

The L7/L12 proteins change their conformation upon interaction of EF-G with ribosomes

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The different functional complexes of ribosomes with elongation factor F (EF-G) were studied by digestion experiments with trypsin. It was found that upon interaction of EF-G with ribosomes the L7/L12 proteins are sensitive to trypsin and are trypsin resistant after dissociation of EF-G from ribosomes. The significance of conformational alterations in the L7/L12 and also in the other proteins in the translation process is discussed.

L7/L12 ribosomal protein Elongation factor G Ribosome trypsinolysis Translation

1. INTRODUCTION

The acidic proteins L7/L12 of *Escherichia coli* ribosomes participate in the functioning of polypeptide chain elongation factors and are localized in the neighbourhood of the binding sites of these factors (see [1–3]), although the functioning and binding of EF-G does not strictly depend on these proteins and can be realized without them [4,5].

A proton magnetic resonance (PMR) study of the L7/L12 proteins in situ has shown the great independent mobility of their C-terminal globular parts [6]. PMR spectra analysis of the complex consisting of EF-G, ribosome and noncleavable analog of GTP has indicated that a considerable immobilization of the L7/L12 proteins occur in such a complex [7]. These results can be connected with structural changes in the proteins, and therefore we carried out trypsin digestion experiments of the ribosome·EF-G complexes with the uncleavable analog of GTP—guanylylmethylene-diphosphonate (GMPPCP) and with GDP and fusidic acid (FA). It was found that in the 70 S·EF-G·GMPPCP complex (ribosomes in the pre-GTP-hydrolysis state) the L7/L12 proteins are digested, whereas in the 70 S·EF-G·GDP·FA complex (ribosomes in the post-GTP-hydrolysis

state) the L7/L12 proteins are trypsin resistant. These results evidence that the conformation of L7/L12 proteins changes upon interaction of ribosomes with elongation factor G.

2. MATERIALS AND METHODS

70 S ribosomes were obtained as in [8] with a minor modification. Before the sedimentation of ribosomes through a sucrose cushion they were pelleted to tube bottom by high speed centrifugation. The association of subunits in this preparation was about 90% as checked by analytical centrifugation. Complex formation of ribosomes with G-factor was checked as in [9]. The following solutions were used in the experiment: 9–12 mg/ml of ribosomes (in 20 mM Tris·HCl (pH 7.5–7.8), 25–50 mM ammonium chloride, 10 mM magnesium acetate); EF-G (11 mg/ml) in a ribosome buffer; trypsin (0.1 mg/ml) in 1 mM HCl; soybean inhibitor (0.2 mg/ml) in water. Ribosomes were mixed with 1.5–2 mol EF-G, 5–10 mol GMPPCP (2 mM of fusidic acid was used with GTP). The mixture was incubated for 15–25 min at 37°C. Trypsin, 0.5 mg per mg of ribosomes, was added to the formed complex and the mixture was incubated for 7–8 min at 37°C. The reaction was terminated with trypsin inhibitor (4 × over

trypsin quantity). Ribosomal proteins were extracted with 66% acetic acid [10], precipitated with 5 vols of acetone and 2-dimensional (2D) electrophoresis was carried out according to system IV in [11].

3. TRYPSINOLYSIS OF RIBOSOMES

3.1. Trypsinolysis of 70 S ribosomes

Fig.1 presents the results of trypsinolysis of 70 S ribosomes. Proteins S6, S3, S19, S21 are digested completely and L27 partially. The L7/L12 proteins are intact (even after 40 min of trypsin treatment). The stability of L7/L12 against trypsin was shown also in the isolated state in the solution ([12] and unpublished). Digestion of S3, S6, S9, S21 and additionally S1, S14, S20 has been reported also in experiments with 30 S subunits [13].

Association of subunits in trypsin-treated ribosomes changed insignificantly (checked by analytical centrifugation). This fact coincides with the results published in [14], but contradicts the

data reported in [15], where there was an insignificant association after treatment of ribosomes with trypsin.

