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

E.S. Bochkareva and A.S. Girshovich

Institute of Protein Research, USSR Academy of Sciences, 142292 Pushchino, Moscow Region, USSR

Received 10 April 1984

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.

Elongation factor G *Ribosomal 50 S subparticle* S. typhimurium *RNase I*

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 ultracentrifugation in 5–20% sucrose gradient in a buffer containing 2 mM $MgCl_2$, 100 mM NH_4Cl, 10 mM Tris–HCl (pH 7.5), 5 mM mercaptoethanol and concentrated by precipitation with polyethylene glycol 6000 [15]. The isolated subparticles were then dissolved in 10 mM $MgCl_2$, 50 mM NH_4Cl, 10 mM Tris–HCl (pH 7.5), 5 mM mercaptoethanol, 0.2 mM EDTA and stored in batches at −70°C. EF-G was isolated as in [16]. The ^{3}H-labeled derivative of EF-G with a photo-activated arylazide residue on the exposed SH-group and the conditions of its cross-linking to the 50 S subparticles are described in [1,11]. Hydrolysis of modified 50 S subparticles with pancreatic RNase (Koch-Light, without proteases) was done under the following conditions: 7 $A_{260\ nm}$ of 50 S subparticles, 3×10^{-3} μg RNase (RNase/RNA ratio about $1:10^5$) in 75 μl buffer containing 3 mM $MgCl_2$, 20 mM NH_4Cl, 10 mM Tris–HCl (pH 7.5) for 1 h at 0°C. The hydrolysis was stopped by addition of 0.1 vol. of 10% SDS. The mixture was diluted with 100 μl buffer containing 0.4 M LiCl, 10 mM EDTA, 50 mM Na–acetate (pH 5.4) and 1 h later it was centrifuged in 5–20% sucrose gradient in the same buffer (Spinco L5-50, rotor SW41, 34000 rpm, 11 h, 12°C). After measuring absorption and radioactivity the fractions were analyzed by SDS-polyacrylamide gel electrophoresis (SDS–PAGE).

A homogeneous preparation of RNase I from *S.*

Published by Elsevier Science Publishers B.V.

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 $MgCl_2$, 20 mM NH_4Cl, 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 A_{260} unit of 50 S subparticles [18] [final buffer contained 10 mM $MgCl_2$, 7 mM NH_4Cl, 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], TI-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

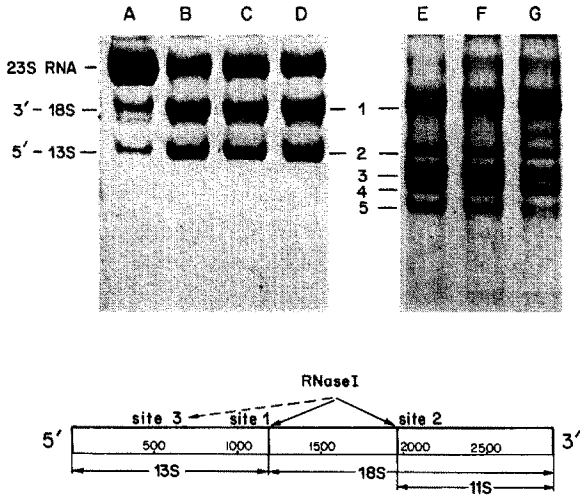

Fig.1. Analysis of the products of hydrolysis of the 50 S subparticle with RNase I by SDS–PAGE under non-denaturing conditions (A–D) and after heat denaturation (E–G). A, initial 50 S subparticle; B and E, 50 S subparticle + RNase I; C and F, 50 S subparticle + EF-G + RNase I; D and G, 50 S subparticle + 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.

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 [^{32}P]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-denaturating 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 subparticles 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 ^{3}H-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 ^{3}H-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

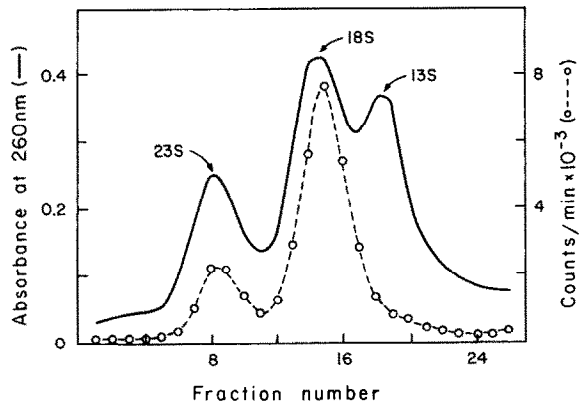

Fig.2. Ultracentrifugation of 23 S RNA after pancreatic RNase limited hydrolysis of the 50 S subparticle affinity labelled with the photoactivated derivative of ^{3}H-labelled EF-G.

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.

ACKNOWLEDGEMENTS

The authors are grateful to Professor A.S. Spirin for critical discussion of the results and to Dr A.M. Kopylov for help in ^{32}P-labelling of the 23 S RNA hydrolyzate. The authors thank Professor B.P. Burma (Banaras, Hindu University, India) for the kind gift of the homogeneous preparation of RNase I from *S. typhimurium*.

