MAMMALIAN MITOCHONDRIAL RIBOSOMES: CHARACTERIZATION OF RIBOSOMAL PROTEINS BY TWO-DIMENSIONAL GEL ELECTROPHORESIS

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Received 11 December 1975

1. Introduction

Ribosomes isolated from mammalian mitochondria were found to sediment at about 55S [1,2]. Investigations on their function as well as their physicochemical properties were performed by several groups in order to characterize these particles. Sensitivity towards some antibiotics, factor exchangeability, and molecular weight favor the idea that mitochondrial ribosomes are of the prokaryotic type [3–5]. The low RNA content and the low negative charge to mass ratio, however, oppose this assumption [6].

Elucidation of the structure of the protein moiety of the 55S ribosome should provide additional information for the classification of these particles. The method preferred for mapping 70S and 80S ribosomal proteins is two-dimensional polyacrylamide gel electrophoresis [7,8]. Ribosomal proteins from mammalian mitochondria, available in minute quantities only, were found to exhibit a lower basicity than 70S or 80S ones. With the introduction of isoelectric focusing the separation of proteins in the first dimension could be improved by replacing the molecular sieve effect and increasing the resolving power.

In this paper we describe a method which combines isoelectric focusing with flat-gel disc electrophoresis in the second dimension for mapping ribosomal proteins. For mitochondrial ribosomal proteins this method yielded 60 dark plus 47 weak protein spots with isoelectric points ranging from 4.0 to 9.5. The amount of sample needed could be reduced to 50 μg of protein.

2. Experimental

2.1. *Preparation of mitochondrial ribosomes and ribosomal proteins*

Rat livers were homogenized in ST-buffer (0.25 M sucrose, 2 mM TES-buffer (tris-(hydroxymethyl)-methyl-2-aminoethane-sulfonic acid), pH 7.4; 1:6 (w/v)). After initial differential centrifugation mitochondria were freed of their outer membrane by digitonin treatment: 1.1 mg digitonin was used per 10 mg mitochondrial protein [9]. The resuspended 'mitoplasts' were lysed using a final concentration of 1.3% Triton X-100. Aliquots of the lysate were layered over 1.0 M sucrose in KMEDT (0.1 M KCl, 10 mM $MgCl_2$, 0.1 mM EDTA, 1 mM dithiothreitol, 2 mM TES-buffer, pH 7.6) and then centrifuged for 3 h at 180 000 *g* in a Spinco 50.2 Ti rotor [6]. The 'crude' ribosomal material obtained as a sediment was further purified using a convex sucrose gradient [10] from 10–23% in KMEDT and centrifuged for 12 h at 53 000 *g* in a Spinco SW 27 rotor. The gradient was then fractionated while the A_{254} profile was recorded using a MICO flow cell adapted to an ISCO UA-4 absorbance monitor (fig. 1). The parts of the gradient containing 55S ribosomes were collected and pooled. Ribosomes were spun down overnight at 110 000 *g*. Proteins were extracted by the acetic acid method, dialyzed stepwise against 10 to 0.2% acetic acid and lyophilized [8].

2.2. *Separation of ribosomal proteins*

The sample was prepared as follows: proteins were dissolved in 8 M urea (100 μg/40 μl), and 20 μl of

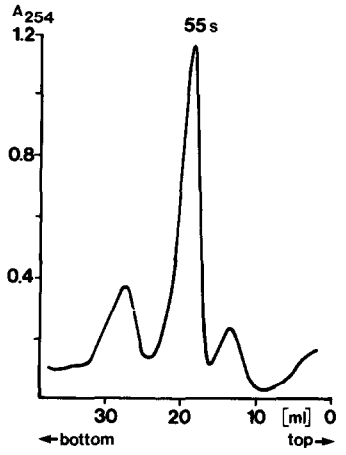

Fig.1. Sucrose density gradient profile of ribosomes isolated from rat liver mitochondria..55S peak: mitochondrial ribosomes, $A_{260/280}$: 1.47.

this solution were mixed with 30 μl of Sephadex G-200 superfine swollen in 20% sucrose containing 8 M urea, 10% mercaptoethanol, and 1% ampholine (LKB). Separation of the mitochondrial ribosomal proteins was performed in the first dimension by isoelectric focusing in polyacrylamide gels carried out according to Klose [11]. Gels and solutions were made up in 6 M urea, the upper electrode solution contained 3 M urea. After this run the cylindrical gels were layered immediately onto a slab gel. Separation in the second dimension was then carried out as described by Kaltschmidt and Wittmann [7] using an apparatus for smaller gel size (10 X 10 X 0.3 cm). These small dimensions led to a reduced voltage (60 V), and a shorter running time (17 h).

