIMENTAL PROCEDURES**

Preparation of Ribosomes—Mitochondrial ribosomes were prepared from bovine liver as described previously (12, 13) with the following modifications. The isolation medium used in homogenizing the liver and washing the mitochondria was 0.34 M sucrose, 1 mM EDTA, and 5 mM Tris-HCl, pH 7.5. After each wash, mitochondria were concentrated by centrifugation in a Beckman JA-10 rotor at 8000 rpm for 10 min. After the first wash, mitochondria were resuspended to a concentration of 20 mg of protein/ml in isolation medium and treated with digitonin at a final concentration of 100 \mu g/ml with constant stirring for 15 min at 4 °C. After diluting this mixture 5-fold in isolation medium, the mitochondria were pelleted as above and washed once more in isolation medium before storage at -70 °C as a concentrated mixture in 0.26 M sucrose, 40 mM KCl, 15 mM MgCl₂, 14 mM Tris-HCl, pH 7.5, 5 mM \beta-mercaptoethanol, 0.8 mM EDTA, 50 \mu M spermine, and 50 \mu M spermidine (buffer A).

To prepare ribosomes, mitochondria were thawed quickly and adjusted with buffer A to a concentration of 20 mg of protein/ml and lysed by the addition of Triton X-100 to a concentration of 1.6%. After centrifugation at 10,000 rpm in a Beckman JA-10 rotor for 45 min, the mitochondrial ribosomes in the supernatant were concentrated by a high-speed centrifugation (100,000 \times g) for 17 h over 34% 20-ml sucrose cushions in buffer B (100 mM KCl, 20 mM MgCl₂, 20 mM Tris-HCl, pH 7.5, 5 mM \beta-mercaptoethanol) containing 1% Triton X-100 in a Beckman Type 35 rotor. In some preparations, ribosomes were concentrated by adsorption on DEAE-cellulose prior to the high speed centrifugation. Ribosomes prepared by either procedure were qualitatively similar.

The crude ribosomes were incubated for 5 min at 37 °C in buffer B containing 1 mM puromycin to disassociate nascent polypeptides (11). The 55 S ribosomes were further purified by sucrose density gradient centrifugation in 10-30% linear gradients in buffer B. Fractions containing the ribosomes were pooled and concentrated by centrifugation. To dissociate the ribosomes, the pellets were resuspended in one of the following buffers: buffer C (200 mM KCl, 2 mM MgCl₂, 10 mM Tris-HCl, pH 7.5, 5 mM \beta-mercaptoethanol), buffer D (300 mM KCl, 5 mM MgCl₂, 10 mM Tris-HCl, pH 7.5, 5 mM \beta-mercaptoethanol), or buffer E (600 mM KCl, 10 mM MgCl₂, 10 mM Tris-HCl, pH 7.5, 5 mM \beta-mercaptoethanol). Subsequently, the subribosomal particles were separated by sedimentation through 10-30% sucrose density gradients in the same buffer.

Using the extinction coefficient \epsilon_{260} = 110 for bovine mitochondrial ribosomes, together with the mass of the monoribosome (2.83 \times 10^9 daltons), large subunit (1.65 \times 10^9 daltons), and small subunit (1.18 \times 10^9 daltons), 1 A₈₂₀ unit corresponds to 32 pmol of 55 S ribosomes, 55 pmol of 39 S subunits, and 84 pmol of 28 S subunits (11).

Bovine liver cytoplasmic ribosomes were prepared by homogenizing beef liver in isolation medium containing 0.34 M sucrose, 10 mM Tris-HCl, pH 7.5, 5 mM MgCl₂, 25 mM KCl, and 5 mM \beta-mercaptoethanol. Mitochondria and cell debris were removed by centrifugation in a Beckman JA-10 rotor at 8,000 rpm for 10 min. Microsomes were pelleted by centrifugation in the JA-10 rotor at 9,000 rpm for 45 min. The microsomes were resuspended in buffer F (100 mM KCl, 5 mM

