THE NUMBER AND SIZE OF THE PROTEINS IN THE SUBUNITS OF HUMAN PLACENTAL RIBOSOMES

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1. Introduction

Several reports were recently published on the number and size of the proteins from eukaryotic ribosomes, isolated mostly from various organs of rodents or *Xenopus laevis* [1–12]. However, since little structural work has been done on human ribosomes, we report here on the number and size distribution of the proteins from both subunits of human placental ribosomes.

Using an improved two-dimensional (2-D) acrylamide gel electrophoresis system, thirty-six cationically moving proteins with molecular weights ranging from 8 900 to 61 900 were found in the large subunit and twenty-eight with molecular weights between 9 600 and 37 600 in the small subunit. Moreover, two proteins, moving anionically at pH 8.6 and which stained very weakly, can be demonstrated in the protein mixture from undissociated ribosomes. They presumably belong to the small subunit.

2. Experimental

2.1. *Preparation of human placental ribosomes*

Ribosomes were prepared from the postmitochondrial supernatant of homogenates in 50 mM Tris–HCl buffer, pH 7.6, 200 mM NH_4Cl, 150 mM KCl, 10 mM $MgCl_2$, 0.5 mM EDTA and 250 mM sucrose treated with 0.6% deoxycholate (DOC) and centrifuged for 240 min at 80 000 *g* in the A130 rotor of a Martin Christ Omega II [13]. The pellets were resuspended in TKM-buffer (10 mM Tris–HCl, pH 7.6, 25 mM KCl, 5 mM $MgCl_2$) and after removing insoluble material by low-speed centrifugation, the ribosome solution was quickly frozen and stored in liquid nitrogen.

2.2. *Preparation of ribosomal subunits*

Ribosomal subunits were obtained by incubating ribosomes (3 mg/ml) for 15 min at 37°C with 0.2 mM puromycin in 50 mM Tris–HCl buffer, pH 7.6, 500 mM KCl, 3 mM $MgCl_2$ [14]. Separation was performed in a BXV zonal rotor of the Omega II on an isovolumetric sucrose gradient of 15 to 39% (w/w) [15] in 50 mM Tris–HCl, pH 7.6, 500 mM KCl, 3 mM $MgCl_2$, using 50 ml sample solution and 200 ml overlay. Centrifugation was at 5°C for 17 hr at 30 000 rpm. The separated subunits were pelleted for 17 hr at 50 000 rpm, resuspended in TKM-buffer and their purity checked by analyzing the extracted RNA on linear sucrose gradients [14].

2.3. *Extraction of ribosomal proteins*

Ribosomes or subunits, suspended in TKM-buffer containing 200 mM $MgCl_2$ were extracted under constant stirring with 2 volumes of glacial acetic acid for 30 min at 4°C. The precipitated RNA was pelleted by centrifugation at 30 000 *g* for 20 min and the supernatant dialyzed during several days against water and finally lyophilized.

2.4. *2-Dpolyacrylamide gel electrophoresis*

The original Kaltschmidt technique [16] requires a relatively large amount of material (~ 2 mg), presumably because the sample gel containing the ribosomal proteins is polymerized before running the first dimension. This seems to cause some abnormalities,

preventing a large proportion of the sample from entering the first dimension gel. In the second dimension, one then sees after staining a large vertical streak in the middle of the gel plate.

Moreover, using this unmodified technique, we only observed two proteins moving anionically at pH 8.6. They stained very weakly and were barely visible on the gels.

In routine runs we therefore limited the separation to the cationic proteins, so that two samples could be run side by side in the second dimension. The first dimension was run in a smaller glass column (9 × 0.6 cm) filled with an 8% gel at pH 8.6 and a small 4% spacer gel. The sample, dissolved in spacer gel buffer pH 8.6 was directly layered on top and electrophoresed for 15 hr at 70 V (3 mA/gel) at room temperature. By omitting a sample gel, the amount of protein in the sample could be reduced at least 5-fold (to ~ 400 μg). Two first dimension gels were then laid on top of the second dimension 18% gel plate (pH 4.1) and run for 10 hr at 105 V (100 mA/gel).

2.5. *Sodium dodecyl sulphate (SDS) gel electrophoresis*

Protein samples were treated for 2 hr at 37°C in 0.1 M phosphate buffer pH 7.1, 1% SDS, 1% 2-mercaptoethanol and 8 M urea and dialyzed overnight at room temperature against 0.01 M phosphate buffer pH 7.1, 0.1% SDS, 0.1% 2-mercaptoethanol. Electrophoresis on 10% polyacrylamide gels containing 0.1 M phosphate buffer pH 7.1, 0.1% SDS and 5 M urea was performed for 4 hr at 8 mA/gel [17]. Gels were stained for 4 hr with 0.1% Coomassie Brilliant Blue R 250 after soaking overnight in 20% sulfosalicyclic acid.