Trypsin-treated ribosomes can bind factor G and [3 H]GMPPCP (checked by filter technique). A same set of proteins were digested in ribosomes with factor G and GDP (electrophoregram not shown).

3.2. Trypsinolysis of the 70 S-EF-G-GMPPCP complex

As known, factor G and the uncleavable analogs of GTP form a strong complex with ribosomes [16]. The results of trypsin treatment of such a complex are presented in fig.2a. In this case the proteins S3, S6, S19, S21 and additionally L7/L12, S15 and S18 are digested (see also table 1). The complex treated with trypsin contains as much [3 H]GMPPCP as the untreated complex (nitrocellulose filter assays). This means that trypsin-treated ribosomes are still bound with factor G.

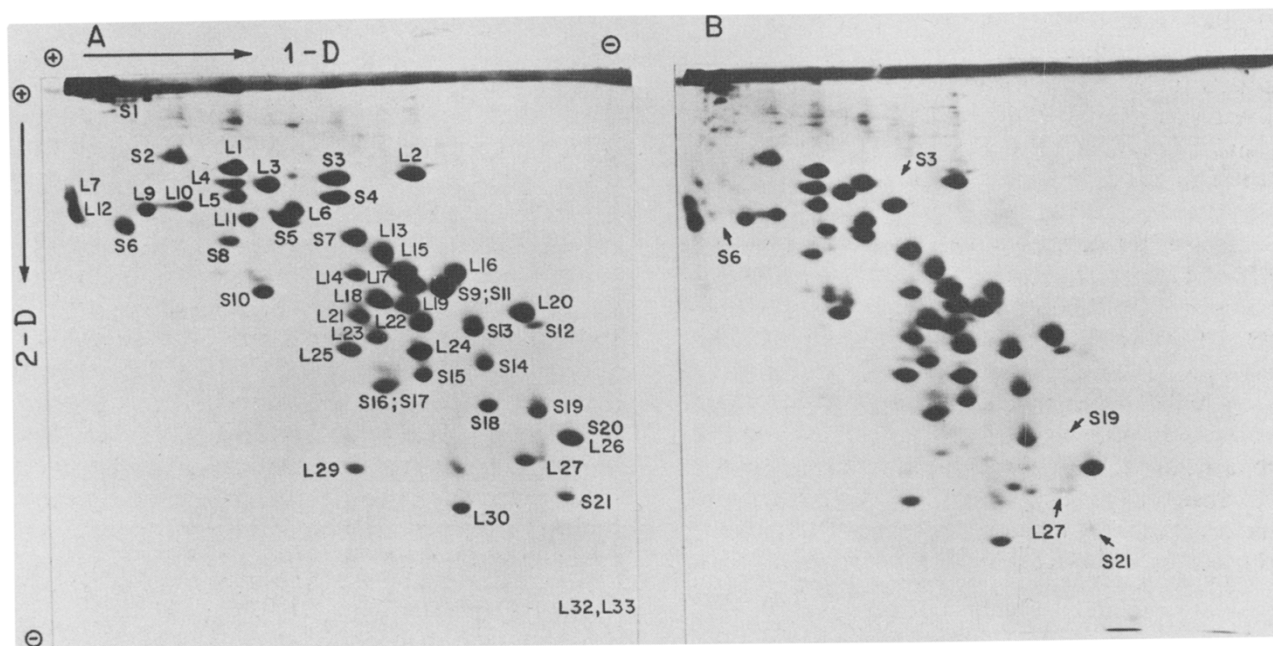


Fig.1. A, 2D-electrophoresis of 70 S ribosomal proteins (control); the amount of proteins loaded corresponds to $\sim 15 A_{260\text{nm}}$ of ribosomes. B, 2D-electrophoresis of 70 S ribosomal proteins after trypsin treatment (7 min); the loaded amount corresponds to $\sim 15 A_{260\text{nm}}$ of ribosomes. Results of trypsin digestion of ribosomes with EF-G and GDP coincide with (A).