The slab gels were fixed in an aqueous solution containing 10% acetic acid, 50% methanol for 1 h and then stained overnight in 0.5% Coomassie brilliant blue R 250, dissolved in 10% acetic acid, 40% methanol. Destaining was carried out in 10% acetic acid, 5% methanol at 50°C for 4 h and then at room temperature while the destaining solution was continuously clarified by pumping over a charcoal filter.

3. Results and discussion

Preliminary mapping experiments carried out in our laboratory with ribosomal proteins in the original Kaltschmidt and Wittmann system, revealed that proteins from rat liver mitochondrial ribosomes exhibit lower isoelectric points than 70S bacterial or 80S cytoplasmic ones. At the basic pH of 8.8 used for the first dimension of this system the majority of proteins from 55S ribosomes migrated anodically. This behavior made it possible to separate proteins of mitochondrial ribosomes using commercially available ampholines stabilizing pH gradients up to pH 11. In contrast to the molecular sieve effect depending on the pore size of the polyacrylamide gels, the isoelectric focusing technique separates proteins only according to their isoelectric points and has a high resolving power. Layering the dissolved proteins on top of the gels in Sephadex was another advantage of this technique, because loss of sample due to copolymerisation between protein and acrylamide could be avoided. Staining with Coomassie blue instead of Amidoschwarz further increased the sensitivity.

An example of a typical two-dimensional protein pattern of 55S rat liver ribosomes is shown in fig.2a. This pattern was obtained with a pH-gradient from 3.5–10 for separation by isoelectric focusing, whereas the 18% acrylamide concentration of the second dimension gel with its smaller pore size, supported resolution of proteins spots largely according to their molecular weights.

The number of clearly visible and distinct spots can be divided into 60 dark and 47 weak ones, a total of 107 spots (fig.2a). This classification, however, does not allow one to draw conclusions on the number of 'truc' mitochondrial ribosomal proteins. Weak spots may also be contaminants of non-ribosomal mitochondrial proteins or derivatives of the main spots. Additionally, this photograph displays some other very small faint spots. This pattern was obtained with a total amount of 80 μg of protein. Reproducible mapping was achieved with as little as 50 μg.

Fig.2b represents a schematic drawing of the pattern shown in fig.2a. To allow estimation of the isoelectric points (pI) of the proteins, pH measurements along the gel were performed. The results have been added to fig.2b. The pI-values for mitochondrial ribosomal proteins were found to range from 4.0 to 9.5, the majority being between pI 7 and 9. Isoelectric focusing experiments performed with another type of ampholines (Servalyt 2-11) revealed that no protein

spots appear above pl 10 [12]. These values differ from those reported for bacterial ribosomal proteins which range from 4.8 to 12, the bulk of protein spots being above pl 10 [13]. The isoelectric points published for eukaryotic 80S ribosomal proteins have been described as being even more basic [14].

Two-dimensional protein patterns from *Xenopus laevis* mitochondrial ribosomes have been described to

