The interaction of ribosomal protein L16 and its fragments with tRNA

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Two large proteolytic fragments of *Escherichia coli* 50 S ribosomal subunit protein L16 were generated by limited hydrolysis with chymotrypsin (missing 9 N-terminal amino acids) and trypsin (missing 16 N-terminal amino acids). It was found that while intact L16 and its chymotryptic fragment both interact with tRNA (K_d = 5.4 × 10^{-7} M), the tryptic fragment does not. These results are interpreted in terms of possible significance of the residues 10–16 in the peptidyl transferase activity.

Protein L16 *tRNA* *RNA–protein interaction*

1. INTRODUCTION

Moore et al. [1] first showed that the peptidyl transferase activity of LiCl-stripped 50 S core particles can be restored by adding ribosomal protein L16. Since then, a number of investigations confirmed and extended this observation [2–5]. According to 50 S ribosomal subunit reconstitution experiments, protein L16 belongs to a group of ribosomal components, essential for the peptidyl transferase reaction [6].

Our earlier experiments revealed that this protein belongs to the tRNA–50 S ribosomal subunit protein complex which can be assembled on an immobilized tRNA column [7]. Below we report that protein L16 interacts with tRNA in solution. Further insight into this interaction was obtained by comparing the tRNA-binding ability of two large proteolytic fragments of L16. Possible functional implications of these results are suggested.

2. EXPERIMENTAL

The isolation of *Escherichia coli* MRE600 ribosomal proteins, tRNA, ^{32}P-labelled tRNA and nitrocellulose filtration of tRNA–protein complexes are described in [7–11]. Individual protein L16 was purified from split protein fractions obtained by treatment of 50 S ribosomal subunits with 2 M LiCl [1]. Protein L16 was identified by 2-dimensional electrophoresis [12]. This protein can be readily identified by SDS–polyacrylamide gel electrophoresis as well as by its violet colour after staining with Coomassie R-250 stain, since protein L15, which migrates close to L16, has a blue colour.

For limited hydrolysis of protein L16 with chymotrypsin (EC 3.4.4.5), L16 (0.2 mg/ml) in 20 mM Tris–HCl (pH 7.5), 1 M NH_4Cl, was incubated with 10 μg of the enzyme (2 h, 0°C). The reaction was stopped with phenylmethylsulphonyl fluoride (0.5 mM). Hydrolysis with trypsin (EC 3.4.4.4) was carried out using enzyme immobilized to CNBr-activated Sepharose 4B (Pharmacia, Uppsala) prepared as in [13]. 1.5 mg of L16 was incubated with trypsin–Sepharose slurry (0.7 ml, 0.5 mg enzyme) in 10 mM Tris–HCl (pH 7.5), 350 mM NH_4Cl (30 min, 4°C). The slurry was passed through GF/B glass-fiber filter to remove trypsin–Sepharose.

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Chymotrypsin- and trypsin-treated L16 was fractionated by Sephadex G-100 chromatography in 6 M urea-containing buffer. Fractions were analysed by SDS–polyacrylamide gel electrophoresis and those containing individual fragments were collected and concentrated on a 0.5-ml CM-cellulose (Whatman) column and eluted with buffer containing 1 M NH_4Cl. The precise sites of cleavage were identified by analyzing N-terminal amino acids of the fragments and from the known primary structure of the protein [14]. The results of the N-terminal analysis of the fragments were also in full agreement with their mobility in SDS–polyacrylamide gel.

3. RESULTS AND DISCUSSION

Fig.1 shows the SDS–polyacrylamide gel electrophoresis picture of protein L16 and its fragments obtained by chymotrypsin (FcL16) and trypsin (FtL16). N-terminal amino acid analysis

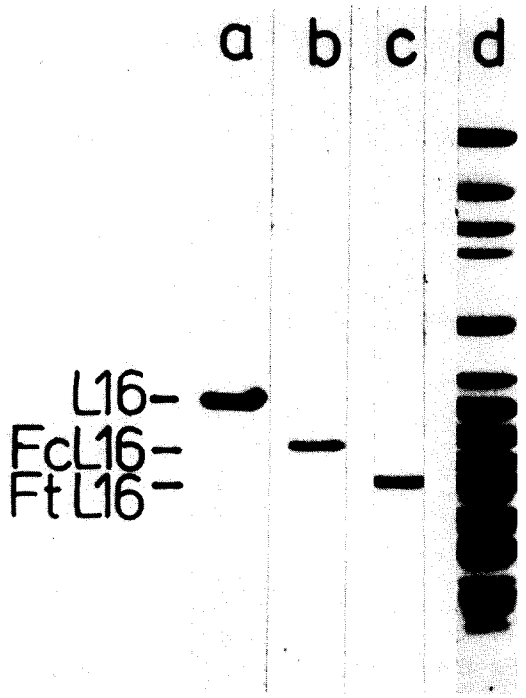

Fig.1. Electrophoresis of protein L16 and its individual fragments in SDS–15% polyacrylamide gel [17]: (a) protein L16; (b) chymotryptic fragment FcL16; (c) tryptic fragment FtL16; (d) 50 S ribosomal subunit proteins.

