THE IDENTIFICATION OF NEW RNA-BINDING PROTEINS IN THE *ESCHERICHIA COLI* RIBOSOME

J. LITTLECHILD, J. DIJK and R. A. GARRETT
Max-Planck-Institut für Molekulare Genetik, Abt. Wittmann, Berlin-Dahlem, Germany

Received 14 December 1976

1. Introduction

Identifying those proteins that bind independently to ribosomal RNAs of the *E. coli* ribosome has proved an important step in the structural characterization of the ribosome. The RNA binding site can subsequently be localised within the RNA sequence and, in some instances, it has been possible to isolate a complex of the protein and its RNA binding site which can then be subjected to detailed structural studies (reviewed in [1,2]).

There is general agreement that proteins S4, S7, S8, S15 and S20 specifically bind to phenol-extracted 16 S RNA and that proteins L1, L2, L3, L4, L6, L16, L20, L23 and L24 bind to the 23 S RNA [1,3]. Conflicting results have appeared on the binding of S13 and S17 to 16 S RNA and L13 and L19 to 23 S RNA [1,3]. Recently, Hochkeppel et al. [4] demonstrated that a further seven proteins from the 30 S subunit, namely S3, S5, S9, S11, S12, S13 and S18, could bind to 16 S RNA that was prepared by a low pH extraction procedure. The binding of these proteins was attributed to the formation of a metastable and more open RNA conformation.

In the present study we have tested 34 ribosomal proteins for specific RNA-binding which were fractionated by very gentle procedures that differed from previous preparation methods in that they avoided any protein denaturing conditions [5]. RNAs, 16 S and 23 S, were prepared by the standard phenol–dodecylsulphate method. The binding results demonstrate that in addition to the well characterized RNA-binding proteins, ribosomal proteins S2, S5, S13 and S19 bind to the 16 S RNA and proteins L11 and L15 bind to 23 S RNA. These results establish that the conformations of the proteins are also very important for attaining stable protein–RNA interactions. Preliminary evidence for the binding of protein–protein complexes to the ribosomal RNAs is also presented.

2. Materials and methods

Ribosomal proteins from *E. coli* A19 (RNAase I^-) were fractionated by a procedure that avoids the use of urea, extreme pH and other denaturing conditions. They were extracted from the 30 S and 50 S subunits, into several groups, by a stepwise increase of LiCl at neutral pH. Further fractionation was obtained by chromatography on CM-Sephadex C-25, using salt gradients in the pH range of 5.5–8.0, followed by gel-filtration on Sephadex G-100 also in buffered salt solutions at pH 5.5–8.0. Purified proteins were concentrated by either pressure ultrafiltration in Millipore concentration cells or by dialysis against dry Sephadex G-150. A detailed account of this procedure will be published elsewhere [5]. The identities of the proteins were established by two-dimensional gel electrophoresis [6] and by dodecylsulphate slab-gel electrophoresis [7,8]. The concentrations of the protein solutions were determined by a fluorescamine assay [9] using lysozyme as a standard. RNAs, 16 S and 23 S, were prepared from *E. coli* A19 by the phenol–dodecylsulphate procedure described earlier [10].

Binding assays [11] were performed with either 65 μg 16 S RNA or 85 μg 23 S RNA in 1 ml of

 North-Holland Publishing Company – Amsterdam

ribosomal reconstitution buffer (0.03 M Tris–HCl, pH 7.4, 0.35 M KCl, 0.02 M $MgCl_2$, 0.006 M 2-mercaptoethanol). Protein was added up to a 3–5-fold molar excess and the mixture was incubated at 42°C for 1 h. 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.

(i) The complex was precipitated with 1.5 vol. ethanol and electrophoresed into 2.25% polyacrylamide gels as described earlier [11]. Gels were stained in duplicate for RNA with toluidine blue and for protein with Coomassie Brilliant Blue [11].

(ii) The complex was precipitated with 5% (w/v) trichloracetic acid in the presence of sodium deoxycholate (50 μg/ml), treated with 1% (w/v) sodium dodecylsulphate and electrophoresed into a 15% polyacrylamide slab-gel containing 0.1% dodecylsulphate [7,8]. Known weights of each protein were co-electrophoresed for quantitation of binding. The protein bands were stained with Coomassie Brilliant Blue and scanned with either a Joyce-Loebl or an Ortec 4310 densitometer.

3. Results and discussion

All binding assays were performed in the ribosomal reconstitution buffer where normally only specific protein–RNA interactions occur. As the main criterion for the specificity of the binding it was demonstrated that, under the binding conditions, the 30 S subunit proteins bound to 16 S RNA but not to the 23 S RNA, whereas the 50 S subunit proteins bound to 23 S RNA but not to 16 S RNA [11].

The following proteins were tested for their capacity to bind to 16 S RNA, namely S2, S3, S4, S5, S8, S10, S13, S14, S15, S19, S20, S21, and to 23 S RNA, namely L1, L2, L3, L6, L7/12, L10, L11, L13, L15, L16, L17, L18, L19, L23, L24, L25, L27, L28, L29, L30, L33 and L34. All the proteins that had previously been shown to bind to the phenol-extracted RNAs, namely S4, S8, S15, S20 and L1, L2, L3, L6, L16, L23 and L24, bound to these RNAs in our experiments. The binding characteristics of these proteins were also similar to those observed earlier [1–3]. In addition, proteins S2, S5, S13 and S19 bound to 16 S RNA and L11 and L15 bound to 23 S RNA. These results were obtained with two or more preparations of each protein and positive binding results were observed with both electrophoretic methods.