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MgCl₂, 20 mM triethanolamine, pH 7.5, and 5 mM β-mercaptoethanol) and lysed with Triton X-100 at a final concentration of 2%. Cytoplasmic ribosomes were concentrated by centrifugation at 96,000 × g in a Beckman Type 50 rotor for 12 h over a 34% sucrose cushion in buffer F. After a 5-min treatment with 1 mM puromycin at 37 °C, the ribosomes were further purified by sucrose density gradient centrifugation 10 rotor F. The 60 S cytoplasmic ribosomes were dissociated into subunits by sedimentation through a sucrose density gradient in high salt buffer G (500 mM KCl, 5 mM MgCl₂, 20 mM triethanolamine, pH 7.5, and 5 mM β-mercaptoethanol). The subunits were concentrated by high-speed centrifugation (230,000 × g) in a Beckman Type 95 rotor and stored frozen at -70 °C until needed.

Radioactive Labeling of Ribosomal Proteins—Ribosomes were resuspended in 20–50 μl of buffer C, D, or E. Solid urea and LiCl were added to adjust the ribosome sample to 9 M urea, 3 M LiCl, and the pH was adjusted to 3.5 by the addition of 3 N HCl for a final volume of 120 μl. The samples were stirred at 5 °C for 12 h and the RNA precipitate was removed by centrifugation at 220,000 × g for 1 h. The RNA pellet was re-extracted by stirring with 9 M urea, 3 M LiCl, pH 3.5, for 6 h. After a second centrifugation, the supernatant fractions were combined and dialyzed in Spectrapore No. 2 tubing against sample buffer (9 M urea, 60 mM potassium acetate, pH 6.7, and 0.01% aminothiazole).

Radioactive Labeling of Ribosomal Proteins—Ribosomal proteins were labeled by reductive methylation using the cyanoborohydride method of Jentoft and Dearborn (14). Two nmol of [²²Na]formaldehyde (42 Ci/mmol, New England Nuclear) were added to 10–20 μg of ribosomal protein in a final volume of 24 μl containing 8 M urea, 3 M LiCl, 20 mM NaN₃, 1 mM Na₂HPO₄, and 20 mM KH₂PO₄, pH 7.2. The labeling reaction was proceeded with constant stirring for 2 h at room temperature. The methylated sample was dialyzed against sample buffer for 3–4 h before the addition of carrier amounts (200 μg) of unlabeled r-proteins. Dialysis was continued for an additional 16 h before applying the samples onto polyacrylamide gels.

Bovine cytochrome c was labeled with [³⁵S] to a specific activity of 2.4 × 10⁶ dpm/pmol using iodogen (1,3,4,6-tetrachloro-3,6-diphenyl-glycoluril) as the catalyst following the procedure of Markwell and Fox (15). The reaction mixture contained 0.5 mg of bovine cytochrome c (Sigma), 100 mM KCl, 10 mM MgCl₂, 10 mM Tris-HCl, pH 7.5, 0.1 mM EDTA, and 1 μM [³⁵S]NaI, 0.16 mgCl (Amersham). The reaction mixture was added to a tube previously coated with 100 μg of iodogen and the reaction was allowed to proceed for 15 min at room temperature. To remove free ¹, the labeled cytochrome c was chromatographed on a small (20 cm × 0.5 cm) Sephadex G-25 column equilibrated with a buffer containing the same ionic composition as the reaction mixture. ³⁵S was counted on a Searle Model 1197 γ counter with 90% efficiency.

Electrophoresis—The electrophoretic system of Leister and Dawid was used, with some modifications (16, 17). The acrylamide concentration of the first dimension separation gel was 4.6% T, 3.2% C (T is the cross-linker, C is the cross-linker, bisacrylamide, in percentage of T%). The first dimension separation gel was prerun overnight in separation gel buffer (9 M urea, 60 mM potassium acetate, pH 4.3) before casting the stacker gel of 4% T, 3.5% C, in 8 M urea, 60 mM potassium acetate, pH 6.7. Proteins were applied to the stacker gel in sample buffer and electrophoresed at 0.2 mA/gel of 3.0-mm diameter until the tracker dye pyronine Y had entered the separation gel. The tank buffer, 35 mM β-alanine, pH 4.5, contained 0.01% aminothiazole. Electrophoresis was carried out at 0.5 mA/gel, until the tracker dye was within 1 cm of the bottom. The first dimension gel was soaked for 10–15 min in a solution of 5 M urea, 2% SDS, 10 mM sodium phosphate, pH 7.2.