REFERENCES

[1] Girshovich, A.S., Pozdnyakov, V.A. and Ovchinnikov, Yu.A. (1980) Bioorgan. Khim. 6, 570–575.
[2] Girshovich, A.S., Bochkareva, E.S. and Gudkov, A.T. (1982) FEBS Lett. 150, 99–102.
[3] Kischa, K., Möller, W. and Stöffler, G. (1971) Nature 233, 62–63.
[4] Brot, N., Yamasaki, E., Redfield, B. and Weissbach, H. (1972) Arch. Biochem. Biophys. 148, 148–155.
[5] Hamel, E., Koka, M. and Nakamoto, T. (1972) J. Biol. Chem. 247, 805–814.
[6] Bernabeau, C., Vazquez, D. and Ballesta, J.P.G. (1976) Eur. J. Biochem. 69, 233–241.
[7] Sander, G., Marsh, R.C., Voigt, J. and Parmeggiani, G. (1975) Biochemistry 14, 1805–1814.
[8] Schrier, P.J. and Möller, W. (1975) FEBS Lett. 54, 130–134.
[9] Highland, J.H. and Howard, G.A. (1975) J. Biol. Chem. 250, 831–834.
[10] Acharya, A.S., Moore, P.B. and Richards, F.M. (1973) Biochemistry 12, 3108–3114.
[11] Girshovich, A.S., Kurtskhalia, T.V., Ovchinnikov, Yu.A. and Vasiliev, V.D. (1981) FEBS Lett. 130, 54–59.
[12] Maassen, J.A. and Möller, W. (1981) Eur. J. Biochem. 115, 279–285.
[13] Sköld, S.-E. (1982) Eur. J. Biochem. 127, 225–229.
[14] Noah, M., Stöffler-Meilicke, M. and Stöffler, G. (1982) in: Electron Microscopy, Int. Congr. Electron Microsc., Hamburg, 3, 101–102.
[15] Expert-Bezancon, A., Guerin, M.F., Hayes, D.H., Lengault, L. and Thibault, J. (1974) Biochimie 56, 77–89.
[16] Alakhov, Yu.B., Motuz, L.P., Stengrevics, O.A., Vinokurov, L.M. and Ovchinnikov, Yu.A. (1977) Bioorgan. Khim. 3, 1333–1345.
[17] Datta, A.K. and Burma, D.P. (1972) J. Biol. Chem. 247, 6795–6801.
[18] Raziuddin, Chatterji, D., Ghosh, S. and Burma, D.P. (1979) J. Biol. Chem. 254, 10575–10578.
[19] Payne, P.I. and Loening, U.E. (1970) Biochim. Biophys. Acta 224, 128–135.
[20] Byasmuni and Burma, D.P. (1982) Biochem. Biophys. Res. Commun. 104, 99–104.

[21] Cahn, F., Schachter, E.M. and Rich, A. (1970) Biochim. Biophys. Acta 209, 512–520.
[22] Hartman, K.A., Amaya, J. and Schachter, E.M. (1970) Nature 171–173.
[23] Allet, B. and Spahr, P.E. (1971) Eur. J. Biochem. 19, 250–255.
[24] Hartman, K.A. and Clayton, N.W. (1974) Biochim. Biophys. Acta 335, 201–210.
[25] Rodgers, A. (1974) Biochim. Biophys. Acta 349, 250–261.
[26] Saha, B.K. (1974) Biochim. Biophys. Acta 353, 292–300.
[27] Spierer, P., Zimmerman, R.A. and Mackie, G.A. (1975) Eur. J. Biochem. 52, 459–468.
[28] Chen-Schmeisser, U. and Garrett, R. (1976) Eur. J. Biochem. 69, 401–410.
[29] Spitnik-Elson, P., Elson, D., Abramowitz, R. and Avital, S. (1978) Biochim. Biophys. Acta 521, 308–323.
[30] Branlant, C., Sriwidada, J., Krol, A. and Ebel, J.-P. (1977) Nucleic Acids Res. 4, 4323–4345.
[31] Branlant, C., Krol, A., Machatt, M.A., Pouyet, J., Ebel, J.-P., Edwards, K. and Kössel, H. (1981) Nucleic Acids Res. 9, 4303–4323.
[32] Noller, H.F., Kop, J., Wheaton, V., Brosius, J., Gutell, R.R., Kopylov, A.M., Dohme, F. and Herr, W. (1981) Nucleic Acids Res. 9, 6167–6189.
[33] Spierer, P., Zimmerman, R.A. and Branlant, C. (1976) FEBS Lett. 68, 71–75.
[34] Sköld, S.-E. (1983) Nucleic Acids Res. 11, 4923–4932.
[35] Stöffler-Meilicke, M., Epe, B., Steinhäuser, K.G., Woolley, P. and Stöffler, G. (1983) FEBS Lett. 163, 94–98.
[36] Steinhäuser, K.G., Woolley, P., Dijk, J. and Epe, B. (1983) Eur. J. Biochem. 137, 337–345.
[37] Traut, R.R., Lambert, Y.M. and Kenny, J.W. (1983) J. Biol. Chem. 258, 14592–14598.
[38] Dijk, J., Garrett, R.A. and Müller, R. (1979) Nucleic Acids Res. 6, 2717–2729.
[39] Schmidt, F.J., Thompson, J., Lee, K., Dijk, J. and Cundliffe, E. (1981) J. Biol. Chem. 256, 12301–12305.
[40] Wower, I., Wower, J., Meinke, M. and Brimacombe, R. (1981) Nucleic Acids Res. 9, 4285–4302.