STUDIES OF THE BINDING SITES OF *ESCHERICHIA COLI* RIBOSOMAL PROTEIN S7 WITH 16S RNA BY ULTRAVIOLET IRRADIATION

B. EHRESMANN, J. REINBOLT, C. BACKENDORF, D. TRITSCH and J. P. EBEL

Laboratoire de Biochimie, Institut de Biologie Moléculaire et Cellulaire du CNRS, 15, rue Descartes, 67000 Strasbourg, France

Received 28 June 1976

1. Introduction

It is now generally agreed that several ribosomal proteins, namely S4, S7, S8, S15, S20, interact directly with the 16S RNA of *E. coli* [1–5]. The studies of the regions of the specific reconstituted protein–RNA complexes protected against mild enzyme digestion, give some informations about the location of some of these proteins on the 16S RNA molecule [6–11].

Further progresses to determine the binding sites between RNA and proteins in the ribosome could be obtained by converting the non-covalent interactions into covalent bonds. Recently photochemical cross-links between proteins and nucleic acids were obtained in the case of ribosomes. Gorelic was able to link covalently to the RNA practically all the proteins by increasing doses of ultraviolet irradiation in the case of the 30S [12] and the 50S subunits of *E. coli* [13].

In a previous paper, we determined the tryptic peptides of protein S4 linked covalently to the 16S RNA in the ultraviolet irradiated 16S RNA–S4 complex [14].

Ribosomal protein S7 is an elongated protein as shown by immune electron microscopy [15], which plays an essential role in Nomura's assembly map [16]. Recently, Möller et al. [17] found that protein S7 was the only 30S protein which was covalently linked to the 16S RNA by low doses of in situ ultraviolet irradiation of the ribosome. These results enabled Rinke et al. [18] to characterize the regions of the 16S RNA crosslinked to protein S7. In this paper we present the structure of the tryptic peptides of protein S7 photochemically linked to the 16S RNA by ultraviolet irradiation of the specific 16S RNA–S7 protein complex.

2. Material and methods

Escherichia coli 16S RNA was extracted from isolated ribosomal 30S subunits as previously described [4,19]. Purified S7 proteins from both *E. coli* B and K strains were kindly given by Professor Wittmann (Berlin). The complex between 16S RNA and protein S7 was prepared using the technique of Garrett et al. [4]. We used about 2 nmol 16S RNA and a 3 to 5 molar excess of protein S7 over the 16S RNA. Under these conditions, we obtained in the complex a molar ratio protein : RNA of approximately 1:1.

The irradiation procedure was described in a previous paper [14]. After irradiation, the covalent complex had to be separated from free S7 protein. The complex was precipitated by ethanol, dissolved under dissociating conditions and then submitted to a Sephadex G-100 chromatography. The fractions containing free 16S RNA and the photochemical cross linked 16S RNA–S7 complex were precipitated by ethanol. After dissolution in water, the protein covalently linked to the 16S RNA was submitted to a total tryptic digestion under the conditions previously described [14]. Free RNA and the 16S RNA on which the S7 tryptic peptides were crosslinked were precipitated by acetic acid at a final concentration of 67%. The free tryptic peptides were separated by a fingerprint technique on thin layer cellulose plates (Polygram Cel 400, Macherey-Nagel, Germany). The

