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Isolation of Eukaryotic Ribosomal Proteins: Purification and Characterization of S25 and L16†

Alan Lin, Tatsuo Tanaka, and Ira G. Wool*

ABSTRACT: Proteins were extracted from rat liver ribosomal subunits with ethanol and ammonium chloride. The extract from the 40S subunit contained mainly S25, but smaller amounts of a number of other proteins were found as well; the extract from the 60S subparticle had L16 in addition to P1, P2, S25, and several other proteins. S25 and L16 had not been purified before. The former was isolated from the ethanol–ammonium chloride extract by stepwise elution from carboxymethylcellulose with LiCl, chromatography on phosphocellulose, and filtration through Sephadex G-75; L16 was purified by elution from carboxymethylcellulose with LiCl (in steps). The molecular weight of the two proteins was estimated by polyacrylamide gel electrophoresis in sodium dodecyl sulfate; the amino acid composition was determined also.

Eighty proteins have been isolated from rat liver ribosomes and characterized (Collatz et al., 1976b, 1977; Tsurugi et al., 1976–1978). The proteins that were purified comprise a number that were not in the original classification (Sherton & Wool, 1972). Moreover, several proteins that were included in the initial group were not isolated; that subset includes the 40S subunit protein S25, and L16 from the 60S subparticle. S25 was identified in group D40 after the initial fractionation of the 40S subunit proteins (Collatz et al., 1976a, 1977), but it could not be purified because there were only small amounts. L16 occurs only occasionally on two-dimensional polyacrylamide gel plates when 60S ribosomal subunit proteins are analyzed (Sherton & Wool, 1972) and it was not encountered during the purification procedures.

Acidic proteins can be extracted from ribosom subunits with ethanol and ammonium chloride (Reyes et al., 1977). The extract from 40S subunits contains relatively large amounts of S25, and that from the 60S subunit has L16 even though the two proteins are basic. S25 and L16 have been resolved from ethanol–ammonium chloride extracts of ribosome subunits by chromatography and the purified proteins characterized.

Experimental Procedure

Preparation of Ribosomes and Ribosomal Subunits. Subunits were prepared from rat liver ribosomes (Martin & Wool, 1969) on a large scale by centrifugation in a zonal rotor (Sherton et al., 1974).

Ethanol–Ammonium Chloride Extraction of Ribosomal Subunit Proteins. The procedure used to extract ribosomal proteins was modified in minor ways from that described by Hamel et al. (1972) and Reyes et al. (1977). Ribosome subunits, either 40 S or 60 S, were suspended (100 A_{260} units per mL) in buffer (1 M NH_4Cl, 20 mM $MgCl_2$, 1 mM β-mercaptoethanol, 10 mM imidazole hydrochloride, adjusted to pH 7.4 with HCl[1]) and 0.5 volume of 95% ethanol was added. The suspension was shaken gently in an ice bath for 15 min; then a second 0.5 volume of 95% ethanol was added and shaking continued for another 5 min. The final concentration of NH_4Cl was 0.5 M, of ethanol 47.5%. The extracted proteins (referred to as EA40 or EA60[2]) were separated from the core particles by centrifugation (15 min at 27000*g*). The core particles were extracted a second time

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[1] The pH of solutions and buffers was determined at 20 °C.

[2] Abbreviations used: EA40 and EA60, ethanol–ammonium chloride extract of the 40S or 60S ribosomal subunits, respectively; EA40 and EA60 core, the core particle remaining after extraction of 40S or 60S ribosomal subunits with ethanol–ammonium chloride, respectively; TP40 and TP60, the total proteins of the 40S and 60S ribosomal subunits, respectively.

