Group Fractionation of Eukaryotic Ribosomal Proteins*  

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The proteins of the subunits of rat liver ribosomes were fractionated by stepwise elution from carboxymethylcellulose with LiCl at pH 6.5. The 40 S ribosomal proteins were separated into five groups containing between 3 and 14 proteins; the 60 S proteins, into seven groups of 3 to 15. Only a comparatively small number of proteins occurred in appreciable amounts in more than one group. The number of relatively acidic proteins associated with the ribosomal subunits was larger than had been reported before; it is not known if they are initiation or translation factors or ribosomal structural proteins. The group fractionation procedure has proven valuable as the initial step in the isolation and characterization of rat liver ribosomal proteins.

The isolation, purification, and characterization of the proteins is a prerequisite for analysis of the structure and function of eukaryotic ribosomes. Few of the individual proteins contained in the organelle have been isolated and purified in appreciable amounts; a start has been made, however, on their characterization (see Ref. 1 for a review and for references). The results would indicate that ribosomal proteins from eukaryotic cells have the same general properties as those from prokaryotic organisms. The number of proteins contained in the subunits of eukaryotic ribosomes (2–7) and their molecular weight (5–8) have been determined. It seems certain that the proteins are heterogeneous—that each ribosome has approximately 70 unique proteins, about 30 in the small sub-particle and 10 in the large.

A great deal is known of the chemistry of prokaryotic ribosomal proteins (cf. Ref. 9 for a review); relatively little, of the proteins from eukaryotic particles. There are a number of reasons why that is so, but perhaps most important eukaryotic ribosomes contain a greater number of proteins. Since the proteins are almost all very basic and relatively similar in molecular weight, the increase in numbers (prokaryotic ribosomes have only 54 proteins) severely complicates the resolution of the proteins, even after separation of the ribosomal subunits. Thus it follows that an efficient group fractionation procedure would facilitate separation and purification of the proteins. The strategy is not a new one: a number of procedures have been tried with prokaryotic (10–12) and eukaryotic (13) ribosomes. In general they suffer the same failings: a great deal of overlap of proteins in the several fractions, or an insufficient number of groups, or both. We describe a different method—the stepwise elution from carboxymethylcellulose with LiCl at pH 6.5—that has proven efficient in resolving the proteins into groups.

**EXPERIMENTAL PROCEDURES**

**Preparation of Ribosomes, Ribosomal Subunits, and Ribosomal Protein**—Subunits were prepared from rat liver ribosomes (14) on a large scale by centrifugation in a zonal rotor (15), and the protein was extracted with 67% acetic acid in 10 mM Tris-HCl containing 67 mM magnesium acetate (16, 17). The proteins were precipitated with 9 volumes of acetone (4). The precipitate was left overnight at 4°, then collected by centrifugation (45 min at 1000 × g). It was dissolved in 8 M urea that had been treated with charcoal (16) and was dialyzed overnight against at least 10 volumes of 8 M urea. The concentration of protein was determined (18) using bovine serum albumin dissolved in 8 M urea as a standard. Disodium EDTA was added to the ribosomal protein solution to a final concentration of 10 mM before it was stored at –20°.

