Isolation of Eukaryotic Ribosomal Proteins

PURIFICATION AND CHARACTERIZATION OF THE 60 S RIBOSOMAL SUBUNIT


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The proteins of the large subunit of rat liver ribosomes were separated into seven groups by stepwise elution from carboxymethylcellulose with LiCl at pH 6.5. Seventeen proteins (L4, L5, L7, L9, L11, L12, L13, L21, L22, L23, L26, L27, L30, L33, L35', L37, and L39) were isolated from three of the groups (B60, D60, G60) by ion exchange chromatography on carboxymethylcellulose and by filtration through Sephadex. The amount of protein obtained varied from 0.5 to 15 mg. Eight of the proteins (L9, L11, L13, L21, L22, L35', L37, and L39) had no detectable contamination; the impurities in the others were no greater than 9%. The molecular weight of the proteins was estimated by polyacrylamide gel electrophoresis in sodium dodecyl sulfate; the amino acid composition was determined.

It is the aim of research on ribosomes to determine the structure and function of the particle, to establish the location of each of the molecules, and to define their contribution to the synthesis of protein. A prerequisite for the analysis of the components is knowledge of the chemistry of the proteins. With that requirement in mind we have undertaken to isolate and characterize eukaryotic (rat liver) ribosomal proteins. Our efforts have been abetted by two auxiliary techniques, an efficient means of prefractionating ribosomal subunit proteins into groups by stepwise elution from carboxymethylcellulose with LiCl at pH 6.5 (1), and a microscale procedure for analysis of the proteins by two-dimensional polyacrylamide gel electrophoresis (2). We reported before (3) the isolation and characterization of 12 of the approximately 40 proteins of the 40 S subunit; we describe here the purification and the properties of 17 of the approximately 40 proteins of the 60 S subunit.

EXPERIMENTAL PROCEDURES

Preparation of Ribosomes, Ribosomal Subunits, and Ribosomal Protein Subunits - The proteins of the large subunit were separated into seven groups (A60, B60, C60, D60, E60, F60, and G60) by stepwise elution from carboxymethylcellulose (Whatman CM32) at pH 6.5 (1). The pH of solutions and buffers was determined at 20º C.

Isolation of 60 S Ribosomal Subunit Proteins - Two groups of proteins, B60-1 and D60, were resolved by chromatography on carboxymethylcellulose. The proteins in Group B60-1 (380 mg) in Buffer A (6 mM urea, 0.02 M H3PO4, 0.05% β-mercaptoethanol, adjusted to pH 6.5 with methylamine) were applied to a column of carboxymethylcellulose (9.6 x 80 cm) that had been equilibrated with the same buffer, and eluted with a linear gradient of 0 to 0.2 M LiCl in Buffer A. Group D60 proteins (900 mg) in Buffer B (6 mM urea, 0.05 M Tris, 0.05% β-mercaptoethanol, 0.1% methylamine, adjusted to pH 10.5 with borate acid) were applied to a column of carboxymethylcellulose (2.6 x 80 cm) and eluted with a linear gradient of 0.03 to 0.15 M LiCl in Buffer B. The flow rate was 48 ml/h, and 15 ml samples were collected.

The proteins in Groups G60 and B60-2, and fractions that were not entirely resolved by ion exchange chromatography, were filtered through Sephadex G-75 (superfine) in 10% acetic acid. The columns were generally 1.6 x 200 cm, but if the amount of protein exceeded 20 mg, a column 2.4 x 180 cm was used. Prior to gel filtration the samples were treated with dithiothreitol and their volumes reduced (3).

Polyacrylamide Gel Electrophoresis - The ribosomal proteins were identified by a modification (2) of the usual two-dimensional polyacrylamide gel electrophoresis procedure (9, 10) in which the first and second dimension gels are miniaturized. The protein in the sample was precipitated with 15% trichloroacetic acid and dissolved in buffer (20 mM Tris/HCl, pH 7.0, 8 mM urea, 20 mM dithiothreitol) before electrophoresis. When the sample was in 10% acetic acid, the protein was lyophilized and dissolved in the same buffer. In some instances the modifications introduced by Lastick and McConkey (11) were used, except that electrophoresis was in a miniaturized gel (2); electrophoresis then was for 210 min at 5 mA per slab in the first dimension and for 220 min at 100 V in the second dimension.