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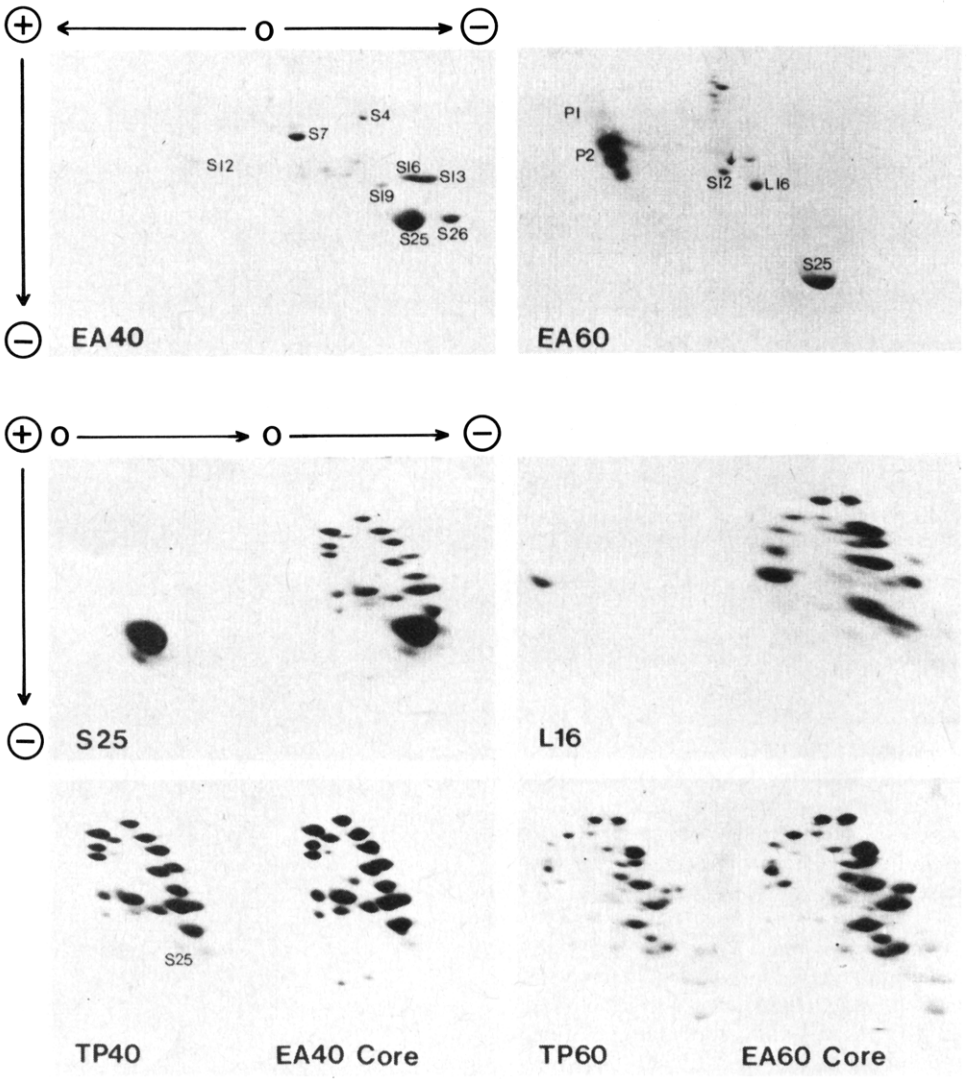

FIGURE 1: Two-dimensional polyacrylamide gel electrophoresis of rat liver proteins. The amount of protein analyzed was: EA40 and EA60, 30 μg; S25, 6 μg; L16, 1.5 μg; TP40 and TP60, 30 μg; EA40 and EA60 core, 90 μg. In the analysis of EA40 and EA60 proteins, the origin was at the top center of the gel and both basic and acidic proteins were separated and identified; electrophoresis in the first dimension was for 85 min for EA40 and 70 min for EA60. The isolated ribosomal proteins S25 and L16 were analyzed alone on the left half of the second dimension gel (origin at top left) to assess their purity, and on the right side (origin at top center) with a small amount (20 μg) of TP40 or TP60 to provide a background to assist in the identification of the proteins. A large amount of the purified proteins (especially of S25) was analyzed so as to facilitate detection of contamination; the satellite material below S25 is more likely artifact than contamination with another ribosomal protein (cf. Figure 5). Electrophoresis was from left to right in the first dimension and from top to bottom in the second. The origin is marked O. The analysis of TP40 and TP60 and of EA40 and EA60 core was only of the basic proteins, i.e., the cathodic portion of the first dimension.

in the same way and the extracts were combined, dialyzed overnight against three changes of 5% acetic acid, and lyophilized.