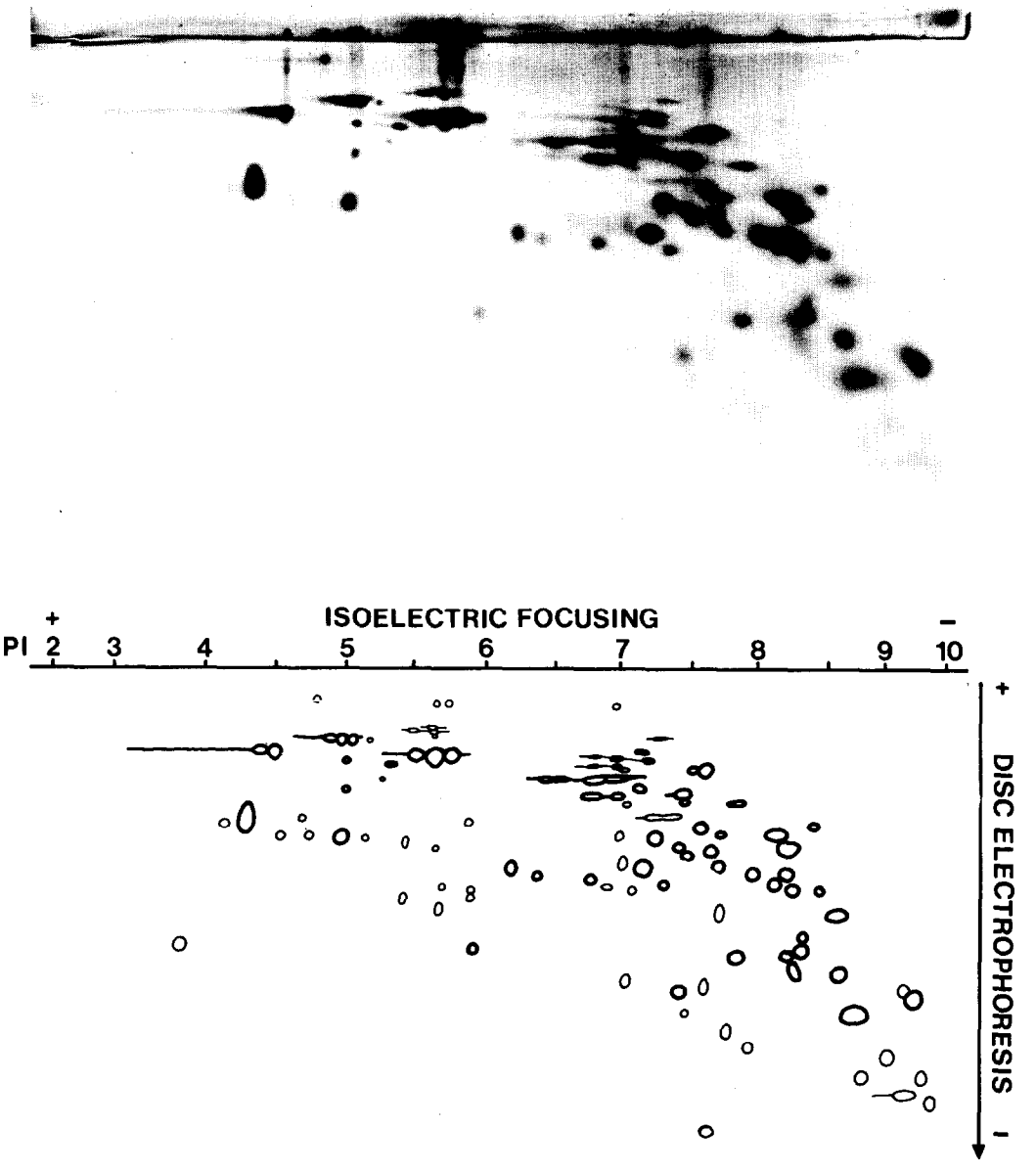

Fig. 2. Two-dimensional protein pattern of 55S mitochondrial rat liver ribosomes. First dimension: Isoelectric focusing, second dimension: slab-gel disc electrophoresis. (a) Photograph of the stained gel, (b) Schematic drawing of (a). The diagrar shows 107 spots, discriminating between 60 dark and 47 weak spots. The isoelectric points of the protein spots are indicated by the scale drawn along the abscissa.

show 77 or 84 ribosomal proteins, depending on evaluation of either the 55S particle or its subunits [15]. Although employing SDS in the second dimension gel – a method expected to yield a distribution of protein spots covering almost the whole slab-gel [16] – this system displays the protein spots in a diagonal arrangement.

Experiments performed with bovine liver mitochondria and using the same technique at least 73 ribosomal protein spots were displayed [17]. These were grouped into 50 major and 23 minor spots. By now the total number of protein spots described by this group has increased to 90 (O'Brien, personal communication).

Calculations performed on the RNA and protein moieties from the buoyant density of the 55S particle suggest that its protein part is of about 1.9×10^6 daltons [5]. In order to estimate the molecular weights of the single proteins an adequate SDS electrophoresis for the second dimension in combination with isoelectric focusing should be employed. Such studies are presently being performed in our laboratory.

Acknowledgements

This work was supported by grants from the Deutsche Forschungsgemeinschaft awarded to Sonderforschungsbereich 29 (Embryonalpharmakologie). We would like to express our appreciation to Mrs Inge Schütte and Miss Gabriele Poutot for their expert technical assistance. We are greatly indebted to Professor H. G. Wittmann for his encouragement during the course of this work and for his fruitful discussions.

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