For the second dimension we used a 1.5-mm thick SDS gel slab of 10% T, 3.5% C in 5 M urea, 0.5% SDS, 0.1 M sodium phosphate, pH 7.2. The first dimension gel was affixed to the top of the slab gel by polymerizing it into a 1.5-cm stacker gel of 4% T, 3.5% C in 5 M urea, 0.5% SDS, 10 mM sodium phosphate, pH 7.2. To allow estimation of the molecular weight of individual ribosomal proteins from their second dimension migration distances, samples of standard molecular weight markers in agarose plugs were positioned on the second dimension slab gel, adjacent to the ends of the first dimension gel. These proteins included: bovine serum albumin (M, = 68,000), ovalbumin (44,000), carbonic anhydrase (30,000), γ-globulin heavy chain (50,000), lysozyme (14,400), and cytochrome c (12,500).

Gel slabs containing ³⁵S-labeled proteins were prepared for fluorography as described by Chamberlain (18) and placed in contact with pre-exposed Kodak XR-5 medical x-ray film as described by Laskey and Mills (19). The radiolabeled proteins were located relative to the stained proteins by superposition of the autoradiogram on the dried gel.

RESULTS

Electrophoresis of Mitochondrial R-Proteins—To study the proteins of bovine mitochondrial ribosomes, conditions for the preparation of the ribosomes were designed to yield ribosomes of high purity. The possibility of contamination by particles with sedimentation coefficients close to those of mitochondrial ribosome subunits was minimized by first isolating the ribosomes as intact 55 S particles and then dissociating them to (more slowly sedimenting) subunits on a second sucrose density gradient.

Ribosomes were dissociated into their subunits with buffer E, a high salt condition (500 mM KCl, 10 mM MgCl₂) commonly used for the dissociation of E. coli ribosomes (20, 21) and eukaryotic-cytoplasmic ribosomes (22, 23). In addition, we used buffers C and D, two other moderate salt conditions (200 mM KCl, 2 mM MgCl₂ and 300 mM KCl, 5 mM MgCl₂) designed to minimize the salt-stripping of bona fide r-proteins from the ribosome. The two-dimensional electrophoretic patterns of the proteins of the large and small subunits prepared under these three buffer conditions are shown in Figs. 1 and 2, respectively.

These subunits of mitochondrial ribosomes appear to contain a large number of different proteins, in comparison with results obtained with other kinds of ribosomes. Seventeen different preparations of salt-washed mitochondrial ribosomes

**Fig. 1.** Separation of the proteins from the large subunit of bovine mitochondrial ribosomes by two-dimensional polyacrylamide gel electrophoresis as described under "Experimental Procedures". The first dimension (left to right) was in urea at pH 4.3, in a separation gel of 4.6% T, 3.2% C, and the second dimension (downward) was in SDS at pH 7.2. A, electropherogram of subunits prepared in buffer C, B, buffer D, and C, buffer E. D, schematic diagram of the reproducibly occurring proteins from the large subunit. The SDS electrophoretic positions of bovine serum albumin (68,000 daltons), human γ-globulin heavy chain (50,000 daltons), ovalbumin (44,500 daltons), carbonic anhydrase (30,000 daltons), lysozyme (14,400 daltons), and cytochrome c (12,500 daltons) are marked at the right of the diagram.
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were analyzed on 54 separate two-dimensional gels to determine which of the protein spots occur with reasonable reproducibility. In this analysis, 52 proteins were found in the large subunit of bovine mt-ribosomes (Fig. 1D) and 33 were found in the small subunit (Fig. 2D). Thirty-nine of the 52 large subunit proteins were always present. The other 13 proteins were analyzed on 54 separate two-dimensional gels to determine which of the protein spots occur with reasonable reproducibility. In this analysis, 52 proteins were found in the large subunit, 33 were found in the small subunit, and 9 r-proteins were present in all samples examined, and 9 r-proteins (S1, S2, S3, S9, S11, S13, S17, S19, and S24) were sometimes absent from the gels.