Group Fractionation of EA40 and EA60. The proteins in EA40 and EA60 were separated into five groups by stepwise elution from a column (1 × 20 cm) of carboxymethylcellulose (Whatman CM 32) at 12–15 °C with LiCl at pH 6.5 (Collatz et al., 1976a); the flow rate was 22 mL/h. Group A proteins did not bind to carboxymethylcellulose; group B was eluted with 0.135 M LiCl; C with 0.185 M; D with 0.25 M; and E with 0.3 M. Protein S25 was in group D (eluted with 0.25 M LiCl), referred to here as EA40-D; L16 was the only protein in group B (eluted with 0.135 M LiCl, designated EA60-B).

Chromatography of Group EA40-D. Group EA40-D contained several small subunit ribosomal proteins besides S25. The proteins, 7.5 mg in buffer C (Collatz et al., 1977), were applied to a column (1.6 × 70 cm) of phosphocellulose (Mannex-P, standard capacity, 0.88 mequiv/g) and eluted with a 2-L linear gradient of 0.2–0.6 M NaCl in the same buffer. Chromatography was at 4 °C.

Protein S25 from ion-exchange chromatography on phosphocellulose was resolved further (i.e., minor contaminants were removed) by filtration through a column (1.3 × 150 cm) of Sephadex G-75 (superfine). The flow rate was 3–4 mL/h and 1.5-mL samples were collected.

Two-Dimensional Polyacrylamide Gel Electrophoresis. The ribosomal proteins were identified by micro-two-dimensional polyacrylamide gel electrophoresis (Lin et al., 1976) using a modification (Lastick & McConkey, 1976) of the more usual procedure (Kaltschmidt & Wittmann, 1970; Sherton & Wool, 1972). The proteins in fractions from ion-exchange chromatography were precipitated with 15% trichloroacetic acid, washed in acetone, and dissolved in sample buffer (Lastick & McConkey, 1976) before electrophoresis; the proteins in fractions from Sephadex filtration were lyophilized and then dissolved in the same buffer.

Polyacrylamide Gel Electrophoresis in Sodium Dodecyl Sulfate. The molecular weight and the purity of the isolated ribosomal proteins were estimated by polyacrylamide gel electrophoresis in sodium dodecyl sulfate using the Laemmli

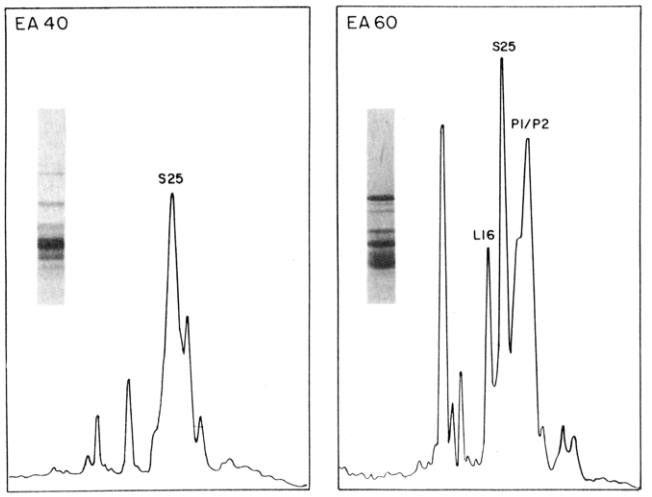

FIGURE 2: Electrophoresis on polyacrylamide gels containing sodium dodecyl sulfate of EA40 and EA60 proteins. The proteins (30 μg) were separated by electrophoresis (see inset) and the gels were scanned at 540 nm. The protein in the first peak in the scan of the EA60 proteins could not be established.

procedure (1970) except that the concentration of polyacrylamide was 15%.

Determination of the Amino Acid Composition of S25 and L16. The isolated ribosomal proteins (1–2 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 were made for incomplete hydrolysis or for decomposition; tryptophan and cysteine were not determined.

