THE PROTEINS OF LIVER AND MUSCLE RIBOSOMAL SUBUNITS: PARTIAL SEPARATION BY CARBOXYMETHYL-CELLULOSE COLUMN CHROMATOGRAPHY

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The exact way in which ribosomes participate in protein synthesis is not known. There is, however, evidence that the ribosomal proteins play an important role in the process [1], and some indication that knowledge of their chemical structure and biological function is necessary for an understanding of regulation of protein synthesis. We report here a partial separation of the proteins of the subunits of liver ribosomes; the study is part of an effort to determine the physical and chemical properties of mammalian ribosomal proteins preparatory to undertaking a study of their function.

The proteins of the 40S subunit of liver ribosomes were partially separated by chromatography on carboxymethyl-cellulose (fig. 1). The elution pattern was reproducible except for peaks I and V: peak I was frequently absent (see fig. 3) and peak V was occasionally split into 2 discrete peaks (fig. 3). The proteins in the fractions obtained by carboxymethyl-cellulose chromatography were compared by split gel electrophoresis with all the proteins of the 40S subunit – the ribosomal proteins of the 40S subunit gave 20 bands when electrophoresed at pH 4.5 (see also reference 7). The split gel technique allows one to determine which of the 40S proteins are found in each of the fractions (fig. 1). The bands that stained darkest are designated by solid lines; they account for at least 80% of the total dye uptake (determined by densitometry of the gels). Staining with amido black is, of course, only an approximate measure of the relative quantities of ribosomal protein [10]. The dashed lines indicate bands that were not detectable by densitometry but were faintly visible to the eye.

The split gel patterns confirmed that some separation of the 40S proteins had been achieved, although none of the fractions was pure. Several of the protein bands, number 11 especially but 9 to 13 also, were eluted by a broad range of NaCl concentrations and appear in gels from several fractions, which would suggest that the original electrophoretic bands contained more than one protein. Thus the band referred to as 11 in the several fractions may in fact be a number of different proteins. (Band 11 did form two components in peaks IX and XIII). Obviously the identity of the band 11 protein or proteins will only be settled by further purification of the carboxymethyl-cellulose fractions. Only bands 6 and 8 are not accounted for; they may be in peaks I, IV, and XVII which were not analysed for lack of sufficient protein.

The proteins of the 60S subunit of liver ribosomes were also chromatographed on carboxymethyl-cellulose (fig. 2) and the proteins in each fraction were analyzed by the split gel technique. (Discontinuous electrophoresis at pH 4.5 of the ribosomal proteins of the 60S subunit gave 24 bands; Low, Wool and Martin [7] found 25, but aside from that discrepancy the pattern was the same.) Once again, some of the bands were eluted over a broad range of NaCl concentrations (for example, numbers 8, 9, 11, 13 and 15). Only bands 1 and 21 were not accounted for. The 60S subunit had three acidic protein bands (electrophoresis at pH 8.7); they were eluted from the carboxymethyl-cellulose in peak I, as was to be expected for acidic proteins on a cation-exchanger.

Carboxymethyl-cellulose chromatography separated the ribosomal proteins approximately as would have been predicted from their electrophoresis