Those proteins which were occasionally absent may represent contaminating or adsorbed nonribosomal proteins. However, for reasons discussed below, we favor an alternative interpretation: rather that these proteins may be ribosomal proteins which were either variably modified, not resolved from adjacent protein spots on particular gels, or lost during the isolation, salt washing, extraction, or electrophoresis. In some of the two-dimensional electropherograms, additional proteins were occasionally present. These infrequently appearing proteins were not counted in the total 85 mt-ribosomal proteins from either the large or the small subunit. The position of less than one molecule of cytochrome c/10 ribosomes. After dissociation and separation into subribosomal particles on the second sucrose density gradient (Fig. 3B), the contamination level of small subunits (1,700 dpm/Azm unit) and large subunits (1,500 dpm/Azm unit) was less than 1 molecule of cytochrome c/90 particles. These subribosomal particles were concentrated by centrifugation in sucrose density gradients and by two-dimensional PAGE of the extracted proteins.

After centrifugation into the first sucrose density gradient (Fig. 3A), the specific radioactivity of the 55 S ribosomes was 7,100 dpm/Azm unit, corresponding to a contamination level of less than one molecule of cytochrome c/10 ribosomes. After dissociation and separation into subribosomal particles on the second sucrose density gradient (Fig. 3B), the contamination level of small subunits (1,700 dpm/Azm unit) and large subunits (1,500 dpm/Azm unit) was less than 1 molecule of cytochrome c/90 particles. These subribosomal particles were concentrated by centrifugation so that their proteins could be extracted (“Experimental Procedures”) and analyzed by two-dimensional PAGE. No additional stained spots were apparent in the two-dimensional gels (data not shown). Furthermore, no radioactive spots were disclosed by fluorography, using conditions adequate to detect spots containing only 50 dpm, corresponding to a contamination level of less than 1 molecule of cytochrome c/1000 ribosomes. In a parallel experiment, [14C]cytochrome c (labeled by reductive methylation) was co-electrophoresed with a carrier amount of r-proteins from either the large or the small subunits. The position

The abbreviations used are: mt-, mitochondrial; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis.
of cytochrome c in these gels, disclosed by fluorography, is just below and to the right of L44 and S29 (Figs. 1D and 2D), demonstrating that bovine cytochrome c does not co-migrate with any of the 85 mt-ribosomal proteins. On the basis of these experiments, it appears unlikely that loosely adsorbed proteins are included among the 85 mt-ribosomal proteins enumerated.

In addition, preliminary experiments with protease inhibitors (L-tosylamido-2-phenylethyl chloromethyl ketone, phenylmethylsulfonyl fluoride, and N-ethylmaleimide) indicate that proteolysis is not a significant factor, since mitochondrial ribosomes prepared in the presence of these inhibitors show the normal distribution of proteins. These observations suggest that the combined effects of proteolysis and adsorption of nonribosomal r-proteins do not contribute appreciably to the large number of proteins in mitochondrial ribosomes.

**Protein Content of Mitochondrial Ribosomes**—The molecular weights of the large subunit proteins range from 8.8 × 10^3 to 49 × 10^3, averaging 21.9 × 10^3; the small subunit contains proteins with molecular weights from 10 × 10^3 to 48 × 10^3, averaging 24.9 × 10^3 (Table I). For comparison, the average molecular weights of *E. coli* ribosomal large subunit and small subunit proteins are 16 × 10^3 and 19 × 10^3, respectively (24). Thus, the 2-fold greater quantity of total protein found in the mitochondrial ribosomes is due partially to a somewhat larger size of the individual proteins and partially to the larger number of proteins. Reported average molecular weights of mammalian cytoplasmic ribosomal proteins range from values similar to those of bovine mt-ribosomal proteins (25–28) to significantly larger values (29, 30).

