STUDIES ON PROTEINS OF ANIMAL RIBOSOMES. XIII. ENUMERATION OF RIBOSOMAL PROTEINS OF RAT LIVER

H.WELFLE, J.STAHL and H.BIELKA

Central Institute of Molecular Biology of the German Academy of Sciences of Berlin, Department of Cell Physiology, 1115 Berlin-Buch, Germany

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1. Introduction

Recently we published results of two-dimensional (2D) polyacrylamide gel electrophoresis of ribosomal proteins of rat liver [1]. We described 34 protein spots specific for the large subunit, 27 for the small one and 15 spots with identical electrophoretic mobility in the proteins of both subunits.

In the meantime other papers about 2D separation of proteins of eukaryotic ribosomes were published. Using sodium dodecylsulfate in the second dimension, Martini and Gould [2] and Hultin and Sjöquist [3] separated ribosomal proteins of rabbit reticulocytes and rat liver, respectively. Huynh-van Tan et al. [4] analyzed rabbit reticulocyte, rabbit liver and rat liver ribosomal proteins. Very recently, Sherton and Wool [5] have determined a number of 68 to 72 proteins in rat liver ribosomes, 30 proteins were found in the small and 39 in the large subunit.

In this paper we present new results about the protein composition of rat liver ribosomes and their subunits on the basis of modified and improved techniques for subunit isolation, protein extraction and electrophoretic separation. The protein mixture from whole ribosomes contained 70 cationically moving proteins. 31 were found to be constituents of the small subunit and 39 of the large one. Besides, two anionically moving proteins of low intensity can be demonstrated in the total ribosomal protein.

2. Material and methods

Ribosomes of rat liver were prepared from post-mitochondrial supernatant of homogenates in 250 mM sucrose, 50 mM Tris-HCl, pH 7.8, 5 mM $MgCl_2$ and 5 mM β-mercaptoethanol by addition of Triton X-100 (2.2%) and $MgCl_2$ (50 mM), essentially following the method of Leitin and Lerman [6]. Ribosomes were sedimented for 50 min at 17 000 rpm in rotor 19 of a Spinco L2 ultracentrifuge, washed twice with a 10 mM $MgCl_2$-solution, containing 5 mM β-mercaptoethanol, resuspended in TKM-buffer (5 mM Tris-HCl, pH 7.8, 50 mM KCl, 1.5 mM $MgCl_2$, 5 mM β-mercaptoethanol) and then intensively dialyzed against the same buffer.

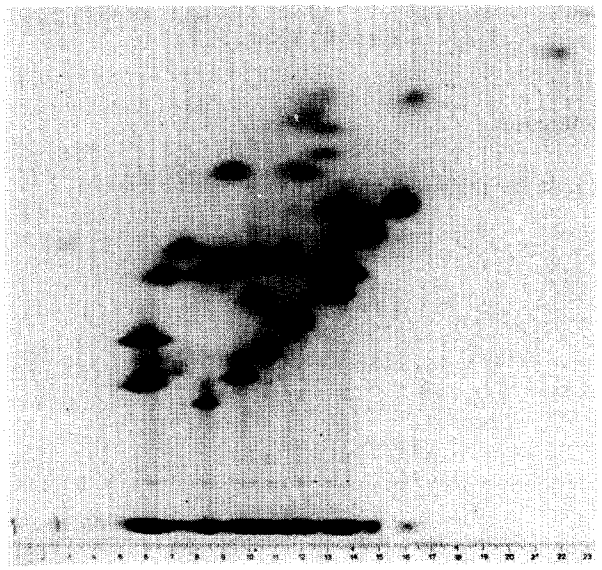

Fig. 1. 2D-pattern of proteins of the small ribosomal subunit. The starting point is on the bottom left. The proteins migrate in the first dimension from the left (+) to the right (−) and in the second dimension from the bottom (+) to the top (−).