**Chromatography of Ribosomal Proteins**—Group fractionation of 40 S and 60 S ribosomal proteins (referred to as TP40 and TP60, for total proteins of the 40 S and 60 S subunits) (4) was performed by stepwise elution from carboxymethylcellulose (Whatman CM32) at 12–15°. A column (2.6 × 40 cm) was packed with carboxymethylcellulose under negative pressure. A preparation of TP40 (200 to 400 mg, in 30 to 50 ml of 8 M urea) was dialyzed against 10 volumes of Buffer A (1.0 M Na2HPO4, 20 mM/urea, 6 M/β-mercaptoethanol, 0.5%—adjusted to pH 6.5 with diethylamine) until the pH and the conductivity of the dialysate were constant. The sample was pumped onto the column at 46 ml/hour. Fractions (10 ml) were collected, and the absorption at 280 nm was determined. The column was washed with Buffer A until just after the concentration of protein in the eluate (calculated from the absorption at 280 nm) was at a maximum, then Buffer A was changed to Buffer B40 (Buffer A containing 0.15 M LiCl), and elution continued until just after the concentration of protein was at a maximum again. In a like manner proteins were eluted with Buffers C40 (Buffer A with 0.15 M LiCl) and D40 (Buffer A with 0.3 M LiCl), and the column was finally washed with Solution E40 (LiCl, 1 M/urea, 6 M/β-mercaptoethanol, 0.05%). The eluted proteins in the samples comprising the separate fractions (A40, B40, C40, D40, and E40) were pooled (Fig. 1) and dialyzed at 4° for 20 to 24 hours against saturated ammonium sulfate. The precipitated proteins were collected by centrifugation (15,000 × g for 45 min), dissolved in a small volume of 8 M urea and treated the same as the acetone-precipitated proteins ("Preparation of Ribosomes, Ribosomal Subunits and Ribosomal Protein").

Chromatography of the large subunit proteins (TP60) was in principle the same as TP40 except for the LiCl concentrations used for the individual steps. The column (5 × 80 cm) was loaded with 3.2 g of TP60 in 170 ml of Buffer A. The flow rate during the packing of the column was 300 ml/hour; it was 150 ml/hour during elution. The composition of the buffers used for elution was: Buffer B60, Buffer A containing 0.185 M LiCl; Buffer C60, Buffer A with 0.23 M LiCl; Buffer 1° The abbreviations used are: TP40 and TP60, the total proteins of the 40 S and 60 S ribosomal subunits. 2° The pH of buffers was determined at 20°.
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D60, Buffer A with 0.27 M LiCl; Buffer E60, Buffer A with 0.3 M LiCl; Buffer F60, Buffer A with 0.4 M LiCl. The column was washed with solution G60: 1 M LiCl, 6 M urea, and 0.05% β-mercaptoethanol.

Two-dimensional Polyacrylamide Gel Electrophoresis—The ribosomal proteins in the individual fractions were identified by two-dimensional polyacrylamide gel electrophoresis (2, 19, 20), except that the first and second dimension gels were miniaturized (21). Electrophoresis in the first dimension was at pH 8.6 on a column (1 x 45 mm) of 8% acrylamide in a 5-cm length of a 200-μl disposable micropipette. The proteins contained in A40 were characterized by analyzing the sample alone on one-half of the gel slab, and in the presence of a small amount of TP40 on TP60 on the other. Only the anodic or the cathodic part of the first dimension gel was analyzed in the second dimension. Since most of the ribosomal proteins migrate toward the anode at pH 8.6, it was the anodic side of the first dimension gel that was generally used. (Exceptions are given in the legends to the figures.) The small amount (20 to 30 μg) of TP40 or TP60 provided a background pattern that assisted in identification of the proteins in the sample, whereas examination of the sample without TP40 or TP60 allowed one to estimate the number of proteins in the fraction.

Cellulose Acetate Gel Electrophoresis—Cellulose acetate gel electrophoresis of ribosomal proteins (22) was in 0.12 M bis(2-hydroxyethyl)iminom-trishydroxyethylmethane/0.12 M 2(N-morpholino)ethanesulfonic acid/8 M urea/0.05% β-mercaptoethanol, pH 6.9. Electrophoresis was for 45 min at 300 volts.

Polyacrylamide Gel Electrophoresis in Sodium Dodecyl Sulfate—Separation of ribosomal proteins by polyacrylamide gel electrophoresis in sodium dodecyl sulfate was performed as described by Fairbanks et al. (23) except that the concentration of acrylamide in the gel was 12%.

RESULTS

Group Fractionation of the Proteins of the 40 S Ribosomal Subunit—Five groups—designated A40 through E40—were obtained when the proteins of the 40 S subunit of rat liver ribosomes (TP40) were chromatographed on carboxymethylcellulose, and elution was at pH 6.5 with stepwise increases in the concentration of LiCl (Fig. 1). On occasion proteins were eluted between the main groups (cf. the small peak between Fractions A40 and B40 in Fig. 1); however, their occurrence was sporadic, and they generally contained only small amounts of protein. The recovery was between 80 and 90% for the group fractionation of six separate preparations of TP40.