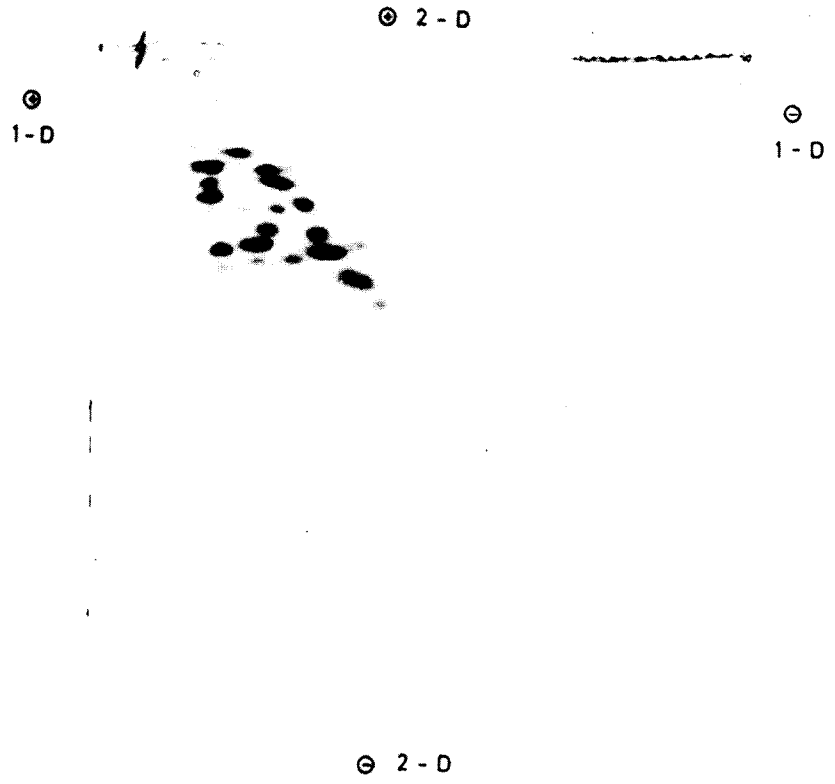

Fig. 1. 2-D pattern of the proteins of the small ribosomal subunit. Starting point is on top left. Migration in the first (1-D) dimension, 8% acrylamide pH 8.6, is from left (+) to right (−) and in the second (2-D) dimension, 18% acrylamide pH 4.1, from top (+) to bottom (−).

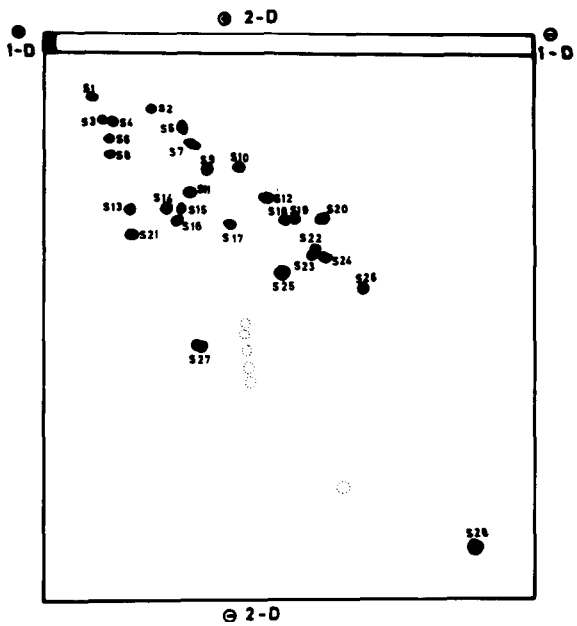

Fig. 2. Scheme and numbering of the proteins of the small subunit. • Main spots. ○ Satellite spots.