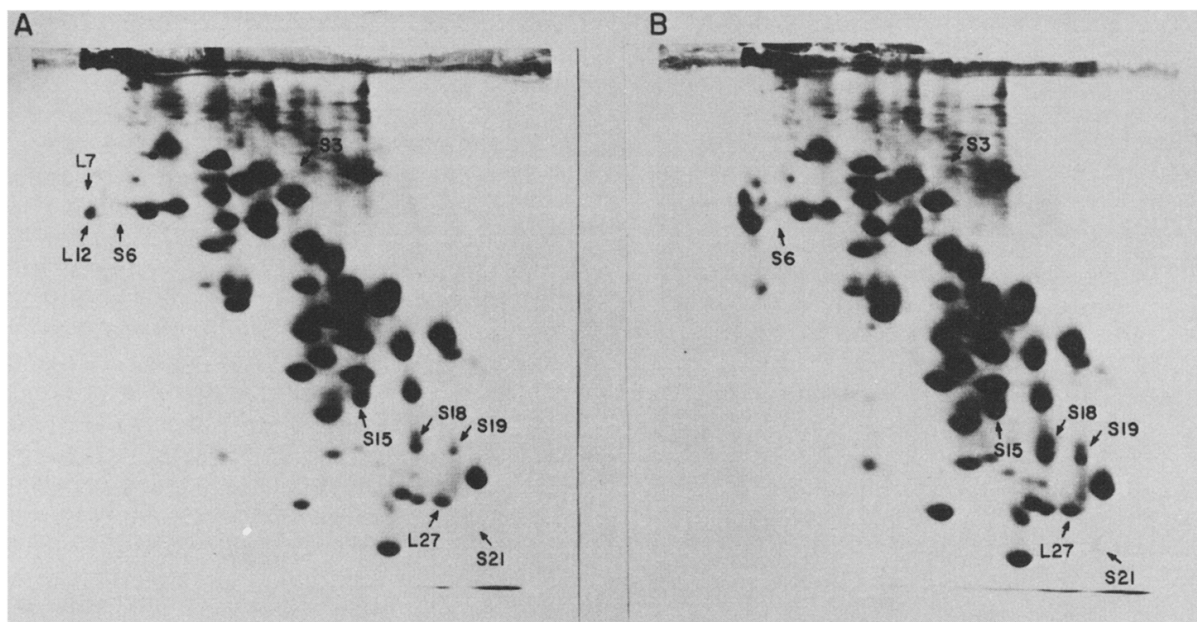


Fig.2. A, 2D-electrophoresis of 70 S ribosomal proteins after tryptic treatment of ribosomes with EF-G and GMPPCP; the loaded amount of proteins corresponds to $\sim 23 A_{260\text{nm}}$ of ribosomes; B, 2D-electrophoresis of 70 S ribosomal proteins after treatment of the complex 70 S · EF-G · GDP · fusidic acid; the loaded amount of proteins corresponds to $\sim 23 A_{260\text{nm}}$ of ribosomes.

Table 1

Proteins digested by trypsin in 70 S ribosomes (electrophoresis results) (for conditions see section 2)

Protein	70 S + trypsin (6 runs)	70 S + EF-G + GDP + trypsin (1 run)	70 S · EF-G · GMPPCP + trypsin (5 runs)	70 S · EF-G · GDP · FA + trypsin (4 runs)
L7	—	—	++++	—
L12	—	—	++++*	—
L27	++	++	+++	+++
S3	++++	++++	++++	++++
S6	++++	++++	++++	++++
S15	—	—	++	+
S18	—	—	++	++
S19	+++	+++	+++	+++
S21	+++	+++	+++	+++

