EUKARYOTIC RIBOSOMAL PROTEINS: THE NUMBER OF PROTEINS IN THE SUBUNITS AND THEIR ISOELECTRIC POINTS

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1. Introduction

Today the number of proteins [1–5] in eukaryotic ribosomal subunits as well as their molecular weights [6] are fairly well determined. Many of these proteins are also isolated and purified [3].

Just like histones, ribosomal proteins are very basic. Both kinds occur naturally in combination with nucleic acids: so it is likely that electrical charges play an important part in the association of these proteins to their respective nucleic acids. As a step towards the understanding of this association it seems therefore necessary to evaluate the electrical charges on the proteins. This may be done globally by measuring their isoelectric points. As the isolation of individual ribosomal proteins is an elaborate and time consuming process, the two-dimensional electrophoresis method of Kaltschmidt [7], which does not require isolation of individual proteins, is used to this end.

2. Methods

2.1. *Preparation of polysomes*

Reticulocytosis is induced in rabbit by daily injection of phenylhydrazine [8]. Reticulocytes are collected by cardiac puncture, washed with 0.9% NaCl and lysed with 5×10^{-3} M $MgCl_2$ [9,10]. The polysomes are obtained by centrifugations at 78 000 *g* for 90 min over a 36% saccharose cushion [11].

2.2. *Preparation of ribosomal subunits*

1000 $O.D._{260}$ units of polysomes are resuspended in buffer and incubated [12] for an hour at 37°C with homologous pH 5 enzyme, to obtain the ribosomal subunits. The subunits are layered on a 7.5–42% hyperbolic sucrose gradient containing 0.3 M KCl, 0.003 M magnesium acetate, 0.02 M Tris–HCl pH 7.5, and 0.010 M β-mercaptoethanol. The gradient is produced by flowing a 45% sucrose solution through a 125 ml mixing chamber containing initially a 7.5% sucrose solution. The gradient is centrifuged for 10 hr at 45 000 rpm in a Ti14 zonal Spinco rotor at 4°C. It is eluted through a 1 mm optical path cell and the absorbance at 252 nm is continuously recorded. Fractions corresponding to the 40 S and 60 S peaks are collected and centrifuged for 17 hr at 55 000 rpm in a Ti 60 Spinco rotor.

2.3. *Preparation of ribosomal proteins*

Proteins are extracted from the subunits by 2 M LiCl, dialyzed against 0.01 M HCl, lyophilized [13] and stored at −20°C.

2.4. *Electrophoresis*

Two-dimensional polyacrylamide electrophoresis in deionized urea [2] is performed according to Kaltschmidt [7] with certain minor modifications. The first dimension is run at 250 V (steady voltage) for 17 hr at 4°C with 1 liter circulating buffer.

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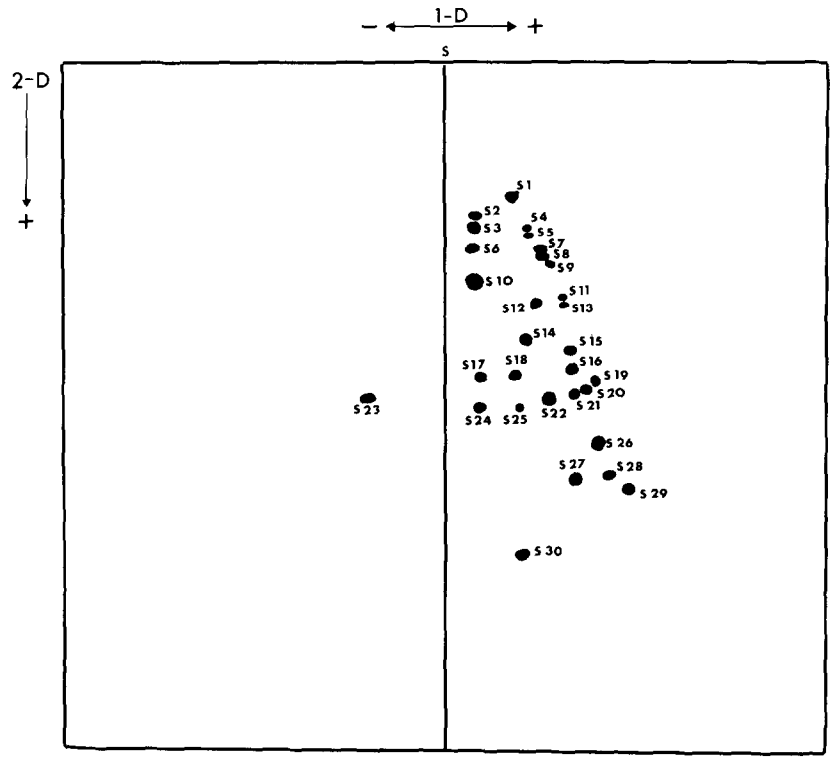

Fig. 1. Pattern of the proteins from the small subunit at pH 8.7 and 8% acrylamide concentration. For other experimental conditions see Methods.