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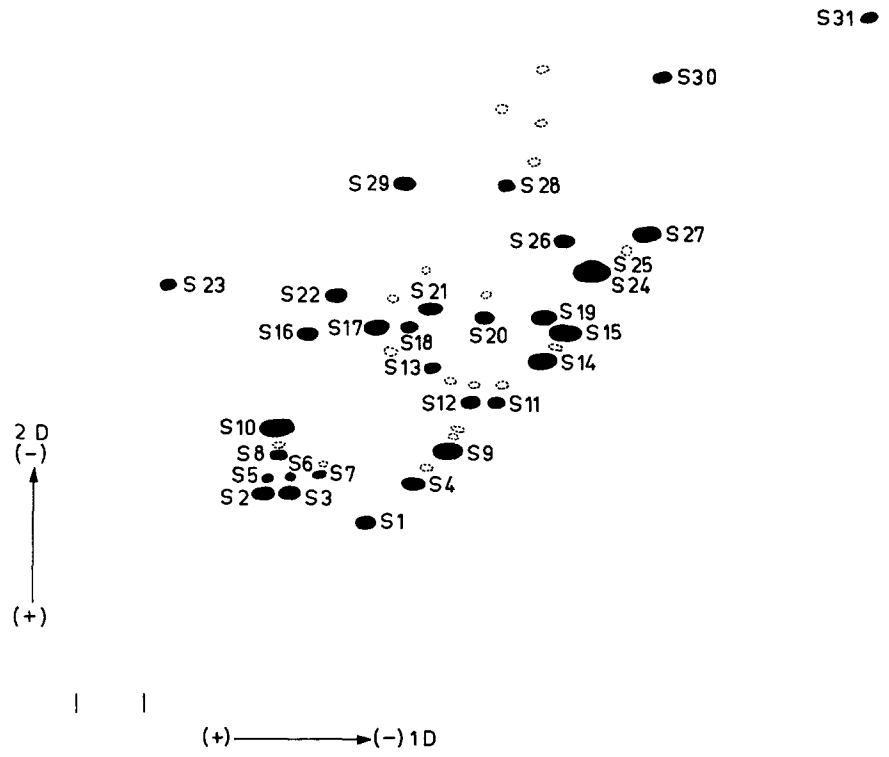

Fig. 2. Scheme of the protein pattern of the small ribosomal subunit. • Main spots; ○ derivation products.

Ribosomal subunits were prepared by treating ribosomes (10 mg/ml) with 30 μg puromycin/ml for 20 min at 0° and 10 min at 37° in a buffer with high KCl-concentration (4.3 mM Tris-HCl, pH 7.8, 550 mM KCl, 1.3 mM $MgCl_2$, 10 mM β-mercaptoethanol), essentially following the method of Blobel and Sabatini [7]. Separation of the subunits was performed in a B XV zonal rotor of a Spinco L2-65B ultracentrifuge on a 800 ml gradient of 7.4% to 38% (w/v) sucrose in 5 mM Tris-HCl, pH 7.7, 500 mM KCl, 5 mM $MgCl_2$, 10 mM β-mercaptoethanol by centrifugration for 3 hr at 35 000 rpm and 20°. The rotor was filled from the outside with 700 ml overlay, 150 ml sample solution and then with the gradient formed according to Eikenberry et al. [8] with a Beckman gradiant pump. The separated small and large subunits were sedimented in rotor 42 for 16 hr at 35 000 rpm. The large subunit fraction was purified by repeating the puromycin treatment and zonal centrifugation.

For preparation of ribosomal proteins, the ribosomes or ribosomal subunits were suspended in TKM-buffer and treated with HCl (0.25 N final conc.) for 30 min at 0°. Aggregates were removed by centrifugation for 10 min at 25 000 *g* and then the protein was precipitated from the supernatant with 5 vol acetone for 4 hr at −20°. The protein precipitate was sedimented for 5 min at 3 500 *g*, washed twice with ice-cold 96% ethanol and dried in vacuum at 0°.