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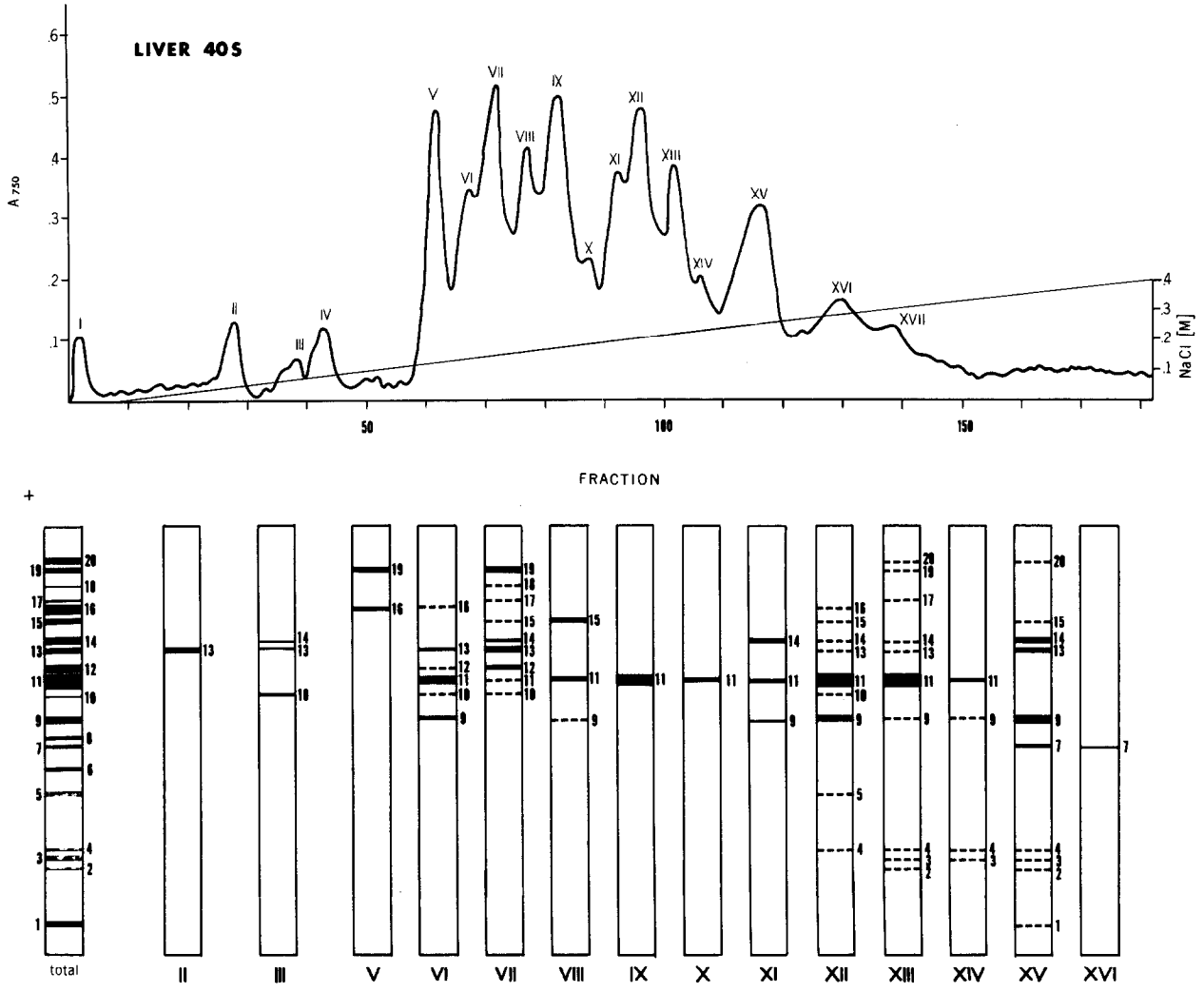