Results and Discussion

Extraction of Proteins from Ribosomal Subunits with Ethanol–Ammonium Chloride. Rat liver 40S ribosomal subunits were treated with ethanol and ammonium chloride: the extract (EA40) contained predominantly S25, but there were also lesser amounts of a number of other proteins including S4, S7, S12, S13, S16, S19, and S26 (Figure 1). It was estimated from the profile obtained by scanning a polyacrylamide gel containing sodium dodecyl sulfate (Figure 2) that S25 accounted for 50% of the protein in the extract. Moreover, analysis by two-dimensional polyacrylamide gel electrophoresis (Figure 1) of the proteins of the core particle (EA40 core) would indicate that S25 is removed quantitatively by extraction with ethanol–ammonium chloride; indeed, it is the only protein entirely removed.

S25 had been identified before as a component of the 40S subunit (Sherton & Wool, 1972). However, the protein is either extracted inefficiently in 67% acetic acid–33 mM magnesium chloride (Hardy et al., 1969; Sherton & Wool, 1974) or is poorly precipitated with acetone, since it was present only in trace amounts in group D40 after fractionation (Collatz et al., 1976a). It would seem that the physical properties of S25 favor its removal from the ribosome in ethanol–ammonium chloride rather than with acetic acid.

The ethanol–ammonium chloride extract prepared from rat liver 60S ribosomal subparticles (EA60) contained P1, P2, and L16; it also had S12, and S25, presumably because the preparation of 60S subunits was contaminated with 40S subparticle (Figure 1). The preparation of EA60 contained about 39% of P1/P2, 23% of S25, and 10% of L16 (Figure 2). While it is difficult to be certain, it appeared that P1 and P2 were completely removed from 60S subunits by the procedure; indeed, no P1 or P2 was obtained in group A (Collatz et al., 1976a; Tsurugi et al., 1978) on chromatography of the

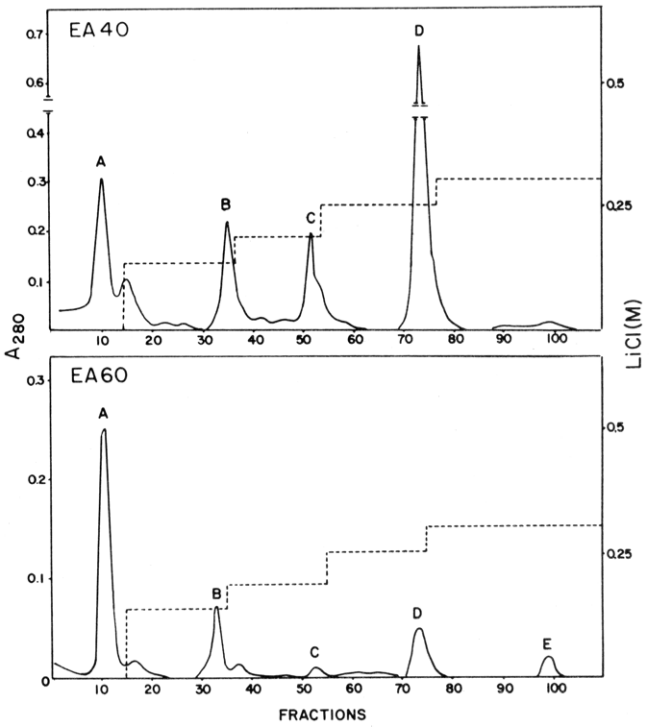

FIGURE 3: Group fractionation of EA40 and EA60 proteins. The proteins (42 mg of EA40 and 28 mg of EA60) were separated into groups by stepwise elution from a column (1 × 20 cm) of carboxymethylcellulose with increasing concentrations of LiCl at pH 6.5. The proteins in group A were not absorbed. The concentrations of LiCl used to elute the other groups of protein were: (B) 0.135 M; (C) 0.185 M; (D) 0.25 M; (E) 0.3 M.

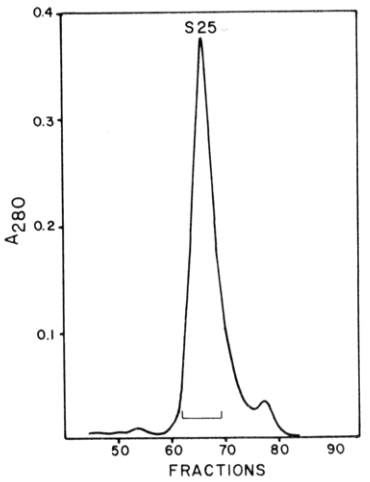

FIGURE 4: Purification of S25 by filtration through Sephadex G-75. The preparation (3.6 mg in 5% acetic acid) containing S25 from phosphocellulose chromatography was filtered through a column (1.3 × 150 cm) of Sephadex G-75 (superfine). The flow rate was 3–4 mL/h and 1.5-mL fractions were collected. The brackets indicate fractions containing purified S25 that were pooled.