Two-dimensional polyacrylamide gel electrophoresis was performed as described earlier [1], with the exception that a 2-fold ionic strength (0.14 M Tris, 3.9 mM EDTA, 0.14 M boric acid) was used in the gels of the first dimension. The length of the gels was 18 cm and the running time at 130 V was 21 hr. Furthermore, we used spacer gels of 1.5 cm length composed of 3% (w/v) acrylamide, 6.7% *N,N'*-methylene-bis-acrylamide, 8 M urea, 0.4 mM EDTA, 52.5 mM boric acid, 0.95 mM *N,N,N',N'*-tetramethyl-ethylenediamine and 0.25 mg riboflavin/100 ml at a pH value of 6.7. For electrophoresis the proteins were dissolved in spacer gel solution without acrylamide and riboflavin.

3. Results and discussion

The protein mixture of the small subunit can be separated into 31 cationically moving proteins (fig.1), designated with the prefix S in fig.2. Besides, some satellite spots were found, marked in fig.2 by open circles. These satellite spots are practically absent in most preparations, but sometimes they appear with relatively high intensities. In such cases their intensities show reversed correlations to the intensities of the main spots, from which they obviously originate. Therefore they are classified as derivation products. This, in particular concerns the spots above S4, S9, S14 and S29, the spot between S21 and S22 and the spot below S27. Separation of the proteins S24 and S25 is very difficult and, only in few experiments, there is clear evidence for two spots.

The protein mixture of the large subunit can be separated into 39 cationically moving proteins (fig.3), marked with the prefix L in fig.4. Also here

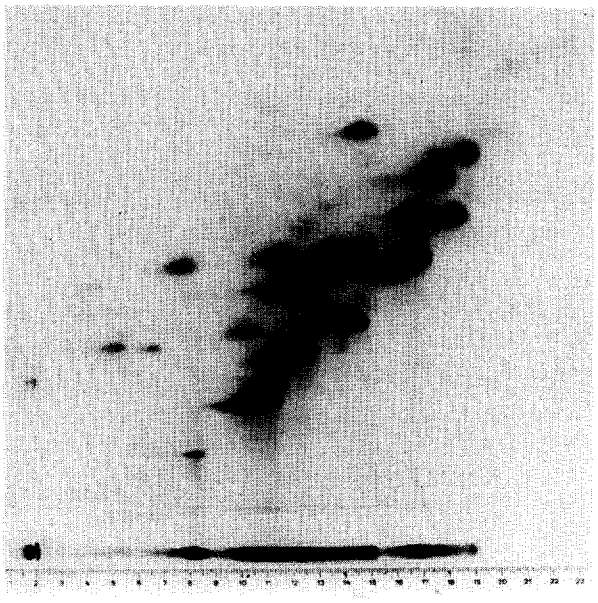

Fig. 3. 2D-pattern of proteins of the large ribosomal subunit.