North-Holland Publishing Company – Amsterdam

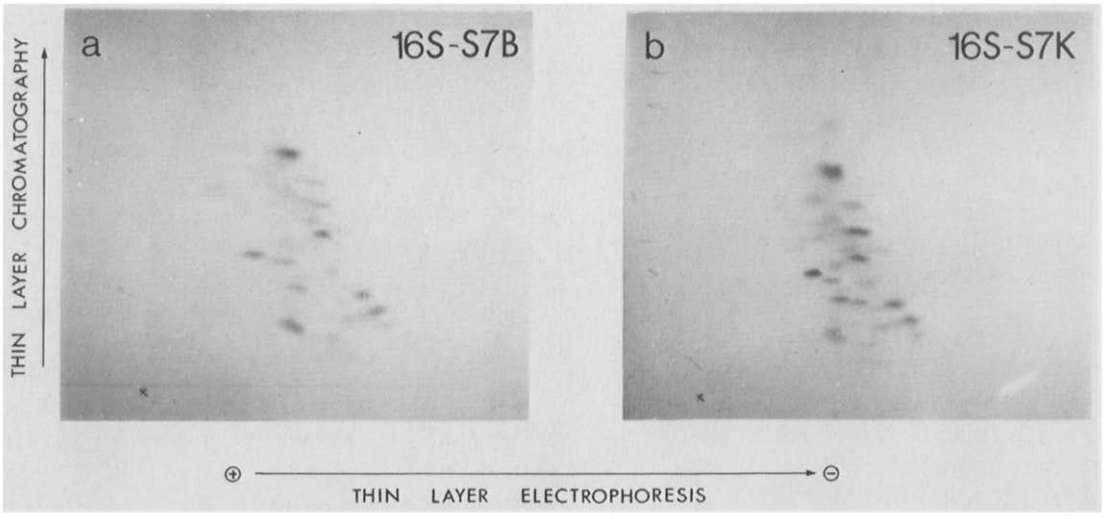

Fig.1. Tryptic fingerprints of protein S7B in the UV irradiated 16S RNA–S7B complex (a) and of protein S7K in the UV irradiated 16S RNA–S7K complex (b).

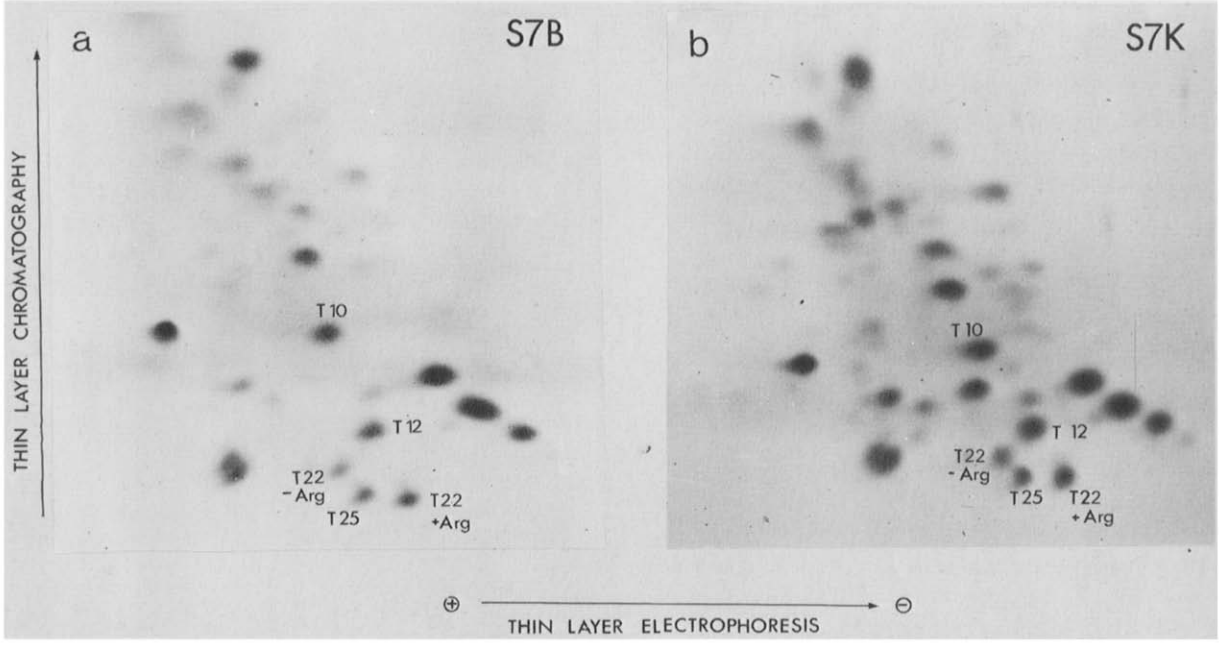

Fig.2. Tryptic fingerprints of protein S7B (a) and S7K (b).

first dimension was an electrophoresis at pH 4.4 in the buffer pyridin/acetic acid/aceton/water (10:20: 75:395, by vol., 400 V, 1.5 H) followed by a chromatography in pyridin/*n*-butanol/acetic acid/water (50:75:15:60, by vol) in the second dimension. The peptides were stained by ninhydrin reagent.

3. Results and discussion

In a previous paper [14] we studied the formation and the specificity of photoinduced covalent bonds between ribosomal protein S4 and the 16S RNA. We showed that specific photochemical crosslinks were obtained only between RNA and a specific protein and only when the stable specific protein–RNA complex was formed before the irradiation. The same conditions are required for the study of the crosslinks between the 16S RNA and protein S7.

The covalently ultraviolet crosslinked 16S RNA–S7 complex was isolated and separated from the free protein as described above. This complex was submitted to a total tryptic hydrolysis and the tryptic peptides were separated by the fingerprint technique as mentioned in Material and methods.