3. Results and discussion

Twenty-eight spots showing a varying but reproducible staining intensity could be detected on the 2-D pattern of the 40 S ribosomal proteins (fig. 1). These were numbered along horizontal lines beginning at the upper left, using the system of Kaltschmidt and Wittmann [18] (S 1 to S 28 in fig. 2). Although most proteins were running as separate spots a doublet S14/S15 and a triplet S22/S23/S24 were observed. These were clearly resolved using a longer electrophoresis time in both directions. Protein S 28 was only visible when 3-fold larger amounts of protein were put on the gel and the electrophoresis time decreased. Under these conditions several weak satellite spots (fig. 2) were observed in the region of S 27. These spots were also seen in the 2-D pattern of the 40 S ribosomal proteins of rat liver [2]. SDS-gel electrophoresis of the protein mixture showed fourteen bands with molecular weights ranging from 9 600 to 37 600 (fig. 5). This pattern was closely comparable with the

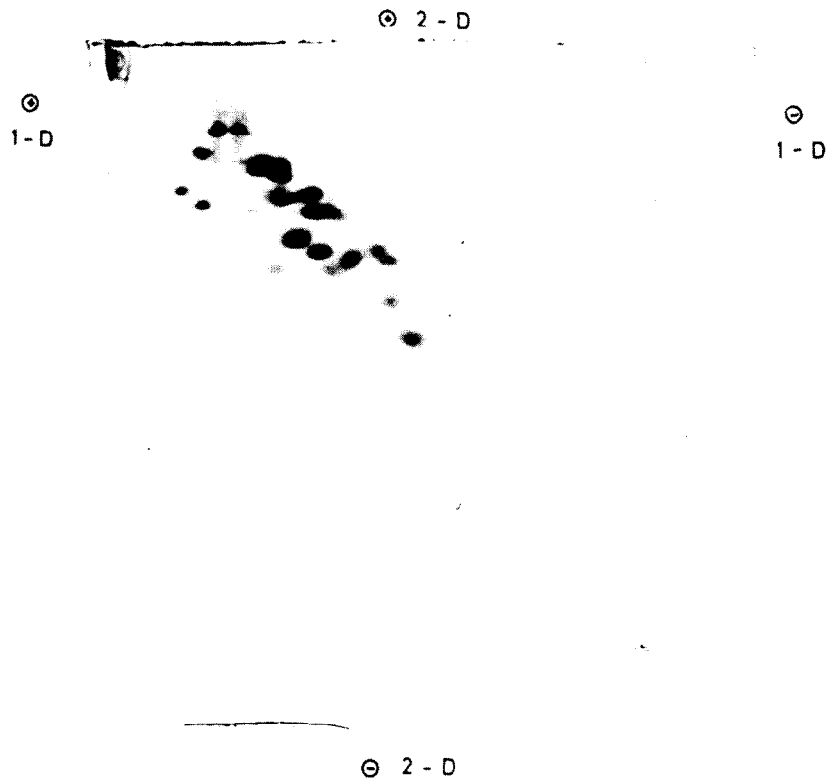

Fig. 3. 2-D pattern of the proteins of the large subunit. For experimental conditions see fig. 1.

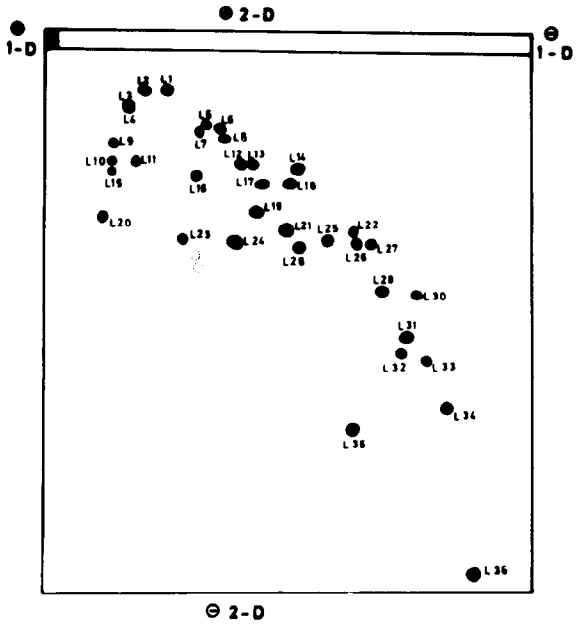

Fig. 4. Scheme and numbering of the proteins from the large subunit. • Main spots. ○ Satellite spots.