The relatively acidic proteins did not bind to carboxymethylcellulose in the conditions that were used and were washed through the column with the starting buffer—they are referred to as group A40. The A40 proteins formed two clusters of diffuse, ill defined spots when analyzed by two-dimensional polyacrylamide gel electrophoresis (Fig. 2a). The proteins stained poorly with amido black or with Coomassie blue (and many other dyes that we tried). One set (designated cluster 1, Fig. 2a) appeared to contain at least four zones, the other (cluster 2) perhaps six. The 40 S ribosomal subunit has been reported (2) to contain only two relatively acidic proteins, S12 and S21 (Fig. 2f). Neither could be located with certainty in A40, although S12 is ordinarily found in the general region of cluster 2.

The proteins contained in A40 were characterized by one-dimensional electrophoresis on cellulose acetate gel strips and on polyacrylamide gels in sodium dodecyl sulfate (Figs. 3a and 4a). Some eight to ten bands were separated by electrophoresis on cellulose acetate (Fig. 3a). The relative and absolute intensity of the bands varied from one preparation to another (Fig. 3a); however, there seemed to be at least three major bands. Some of the bands in A40 migrated the same distance as proteins in TP40; however, at least three of the most acidic bands, including one that was prominent, did not correspond with bands in TP40. At least eight bands were also seen when the A40 proteins were separated by electrophoresis in polyacrylamide in sodium dodecyl sulfate (Fig. 4e). Four of the bands were especially prominent. Several of the protein bands in A40 did not correspond to any in TP40. The molecular weights of the proteins in the more prominent bands were 28,500, 26,000, 20,900 and 14,600. The molecular weights of the proteins in the other bands were 38,000, 35,900, 34,200, and 11,500.

Group B40 always contained appreciable amounts of five proteins—S3, S5, S17, S20, and S28 (Fig. 2b and Table I). In addition, S10 was contained in group B40 or C40 but not in both; in the results illustrated in Fig. 2, S10 was in group C40. Small amounts of S1 were found either with the B40 or C40 proteins; appreciable amounts of S1 were never recovered. Protein S14 was found mainly in C40 but could be detected also in B40 and D40; it was the one 40 S ribosomal protein that eluted with several groups.

Eight proteins—S2, S3, S4, S7, S10, S14, S16, and S17—were eluted in appreciable amounts with group C40 (Fig. 2c and Table I). S19 was present in small amounts. Protein S20 sometimes was present in substantial amounts in group C40. (It is always found in B40.) Finally, C40 contained three proteins which were not described before (2, 3). The three are designated S3’, S14’, and S15’ for their three nearest neighbors. S3’ and S15’ may be the same as rat liver ribosomal proteins A and B with 0.3 M LiCl.
FIG. 2. Two-dimensional polyacrylamide gel electrophoretograms of proteins of the 40 S ribosomal subunit. The samples analyzed (5 to 40 μg of protein were: a, A40; b, B40; c, C40; d, D40; e, E40; f, TP40. The anode was at the left in the first dimension and at the top in the second. In a only the anodic half of the first dimension gel was used for proteins S4 and S20 in nomenclature used by Terao and Ogata (7); alternatively, S15' may be protein L25 (see below).

The greatest number of proteins, 14, was in group D40 (Fig. 2d and Table I). That group included appreciable amounts of S6, S8, S9, S11, S13, S15, S18, S23, S24, S25, and S26. Proteins S14 and S14' were present in small amounts. Protein S29 was not always recovered in appreciable amounts, but when it was found, it was in fraction D40.

