

THE RNA BINDING PROPERTIES OF 'NATIVE' PROTEIN-PROTEIN COMPLEXES ISOLATED FROM THE *ESCHERICHIA COLI* RIBOSOME

Jan DIJK, Jenny LITTLECHILD and Roger A. GARRETT

Max-Planck-Institut für Molekulare Genetik, Abt. Wittmann, Berlin-Dahlem, Germany

Received 23 March 1977

1. Introduction

The assembly of the *Escherichia coli* ribosome is based on many specific interactions between the ribosomal components. Direct evidence for single protein-RNA interactions has been well established (reviewed in ref. [1]). Various observations provide circumstantial support for the existence of protein-protein complexes in the ribosome. For example, cooperative protein binding effects have been observed during 30 S subunit reconstitution [2] and in protein assembly to 5 S and 23 S RNA [3,4]. Moreover, many protein pairs have been cross-linked with relatively short reagents [5,6], one pair of which was subsequently shown to be active in reconstitution experiments [7]. The first direct evidence, however, for the occurrence of a specific protein-protein interaction came from the isolation of a very stable complex between proteins L7/12 and L10 from the 50 S subunit [8].

Conventional purification procedures for ribosomal proteins purposely use strong dissociating conditions [9], which would disrupt any native protein complex. Isolation of ribosomal proteins under non-denaturing conditions, however, has made it possible to purify several putative 'native' protein complexes from both ribosomal subunits [10]. This paper deals with two of these potential complexes, namely S13-S19 and L7/12-L10. They bound specifically to 16 S and 23 S RNA, respectively, whereas their constituent proteins showed little (S13 and S19) or no (L7/12 and L10) binding to RNA. We consider that the specific RNA binding properties of these two protein complexes provide direct evidence that they are biologically significant complexes.

2. Materials and methods

Ribosomal proteins from *E. coli* A19 (RNase I⁻) were prepared by a gentle procedure, avoiding the use of urea, acetic acid, lyophilization and other strong denaturing conditions [10,11]. The putative complex S13-S19 was purified, after LiCl-extraction, by consecutive CM-Sephadex and Sephadex G-100 chromatography, whereas the L7/12-L10 complex was obtained by chromatography on CM-Sephadex, DEAE-Sephadex and Sephadex G-100. These methods will be described in detail elsewhere [10]. The identities of the proteins were established by two different two-dimensional gel electrophoresis systems [12-14] and by dodecylsulphate slab-gel electrophoresis [15,16]. The concentrations of the ribosomal proteins were determined by a nitrogen assay [17], which gave 20-50% lower values than were obtained with a fluorescamine assay previously [11]. The results from this nitrogen assay are in agreement with those from amino acid analyses and represent absolute protein concentrations. Determination of ribosomal protein concentrations according to Lowry et al. [18] were generally lower than those from the nitrogen assay. The following molecular weight values were taken from sequence data (reviewed in ref. [19]): S13 - 12 900; L7/12 - 12 200; L10 - 17 700; S7 and S19 were taken as 20 000 and 10 000 respectively. Ribosomal RNAs were prepared by a phenol-dodecylsulphate procedure [20].

Binding assays [21] were performed with either 65 µg 16 S RNA or 85 µg 23 S RNA in 1 ml reconstitution buffer (30 mM Tris-HCl, pH 7.4, 0.35 M KCl, 20 mM MgCl₂, 6 mM 2-mercaptoethanol) to which the proteins were added in the appropriate

molar excess. After incubating for 1 h at 42°C the complex was separated from unbound protein on an agarose column (Biorad Bio-Gel A-0.5 m) and assayed for complex formation by two different methods:

(1) The complex was precipitated with 5% (w/v) trichloroacetic acid in the presence of sodium deoxycholate and electrophoresed on 15% polyacrylamide gels containing sodium dodecylsulphate [15,16].

(2) The complex was precipitated with 1.5 vol ethanol and electrophoresed on a 2.25% polyacryl-

amide gel in the presence of 1 mM MgCl_2 as described earlier [21].

Gels were stained and scanned as previously described [11].