The sum of the molecular weights of all of the ribosomal proteins (measured by their mobilities in the second electro-

### Table 1

| Protein content of bovine mitochondrial ribosomes |
|--------------------------------------------------|
| Data used in calculation | Protein content × 10^-6 daltons |
|--------------------------|-------------------------------|
|                         | Large subunit | Small subunit | Total |
| Buoyant density of subunit (11, 8) | 1.10 | 0.71 | 1.81 |
| Molecular weight of rRNA (11, 6) | 1.51 | 0.75 | 1.86 |
| Molecular weights of individual proteins | 1.14 | 0.82 | 1.96 |
which are found in greater amounts than their corresponding pairs are S6 and L26. If L4 is excluded from the set of large subunit proteins, the sum of the molecular weights of the proteins in this subunit would be reduced from $1.14 \times 10^9$ to $1.10 \times 10^9$, a value identical with that obtained by the other independent physical-chemical measurements (Table II). The aggregate protein content of the small subribosomal particle is unaltered by this manipulation, since protein Sh is not within the set of reproducible proteins for this particle.

Comparison with the Proteins of Bovine Cytoplasmic Ribosomes—Bovine mitochondrial and cytoplasmic ribosomes are physically very different kinds of ribosomes, residing in the same cells, and the proteins of both are made largely on cytoplasmic ribosomes. A direct comparison of the electrophoretic properties of these different sets of proteins should disclose proteins in both ribosomes that have identical mobilities, raising the possibility that these may be shared ribosomal proteins or common, nonribosomal contaminants.

To obtain an accurate relative positioning of the two protein patterns, the cytoplasmic protein samples were labeled with $^{14}$C by reductive methylation and co-electrophoresed with a much larger, stainable quantity of r-proteins from the mito-
chondrial ribosomes ("Experimental Procedures"). As shown in Fig. 5, all of the mt-ribosomal proteins are electrophoretically distinct from those of the corresponding subunit of the cytoplasmic ribosome. Comparing proteins of similar molecular weights (similar positions in the second electrophoretic dimension) that migrate in the cytoplasmic ribosomal proteins migrate more rapidly in the first dimension than do the mt-ribosomal proteins. This tendency, which is most evident in the comparison of the large subunits (Fig. 5E), implies that the cytoplasmic ribosomal proteins bear a greater positive charge at pH 4.3 than the mt-ribosomal proteins. It follows that the isoelectric points of many of the cytoplasmic ribosomal proteins are higher than those of the mt-ribosomal proteins.

**DISCUSSION**

The Number of Proteins in Bovine Mitochondrial Ribosomes—The number of proteins in bovine mitochondrial ribosomes is considerably larger than the number in bacterial ribosomes (31), or even in mammalian cytoplasmic ribosomes (32). Our best estimate at present, about 85 proteins, is consistent with the large numbers reported for mitochondrial ribosomes of other vertebrate species (7, 33, 34). In fact, our number of 85 is not much larger than one would calculate from the total protein content determined by ultracentrifugal and physical-chemical data and the average molecular weight of the individual proteins. In the large subunit with a protein content of 1.11 x 10^8 daltons (Table II) and an average protein molecular weight of 21.9 x 10^3, one would expect 1.11 / 0.0219 or 51 proteins. This is in excellent agreement with the 52 reproducible proteins found in the present work. Similarly, for the small subunit the total number, 33 proteins, is in good agreement with the theoretical number, 30, calculated by dividing the total protein content (0.76 x 10^6 daltons) by the average protein molecular weight of 24.9 x 10^3. Thus, the number of proteins found in two-dimensional PAGE maps agrees well with the number of proteins predicted from physical-chemical data.

The determination of the number of proteins in any ribosome by two-dimensional electrophoresis is complicated by a number of factors. The number of ribosomal proteins may be underestimated because of losses of some proteins during the isolation and salt-washing of the ribosomes or during the extraction of the proteins, or because the electrophoretic system fails to resolve all of the proteins from each other. Other factors may lead to an overestimate of the number of proteins. For example, nonribosomal proteins are often found to be associated with bacterial or eukaryotic cytoplasmic ribosomes that have not been salt-washed. However, in the present study, the mitochondrial ribosomes were isolated using conditions which prevent the significant adsorption of proteins like cytochrome c. In addition, all of the mitochondrial ribosomes used in this study were further washed when the monosomes were dissociated into subunits by buffers containing 0.2-0.5 M KCl and separated in sucrose density gradients. Therefore, adsorption of nonribosomal proteins, including initiation and elongation factors, is not expected to be a significant factor in the high protein content of these ribosomes.