Two or three proteins were in group E40 (Fig. 2c and Table I): S27 and S30 were always found there in appreciable amounts, whereas the presence of S8 was variable. As has been reported before (2), S27 formed a group of satellite spots of variable intensity. Group E40 (which was eluted with 1 M LiCl) also contained trace amounts of as many as 20 additional proteins; some of the spots correspond to the positions occupied on two-dimensional polyacrylamide gel slabs by proteins of the 60 S ribosomal subunit. A few of the proteins could not be identified.

Twenty-three of the thirty proteins of the 40 S ribosomal subunit that were identified were in only one group. Only three proteins—S3, S14', and S17—always were in two groups. S8, S10, and S20 were sometimes present in a second group. Protein S14 was consistently found in three groups, although in appreciable amounts only in C40. All but three of the proteins—S12, S21, and S22—were accounted for. The relatively acidic proteins, S12 and S21, which should be in group A40, could not be identified with certainty. S22 is only resolved from S23 and S24 (which are in group D40) when the conditions of electrophoresis are altered (2); no special efforts were made to resolve S22 from S23 and S24. There was some uncertainty as to whether S16 and S18 were entirely segregated in groups C40 and D40, respectively, since they migrate very close to one another on two-dimensional gel electrophoresis. Occasionally spots were seen that corresponded to large subunit proteins.

Group Fractionation of the Proteins of the 60 S Ribosomal
The A60 group of proteins was separated by electrophoresis on cellulose acetate gel strips into six to eight bands (Fig. 3b); of those only two or three were seen when TP60 was analyzed and then only if large amounts were subjected to electrophoresis. Electrophoresis on polyacrylamide gels in sodium dodecyl sulfate of the A60 proteins gave (Fig. 4b) two prominent bands with molecular weights of 14,100 and 15,700, and a number of less intense bands whose molecular weights ranged from 9,400 to 38,300. The characterization of the A60 group was considerably impeded by the poor staining quality of the proteins, by the small amount of protein available (the A60 group constitutes only 2 to 3% of the TP60 that was recovered), and by the variability of the behavior of the proteins.

Group B60 contained eight ribosomal proteins (Fig. 6b and Table II): L5, L9, L11, L12, L20, L21, L25, and L30; L9, L11, and L12 were present in the greatest amounts. Only L5 and L25 occurred in another group. L25 and SL5 migrate to appreciably the same position on two-dimensional gel plates. Since the putative L25 is present in B60 in only small amounts, the identity of the protein is not certain.

There were eight or nine proteins in group C60 (Fig. 6c and Table II): L5, L7, L8, L17, L19, L25, L31, and L38; protein L10 occurred in C60 or D60, but not in both. Proteins L5, L8, and L31 were found in the largest amounts in this group; only traces of L38 were recovered in C60. The identification of the putative L13 is not certain, since only small amounts were in C60, and the protein occupies nearly the same position on two-dimensional gel slabs as S11; moreover, L19 occurs in C60. Of the group C60 proteins only L10 and L38 never are in more than one fraction.

Ten or eleven proteins were in group D60 in appreciable amounts (Fig. 6d and Table II): L3, L4, L7, L8, L13, L14, L17, L23, L26, and L31; L10 was in D60 or C60 (see above). Group D60 also contained small amounts of L27, L35, and L36; most of L35 and L36 were to be found in F60. Only L3, L10, and L14 of the group D60 proteins are unique to one fraction.

There were appreciable amounts of seven proteins in group E60 (Fig. 6e and Table II): L3, L4, L7, L8, L13, L23, L26, and L27; there were small amounts of six others—L8, L13, L18, L24, L28, and L31. Proteins L32 and L33 were also sometimes found in E60 in small amounts, although they were consistently found in appreciable quantities in F60. No protein was unique to group E60.

Group F60 had 12 proteins in quantity (Fig. 6f and Table II): L6, L7, L18, L19, L27, L28, L29, L30, L31, L33, L34, L35, and L36. Protein L29 was not always recovered, but when it was it was in E60. This group had small amounts of L13, L15, and L24, L29 and L34, and perhaps L35 and L36 (see above), were found only in F60.