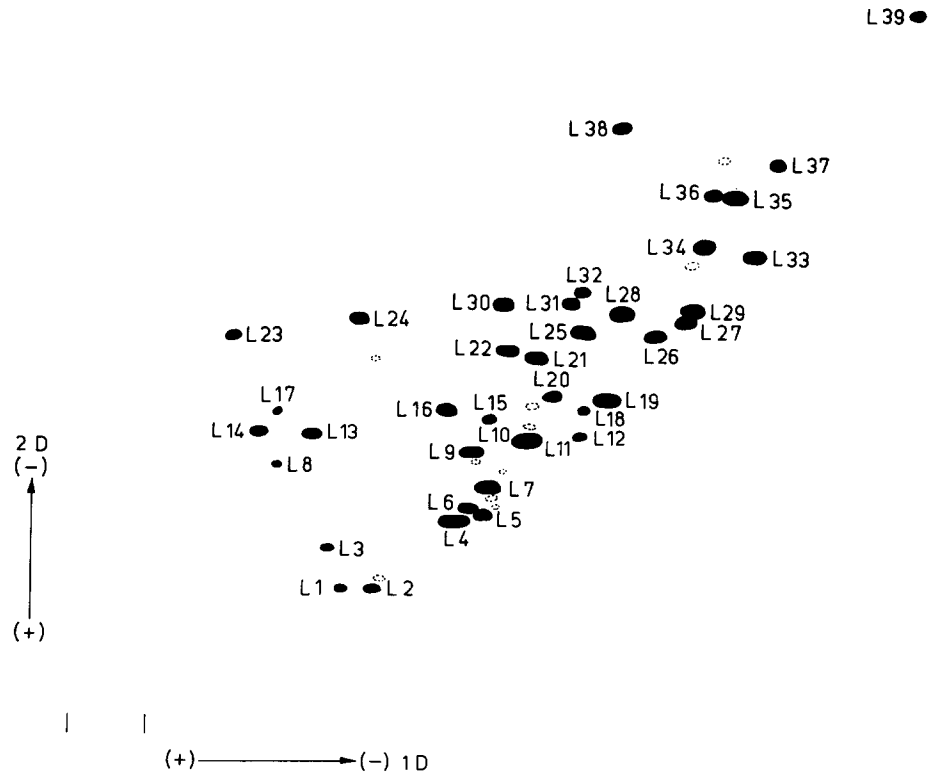

Fig. 4. Scheme of the protein pattern of the large ribosomal subunit. • Main spots; ◌ derivation products.

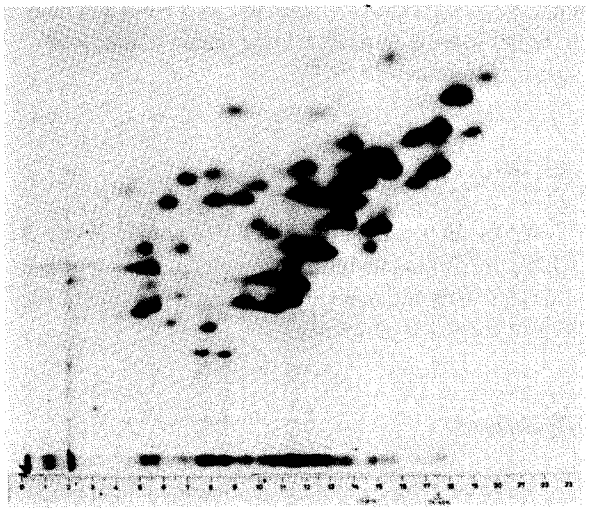

Fig. 5. 2D-pattern of total ribosomal protein.

some derivation products can be demonstrated. The components L10 and L11 appear in most cases in form of one spot only.

Considerable differences in the intensities of the Amidoblack staining of the various spots might be due either to differences in the number of protein copies per ribosome, or to a loss of some material during the preparation steps. The repeated treatment of the large subunit with high KCl-concentrations at relatively low $MgCl_2$ concentrations seems to split off a part of some proteins. This becomes evident from reduced intensities of the spots L5, L8, L12, L13, L14, L17, L23 and L39 in the protein pattern from purified large subunits in comparison to protein pattern from the unpurified large subunit and from whole ribosomes.

Analyzing the pattern of total ribosomal protein, altogether 68 main spots can be demonstrated (figs.5 and 6). Because two spots, containing the proteins