A single protein may appear as more than one spot on a gel if some of the protein has been modified post-translationally, as the result of either a normal in vivo process or an artifact of the isolation and electrophoretic procedure. Thus, E. coli L7 is actually identical with L12, except that it has been acetylated at the NH2 terminus in vivo (35). Similarly, the rat liver cytoplasmic ribosomal protein S6 sometimes appears as multiple electrophoretic species due to in vivo phosphorylation (36). Two electrophoretically distinct forms, thought to represent different states of oxidation, have been observed for each of the E. coli proteins S11, S12, and S17 (37). Carbamylation of proteins by cyanate ions formed spontaneously in urea solutions can also alter their electrophoretic mobilities (38).

In the electrophoretic system used in the present experiments, these kinds of chemical modifications could result in a small alteration of a protein's mobility in the first electrophoretic dimension (in urea at pH 4.5), but are not expected to affect the migration in the second dimension (in SDS). Some groups of spots which do show this electrophoretic pattern include L42, L43, and L44; and S28 and S29 (Figs. 1 and 2). Whether each of these groups actually does represent a single protein with varying degrees of chemical modification is a question which cannot be answered until these proteins have been analyzed further by tryptic peptide maps and/or immunochromatographic techniques.

The most likely way in which the present results could represent an overestimate of the actual number of proteins in these ribosomes is related to the observation that two of the large subunit proteins are electrophoretically indistinguishable from a set of corresponding proteins in the small subunit (Fig. 4). It seems probable that the protein pairs are actually single proteins which fail to bind exclusively to either one of the subunits when the monosome is dissociated under our experimental conditions (treatment with puromycin and high salt). This phenomenon has been observed in E. coli ribosomes: the small subunit protein S20 has been shown to be identical with the large subunit protein L26 by immunochromatographic and genetic studies (39, 40), and significant quantities of S5 are found in the large subunit as well (39). Likewise, three proteins of rat cytoplasmic ribosomes may be shared between the dissociated subunits (22). In any case, more discriminating tests, such as immunochromatographic and protein-chemical analyses of the isolated proteins, will be required to establish the identity or nonidentity of these pairs of overlapping mt-ribosomal proteins.

**Structure of Bovine Mitochondrial Ribosomes—The remarkably large number (and total quantity) of proteins in these ribosomes raises some interesting questions about the similarities and differences that must exist among these particles and other structural kinds of ribosomes, with respect to their biosynthesis, assembly, and detailed functional activities. For example, it seems probable that these ribosomes, in which the ratio of RNA to protein is only 1:2, are held together predominately by different kinds of intermolecular bonding interactions than are found in E. coli ribosomes, which have an RNA:protein ratio of about 1:6. Clearly, the structure of the mt-ribosome must involve more protein:protein interactions and fewer protein-RNA interactions than that of the E. coli ribosome. Accordingly, these mt-ribosomal proteins are expected to be less basic and more hydrophobic than the proteins of other, RNA-rich ribosomes.

The pattern of proteins obtained from bovine mt-ribosomes shows no obvious similarities to that obtained from E. coli ribosomes,1 cytoplasmic ribosomes (Fig. 5), or even to Xenopus mt-ribosomes (7). This is especially interesting in view of the fact that these ribosomes are all performing the identical enzymological function. The remarkably large number of proteins in the mt-ribosome raises interesting questions about the structural and functional activities of each of the individual proteins. Those sequences and structures which have been preserved through evolution are expected to be those most critical for the functional activity of the ribosome. Although

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1 D. E. Matthews and T. W. O'Brien, unpublished observation.
the protein complement varies in the diverse ribosome types, both in number and total mass, at least the binding sites for the various macromolecules interacting in protein synthesis must be functionally (and probably structurally) equivalent. One would, therefore, expect some amino acid sequence homology among functionally important proteins in bacterial and mitochondrial ribosomes, and these might be detected using immunological techniques. Once the amino acid sequences of the conserved structures in mitochondrial ribosomes are known, it may be possible, by comparison to known sequences of *E. coli* r-proteins, to identify molecular domains critical for protein synthesis in each of these different kinds of ribosomes.

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