Fig. 1. Carboxymethyl-cellulose column chromatography of proteins from the 40S subunit of liver ribosomes. Ribosomes prepared from liver of 150–200 gm male Sprague-Dawley rats [2] were stripped of nascent peptide and endogenous messenger RNA by incubation in the same media used to assay protein synthesis [3] and with 50 μg/ml of puromycin. Ribosomes were dissociated [4] and the subunits were collected from sucrose gradients; the subunits were precipitated by allowing them to stand overnight at 4°C after adding 50% trichloroacetic acid (final concentration 10%). Protein was extracted from ribosomes and from ribosome subunits by the acetic acid method of Waller and Harris [5], modified as follows: The trichloroacetic acid precipitated ribosomal subunits were resuspended in 0.01 M Tris-HCl (pH 7.8), 0.1 M magnesium acetate to a final ribosome concentration of 30 mg per ml. Two volumes of glacial acetic acid were added and the mixture was stirred at 4°C for 20 min. The precipitated RNA was then removed by centrifugation at 5,000 X g for 20 min. The supernatant was dialyzed for 4 hr against several changes of deionized water, then overnight against 1,000 volumes of 6 M urea in 0.005 M sodium acetate, pH 5.6 (urea buffer). The recovery of protein which was generally 85–90% (although rarely only 50% was recovered from the 60S subunit), was better and more consistent than when the urea-LiCl method [6] was used (recovery 30–80%). Preswollen carboxymethyl-cellulose (Reeve Angel, Whatman CM 52) was equilibrated with urea buffer and fines were removed. The carboxymethyl-cellulose was packed in columns (0.6 X 25 cm) under a pressure of 0.4 kg/cm [2]. Ribosomal protein (11 mg) from the 40S subunit was placed on the column and eluted at room temperature with 400 ml of NaCl in urea buffer; the concentration of the NaCl was increased linearly from 0 to 0.4 M. The rat of flow was kept at 6–7 ml per hr with a peristaltic pump. Fractions were collected every 20 min. The concentration of protein in the fractions, given by the absorbancy at 750 mμ, was determined by a modification [7] of the method of Lowry et al. [8]. The recovery of protein was 85–90%. Discontinuous polyacrylamide gel electrophoresis was by the method of Leboy, Cox and Flaks [4]. The acrylamide concentration in the separating gel was 10% (w/v) for gels run at pH 4.5 and 7.5% for gels run at pH 8.7. The bisacrylamide concentration was 0.2%. Electrophoresis was at a constant current of 3 ma per tube of 4°C for 150 to 180 min. Fractions from carboxymethyl-cellulose chromatography were analyzed by the split gel technique. The amount of protein in the fractions was not constant but the Rf's of ribosomal proteins do not vary significantly with change in concentration [10]. The solid lines designate prominent bands, dashed lines faint but visible bands (the latter were not detectable by densitometry). The amount of protein in peaks I, IV, XVII was too small for electrophoretic analysis.