The last group, G60, contained significant quantities of only two proteins—L37 and L39 (Fig. 6g and Table II). Those two proteins were recovered only in group G60. This fraction, which was eluted with 1 M LiCl, had trace amounts of a number of proteins just as did E40.

Fourteen of the 35 60 S ribosomal proteins that were identified with certainty after fractionation always were eluted in only one group (L3, L9, L10, L11, L12, L14, L20, L21, L29, L30, L34, L37, L38, and L39). Proteins L19, L35, and L36 also may be in only one group (F60), since their presence in a second fraction is not certain (Table II). Two proteins occurred only occasionally in a second group in small quantities (L32 and L33). Five additional proteins (L5, L17, L18, L25, and L28) were in one group in large amounts and in a second in only...
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FIG. 1. Sodium dodecyl sulfate electrophoretograms of the acidic proteins of rat liver ribosomal subunits. Two separate preparations of 15 µg of the A40 acidic proteins, and two of about 20 µg of A60, were analyzed and compared with TP40 (45 µg) and TP60 (60 µg). The conditions of electrophoresis were as described before (23) except that the concentration of acrylamide was 5%.

The molecular weights were estimated from standards analyzed at the same time: ovalbumin (43,000); yeast alcohol dehydrogenase (37,000); α-chymotrypsinogen (26,700); myoglobin (17,200); and lysozyme (14,700).

small quantities. Finally, it is likely that proteins L40 and L41 (24), which were not positively identified, are in only A60. Thus, given the most favorable conditions and the most favorable interpretation, 24 of the 35 TP60 proteins (26 of 37, if L40 and L41 are included) are found in appreciable amounts in one group. Six proteins were always found in two groups in more or less equal amounts (L4, L6, L15, L23, L24, and L26). Five proteins were poorly separated: L8, L13, L27, and L31 occurred in three groups, and L7 in four. Only small quantities of proteins L15, L20, L21, L24, L30, and L38 were recovered. Six of 41 proteins of the 60 S subunit (L1, L2, L16, L22, L40, and L41) that had been identified before (3) could not be positively accounted for in any of the groups. The acidic proteins L1 and L2 were originally seen (2) only when an excess of TP60 was analyzed, and it was not certain whether they were ribosomal structural proteins or factors.

DISCUSSION

The isolation of ribosomal proteins is rendered difficult by the large number contained in the organelle; by the strong tendency of the proteins, most of which are basic, to aggregate when contained in mixtures; and by the relatively narrow range of molecular weights. Because of the difficulties, methods have been sought to fractionate the ribosomal proteins into more manageable groups. The simplest procedure, and the one most often employed, is to dissociate ribosomes into subunits either by lowering the magnesium (25), or by raising the potassium concentration (26, 27). By that method prokaryotic ribosomal proteins are separated into two subsets of 21 and 33 each (cf. Ref. 9 for references), and eukaryotic ribosomal proteins, into groups of about 30 and 40 each (see below). The 21 proteins of the small subunit of Escherichia coli ribosomes were isolated and purified without prefractation (16, 28). However, the other subsets are too large to be resolved efficiently without some preliminary separation. Spitnik-Elson (10) was able to effect the fractionation of the proteins of the large subunit of E. coli ribosomes by absorbing the particles, after they had been unfolded by dialysis against EDTA, to DEAE-cellulose. The proteins were then eluted from the rRNA bound to the matrix with a linear sodium phosphate gradient in 6 M urea at pH 6.5. Although some fractionation was certainly obtained, the efficiency of the procedure cannot be evaluated since the proteins were not identified.

Ammonium sulfate precipitation was employed by Mora et al. (12) to fractionate the proteins of the 50 S subunit of E. coli ribosomes into five groups prior to chromatography. There was considerable overlap of proteins between groups. Hindennach et al. (11) tried the same procedure. Each of the 33 50 S subunit proteins was found in three or more of seven groups, and for that reason the method was recommended only for very special purposes. Our experience with the use of ammonium sulfate for group fractionation of eukaryotic ribosomal proteins was equally disappointing. The procedure that was adopted by Hindennach et al. (11) was fractionation into three groups by treatment of the 50 S ribosomal proteins with different concentrations of LiCl in the presence or absence of urea. While the several procedures have proven of value—they did, after all, facilitate isolation of the proteins of the large subunit of E. coli ribosomes (11, 12)—they cannot be said to be ideal since so many of the proteins were contained in more than one group.