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The molecular weight and the purity of the isolated ribosomal proteins were estimated by polyacrylamide gel electrophoresis in sodium dodecyl sulfate by means of the procedure of Laemmli (12) with minor modifications. The concentrations of acrylamide and bisacrylamide were 15% and 0.31%, respectively. Electrophoresis was in gel slabs (10 x 13 x 0.2 cm) usually for 6 h at 20 mA per slab. The proteins used as standards to estimate the molecular weight of ribosomal proteins and the procedure for the estimation of the extent of contamination of the isolated proteins were described before (3).

**RESULTS AND DISCUSSION**

Isolation of 60 S Ribosomal Subunit Proteins in Groups B60-1 and B60-2—The proteins of the large subunit were fractionated into seven groups by stepwise elution from carboxymethylcellulose with LiCl at pH 6.5 (Fig. 1). The results were the same as reported before (1) except that B60 separated into two fractions (B60-1 and B60-2, see Fig. 1).

Group B60-1 contained eight proteins (L9, L11, L12, L20, L22, L23, L25, and L30). The mixture (380 mg) was applied to a column of carboxymethylcellulose and eluted with a 1-liter linear gradient of 0 to 0.2 M LiCl at pH 6.5 (Fig. 2). The identity of the proteins in the fractions was determined by two-dimensional polyacrylamide gel electrophoresis. Peak I had only L12 and Peak II (actually II', and II") only L11 (Fig. 2).

![Figure 1. Group fractionation of 60 S ribosomal subunit proteins.](http://www.jbc.org) The proteins (2.3 g of TP60) were separated into groups by stepwise elution from a column (5 x 70 cm) carboxymethylcellulose with increasing concentrations of LiCl at pH 6.5 (1). The proteins in Group A60 were not adsorbed to carboxymethylcellulose at pH 6.5. The concentrations of LiCl used to elute the other groups of proteins were: B60, 0.185 M; C60, 0.23 M; D60, 0.27 M; E60, 0.3 M; F60, 0.4 M; G60, 1.0 M.

The molecular weight and the purity of the isolated ribosomal proteins were estimated by polyacrylamide gel electrophoresis in sodium dodecyl sulfate by means of the procedure of Laemmli (12) with minor modifications. The concentrations of acrylamide and bisacrylamide were 15% and 0.31%, respectively. Electrophoresis was in gel slabs (10 x 13 x 0.2 cm) usually for 6 h at 20 mA per slab. The proteins used as standards to estimate the molecular weight of ribosomal proteins and the procedure for the estimation of the extent of contamination of the isolated proteins were described before (3).

**Determination of Amino Acid Composition of Ribosomal Proteins—**The isolated ribosomal proteins (1 to 2 nmol) were hydrolyzed in 6 M HCl for 24 h at 110°C. The concentration of the amino acids in the hydrolysate was determined with a Durrum D500 analyzer. No corrections for incomplete hydrolysis or for decomposition were made; tryptophan and cysteine were not determined.

![Figure 2. Chromatography on carboxymethylcellulose of 60 S ribosomal subunit proteins in B60-1.](http://www.jbc.org) The proteins (380 mg) were eluted from a column (2.6 x 80 cm) of carboxymethylcellulose with a 1-liter linear gradient of 0 to 0.2 M LiCl at pH 6.5 (Fig. 2). The proteins in the fractions (15 ml) were identified by micro-two-dimensional polyacrylamide gel electrophoresis, and the results are given on the chromatogram. The proteins are listed in order, from top to bottom, of decreasing relative amounts.

The other peaks contained more than one protein, and some proteins were eluted in more than one peak (Fig. 2). However, a number of proteins could be resolved from the mixtures in the separate peaks by filtration through Sephadex G-75 (Fig. 3). Protein L9 was purified in that way from Peaks V (which also had L22), VI (which also had L25 and L30), VII (which also had S2, a contaminant from the 40 S subunit), and VIII (Fig. 3). L22 was isolated from Peak V and L30 from VI and VIII (Fig. 3). The identity of the proteins (L9, L11, L12, L22, and L30) was verified and their purity assessed by two-dimensional polyacrylamide gel electrophoresis (Fig. 4) and by sodium dodecyl sulfate electrophoresis (Fig. 5).

Proteins L23 and L33 were in Group B60-2; 24 mg of the mixture were applied to a column of Sephadex G-75 (Fig. 3). The proteins were eluted in a single peak, but the leading edge had L23 and the trailing shoulder L33. L23 seemed pure from the two-dimensional electropherogram (Fig. 4); however, when analyzed by electrophoresis on gels containing sodium dodecyl sulfate (Fig. 5) the preparation of L23 was seen to contain three additional bands which in sum constituted 4% of the protein. The results would seem to confirm our impression that, in general electrophoresis in gels having sodium dodecyl sulfate is, in addition to being more easily quantitated, a more sensitive test of the purity of the ribosomal proteins than two-dimensional polyacrylamide gel electrophoresis (however, see
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amide was 15%. The analysis was of 3 to 5 μg of purified single proteins and 40 μg of TP60.

as described by Laemmli (12) except that the concentration of acrylamide was 15%. The analysis was of 3 to 5 μg of purified single proteins and 40 μg of TP60.