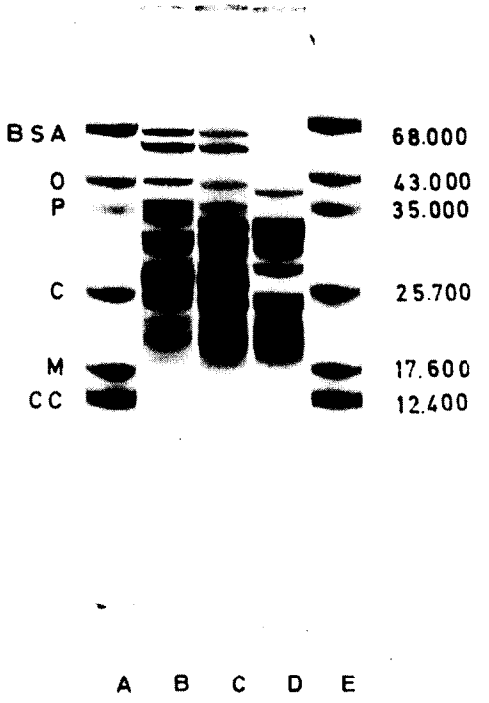

Fig. 5. Molecular weight distribution of the proteins from the ribosomal subunits resolved in SDS-gels. Electrophoresis was performed as described in the Experimental section. Standard proteins are: BSA, bovine serum albumin (mol. wt = 68 000); O, ovalbumin (mol. wt = 43 000); P, pepsin (mol. wt = 35 000); C, chymotrypsin (mol. wt = 25 700); M, myoglobin (mol. wt = 17 600) and CC, cytochrome *c* (mol. wt. = 12 400).

molecular weight distribution of the 40 S ribosomal proteins from rabbit reticulocytes [6, 7].

The protein mixture of the large subunit could be separated into thirty-six cationically moving proteins (fig. 3) marked with the prefix L (fig. 4). Here also the doublets L3/L4, L12/L13 and the triplet L22/L26/L27 could be resolved by increasing the running time of the electrophoresis. Very weakly stained spots L32, L33, L34 and L36 were clearly detected putting a double amount of protein on the gel, which also made 2 satellite spots become visible close to L23. On SDS-gels seventeen protein zones were separated from the large subunit. These had molecular weights between 8 900 and 61 900 (fig. 5). Two large proteins L1 (mol. wt $\simeq$ 61 900) and L2 (mol. wt $\simeq$ 56 300) were clearly separated from the rest which had a molecular weight distribution from 8 900 to 45 500. This separation was only slightly different from that reported for the 60 S ribosomal proteins of rabbit reticulocytes by King et al. [6].

In order to determine whether all proteins from both subunits migrated at unique positions in the 2-D gels, a mixture was electrophoresed under standard conditions. Proteins S10 and L12, S18 and L21, S23 and L28 were observed to form pairs migrating almost as single spots, whereas all other proteins moved as distinct spots. Such overlapping proteins of both subunits were also found in rat liver and rabbit reticulocyte ribosomes [2, 8].

We conclude that human placental ribosomes are composed of sixty-four cationically moving proteins, twenty-eight in the small subunit and thirty-six in the large one. In addition, two anionically moving proteins are reproducibly detected in several preparations of proteins from undissociated ribosomes. We classify them with the 40 S proteins, because in overloaded one-dimensional gels at pH 8.6 with reversed polarity, two clear bands are observed with the 40 S proteins, and none with the 60 S ones. Proteins running to the anode at pH 8.6 and appearing in the same position

on 2-D gels were also observed in Ehrlich ascites ribosomes [10], whereas the number of these proteins in rat liver ribosomes ranged from 0 to 3 [1, 3] and amounted even to five in rabbit reticulocyte ribosomes [8].

In *E. coli* the anionically moving proteins stain almost as well as the cationic ones. Four are located in the small subunit, and seven in the large one [18]. Among these, L7 and L12, with pK_a-values of 4.8, 4.9 respectively, may be implicated in the translocation function [20]. In contrast to *E. coli*, the eukaryotic anionic proteins reported on in the literature and in this work usually yield very much weaker staining intensities than the cationic ones, and they are much fewer in number.

In view of what is known for *E. coli*, it seems hard to accept that the pK_a-differences alone could be responsible for the weaker staining intensities. Moreover, Racusen [19] found only slight variations in Amido Black dye uptake for a series of proteins with strongly divergent pK_a-values. One is tempted to conclude that these eukaryotic anionic proteins are either non-ribosomal impurities or stoechiometrically present on only a small fraction of all ribosomes.

Our results are in close agreement with the reported number of proteins detected by 2-D electrophoresis in ribosomes of several animal tissues [1–3, 7–10, 12] and the molecular weight distributions are comparable [4, 6, 9].

There exists a remarkable degree of similarity in the 2-D patterns of human ribosomal proteins and those of rat liver and rabbit reticulocytes [1–3, 8]. Delaunay et al. [21] have also noted large interspecies similarities.

It is also interesting that the number of different proteins in eukaryotic 40 S subunits is considerably larger than in bacterial 30 S subunits, whereas the number of proteins in both larger subunits is practically the same. This may perhaps reflect the specialized role of the 40 S subunit in the more complicated regulatory processes of eukaryotic protein synthesis.

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