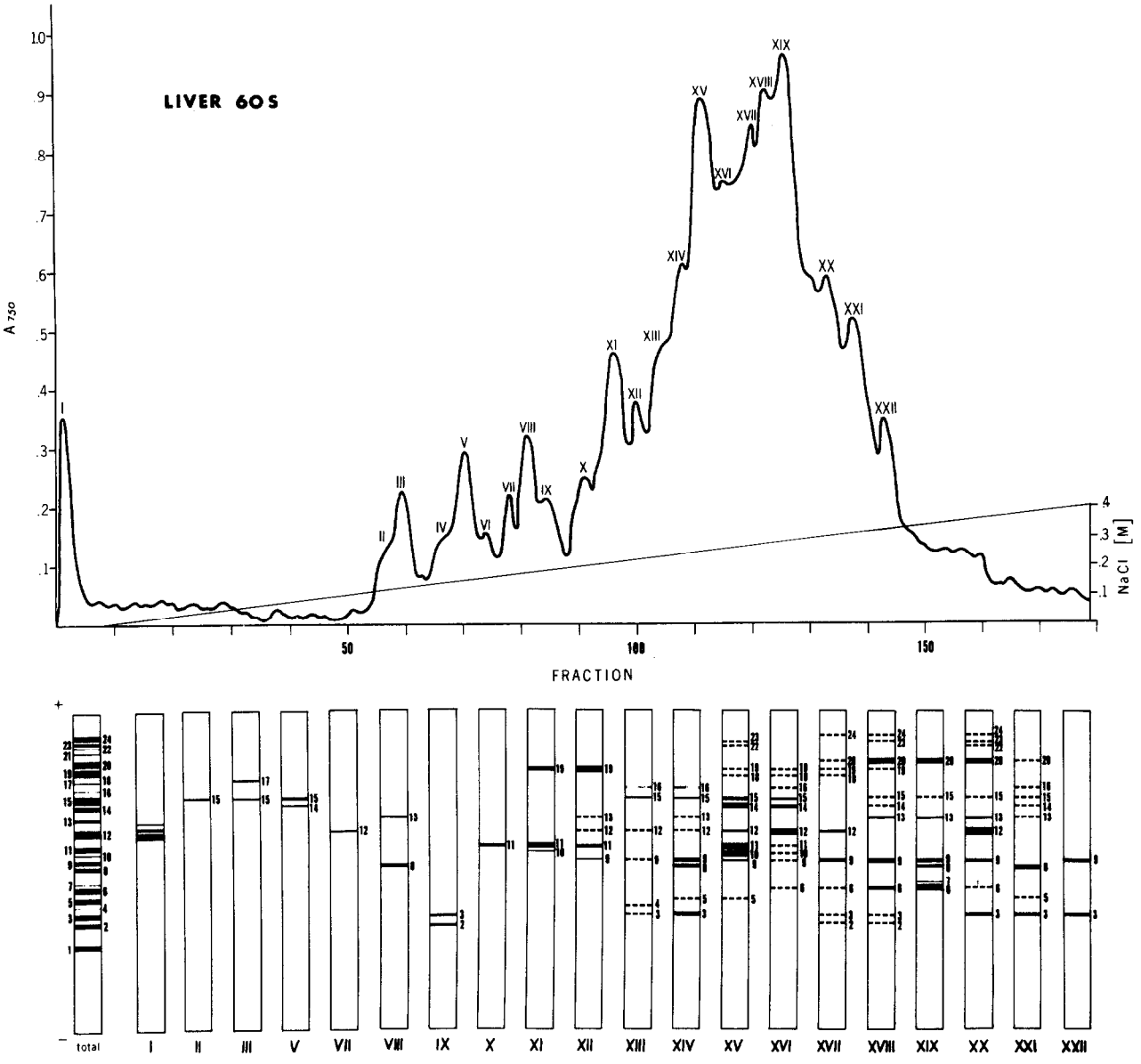

Fig. 2. Carboxymethyl-cellulose chromatography of proteins from the 60S subunit of liver ribosomes. The conditions were the same as in fig. 1 except that 17 mg of proteins from the 60S subunit of rat liver ribosomes were chromatographed. Electrophoresis of the proteins in peak I was at pH 8.7. The amount of protein in peaks IV and VI was too small for electrophoretic analysis.

on polyacrylamide gels – the greater the mobility, or the more basic a protein, the higher the ionic strength at which it was eluted.

We have attempted to determine whether the proteins of the subunits of ribosomes from liver and muscle were the same. Proteins from the 40 or 60S subunits of muscle ribosomes were mixed with small amounts of very radioactive proteins from the corresponding subunit of liver and chromatographed simultaneously on carboxymethyl-cellulose (fig. 3). The content of muscle ribosomal proteins in the fractions eluted from the carboxymethyl-cellulose was deter-