The binding of proteins S2, S13 and S19 to the 16 S RNA was weak, reaching a molar protein:RNA ratio of 0.1–0.5:1. Protein S5 generally bound more strongly at 1:1 or slightly higher. Proteins L11 and L15 both bound strongly to the 23 S RNA. The binding ratio of protein L11 was approximately 0.8:1, whereas L15 bound at more than a 1:1 molar ratio in the presence of a large molar excess of protein. This was probably due to some binding of protein aggregates to the RNA, as shown by chemical cross-linking studies of the RNA–protein complex (unpublished results). None of these proteins bound to the RNAs when they were fractionated by standard procedures under conditions where protein denaturation can occur (reviewed in [12]). The exception to this is protein S13 that bound to 16 S RNA when fractionated by certain of these procedures [1].

Two of the new binding proteins, namely S5 and S13, were reported to bind to 16 S RNA that had been freshly extracted with an acetic acid/urea mixture containing 75% acetic acid and 1 M urea [4]. This RNA exhibits a metastable conformation that is probably related to that studied earlier by Cox, Katchalsky and co-workers [13,14]. Hochkeppel et al. [4] claim that the RNA is in a more open conformation such that the binding sites of the proteins are more accessible. Our results indicate that these two proteins can also associate with the more commonly used phenol-extracted 16 S RNA when they are prepared under non-denaturing conditions. This emphasizes that the conformations of the proteins are also important for stable protein–RNA interactions.

It is probable that other proteins bind directly with the ribosomal RNAs in the ribosome. The binding assays employed here detected protein binding in non-equilibrium conditions where proteins with low affinities for the RNA may have already dissociated. Moreover, the results in no way violate the concept of cooperative protein–RNA assembly effects first observed by Mizushima and Nomura [15] for the 16 S RNA and by Feunteun

et al. [16] for the 5 S RNA, in which the binding constant of one protein for the RNA is increased by the presence of another RNA-binding protein. In one respect they support these findings, since a protein–protein complex of two of the new binding proteins, namely S13 and S19, bound more strongly at higher protein:RNA molar ratios when complexed to the 16 S RNA than when the individual proteins were bound separately [17]. Furthermore, whereas proteins L7/L12 and L10 did not bind to 23 S RNA, a complex of the two proteins [18] bound strongly to the 23 S RNA [17].

Acknowledgements

We thank Mrs Christa Hennig and Mr Jack Hunter for experimental help and Mrs I. Ackermann and Miss A. Malcolm for assistance in preparing and characterizing the ribosomal proteins. This research was supported by 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] Brimacombe, R., Nierhaus, K. H., Garrett, R. A. and Wittmann, H. G. (1976) in: Progress in Nucleic Acid Research and Molecular Biology (Cohn, W. ed) Vol. 18, pp. 1–44, Academic Press.
[3] Garrett, R. A., Müller, S., Spierer, P. and Zimmermann, R. A. (1974) J. Mol. Biol. 88, 553–557.
[4] Hochkeppel, H.-K., Spicer, E. and Craven, G. R. (1976) J. Mol. Biol. 101, 155–170.
[5] Littlechild, J. and Dijk, J. (1977) manuscript in preparation.
[6] Kaltschmidt, E. and Wittmann, H. G. (1970) Anal. Biochem. 36, 401–412.
[7] Laemmli, U. K. (1970) Nature 227, 680–685.
[8] Studier, F. W. (1973) J. Mol. Biol. 79, 237–248.
[9] Böhlen, P., Stein, S., Dairman, W. and Udenfriend, S. (1973) Arch. Biochem. Biophys. 155, 213–220.
[10] Folkhard, W., Pilz, I., Kratky, O., Garrett, R. A. and Stöffler, G. (1975) Eur. J. Biochem. 59, 63–71.
[11] Schulte, C., Morrison, C. A. and Garrett, R. A. (1974) Biochemistry 13, 1032–1037.
[12] Wittmann, H. G. (1974) in: Ribosomes (Nomura, M., Tissières, A. and Lengyel, P. eds) pp. 93–114, Cold Spring Harbor.
[13] Cox, R. A. and Katchalsky, A. (1972) Biochem. J. 126, 1039–1054.
[14] Revzin, A., Neumann, E. and Katchalsky, A. (1973) J. Mol. Biol. 79, 95–114.
[15] Mizushima, S. and Nomura, M. (1970) Nature 226, 1214–1218.
[16] Feunteun, J., Monier, R., Garrett, R. A., Le Bret, M. and Le Pecq, J. B. (1975) J. Mol. Biol. 93, 535–541.
[17] Littlechild, J., Dijk, J. and Garrett, R. A. (1977) manuscript in preparation.
[18] Pettersson, I., Hardy, S. J. S. and Liljas, A. (1976) FEBS Letters 64, 135–138.