3. Results

During the fractionation of the 30 S subunit proteins, S13 and S19 co-migrated on both CM-

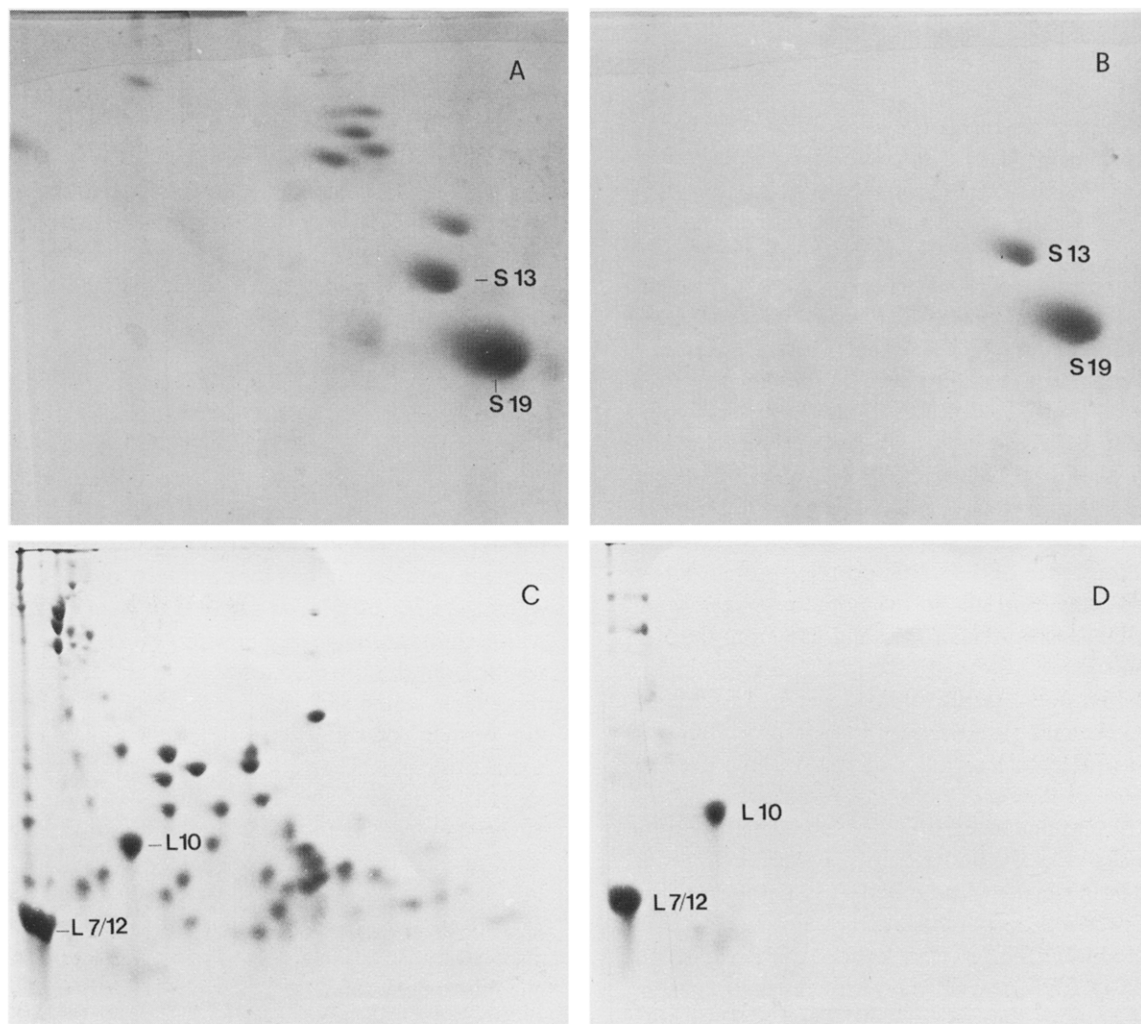


Fig.1. Two-dimensional gel electrophoresis. A and B: Complex of proteins S13 and S19 run with and without a background of total 30 S subunit proteins using the system of Kaltschmidt and Wittmann [12]. C and D: Complex of proteins L7/12 and L10 run with and without a background of total 50 S subunit proteins using the system of Mets and Bogorad [13,14] which gave better resolution of L10.

Sephadex and Sephadex G-100 columns. In the ion-exchange chromatography, varying amounts of S13 were separated from S19 and subsequently eluted at a different position. The comigration of the majority of the S13 with S19 strongly suggested an interaction between the proteins. Figure 1 A,B shows two-dimensional (2-D) electrophoresis gels of the protein pair first with a background of total 30 S subunit proteins to establish their identity and second without marker proteins to confirm their purity. Proteins L7/12 and L10, from the 50 S subunit, also were eluted from CM-Sephadex, DEAE-Sephadex and Sephadex G-100 columns with a constant protein : protein molar ratio throughout the elution peak. The position of the peak on Sephadex G-100 was different from that of the constituent proteins. These proteins were also identified and checked for purity by 2-D gel electrophoresis (fig.1 C,D).