... procedure for the detection of the contamination.

... have the same molecular weight (Table I). In this unusual case two-dimensional electrophoresis is actually the better procedure for the detection of the contamination.

Isolation of Group D60 Proteins — About 900 mg of the ten proteins in D60 (L4, L5, L7, L13, L14, L18, L21, L26, L27, and L27') were applied to a column of carboxymethylcellulose and eluted with 10 liters of a linear gradient of 0.03 to 0.15 M LiCl at pH 10.5 (Fig. 6). (Attempts to resolve the D60 proteins on carboxymethylcellulose at pH 6.5 or on phosphocellulose (Whatman P11) at pH 9 or 10 had not been successful.)

Four of the eight peaks contained predominantly one protein (Fig. 6); however, each was sufficiently contaminated to necessitate further purification by filtration through Sephadex G-75 (Fig. 7). In that way L5 was prepared from I, L35' from V, L7 from VI, and L13 from VII; L13 was also obtained from Fraction VII (Fig. 7).

Proteins L21, L26, and L27, which eluted in a broad peak, were divided into three fractions (II, III, and IV, see Fig. 6) and applied to columns of Sephadex G-75 (Fig. 7). Proteins L21 and L27 were isolated from each of the three fractions (Fig. 7). L26 was purified from Fraction IV by rechromatography of the portion designated a (Fig. 7; D60 IV and IVa). L4 was prepared in a similar manner from Peak VII by two cycles of filtration through Sephadex C-75 (Fig. 7; D60 VII and VIIa).

The identity of the proteins was confirmed by two-dimensional polyacrylamide gel electrophoresis (Fig. 4) and their purity assessed by sodium dodecyl sulfate electrophoresis (Fig. 5). The identification of three proteins, L7, L21, and L23, posed special problems. L7 is not ordinarily clearly resolved from L6 on two-dimensional electropherograms. The identification of the isolated protein was greatly facilitated by the use of the conditions recommended by Lastick and McConkey (11) and by extending the electrophoresis in the second dimension from 220 min to 10 h. In those circumstances L6 and L7 are resolved, and the protein that was isolated is seen to be L7 (see the inset in the panel displaying L7 in Fig. 4).

L21 and L23 are generally not entirely separated by two-dimensional polyacrylamide gel electrophoresis (9, 13); the separation is even less satisfactory on micro-two-dimensional gels. Two separate proteins that migrated during electrophoresis in the region of L21 and L23 were isolated; they were shown to be distinct by differences in molecular weight (one was 15,600 and the other 20,300) and in amino acid composition (Table I). Moreover, they could be separated by two-dimensional polyacrylamide gel electrophoresis if the conditions were altered (11). We had previously determined that the mass of L21 was greater than that of L23 (14). Of the two isolated proteins, the putative L21 can be seen from electrophoresis on gels containing sodium dodecyl sulfate (Fig. 5) to have a molecular weight greater than that of the protein that is likely to be L23. In the circumstances we cannot be certain which protein conforms to the original name L21 (9); the problem is even more difficult with respect to L23, which may actually correspond to a protein not previously identified on two-dimensional electropherograms, but which is now some...
times found when preparations of TP60\textsuperscript{a} are analyzed. It should be noted in this connection that a previous distinction between the two proteins (1) may not have been correct. For that reason, the proteins are herein defined operationally: L21 is the $M_r = 20,300$ species, having the amino acid composition given in Table I; L23 is the $M_r = 15,600$ protein, having the amino acid composition described in Table I.

Protein L35\textsuperscript{a} was originally thought to be a satellite (i.e. a chemically altered form) of L35 (13). However, they now appear distinct proteins: L35\textsuperscript{a}, which was isolated (Fig. 4), occurs in group D60; L35, which has not yet been purified, is in F60 (1).

Isolation of Group G60 Proteins—L37 and L39, the two proteins in group G60, were separated by filtration of the mixture (about 20 mg) through Sephadex G-75 (Fig. 8); the identity (Fig. 4) and purity (Fig. 5) of the two proteins were determined.