There is general agreement that there are about 30 proteins
TABLE I

Group fractionation of the proteins of rat liver 40 S ribosomal subunits

| Protein | B40 | C40 | D40 | E40 |
|---------|-----|-----|-----|-----|
| S1      | (+)* | (+)* |     |     |
| S2      |     |     |     |     |
| S3      |     |     |     |     |
| S3'     |     |     |     |     |
| S4      |     |     |     |     |
| S5      |     |     |     |     |
| S6      |     |     |     |     |
| S7      |     |     |     |     |
| S8      |     |     |     |     |
| S9      |     |     |     |     |
| S10     |     |     |     |     |
| S11     |     |     |     |     |
| S12     |     |     |     |     |
| S13     |     |     |     |     |
| S14     | (+) |     | (+) |     |
| S14'    |     |     |     |     |
| S15     |     |     |     |     |
| S15'    |     |     |     |     |
| S16     |     |     |     |     |
| S17     |     |     |     |     |
| S18     |     |     |     |     |
| S18'    |     |     |     |     |
| S19     |     |     |     |     |
| S20     |     |     |     |     |
| S21     |     |     |     |     |
| S22     |     |     |     |     |
| S23     |     |     |     |     |
| S24     |     |     |     |     |
| S25     |     |     |     |     |
| S26     |     |     |     |     |
| S27     |     |     |     |     |
| S28     |     |     |     |     |
| S29     |     |     |     |     |
| S30     |     |     |     |     |

* The symbols used are: +, present in appreciable amounts; (+), present in small amounts.
* Present in one fraction or another but not in both.
* Three previously undescribed proteins are designated S 3', S 14', and S 15' after their nearest neighbors.
* Occurrence in the fraction varied.

Approximately eight acidic proteins were found to be associated with the 40 S subunit, and perhaps an equal number with the large subparticle; far more than had been identified before. There is no way at the moment to determine which are initiation or elongation factors or even contaminants that have no means certain that the several groups of investigators are counting exactly the same proteins. Indeed, for a small number in each subunit there must remain some doubt whether they are authentic structural proteins of the ribosome, just as there is no assurance that all the structural proteins have been identified. These reservations are especially relevant with regard to the acidic ribosomal proteins which provide particular difficulties (see below).

Only a few attempts have been made (29, 30) to separate the complex mixtures of eukaryotic ribosomal subunit proteins by column chromatography without some preliminary fractionation into groups; and then with little (29) or only moderate success (30). Westermann and Bielka (13) bound rat liver 40 S ribosomal subunits to DEAE-Sephadex A-25 and eluted the proteins into four groups with a continuous gradient of LiCl. However, the procedure cannot be reckoned very efficient since only three proteins were in a single group and 13 of the 31 were in three of the four groups. The procedure which is described here—stepwise elution from carboxymethylcellulose with increasing concentrations of LiCl at pH 6.5—is convenient and efficient. The proteins of the 40 S subunit are separated into five groups of 3 to 14 proteins: 23 of the 30 proteins that were identified were in only one of the groups. Moreover, only four proteins were always in two or more groups. Group fractionation of the large subunit proteins was almost equally successful. They were resolved into seven groups of 3 to 15 proteins; at least 14, and in the best circumstances as many as 24, of the 35 60 S ribosomal subunit proteins that were identified were in one group. We have not yet had occasion to test the assumption we see no reason why the same procedure should not be helpful in group fractionation of the ribosomal proteins from other eukaryotes and from prokaryotes as well. However, it is to be noted that it is a limitation of the procedure that the same property (separation by charge) is exploited for the preliminary group fractionation as is likely to be used for the subsequent isolation of the proteins by chromatography.
Fig. 6. Two-dimensional polyacrylamide gel electrophoretograms of proteins of the 60 S ribosomal subunit. The samples analyzed (30 to 200 μg of protein) were: a, A60; b, B60; c, C60; d, D60; e, E60; f, F60; g, G60; h, TP60. The anode was at the left in the first dimension, at the top in the second. In a only the anodic half of the first dimension gel was used for electrophoresis in the second dimension; in b through h the cathodic half was used, and in h the entire first dimension gel was used. The conditions of electrophoresis were as in Fig. 2. There is uncertainty whether the spots designated L19 and L25 in c are not actually S 14 and S 15, respectively.
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Table II