Of these four proteins, two, namely S13 and S19, have recently been shown individually to bind weakly to 16 S RNA [11] while the other two, L7/12 and L10, showed no binding to 23 S RNA [11,21]. We tested the RNA binding capacities of the putative complex S13-S19 and the complex L7/12-L10 under standard ribosomal subunit reconstitution conditions, reasoning that if specific binding occurred between the protein pairs and the RNA, then this was an excellent criterion for the specificity of the protein-protein complexes.

For the S13-S19 protein complex two binding methods were used. In one method the precise amount of each protein bound to the RNA was determined. In the second method only the total amount of bound protein complex was assayed. For the L7/12-L10 complex only the former method could be employed because the second method involved precipitation of the protein-RNA complex with ethanol which resulted in the dissociation of the latter proteins from the RNA. This finding is in agreement with earlier studies on the interaction of L7/12 with the ribosome [23].

Three criteria were invoked for the specific binding of the protein complexes to the RNAs:

- (1) That the proteins bound exclusively to their cognate RNA and not to the other RNA.
- (2) That an integral protein : protein molar ratio occurred in the protein-RNA complex.
- (3) That saturation of binding occurred at approxi-

mately a 1 : 1 molar protein : RNA ratio or less.

Criteria (1) and (3) were testable by both binding assay methods, criterion (2) could only be tested by method (1).

The binding capacity of each of the protein complexes to the 16 S and 23 S RNA was tested. The S13-S19 complex was shown by both binding methods to bind exclusively to 16S RNA. The L7/12-L10 complex was tested by method (1); it bound strongly to 23 S RNA and only very weakly to 16 S RNA (for an input molar ratio of 5 : 1 the protein bound at less than 0.1 : 1). The dodecylsulphate gels showing the protein complexes before and after complexing with the RNAs are given in fig.2. For the different S13-S19 protein complexes a variable excess of protein S19 was present. This was determined quantitatively from fig.2A and corrected for in



Fig.2. Dodecylsulphate-polyacrylamide slab-gels showing: A, Protein complex S13-S19; B, Protein complex S13-S19 after binding and re-extracting from the 16 S RNA; C, Protein complex L7/12-L10; D, Protein complex L7/12-L10 after binding and re-extracting from the 23 S RNA. Both protein complexes were present at a 5 : 1 molar excess during incubation with the RNA. The L7/12-L10 complex contains small amounts of aggregates which were assumed to be artifacts of the electrophoretic method and small amounts of degradation products were seen which increased after incubation with the RNA.

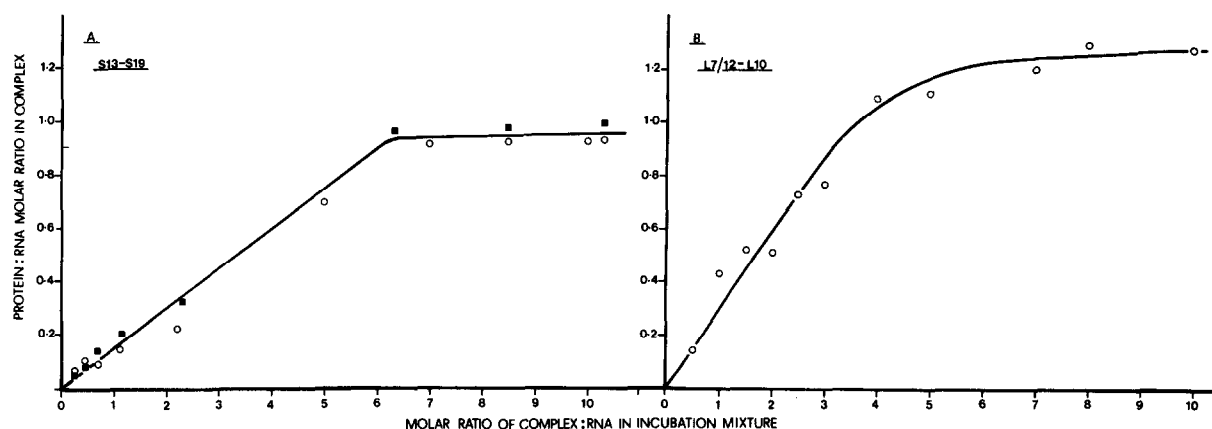


Fig.3. Protein-RNA binding saturation curves. A, S13-S19 binding to 16 S RNA. B, L7/12-L10 binding to 23 S RNA. (■—■) Indicates results from the standard disc-gel method [20] and (○—○) gives results from the dodecylsulphate-gel method [11].

the binding assays. The S13 : S19 molar ratio in the RNA-protein complex was estimated at about 1 : 1 in each complex examined. When high molar excesses of the S13-S19 complex were incubated with the 16 S RNA, a contaminant protein, S7, that was hardly visible in the starting material (fig.2A) was present at low molar ratios in the 16 S RNA complex (fig.2B) (see below). For the L7/12-L10 complex the molar ratio between the two proteins was constant at about 4 : 1 before and after binding to the RNA. This is compatible with the extreme stability of this complex reported earlier [8].

