COMPARATIVE STUDIES ON RIBOSOMAL PROTEINS OF *ESCHERICHIA COLI* 113-3 AND STRAINS B, C AND K_{12}

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1. Introduction

The incorporation of a nucleotide fragment into the corrin ring in vitamin B_{12} biosynthesis involves the participation of ribosomes.

Walerych et al. [1] came to this conclusion, when using in their investigations on the vitamin B_{12} biosynthesis a model system containing as one of non-alternative factors, ribosomes or one of ribosomal proteins. Pezacka-Fojudzka and Walerych [11] isolated from *E. coli* 113-3, a strain incapable of synthesising the vitamin by itself and not being an auxotroph in regard to it. The observed phenomenon prompted the authors to detailed studies on the ribosomal proteins isolated from this mutant.

The development of two-dimensional polyacrylamide gel electrophoresis procedure [2,3] of separation of the extraordinarily complex mixture of ribosomal proteins has stimulated detailed studies on the structure and function of *E. coli* ribosomes [4]. Kaltschmidt and Wittmann [3] described the results of this procedure for the separation of proteins derived from 30 S and 50 S subunits, as well as 70 S ribosomes of *E. coli*. They found that the 30 S subunits contain 21 proteins, at the 50 S subunit as many as 34 proteins. A numbering system was proposed based on the position of protein spots on the 2D-electropherogram.

Employing this method, Kaltschmidt et al. [5] performed an analysis of the ribosomal proteins from *E. coli* strains B, C, K_{12} and MRE 600 and showed different physico-chemical properties of proteins S5 and S7 derived from those four strains. Up till now, no map has been made of the ribosomal proteins derived from the *E. coli* 113-3 mutant, of interest because of their functions in vitamin B_{12} synthesis.

2. Materials and methods

E. coli 113-3 was grown in a vitamin-free medium in contrast to the strains *E. coli* B, C and K_{12} grown in normal rich medium. The cells were harvested at 2/3 log phase. The 70 S ribosomes were obtained by differential centrifugation and washed with 0.5 M NH_4Cl and 2 mM Mg^{2+} [6]. The subunits were separated by sucrose gradient (6–30% sucrose with 2 mM spermidine) centrifugation in a Beckmann Ti 14 zonal rotor [7].

Proteins were extracted from ribosomes and subunits by treatment with three methods.

(A) With 67% acetic acid in the presence of 30 mM Mg^{2+} and 10 mM mercaptoethanol [8].

(B) With 0.25 N (final concn.) HCl in the presence of 50 mM $MgCl_2$ and 10 mM mercaptoethanol [9].

(C) With 5% trichloroacetic acid in the presence of 10 mM mercaptoethanol and with simultaneous thermal disintegration of possible rRNA–protein complexes.

After this procedure the proteins were precipitated with acetone at $-20^{\circ}C$ for 4 h and after centrifugation washed with ice-cold 96% ethanol and dried in a vacuum. For electrophoresis the proteins were dissolved in spacer gel solution without acrylamide and riboflavin [9]. The acrylamide concentration in the first dimension was 5% in Tris–EDTA–boric buffer, pH 8.3. The electrophoretic conditions in the second dimension were always 15% acrylamide gel in acetate buffer, pH 3.2.

3. Results and discussion

Two-dimensional electrophoresis was performed. The ribosomal proteins were isolated by three methods as, using 0.25 N HCl, for example, in separating the proteins from rRNA, protein L 29 remained bound. While using 5% trichloroacetic acid for this purpose, the aggregation of L 18 and L 19 was observed. On the other hand, the use of 67% acetic acid did not allow L 31 and L 34 to be identified.

The electropherograms were successively made of 30 S, 50 S and 70 S ribosomal proteins from *E. coli* 113-3 and of 70 S ribosomes from *E. coli* B, C and K_{12}. The maps of the ribosomal proteins from wild strains were made for identification and comparison, as our conditions of 2D electrophoresis are somewhat different from those used by Kaltschmidt and Wittmann [3]. The map of ribosomal proteins published by those authors and their numbering system were the form adopted by us in classifying the proteins from *E. coli* 113-3 ribosomes.

3.1. *30 S subunit of ribosomes from* E. coli *113-3*

The electropherogram reveals the presence of 21 proteins, three of which have acidic character. One of them, S 1, undergoes a considerable degradation, no matter what method of protein isolation from the 30 S subunit is employed. This phenomenon was not observed in protein S 1 from wild strains of *E. coli*, which makes it seem to be a feature characteristic of the protein isolated from the mutant ribosomes (fig.1).

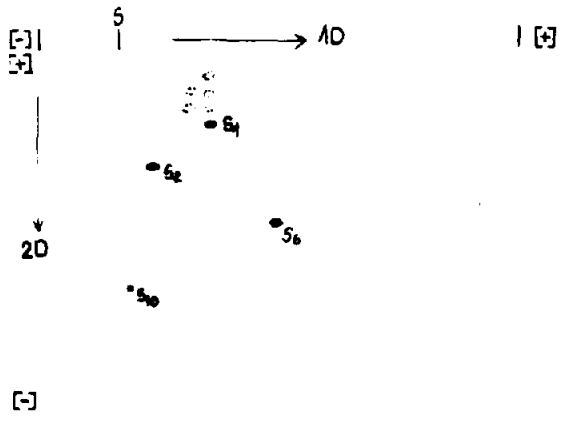

Fig.1. The protein pattern of the small ribosomal subunit. (●) Main spots (⁘) Derivative products.