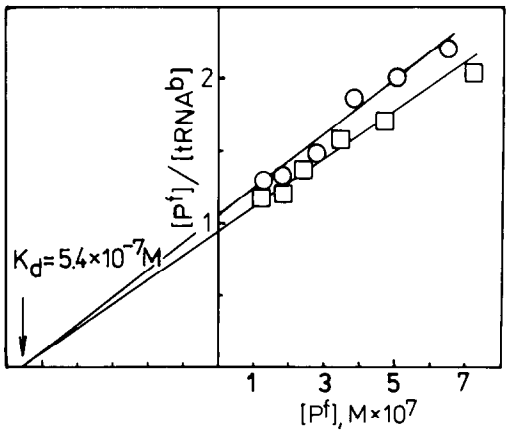

Fig.2. Determination of complex formation between tRNA and protein L16 (○—○) and FcL16 (□—□) on nitrocellulose filters. ^{32}P-labelled deacylated tRNA (4.5×10^{-7} M) was titrated with increasing amounts of protein in 10 mM Tris–HCl buffer (pH 7.4) containing 10 mM $MgCl_2$, 100 mM NH_4Cl and 6 mM 2-mercaptoethanol. Samples of 50 μl were incubated for 10 min at 30°C and filtered through nitrocellulose filters. [tRNA^b], concentration of bound tRNA; [P_f], concentration of free protein (L16 or FcL16). [P_f] was calculated from the known amount of protein in the assay and the known amount of tRNA bound onto the filter, assuming a 1:1 ratio between protein and tRNA.

revealed the presence of Arg at the end of FcL16 and Asn at the end of FtL16, which, together with the mobility of these fragments (using TP50 M_r reference), leads to the conclusion that compared with L16, FcL16 has lost 9 and FtL16 16 N-terminal residues.

Titration of a constant amount of ^{32}P-labelled bulk tRNA with increasing amounts of protein L16, FcL16 and FtL16 is shown in fig.2. Linear transformation of binding isotherms indicate almost identical affinities of tRNA to L16 and FcL16. In contrast, the fragment generated by trypsin which lacks 16 residues has lost its ability to form a stable complex with tRNA. Notably, the sequence of protein L16 [14] between amino acid residues 10 and 16, Arg–Lys–Met–His–Lys–Gly–Arg, is enriched in basic amino acids and may well provide a binding site for an RNA. This sequence contains the only histidyl residue of protein L16, which is presumably important in the functioning of the peptidyl transferase center. Thus, it was found that modification of His-13 leads to loss

of the ability of L16 to restore peptidyl transferase activity [4,5]. It is not clear whether His-13 is directly involved in the catalytic mechanism, since it was suggested that photoinactivation of the peptidyl transferase activity, involving modification of histidyl residues, abolishes the interaction of the 3′-end of amino-acylated tRNA with the ribosomal A site [15]. The results presented in this paper show that outside the ribosome protein L16 interacts with tRNA, while the fragment which lacks His-13 does not.

It would be premature to extrapolate our results to the situation within the ribosome. For technical reasons, the method employed by us does not allow simultaneous measurements of the interaction of protein L16 with 23 S RNA and tRNA. Since L16 is known to bind individually to 23 S RNA [16], there remains a possibility that an RNA-binding site of L16, which possibly lies within residues 10–16, is not specific for tRNA. Nevertheless, our results do indicate that the loss of the peptidyl transferase activity of 50 S ribosomal particles containing modified L16 might be connected with its inability to interact with certain regions of tRNA.

REFERENCES

[1] Moore, V.G., Atchison, R.T., Thomas, G., Moran, M. and Noller, H.R. (1975) Proc. Natl. Acad. Sci. USA 72, 844–848.
[2] Dohme, F. and Fahnestock, S.R. (1979) J. Mol. Biol. 129, 63–81.
[3] Teraoka, H. and Nierhaus, K.H. (1978) FEBS Lett. 88, 223–226.
[4] Baxter, R.M. and Zahid, N.D. (1978) Eur. J. Biochem. 91, 49–56.
[5] Baxter, R.M., White, V.T. and Zahid, N.D. (1980) Eur. J. Biochem. 110, 161–166.
[6] Hampl, H., Schulze, H. and Nierhaus, K.H. (1981) J. Biol. Chem. 256, 2284–2288.
[7] Ustav, M., Villems, R., Saarma, M. and Lind, A. (1977) FEBS Lett. 83, 353–356.
[8] Ustav, M., Villems, R. and Lind, A. (1977) FEBS Lett. 82, 259–262.
[9] Ustav, M., Remme, J., Lind, A. and Villems, R. (1979) Bioorg. Khim. 5, 365–369.
[10] Metspalu, E., Maimets, T., Ustav, M. and Villems, R. (1981) FEBS Lett. 132, 105–108.
[11] Sedman, J., Maimets, T., Ustav, M. and Villems, R. (1981) FEBS Lett. 136, 251–254.
[12] Howard, G. and Traut, R.R. (1974) Methods Enzymol. 30/F, 529–536.
[13] March, S.C., Parikh, J. and Cuatrecasas, P. (1974) Anal. Biochem. 60, 149–152.
[14] Brosius, J. and Chen, R. (1976) FEBS Lett. 68, 105–109.
[15] Černa, J. and Rychlik, I. (1979) FEBS Lett. 102, 277–281.
[16] Stöffler, G., Daya, L., Rak, K.H. and Garrett, R.A. (1971) Mol. Gen. Genet. 114, 125–133.
[17] Polyacrylamide Gel Electrophoresis (1980) Pharmacia Fine Chemicals Publication, p.28, Pharmacia, Uppsala.