The saturation binding curves for the two complexes are shown in fig.3. They indicate that saturation of binding occurs between 0.9 and 1 : 1 for S13-S19 and at 1.25 : 1 for the L7/12-L10 complex, respectively. Error limits were estimated at $\pm 15\%$. The molar ratio of S7 : 16 S RNA (fig.2B) was estimated at 0.1 : 1 at the onset of saturation of the S13-S19 complex (see fig.3A) and at 0.2 : 1 when the 16 S RNA was incubated with a 10-fold molar excess of S13-S19 complex (fig.3A). The data from the disc-gel method were corrected for this.

Each criterion for specificity was satisfied for the two complexes. The very weak binding of the L7/12-L10 complex to the 16 S RNA is difficult to explain, unless there is some weak specific affinity of this complex for 16 S RNA which may arise from the location of this complex in the ribosomal subunit interface [24].

4. Discussion

Several lines of evidence support the existence of a complex between S13 and S19. These can be summarized as:

- (1) The two proteins co-migrate on an ion-exchange column in a different position from protein S13.
- (2) The protein pair binds much more strongly to 16 S RNA than the individual proteins [11].
- (3) Saturation of binding to the RNA occurs at about a 1 : 1 protein complex : RNA ratio.
- (4) The proteins are present in the RNA complex in a 1 : 1 molar ratio.

This is the first direct evidence for the existence of a specific protein-protein complex in the 30 S subunit and is compatible with the following observations:

- (1) These proteins have been identified in cross-linked complexes isolated from 30 S subunits [6,25] which indicates that they are neighbours in the ribosome.
- (2) Electron microscopy studies of Lake et al. [26], using antibody markers, show that S13 and S19 are located side by side on the head of the 30 S

subunit, although they were found to be more separated in a similar study by Tischendorf et al. [24].

- (3) Huang et al. [27] established the close proximity of S13 and S19 by singlet energy-transfer using fluorescent probes.
- (4) Both S13 and S19 have been identified in cross-linked complexes with initiation factors IF-2 and IF-3 [28,29].
- (5) Finally, 30 S subunit assembly studies [30] showed that protein S7 stimulated the separate incorporation of proteins S13 and S19. Variations in the amount of S7 in the starting material did not affect the extent of binding of S13–S19 to the RNA; when the RNA binding of the complex reached saturation the molar ratio of bound S7 was low. Therefore we conclude that there is no direct effect of protein S7 on the binding of complex S13–S19 to 16 S RNA.

Direct evidence for the existence of a L7/12–L10 complex was obtained earlier by the isolation and in vitro reconstitution of a complex, consisting of proteins L7/12 and L10 in a 4 : 1 molar ratio [8,31]. The RNA-binding properties of this complex, presented here, establish that this is indeed biologically significant. This conclusion is also compatible with the following biochemical data:

- (1) L7/12 and L10 have been cross-linked in the intact ribosome [32].
- (2) Functional studies have shown that they both interact with elongation factors during protein synthesis [33].
- (3) During reassembly experiments it was observed that the presence of L10 on the subunit is required for the reassembly of L7/12 [34,35].

We are currently investigating other putative 'native' protein–protein complexes for their capacities to bind specifically to ribosomal RNAs.

Acknowledgements

We thank Mrs Kathrein Farnung, Mrs Christa Hennig and Mr Jack Hunter for their excellent help with the RNA binding experiments and Mrs I. Ackermann and Ms A. Malcolm for expert assistance in preparing and characterizing the ribosomal proteins. Dr A. R. Subramanian identified proteins L7/12 and L10 by two-dimensional gel electrophoresis. This study was supported by research grants from the Deutsche Forschungsgemeinschaft.

