Elongation factor G protects a nuclease-sensitive site of 23 S RNA within the ribosome

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Elongation factor G is shown to protect the nuclease splitting off of the 3'-terminal 11 S fragment from the 23 S RNA within the ribosomal 50 S subparticle.

1. INTRODUCTION

Studies of the elongation factor G (EF-G) interaction with the ribosome are very important for understanding the mechanism of its functioning. The use of photoaffinity cross-linking revealed the 23 S RNA as the main component of the EF-G-binding center of the ribosome [1,2]. In the following experiments it was shown that EF-G has a specific affinity for isolated 23 S RNA [2]. In addition, the proteins L10/(L7/L12)4 and L11 stimulating the interaction of EF-G with the ribosome [3–9] and located close to the EF-G-binding center [10–14] increase the cross-linking yield of the factor to the isolated 23 S RNA. Hence, an assumption was made that the affinity of EF-G for some exposed site of 23 S RNA is essential for the factor interaction with the ribosome [2].

Here we present data in favour of the above assumption. It has been shown that the formation of a stable complex of EF-G with the ribosomal 50 S subparticle protects one of the most nuclease-sensitive sites of 23 S RNA which is located within the 3'-terminal 18 S fragment.

2. MATERIALS AND METHODS

Subparticles of 50 S were isolated from tight couples of E. coli MRE-600 ribosomes by ultracen-
typhimurium [17] was dialysed against 50 mM Tris–HCl (pH 7.5), 5 mM mercaptoethanol, 10% glycerin and stored in batches at −70°C. The conditions for the formation of the complex of 50 S subparticles with EF-G and for its hydrolysis with RNase I were the following: 0.4 nmol of 50 S subparticles, 50 nmol GTP, 450 nmol fusidic acid (mixture 1, control) or 0.4 nmol of 50 S subparticles and 2 nmol EF-G (mixture 2, control), or mixture 1 plus 2 nmol EF-G (mixture 3, experiment) in 100 μl buffer containing 20 mM MgCl₂, 20 mM NH₄Cl, 15 mM Tris–HCl (pH 7.5), 5 mM mercaptoethanol were incubated for 10 min at 25°C and cooled in ice for 1 h. Cooled RNase I was added to the samples of mixture 1–3 in a ratio of 4–4.5 units enzyme per A₂₆₀ unit of 50 S subparticles [18] [final buffer contained 10 mM MgCl₂, 7 mM NH₄Cl, 30 mM Tris–HCl (pH 7.5), 5 mM mercaptoethanol]. The mixtures obtained were incubated in ice for 1–5 min and hydrolysis was stopped with 0.1 vol. of 10% SDS. After the addition of EDTA to 30 mM the samples were divided in two equal parts: one was directly analysed by SDS–PAGE and the other was analysed after heat denaturation at 78°C for 5 min and rapid cooling.

Electrophoresis was done in 3% polyacrylamide gel containing 0.1% SDS in the gel and in the running buffer [19].

3. RESULTS AND DISCUSSION

As mentioned in section 1, this study is based on the assumption that the interaction of EF-G with the ribosome is governed by the affinity of the factor for some exposed site of 23 S RNA [2]. In accordance with the position of EF-G on the ribosome [11], this site should be located on the 50 S subparticle interface at the base of the L7/L12 stalk. A possible exposure of 23 S RNA in this region was indicated by the data obtained in [18]: mild hydrolysis of the 50 S subparticles with RNase I from S. typhimurium leads to the ‘cutting off’ of the L7/L12 stalk (the subparticle loses a strictly determined set of proteins; i.e., all 4 copies of L7/L12 and its neighbours L10 and L4). In addition, it is shown that the sensitivity to RNase I is a consequence of the presence of protein L7/L12 in the 50 S subparticle [20]: the hydrolysis is significantly inhibited on removal of this protein as in [5] and is restored when the protein is inserted into the deficient subparticle.

The above data suggest that the region of 23 S RNA, highly sensitive within the 50 S subparticle to RNase I and exposed somewhere at the base of the L7/L12 stalk, can be a part of the binding site for EF-G. If this suggestion is valid, it should be expected that the formation of a stable EF-G–50 S subparticle complex would protect the action of RNase.

3.1. Hydrolysis of the 50 S subparticle with RNase

As known, 23 S RNA within the 50 S subparticle of E. coli ribosomes apparently has 3 exposed sites which are most sensitive to the action of different ribonucleases specific to single-stranded regions of the polynucleotide chain (pancreatic RNase [21–29], T1-RNase [30] and endogenous ribosomal RNase from E. coli [26]). One of these sites (site 1, see scheme in fig.1) is positioned at a distance of about 1171–1178 nucleotides from the 5′-end of 23 S RNA [31,32] and its hydrolysis gives two fragments detected by SDS–PAGE.

![Fig.1. Analysis of the products of hydrolysis of the 50 S subparticle with RNase I by SDS–PAGE under non-denaturing conditions (A–D) and after heat denaturation (E–G). A, initial 50 S subparticle; B and E, 50 S subparticle + RNase I; C and F, 50 S subparticle + EF-G + RNase I; D and G, 50 S subparticle + EF-G + GTP + fusidic acid + RNase I. Bottom, scheme showing the location in 23 S RNA of regions sensitive to the action of RNase I within the 50 S subparticle.](image-url)
without denaturation of the RNA secondary structure: the 3'-terminal 18 S and 5'-terminal 13 S fragments [26-28]. Electrophoresis of the hydrolyzate after preliminary denaturation of the RNA (5 min at 75-80°C, 0.2% SDS [24] or 6.6-8.0 M urea [25,30]) reveals another specific cleavage site (site 2 in the scheme) at a distance of about 1000 nucleotides from the 3'-end of 23 S RNA with formation of the 3'-terminal 11 S [28,32] (or 12 S [30,33]) fragment. The third cleavage site (site 3 in the scheme) detected also only in a denatured sample is located somewhere within the 5'-terminal 13 S fragment [24,25].