| Group fractionation of the proteins of rat liver 60 S ribosomal subunits |
|--------------------------------------------------|
| **Protein** | **Group** |
| L1 | B60 C60 D60 E60 F60 G60 |
| L2 |  |
| L3 | +  |
| L4 | + +  |
| L5 | (+) + + +  |
| L6 | + + + (+)  |
| L7 | (+) + + +  |
| L8 | + + (+)  |
| L9 | + + +* +*  |
| L10 | (+') + + +  |
| L11 | + + + +  |
| L12 | + + + (+)  |
| L13 | + + + +  |
| L14 | + (+) (+)  |
| L15 | + (+) (+)  |
| L16 |  |
| L17 | (+) + + +  |
| L18 | (+') + + +  |
| L19 | (+') + + +  |
| L20 | (+) (') + +  |
| L21 | (+)  |
| L22 |  |
| L23 | + +  |
| L24 | (+) (') (')  |
| L25 | (+') + + +  |
| L26 | + + +  |
| L27 | (+) + +  |
| L28 | (') + +  |
| L29 | + + +  |
| L30 | (+) + + +  |
| L31 | + + +  |
| L32 | + (') + +  |
| L33 | + (') + +  |
| L34 | + + +  |
| L35 | + + +  |
| L36 | (') + +  |
| L37 | + + +  |
| L38 | (') + +  |
| L39 | + + +  |

* The symbols used are: +, present in appreciable amounts; (+), present in small amounts.

* Present in one fraction or another but not in both.

* Identity questionable: L19 might be contaminated with S11, L25 with S15.

* Occurrence in the fraction varied.

The proteins stain intensely even when large amounts are analyzed by polyacrylamide gel electrophoresis, which suggests they may be contaminants originally present on the ribosome in trace amounts but concentrated in the A40 and A60 fractions. On the other hand, it may be an intrinsic property of the relatively acidic ribosomal proteins that they stain poorly. Proteins L40 and L41 are almost certainly ribosomal structural proteins (24), yet they stain weakly at best. Their poor staining properties make the acidic proteins difficult to identify. The characterization of the putative acidic ribosomal proteins is an important problem to be resolved.

The group fractionation procedure described here has already proven its value as the first step in the purification of a number of the proteins of rat liver ribosomes. Significant amounts of the small subunit proteins S3, S4, S5, and S7 were isolated from groups B40 and C40; S6, S9, S13, and S23/24, from D40; and S8 and S27, from E40. In addition, a number of large subunit proteins have been purified after group fractionation. They include: L5, L9, L11, L12, L21, and L30 from B60; and L30 and L39, from G60. We had not been able to purify any of these proteins by direct chromatography of unfractionated TP40 or TP60. Moreover, group fractionation has provided a means of characterizing the acidic ribosomal proteins (see above). That had not previously been possible since they constitute such a small part of the whole. The acidic proteins of the 60 S subunit, for example, are only 2 to 3% of TP60. Because the acidic proteins are present in small amounts, and because they stain poorly, it is hard to separate and identify them by gel electrophoresis without prefractionation. A number of other valuable uses of the group fractionation procedure seem likely. For example, one could complete affinity columns with the proteins in a group and then enrich antisera against TP40, or TP60, or TP80 (24) for the antibodies against the proteins in that group. Finally, groups of a small number of proteins can be useful in searching for the altered protein in ribosomal mutants, or for reconstitution of subunits from core particles, or for in vitro studies of rRNA-ribosomal protein interaction.