References

- [1] Zimmermann, R. A. (1974) in: *Ribosomes* (Nomura, M., Tissières, A. and Lengyel, P. eds) pp. 225–269, Cold Spring Harbor.
- [2] Mizushima, S. and Nomura, M. (1970) *Nature* 226, 1214–1218.
- [3] Feunteun, J., Monier, R., Garrett, R. A., Le Bret, M. and Le Pecq, J. B. (1975) *J. Mol. Biol.* 93, 535–541.
- [4] Spierer, P. and Zimmermann, R. A. (1976) *J. Mol. Biol.* 103, 647–653.
- [5] Lutter, L. C., Kurland, C. G. and Stöffler, G. (1975) *FEBS Lett.* 54, 144–150.
- [6] Sommer, A. and Traut, R. R. (1976) *J. Mol. Biol.* 106, 995–1015.
- [7] Lutter, L. C. and Kurland, C. G. (1973) *Nature* 243, 15–17.
- [8] Pettersson, I., Hardy, S. J. S. and Liljas, A. (1976) *FEBS Lett.* 64, 135–138.
- [9] Wittmann, H. G. (1974) in: *Ribosomes* (Nomura, M., Tissières, A. and Lengyel, P. eds) pp. 93–114, Cold Spring Harbor.
- [10] Littlechild, J. and Dijk, J. (1977) manuscript in preparation.
- [11] Littlechild, J., Dijk, J. and Garrett, R. A. (1977) *FEBS Lett.* 74, 292–294.
- [12] Kaltschmidt, E. and Wittmann, H. G. (1970) *Anal. Biochem.* 36, 401–412.
- [13] Mets, L. J. and Bogorad, L. (1974) *Anal. Biochem.* 57, 200–210.
- [14] Kyriakopoulos, A. and Subramanian, A. R. (1977) *Biochim. Biophys. Acta* 474, 308–311.
- [15] Laemmli, U. K. (1970) *Nature* 227, 680–685.
- [16] Studier, F. W. (1973) *J. Mol. Biol.* 79, 237–248.
- [17] Jaenicke, L. (1974) *Anal. Biochem.* 61, 623–627.
- [18] Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) *J. Biol. Chem.* 193, 265–275.
- [19] Stöffler, G. and Wittmann, H. G. (1977) in: *Protein Biosynthesis* (Weissbach, H. and Pestka, S. eds) pp. 117–202, Academic Press, New York.
- [20] Folkhard, W., Pilz, I., Kratky, O., Garrett, R. A. and Stöffler, G. (1975) *Eur. J. Biochem.* 59, 63–71.

- [21] Schulte, C., Morrison, C. A. and Garrett, R. A. (1974) *Biochemistry* 13, 1032–1037.
- [22] Stöffler, G., Daya, L., Rak, K. H. and Garrett, R. A. (1971) *Molec. Gen. Genet.* 114, 125–133.
- [23] Hamel, E., Koka, M. and Nakamoto, T. (1972) *J. Biol. Chem.* 247, 805–814.
- [24] Tischendorf, G. W., Zeichhardt, H. and Stöffler, G. (1975) *Proc. Natl. Acad. Sci. USA* 72, 4820–4824.
- [25] Lutter, L. C., Bode, U., Kurland, C. G. and Stöffler, G. (1974) *Molec. Gen. Genet.* 129, 167–176.
- [26] Lake, J. A. and Kahan, L. (1975) *J. Mol. Biol.* 99, 631–644.
- [27] Huang, K. H., Fairclough, R. H. and Cantor, C. (1975) *J. Mol. Biol.* 97, 443–470.
- [28] Bollen, A., Heimark, R. L., Cozzone, A., Traut, R. R., Hershey, J. W. B. and Kahan, L. (1975) *J. Biol. Chem.* 250, 4310–4314.
- [29] Heimark, R. L., Kahan, L., Johnston, K., Hershey, J. W. B. and Traut, R. R. (1976) *J. Mol. Biol.* 105, 219–230.
- [30] Green, M. and Kurland, C. G. (1973) *Mol. Biol. Reports* 1, 105–111.
- [31] Österberg, R., Sjöberg, B., Pettersson, I., Liljas, A. and Kurland, C. G. (1977) *FEBS Lett.* 73, 22–24.
- [32] Expert-Bezançon, A., Barritault, D., Milet, M. and Hayes, D. H. (1976) *J. Mol. Biol.* 108, 781–787.
- [33] Acharya, A. S., Moore, P. B. and Richards, F. M. (1973) *Biochemistry* 12, 3108–3114.
- [34] Schrier, P. I., Maassen, J. A. and Möller, W. (1973) *Biochem. Biophys. Res. Commun.* 53, 90–98.
- [35] Stöffler, G., Hasenbank, R., Bodley, J. W. and Highland, J. H. (1974) *J. Mol. Biol.* 86, 171–174.