Throughout the experiments internally ground glass tubes with a length of 220 mm are used to prevent the gel from slipping out. This slipping occurs at a pH greater then 11. The gel length is kept at 180 mm. The glass tubes are broken in a wrench to get the gels out before running the second dimension in the usual conditions [14]. The sample gel contains 0.3–2 mg of proteins. The acrylamide concentration ranges from 4% to 12% and the pH of the buffer from 4.9 to 12.3. The second dimension in SDS is run according to Martini and Gould [1].

3. Results

3.1. *Proteins of the small subunit*

Fig. 1 schematically shows the pattern of the 30 proteins from the small subunit. Only spots that can be reproduced from gel to gel are taken into account. Proteins S19, S20 and S21 can be best separated with 4% acrylamide gel. Proteins S4 and S5, S7, S8 and S9, S11 and S13 are separated only when a small amount (0.3 mg) of proteins is electrophoresed. With a larger amount (1 mg) of proteins, nine additional spots are visible; however, they are not reproducible and are not included in the total number. Table 1 gives the isoelectric points of the proteins as determined from the variation of pH and of acrylamide concentration (according to Kaltschmidt's method [7]). No proteins have a pH lower than 7, although S23 moves anionically at pH 8.7. Seven proteins have a pH_i lower than 10 and 23 over 10.

3.2. *Proteins of the large subunit*

The 46 proteins of the large subunit are portrayed schematically in fig. 2. Proteins L3 and L4, L5 and L6 are best separated with 4% acrylamide gel. Proteins L14, L19, L20, L21 are seen well only with a large amount (2 mg) of proteins. With that amount of proteins other spots also become apparent but they

Table 1
pH_i of proteins from the small subunit

no.	pH_i	no.	pH_i	no.	pH_i
1	> 11	11	> 12	21	> 11
2	9.6	12	11.2	22	11.0
3	9.4	13	> 12	23	< 7.6
4	10.7	14	10.5	24	9.3
5	10.8	15	12.0	25	10.3
6	9.3	16	11.3	26	> 11
7	11.6	17	9.3	27	10.7
8	11.7	18	10.5	28	ND*
9	11.8	19	> 11	29	> 12
10	9.3	20	> 11	30	10.3

* ND = not determined.

are not counted as they are not reproducible.

Table 2 gives the isoelectric points of the proteins. At pH 8.7 four proteins (L1, L2, L8, L25) move anionically, although none have a pHi lower than 7. Twenty six proteins have their pH_i over 10.

Table 2
pH_i of proteins from the large subunit

no.	pH_i	no.	pH_i	no.	pH_i
1	7.2	16	11.5	31	11.2
2	7.7	17	9.9	32	11.9
3	10.8	18	9.6	33	11.5
4	10.5	19	10.8	34	9.6
5	9.6	20	ND*	35	11.2
6	9.6	21	10.8	36	ND*
7	11.8	22	ND*	37	ND*
8	7.9	23	11.7	38	11.6
9	11.8	24	> 12	39	> 12
10	> 12	25	8.3	40	> 12
11	> 12	26	11.5	41	11.5
12	9.0	27	11.0	42	ND*
13	> 12	28	12.3	43	ND*
14	11.2	29	9.6	44	10.5
15	ND*	30	10.5	45	ND*
				46	ND*

* ND = not determined.