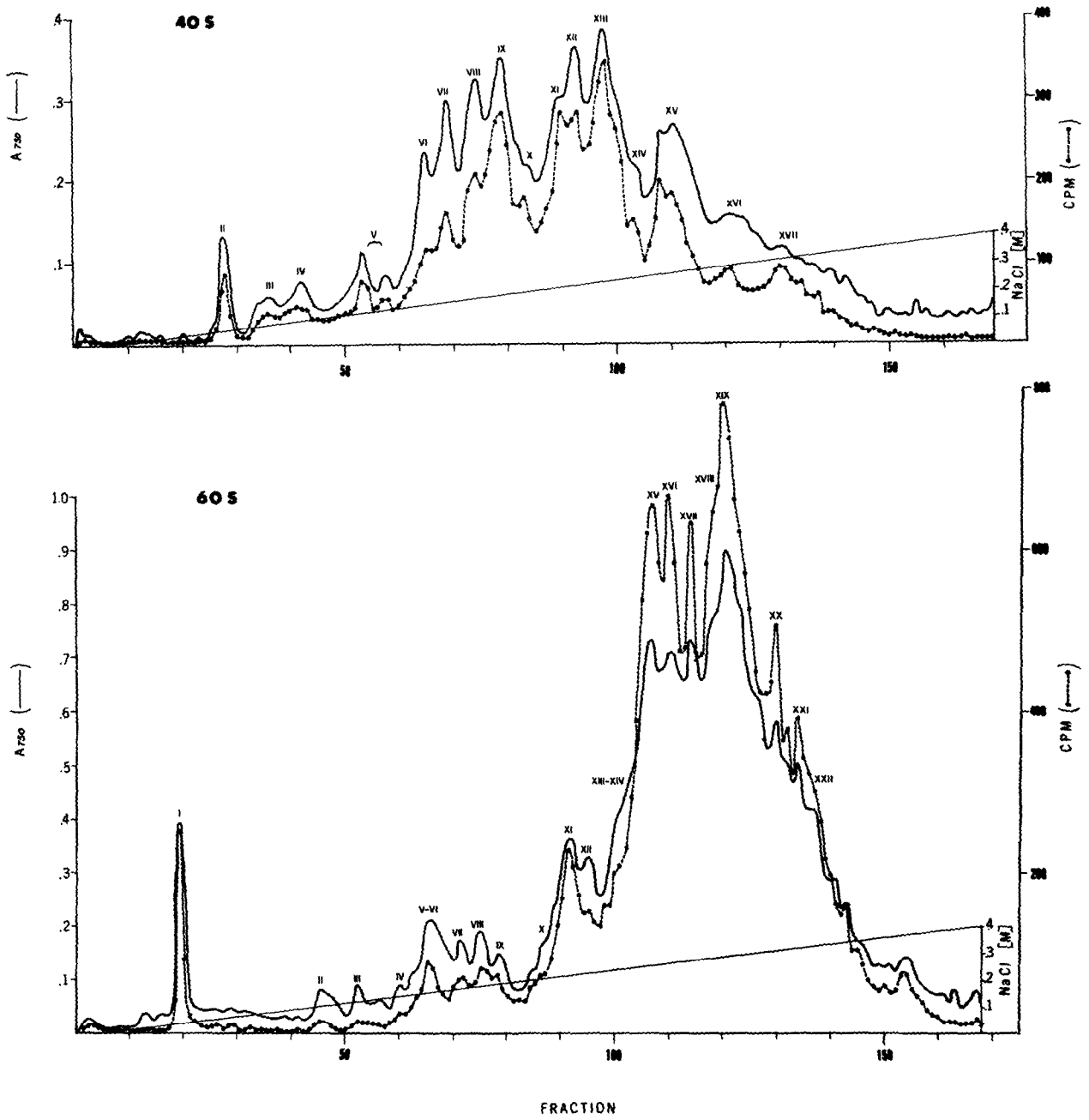

Fig. 3. Chromatography on carboxymethyl-cellulose of unlabelled protein from subunits of muscle ribosomes and ^{3}H-labelled proteins from subunits of liver ribosomes. The preparation of muscle ribosomes has been described before [2]. For the preparation of radioactive liver ribosomal proteins, 4 rats were partially hepatectomized [11] and 12 and 24 hr later injected intravenously with 1.25 mC of ^{3}H-lysine (252 mC/mmole); 48 hr after hepatectomy, the animals were killed, the livers were removed, and ribosomes and ribosomal subunits were prepared. The synthesis of ribosomes is ten times the normal rate in regenerating rat liver [12]. Lysine is the commonest amino acid in rat liver ribosomal proteins [7]. The radioactivity of samples (0.5 or 1 ml) from carboxymethyl-cellulose chromatography was determined in a Packard TriCarb liquid scintillation spectrometer. 10 mg of protein from the 40S subunit of muscle ribosomes were mixed with 0.9 mg of ^{3}H-labelled protein from the 40S subunit of liver ribosomes (1.33×10^{5} cpm per mg protein); for comparison of 60S proteins the mixture was of 19.2 mg from muscle and 1.3 mg of ^{3}H-labelled protein from liver. The conditions for chromatography were the same as in fig. 1.

mined by the Lowry method [8]; the liver ribosomal proteins were estimated from the radioactivity in the fractions. The patterns determined by chemical analysis and by assay of the radioactivity of the fractions were congruent for both 40 and 60S ribosomal proteins (fig. 3). The muscle ribosomal proteins in each fraction from the 40 and 60S subunits were also analyzed by discontinuous polyacrylamide gel electrophoresis; the patterns were the same as for the corresponding fractions of liver ribosomal subunits. The results accord with the conclusion [7] that the ribosomal proteins of muscle and liver are the same. Proof of that proposition requires purification and characterization of the individual ribosomal proteins.

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