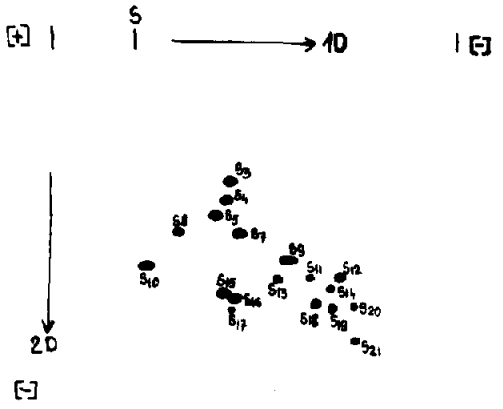

Fig.2. The protein pattern of the small ribosomal subunit.

One of the proteins on the electropherograms discussed, namely protein S 10, shows an electroneutral character (figs.1,2). This fact is true in the picture of the map of ribosomal proteins obtained by us from the wild strains of *E. coli*.

The other of the 21 proteins have their isoelectric points at pH > 8.3 (fig.2), and proteins S 5 and S 7 take positions on the electropherogram characteristic of the proteins separated from strain B of *E. coli* [5].

3.2. *50 S subunit of ribosomes from* E. coli *113-3*

The electropherograms of the proteins of the large subunit of ribosomes reveal the presence of 34 proteins. Four of them have their isoelectric points at pH < 8.3. They are L 8, L 9, L 7 and L 12 (fig.3). Under the electrophoretic conditions used, proteins L 8 and L 9

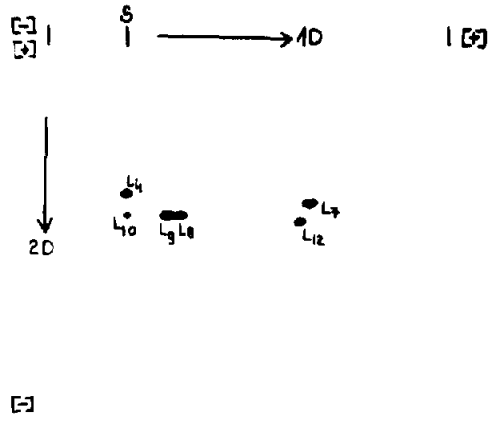

Fig.3. The protein pattern of the large ribosomal subunit.

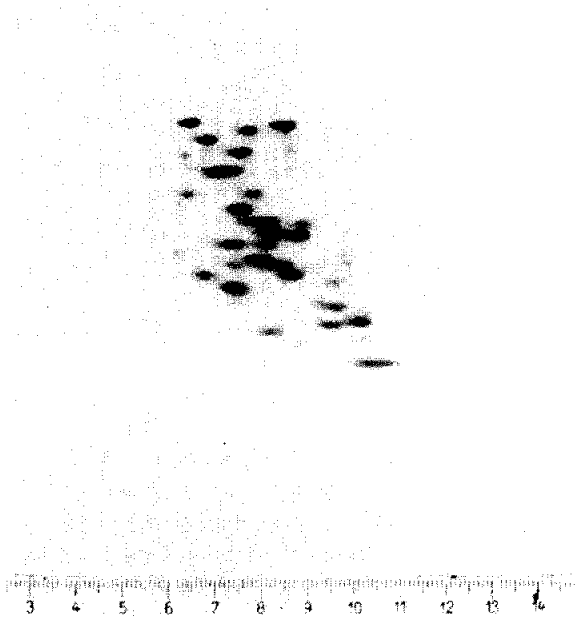

Fig.4. The protein pattern of the large ribosomal subunit.

separate as two clearly discernible spots both in the material derived from wild strains of bacteria as well from the mutant strain. According to Kaltschmidt et al. [3], these proteins become separated in the course of the electrophoresis at pH 9.6.

Under the electrophoretic conditions used, proteins L 4 and L 10 show an electroneutral character (figs.3 and 4). Protein L 21, which according to Kaltschmidt and Wittmann [3] is slightly acidic, in the material from K_{12} and B strains in our electrophoretic conditions was electroneutral, while in that from the 113-3 strain was slightly basic (fig.4).

On the electropherogram, protein L 11 from *E. coli* 113-3 ribosomes takes up a position characteristic of the protein from strains B and C [3,5].

The position of protein L 34 is easy to identify on the electropherogram of the material from strain 113-3 (fig.5), in contrast to the analogous system from strains B, C and K_{12} [3]. Analysing the schemes of electropherograms of the proteins separated from 70 S ribosomes we notice a high degree of degradation of protein S 1, a slightly basic character of protein L 21 and the possibility of identifying protein L 34 with no spot in the position of L 29 (fig.5).

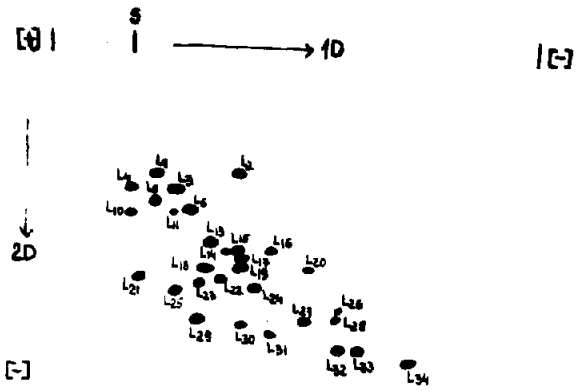

Fig.5. 2D-pattern of proteins of total ribosomal subunit. The starting point is on the top left. The proteins migrate in the first dimension from the left (+) to the right (−), and the second dimension from the top (+) to the bottom (−).

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