proteins of the ethanol–ammonium chloride core particle (results not shown). The amount in the EA60 core particle of L16 was not appreciably decreased (Figure 1). Proteins P1 and P2 had been purified from group A 60 (Tsurugi et al., 1978); however, L16 had not been found in any of the group fractions during the isolation procedure. L16 had been identified on two-dimensional gels after electrophoresis of 60S ribosomal proteins from rat liver (Sherton & Wool, 1972; Terao & Ogata, 1975), rabbit reticulocytes (Howard et al., 1975), and chicken liver (Ramjoué & Gordon, 1977), but not from HeLa cells (Lastick & McConkey, 1976), nor in one study of rat liver (Welfle et al., 1972). Once again it would

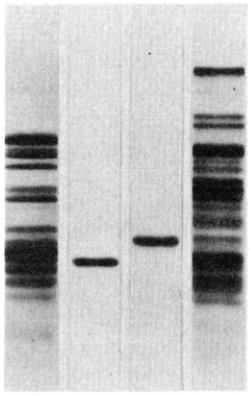

FIGURE 5: Polyacrylamide gel electrophoresis in sodium dodecyl sulfate of purified S25 and L16. The analysis was of 4 μg of the purified proteins and 20 μg of TP40 and TP60.

Table I: Molecular Weight and Amino Acid Composition of S25 and L16[a]

	S25	L16
M_r (× 10^{-3})	17.0	18.7
Asp	12.7	10.5
Thr	3.2	4.9
Ser	6.3	7.2
Glu	6.2	11.1
Pro	3.2	6.4
Gly	10.7	10.0
Ala	10.5	9.3
Val	6.6	6.9
Met	0.0	0.0
Ile	2.9	6.7
Leu	8.8	7.5
Tyr	1.8	1.2
Phe	1.0	1.6
His	0.8	2.0
Lys	20.3	8.5
Arg	5.0	6.0

[a] The value for amino acids are in moles percent.

seem the physical properties of L16 make the preparation of the protein in the usual way unreliable and probably account for the conflicting reports concerning whether it is a component of the 60S ribosomal subunit.

Purification of Ribosomal Proteins S25 and L16. The proteins in EA40 were separated into groups by elution from carboxymethylcellulose with increasing concentrations of LiCl (added in steps). S25 was eluted with 0.25 M LiCl in group D (EA40-D) (Figure 3). Since EA40-D contained other proteins, in addition to S25, it was resolved further by chromatography on phosphocellulose with a linear gradient of 0.2–0.6 M NaCl. S25 was eluted at 0.32 M NaCl,[3] but again there was a minor contaminant so the fraction containing S25 was filtered through Sephadex G-75 (Figure 4).

The five proteins in EA60 were fractionated into groups by stepwise elution from carboxymethylcellulose at pH 6.5. L16 was eluted alone with 0.135 M LiCl in group B (Figure 3).

Characterization of Ribosomal Proteins S25 and L16. The identity of the isolated proteins, S25 and L16, was confirmed by two-dimensional polyacrylamide gel electrophoresis (Figure 1); the purity was assessed after electrophoresis in gels containing sodium dodecyl sulfate by scanning at 540 nm (Figure 5). The impurities in S25 did not exceed 3%, and in L16 were about 5%. The molecular weights of S25 (17 000) and of L16 (18 700) were estimated from the gels by comparison with standards (Table I). The amino acid composition was also determined (Table I).

The isolation of S25 and L16 brings to 82 the number of proteins purified from rat liver ribosomes: 33 from the small, and 49 from the large, subunit. It remains to be established that each is chemically unique, and that each is a structural component of the ribosome, although it is certain most are.

[3] The elution profile for the chromatography of EA40-D is not shown because the proteins in the fractions were too dilute to determine the absorption at 280 nm; instead, S25 was located by analyzing every fifth fraction by electrophoresis in gels containing sodium dodecyl sulfate.

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