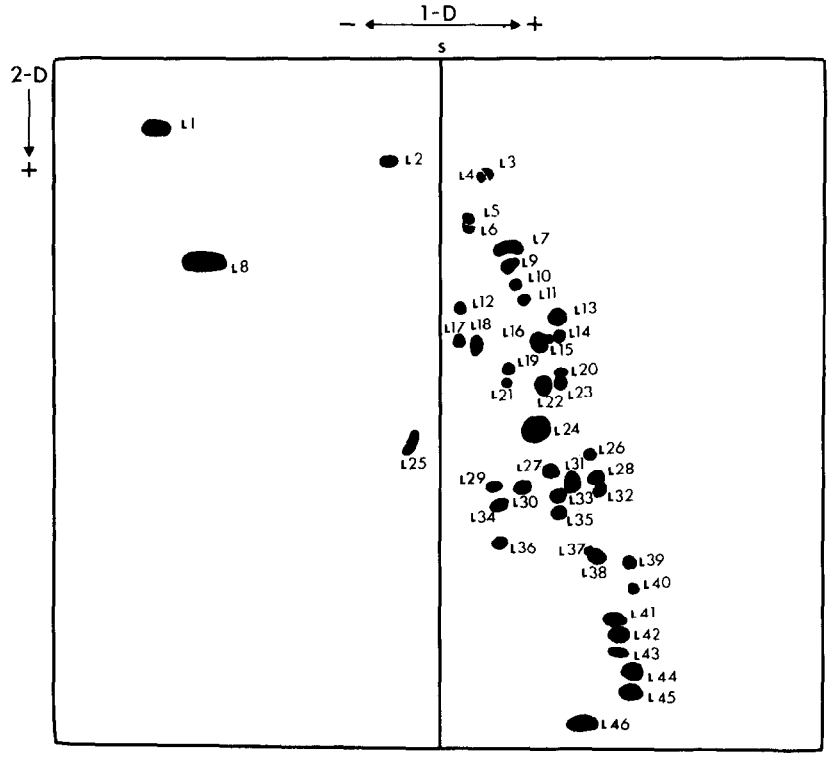

Fig. 2. Pattern of the proteins from the large subunit. Same experimental conditions as in fig. 1.

4. Discussion

4.1. *Number of proteins in the subunits*

L1 and L2 are observed sometimes with the small subunit but most of the time with the large subunit. In any case, they are never found in both preparations at the same time. So we included them among the proteins of the large subunit. Chatterjee et al. [4] estimated the molecular weights of these two proteins to be 60 000, out of the attributed range of the molecular weights of the ribosomal proteins. In running the second dimension in SDS we determined the molecular weights of these proteins to be 37 000 and 32 000 respectively which are well within the range of the molecular weights of the proteins. So we count them as ribosomal proteins.

At pH 8.7 we also find five anionically moving proteins, L1, L2, L8, L25 and S23, although for some of them, the locations on the subunits and on the electrophoretograms differ from [4]. Other discrepancies may also be observed which may be partially explained by the different experimental conditions as well as by a possible different distribution of proteins between the subunits during isolation.

On the whole there is an absolute agreement on the location of 18 spots for the small subunit and 32 spots for the large subunit.

By shortening the electrophoresis time down to half the usual time in both directions, no more protein can be detected. Furthermore by running simultaneously the proteins from both subunits we find only 75 spots (one pair of proteins, S10 and L12 are overlapping). Therefore under our conditions, there are 30 proteins in the small subunit and 46 in the large one.

Table 3 shows the number of proteins in eukaryotic ribosomal subunits reported by various laboratories. The results are quite close to each other; however, some uncertainties about the location of several proteins on the subunits and on the electrophoretograms remain to be solved.

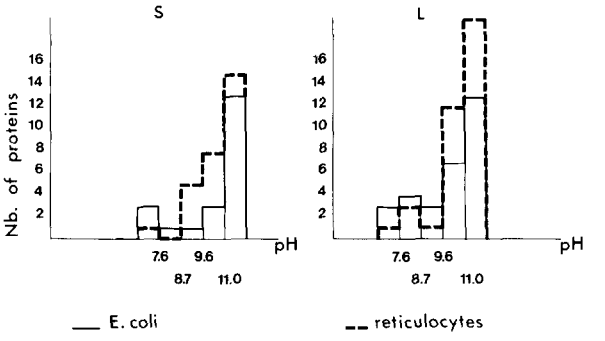

Fig. 3. Comparison of pH_i of ribosomal proteins from *E. coli* and rabbit reticulocytes. The data for *E. coli* come from [7].

4.2. *Isoelectric points*

In eukaryotes, ribosomal proteins are less easily dissociated from their RNA by monocationic salts than in prokaryotes. This fact may be correlated with the finding that there are more basic proteins in eukaryotes as shown by the graphs in fig. 3. The shapes of the graphs look similar for *E. coli* and the rabbit. The same similarity is also found for the molecular weights of the proteins (F. Creusot, personal communication). This suggests that ribosomes from both organisms are built up along the same principles and that most of their proteins have homologous functions. This homology has been shown for two proteins [15].

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Table 3
Number of proteins in ribosomal subunits

References	[1]	[2]	[3]	[4]	[5]	[6]	This article
Small subunit	26	30	31	33	28	32	30
Large subunit	37	39	39	40	36	39	46

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