Yield and Purity of Isolated Proteins—The amount of the individual ribosomal proteins that was obtained varied from 0.5 to 15 mg (Table II); the yield was determined in the first instance by the number of steps required for the isolation, but also by the quantity of the starting material (i.e. the amount in the group fractions) and the quality of the chromatography. The purity of the isolated proteins was assessed (3) from the profiles obtained by scanning the gels at 540 nm after electrophoresis in polyacrylamide gels containing sodium dodecyl sulfate (Fig. 5 and Table II). Several proteins (L9, L11, L13, L21, L22, L35, L37, and L39) had no detectable contamination; the impurities in the others were no greater than 9%.

Molecular Weight—The molecular weight of each purified protein was determined by electrophoresis in polyacrylamide gels containing sodium dodecyl sulfate (Fig. 5 and Table I).

The apparent molecular weight of proteins can be influenced by the conditions used in the analysis. A comparison was made of the molecular weights obtained for the ribosomal proteins with the Laemmli (12) technique (which are recorded in Table I) and with the procedure of Fairbanks et al. (18) which employs a higher degree of cross-linking and a greater concentration (e.g. 1%) of sodium dodecyl sulfate. The latter gave values for some proteins (L4, L5, L7, L9, L11, L12, and L13) that were 5 to 10% greater, whereas the result for the others (L21, L22,
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Molecular weight and amino acid composition of isolated 60 S subunit proteins

<table>
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<th>Molecular Weight (x 10^3)</th>
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<th>Pro</th>
<th>Gly</th>
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<th>Val</th>
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The molecular weights are the averages of four determinations. The values for amino acids are in moles percent.

FIG. 8. Separation of 60 S ribosomal subunit proteins in Group G60 by filtration through Sephadex G-75. The mixture of L37 and L39 (20 mg in 10% acetic acid) was filtered through a column (1.6 x 200 cm) of Sephadex G-75, and 1.4-ml fractions were collected. The proteins in the fractions were identified by two-dimensional polyacrylamide gel electrophoresis, and the results are on the chromatogram.

Table II

Yield and purity of isolated 60 S ribosomal subunit proteins

Electrophoresis of the isolated proteins (3 to 5 µg) was in polyacrylamide gels containing sodium dodecyl sulfate (Fig. 5). The extent of the contamination was estimated after scanning the gels at 540 nm; the part of the total absorption that did not derive from the main band (in a sample shown to contain one, or predominantly one, spot after two-dimensional polyacrylamide gel electrophoresis; see Fig. 4) was taken to give the percentage of contamination.

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<thead>
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<th>Protein</th>
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<tr>
<td>L39</td>
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</table>

* No contamination was detected.

† The extent of the contamination of L33 with L23 cannot be determined since they have the same molecular weight; L33 is not contaminated with any other protein.
the difference is conditioned by the mass of the proteins. The molecular weight of histone proteins, estimated from polyacrylamide gel electrophoresis in sodium dodecyl sulfate, is greater than the actual value determined from the amino acid sequence (19, 20). Since ribosomal proteins are in general relatively small and basic, like the histones, the molecular weight determined by sodium dodecyl sulfate gel electrophoresis may also exceed their true values. Indeed, that has been shown to be the case for *Escherichia coli* ribosomal protein S21 (21).

**Amino Acid Composition**—The amino acid composition of the several large subunit proteins tended to be similar, although the actual pattern for each of the 17 proteins was in fact unique (Table I).

Westermann et al. (22) reported the isolation of 31 proteins from rat liver 80 S ribosomes; of those 14 were said to be from the large subunit. The proteins were not identified by two-dimensional polyacrylamide gel electrophoresis, thus precluding comparison with our results. The putative large subunit proteins, however, had amino acid compositions that were more similar to each other than we found.

A determination of the structure and function of eukaryotic ribosomes is contingent on knowledge of the chemistry of the components. A great deal is known of the structure of eukaryotic ribosomal RNA, far less of the proteins, although a beginning has been made on their characterization (see Ref. 23 for a review and the original references). Eukaryotic ribosomes have approximately 70 different proteins, about 30 in the small subunit and 40 in the large. Heretofore, only two proteins had been isolated from the 60 S ribosomal subunit (from the brine shrimp *Artemia salina*) and characterized (24). The proteins may be homologous to *E. coli* ribosomal proteins L7 and L12 (23-25). We report now the purification and characterization of 17 of the 40 proteins of the large subunit of eukaryotic ribosomes; the amounts obtained (up to 15 mg) have allowed a beginning to be made on sequence analysis, provided material to characterize antisera against eukaryotic ribosomes, and will in the future support other studies of the structure and function of eukaryotic ribosomal proteins.

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