++++, protein is digested completely; +++, a trace of protein can be seen; ++, protein is cleaved by about 50%; +, noticeable cleavage; —, protein is trypsin resistant. Complex formation checked by nitrocellulose technique corresponded to: 70 S · EF-G · [^3H]GMPPCP, 60–75%; 70 S · EF-G · [^{14}C]GDP · FA, 50–65%. *, in 2 of the 5 cases traces of L12 can be seen

3.3. Trypsinolysis of the 70 S · EF-G · GDP · FA complex

Incubation of ribosomes with factor G, GTP and fusidic acid leads to hydrolysis of GTP, but

the G-factor with GDP and fusidic acid remains bound with the ribosomes [17]. The results of trypsin treatment of this complex are shown in fig.2b. In this case, in addition to the proteins in the free

ribosomes, the proteins S15 and S18 are cleaved, while the L7/L12 proteins are not (see also table 1).

4. DISCUSSION

It follows from the experimental data that in the complex of factor G with ribosomes the accessibility to trypsin digestion changes only for the L7/L12, S15 and S18 proteins.

It must be noted that due to the high specificity of trypsin there may be changes in other proteins also, the more so that we have analyzed only the proteins whose spots, at electrophoresis, disappear in the gel completely or to a significant extent. Therefore, the use of other proteases with other specificities would be fruitful.

Several new spots (see figs 1,2), one at S8, three above S10 and two or three to the left of L27 and one large spot to the right of L3 (probably of S3 origin) do not interfere with the interpretation of the electrophoresis results.

The L7/L12 proteins are digested after 7 min at the chosen conditions [such a high rate of cleavage is inherent only to oxidized, monomeric L7 (unpublished)] only in the ribosome with EF-G and GMPPCP complex (the G-factor is bound, the functioning state of ribosomes is that prior to GTP hydrolysis, see table 1, column 4). In the complex with fusidic acid (table 1, column 5) the proteins L7/L12 are trypsin resistant when factor G is bound but GTP has already been cleaved.

It is known that factor G without L7/L12 proteins can function and bind with the ribosomes (or even with 23 S RNA) [2-5] although less effectively than with intact ribosomes. Therefore, the whole set of data permits one to believe that the conformational change of L7/L12 proteins is not the result of the primary interaction of EF-G with these proteins, but is the result of a conjugated alteration of some other ribosomal components (including 23 S RNA [5,18]) after EF-G and GTP binding to ribosomes. GTP hydrolysis normally occurs after such alterations of L7/L12.

After GTP hydrolysis the L7/L12 proteins take the initial conformation or, in any case, some other one and become trypsin resistant. Such a situation is modeled on the 70 S·EF-G·GDP·FA complex, but in this case EF-G remains bound to the ribosome and the S15 and S18 proteins are still

accessible for trypsin (table 1, column 5) because ribosomes do not return completely to the initial state.

The ribosomes return to the initial state only after GTP hydrolysis and dissociation of EF-G with GDP (compare columns 1 and 2, table 1).

The S15 and S18 proteins are localized on the opposite side of the 30 S subunit [19] remote from the binding site of EF-G [3]. Therefore, the apparent accessibility of S15 and S18 proteins for trypsin is impossible to explain by the direct interaction of EF-G with these proteins. But the binding of EF-G with GMPPCP induces translocation [20] and if the hypothesis of mutual rearrangement of the ribosome subunits during translocation is correct [21] then the increased accessibility of the S15 and S18 proteins is not surprising.

The reproducible digestion of S3, S6, S19, S21 and L27 in all cases can be an internal control of trypsinolysis experiments.

In conclusion, it can be said that EF-G interaction with ribosomes leads to conformational changes, not only of neighbouring L7/L12 proteins, which probably participate in GTP hydrolysis, but in other ribosomal sites (S15, S18), and this can indicate conformational changes or mutual rearrangements of ribosomal subunits in the translation process.

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