Comparison of the peptides maps of the tryptic peptides from crosslinked proteins both S7B and S7K to 16S RNA (see figs.1a, b) with those of free proteins S7B and S7K (fig.2a, b) enabled us to find out which tryptic peptides were covalently bound to the 16S RNA. The peptides T10, T12, T22 and T25 stained very weakly with the ninhydrin reagent on the fingerprints of crosslinked proteins S7B and S7K. Therefore these peptides must be covalently crosslinked to the 16S RNA by UV irradiation. Their sequences have been determined:

T10: Gly-Thr-Ala-Val -Lys
T12: Ser-Gly-Lys-
T22: Arg-Gly-Asp-Lys
T25: Glu-Asp-Val-His- Arg

It is interesting to observe that the crosslinked peptides have identical sequences in both S7B and S7K proteins. The polypeptide chain S7K is longer than that of S7B. The alterations between S7B and S7K are located in the C-terminal part of the sequence (J. Reinbolt and D. Tritsch, unpublished results). Therefore it seems that this C-terminal part of the polypeptide chain of either S7B or S7K does not play any role in the molecular interaction between both S7B and S7K proteins and the 16S RNA.

In our previous study of the binding sites of protein S4 with 16S RNA, we observed that the peptides interacting with 16S RNA are located in clusters of basic regions [14]. In the case of protein S7, the present state of the sequence of this protein does not allow to determine the structure of the peptides surrounding those which are crosslinked to the RNA and therefore to draw more precise conclusions concerning the nature of the aminoacids or of peptide sequences involved in the RNA-protein recognition process or in the mechanism of crosslinkage between these components.

Acknowledgements

We thank Professor H. G. Wittmann (Berlin) for his gift of both S7 proteins used in this work. This work was supported by the Délégation à la Recherche Scientifique and the Commissariat à l'Energie Atomique

References

[1] Mizushima, S. and Nomura, M. (1970) 226, 1214–1218.
[2] Schaup, H. W., Green, M. and Kurland, C. G. (1970) Mol. Gen. Genet. 109, 193–205.
[3] Schaup, H. W., Green, M. and Kurland, C. G. (1971) Mol. Gen. Genet. 112, 1–8.
[4] Garrett, R. A., Rak, K. H., Daya, L. and Stöffler, G. (1971) Mol. Gen. Genet. 114, 112–124.
[5] Zimmermann, R. A., Muto, A., Fellner, P., Ehresmann, C. and Branlant, C. (1972) Proc. Natl. Acad. Sci. USA 69, 1282–1286.
[6] Muto, A., Ehresmann, C., Fellner, P. and Zimmermann, R. A. (1974) J. Mol. Biol. 86, 411–432.
[7] Zimmermann, R. A., Muto, A. and Mackie, G. A. (1974) J. Mol. Biol. 86, 433–450.
[8] Schaup, H. W., Sogin, M., Woese, C. and Kurland, C. G. (1972) Mol. Gen. Genet. 114, 1–12.
[9] Schaup, H. W., Sogin, M. L., Kurland, C. G. and Woese, C. R. (1973) J. Bacteriol. 115, 82–87.
[10] Zimmermann, R. A., Mackie, G. A., Muto, A., Garrett, R. A., Ungewickell, E., Ehresmann, C., Stiegler, P., Ebel, J. P. and Fellner, P. (1975) Nucleic Acid Res. 2, 279–302.

[11] Ungewickell, E., Garrett, R., Ehresmann, C., Stiegler, P. and Fellner, P. (1975) Europ. J. Biochem. 51, 165–180.
[12] Gorelic, L. (1975) Biochemistry 14, 4627–4633.
[13] Gorelic, L. (1975) Biochim. Biophys. Acta 390, 209–225.
[14] Ehresmann, B., Reinbolt, J. and Ebel, J. P. (1975) FEBS Lett. 58, 106–111.
[15] Tischendorf, G. W., Zeichhardt, H. and Stöffler, G. (1975) Proc. Natl. Acad. Sci. USA 72, 4820–4824.
[16] Held, W. A., Ballon, B., Mizushima, S. and Nomura, M. (1974) J. Biol. Chem. 249, 3103–3111.
[17] Möller, K. and Brimacombe, R. (1975) Mol. Gen. Genet. 141, 343–355.
[18] Rinke, J., Yuki, A. and Brimacombe, R. (1976) Europ. J. Biochem. 64, 77–89.
[19] Branlant, C., Krol, A., Sri Widada, J., Fellner, P. and Crichton, R. R. (1973) FEBS Lett. 35, 265–272.