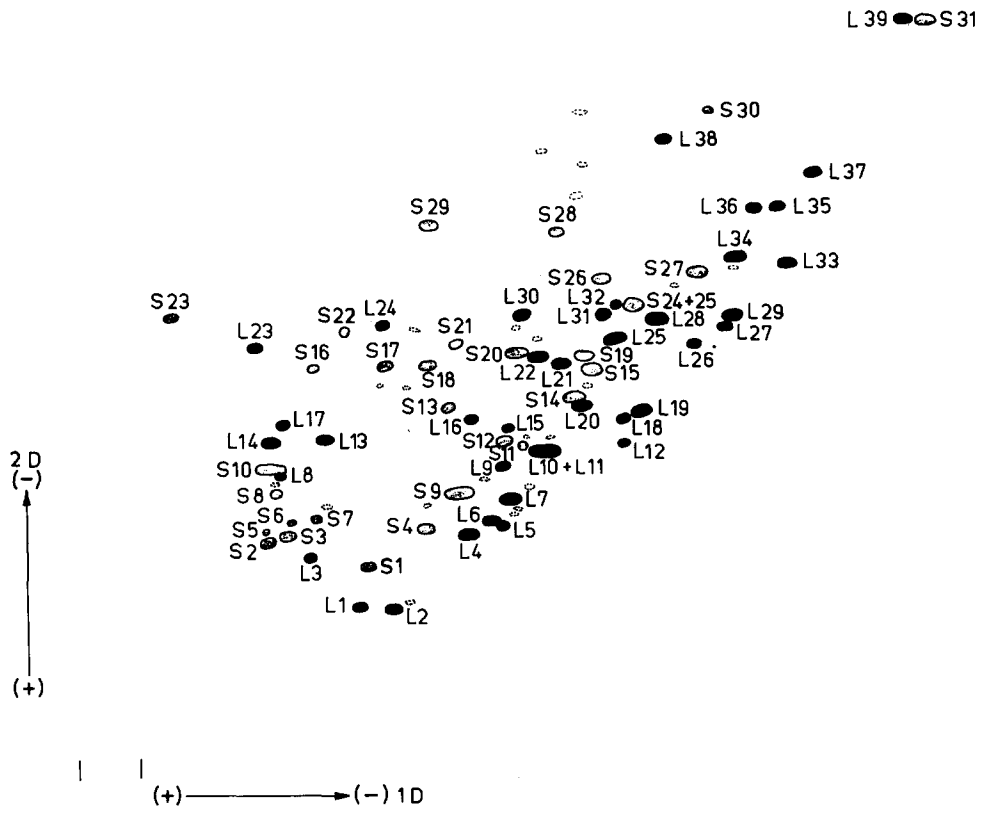

Fig. 6. Scheme of the protein pattern of total ribosomal protein. ○ Proteins of the small subunit; ● proteins of the large subunit; ◌ derivation products.

L10 and L11 as well as S24 and S25, are not separated in the pattern of total ribosomal protein, these 68 spots represent 70 cationically moving proteins.

There are also some difficulties with regard to the disbandment of the very closely neighboured spots S10 and L8, S11 and L10 and S31 and L39, but nevertheless, they were found in different positions in the 2D-pattern. All other spots found in the protein pattern of isolated subunits can be identified quite clearly in the pattern of total ribosomal protein at unique positions. There are no proteins of identical electrophoretic mobility in the small and in the large subunit. The earlier finding [1] of proteins with identical mobilities in both subunits was obviously due to cross contaminations of the subunit preparations.

In addition to the cationically moving proteins, two very weak spots were found, when total ribosomal protein was analyzed for anionically moving proteins. When proteins from the subunits were checked, these two proteins (L40 and L41) were found only in few cases in the protein preparation of the large subunit.

Altogether, we found 72 proteins in rat liver ribosomes, from which 70 were moving cationically and two anionically. This result is in essential agreement with the results of Sherton and Wool [5], but in detail some questions remain open, which need further investigation.

Acknowledgements

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References

[1] H.Welfle, J.Stahl and H.Bielka, Biochim.Biophys.Acta 243 (1971) 416.
[2] O.H.W.Martini and H.J.Gould, J.Mol.Biol.62 (1971) 403.
[3] T.Hultin and A.Sjöquist, Analyt.Biochemistry 46 (1972) 342.
[4] Huynh-van Tan, J.Delaunay and G.Schapira, FEBS Letters 17 (1971) 163.
[5] C.C.Sherton and I.G.Wool, personal communication.
[6] V.L.Leitin and M.I.Lerman, Biokhimiya 34 (1969) 839.
[7] G.Blobel and D.Sabatini, Proc.Natl.Acad.Sci.U.S. 68 (1971) 390.
[8] E.F.Eikenberry, T.A.Bickle, R.R.Traut and C.A.Price, European.J.Biochemistry 12 (1970) 113.