REFERENCES

1. Wool, I. G., Stöfler, G. (1974) in Ribosomes (Nomura, M., Tissières, A., and Lengyl, P., eds) pp. 417-460, Cold Spring Harbor Laboratory, New York.
2. Sherton, C. C., and Wool, I. G. (1972) J. Biol. Chem. 247, 4460-4467.
3. Sherton, C. C., and Wool, I. G. (1974) J. Biol. Chem. 249, 2258-2267.
4. Woffle, H., Stahl, J., and Bielka, H. (1972) FEBS Lett. 26, 228-232.
5. Howard, G. A., Traua, J. A., Crock, E. A., and Traut, R. T. (1975) J. Mol. Biol. 93, 391-404.
6. Martini, O. H. W., and Gould, H. J. (1975) Mol. Gen. Genet., in press.
7. Terak, K., Ogata, K. (1975) Biochim. Biophys. Acta 402, 214-221.
8. Lin, A. and Wool, I. G. (1974) Mol. Gen. Genet. 134, 1-6.
9. Wittmann, H.-G. (1974) in Ribosomes (Nomura, M., Tissières, A., and Lengyl, P., eds) pp. 93-114, Cold Spring Harbor Laboratory, New York.
10. Spinak-Ellson, P. (1970) FEBS Lett. 7, 214-216.
11. Hindennach, I., Kaltschmidt, E., and Wittmann, H.-G. (1971) Eur. J. Biochem. 23, 12-16.
12. Mora, G., Donner, D., Thammanna, P., Lutter, L., and Kurland, C. G. (1971) Mol. Gen. Genet. 112, 229-243.
13. Westermann, P., and Bielka, H. (1973) Mol. Gen. Genet. 126, 349-356.
14. Martin, T. E., and Wool, I. G. (1969) J. Mol. Biol. 43, 151-161.
15. Sherton, C. C., DiCamelli, R. F., and Wool, I. G. (1974) Methods Enzymol. 30(F), 354-367.
16. Hardy, S. J. S., Kurland, C. G., Vojnow, P., and Mora, G. (1969) Biochemistry 8, 2897-2906.
17. Sherton, C. C., and Wool, I. G. (1974) Mol. Gen. Genet. 135, 97-112.
18. Lowry, 0. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265-275.
19. Kaltschmidt, E., and Wittmann, H.-G. (1970) Anal. Biochem. 36, 401-412.
20. Sherton, C. C., and Wool, I. G. (1974) Methods Enzymol. 30(F), 506-526.
21. Lin, A., Collatz, E., and Wool, I. G. Mol. Genet., in press.
22. Stöfler, G. (1967) Mol. Gen. Genet. 100, 374-377.
23. Fairbanks, G., Steck, T. L., and Wallach, D. F. H. (1971) Biochemistry 10, 2606-2617.
24. Stöfler, G., Wool, I. G., Lin, A., and Rak, K.-H. (1974) Proc. Natl. Acad. Sci. U.S.A. 71, 4723-4726.
25. Tissières, A., Schiessinger, D., and Gros, F. (1969) Proc. Natl. Acad. Sci. U.S.A. 46, 1450-1456.
26. Martin, T. E., and Wool, I. G. (1968) Proc. Natl. Acad. Sci. U.S.A. 60, 569-574
27. Martin, T. E., Rolleston, F. S., Low, R. B., and Wool, I. G. (1969) J. Mol. Biol. 43, 135-149
28. Hindennach, I., Stöffler, G., and Wittmann, H.-G. (1971) Eur. J. Biochem. 23, 7-11
29. Kanai, K., Castles, J. J., Wool, I. G., Stirewalt, W. S., and Kanai, A. (1969) FEBS Lett. 5, 68-72
30. Terao, K., and Ogata, K. (1972) Biochim. Biophys. Acta 285, 473-482
Group fractionation of eukaryotic ribosomal proteins.
E Collatz, A Lin, G Stöffler, K Tsurugi and I G Wool

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