The data presented in fig.1 show that RNase I from S. typhimurium has an analogous action. Under non-denaturing conditions the hydrolyzate of the 50 S subparticle (column B) gives two bands (1 and 2) corresponding to the 18 S and 13 S fragments. Traces of these fragments are present in the control sample (column A) as a result of the action of endogenous ribosomal RNase from E. coli. The denaturation of the hydrolyzate leads (column E) to a decrease of the intensity of bands 1 and 2 (and of the initial 23 S RNA) and to the appearance of 3 smaller fragments of about 1000, 860 and 730 nucleotides (bands 3, 4 and 5, respectively). In analogy with the hydrolysis of the 50 S subparticles by other ribonucleases (for references see above), bands 3 and 5 originate from the 3'-terminal 18 S fragment and band 4 from the 5'-terminal 13 S fragment of 23 S RNA. The labeling of the 23 S RNA hydrolyzate with [32P]pCp in the presence of T4 RNA ligase confirms such an identification: the radioactive label is found only in bands 1 and 3 corresponding to the 3'-terminal 18 S and 11 S (12 S) fragments.

Thus, the action of RNase I does not differ from the action of other nucleases and is explained by the presence in the 50 S subparticle of 3 exposed sites of the 23 S RNA which are highly sensitive to the action of different nucleases.

3.2. Effect of EF-G on hydrolysis of the 50 S subparticles with RNase I

The results are shown in fig.1. Columns A–D give the electrophoretic analysis of the initial sample and hydrolyzates under non-denaturing conditions and columns E–G show the analysis of hydrolyzates after heat denaturation (see section 2). It is seen that preincubation of the 50 S subpar-

ticles only with EF-G (or only with GTP and fusidic acid, not shown) does not affect the hydrolysis picture (cf. columns C with B, or F with E). A principally different pattern is observed for the complex of the 50 S subparticles with EF-G in the presence of GTP and fusidic acid. It is clearly seen that stabilization of site 2 within the 3'-terminal 18 S fragment takes place: band 1 (the 18 S fragment) in column G is significantly more intense than in the control columns E and F; consequently, bands 3 and 5 (the products of hydrolysis of the 18 S fragment at site 2) are essentially less intense in column G. The sensitivity of the other sites practically does not differ in the experiment and in controls: bands 1 and 2 in columns B–D (hydrolysis of site 1) and bands 2 and 4 in columns E–G (hydrolysis of site 3) have an approximately equal intensity.

The cross-linking of the photoactivated derivative of 3H-labeled EF-G with the arylazide residue on the exposed SH-group to the 50 S subparticle corroborates the location of the EF-G within the 3'-terminal 18 S fragment of 23 S RNA. The result of limited hydrolysis of the 50 S subparticle with the cross-linked factor by pancreatic RNase is given in fig.2. The figure shows that the radioactive label, i.e., the cross-linked 3H-labeled EF-G, is present only in the peaks corresponding to the initial 23 S RNA and the 18 S fragment. The electrophoretic analysis with subsequent fluorography of gels confirmed the presence
of radioactivity in 23 S RNA and the 18 S fragment and its absence in the 13 S fragment.

Thus, it may be concluded that in the stable complex with the 50 S subparticle EF-G contacts (at least, its region including the exposed SH-group) the 3'-terminal 18 S fragment of the 23 S RNA and protects, within this fragment, the nuclease-sensitive single-stranded site at a distance of about 1000 nucleotides from the 3'-terminus of 23 S RNA (site 2 in the scheme, fig.1). In accordance with the position of EF-G on the ribosome [11], this site 2 should be apparently exposed on the interface of the 50 S subparticle at the base of the L7/L12 stalk. The correlation of this result with the data in [18,20] allows us to understand better the mechanism of stimulation by protein L7/L12 of the interaction of EF-G with the ribosome. It is likely that the role of this protein is to expose site 2 in 23 S RNA for the binding of EF-G.

The tertiary structure of 23 S RNA in the region of the EF-G-binding center is of special interest. It is known that proteins L7/L12, L10, L11, L14 and L6 are located in or near this center, can be cross-linked with EF-G and are nearest neighbours (e.g., [10–14, 35–37]). At the same time they bind to remote regions of the 23 S RNA sequence: proteins L7/L12 + L10 and L11 bind to the 5'-terminal 13 S fragment [38,39], protein L14 to the central 8 S fragment [28] and protein L6 to the 3’-terminal 11 S (12 S) fragment [33,40]. Besides, according to our data, EF-G is photoaffinity cross-linked with the 3’-terminal 18 S fragment and protects site 2 within this fragment from the nuclease whereas diepoxybutane treatment cross-links the factor with the 5'-terminal 13 S fragment near site 1 (sequence 1055–1081) [34]. Thus the environment of the EF-G-binding center of the 50 S subparticle seems to be formed with the participation of practically all the 23 S RNA domains. Their drawing together at the base of the L7/L12 stalk (region of EF-G localization [11]) can be important for the understanding of the role of 23 S RNA in the functioning of the ribosome.

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REFERENCES