RANDOM EXCHANGE OF RIBOSOMAL PROTEINS IN EDTA SUB-PARTICLES

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1. Introduction

The use of EDTA to 'unfold' ribosomal sub-particles has been widespread over the last few years (see e.g. [1] for review). The EDTA particles retain most if not all of the ribosomal proteins [2], but it is not yet known whether the proteins in the unfolded particle retain their specific locations on the ribosomal RNA or whether their distribution becomes random. This is obviously an important consideration, when EDTA particles are used for studies on the organization of the ribosomal components. In particular, several authors have reported the isolation of specific ribo-nucleoprotein fragments from EDTA-treated [3–5] or de-salted [6] particles, which would suggest that the proteins remain specifically located on the RNA. However, in our own studies on specific fragments from *E. coli* ribosomes, we have been unable to obtain fragments from either the 30S or 50S sub-particles in the presence of EDTA which satisfy our rigorous criteria for specificity [7,8]. This has led us to suspect that in EDTA the proteins have lost their specific sites on the RNA, and in this paper we demonstrate that this is indeed the case, by examining the ability of the proteins to exchange between different ribosomal RNA's or sub-particles.

2. Materials and methods

Ribosomal sub-particles from *E. coli*, either unlabelled or labelled with [^{14}C] amino acids and [^{3}H] uridine were prepared as described [7]. [^{3}H] 23S RNA or 16S RNA were prepared by phenol extraction of 50S or 30S sub-particles, followed by ethanol precipitation. Sub-particles and RNA were mixed together at 0°C under various conditions (see text and legends to figures), and were then immediately separated by electrophoresis on a 3% polyacrylamide/agarose gel slab, containing 20 mM KCl, 1 mM magnesium acetate, and 25 mM Tris buffer, as described [7]. Samples were run in adjacent 1 cm slots, and electrophoresis was continued until the Bromophenol Blue dye marker had run 6–7 cm. 16 X 2.5 mm slices were cut from each sample strip and counted, after standing overnight in a toluene scintillation fluid containing 8% Soluene (Packard). ('Standard buffer' is 10 mM Tris–HCl pH 7.8, containing 0.3 mM magnesium acetate and 6 mM β-mercapto ethanol).

3. Results and discussion

If in the EDTA particle the proteins are randomly distributed along the RNA, then one would expect the proteins to be exchangeable with a heterologous RNA. To test this idea, we mixed together 30S sub-particles (labelled in both RNA and protein) with labelled 23S RNA. The mixture, with or without added EDTA (5 mM), was then separated on a gel in order to examine the distribution of 30S proteins between the two RNA species. A typical result is shown in fig.1. Figs.1A and 1B show the positions in the gel of the individual 23S and 30S components, in the presence of EDTA. In fig.1C, the two components were mixed together in the absence of EDTA; it can be seen that no significant transfer of protein from the 30S particle to the 23S RNA has occurred. However, when the same mixture was treated with EDTA (fig.1D), it is clear that the proteins become distributed almost equally between the 16S and 23S RNA. Further, a similar

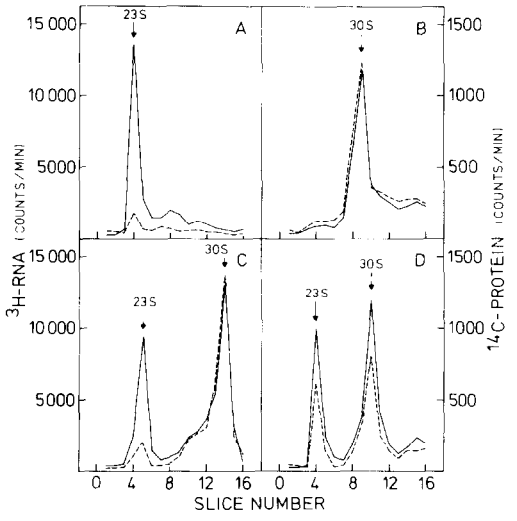

Fig.1. Gel electrophoresis patterns of 30S ribosomal sub-particles mixed with 23S RNA in standard buffer (see Materials and methods), with or without added EDTA (5 mM, disodium salt). Each sample was 20 μl. concentration approx. 50 A_{260} units/ml. A) 23S RNA alone, plus EDTA. B) 30S sub-particles alone, plus EDTA. C) 30S sub-particles mixed with 23S RNA, minus EDTA. D) 30S sub-particles mixed with 23S RNA, plus EDTA. (———) [^{3}H] RNA; (– – – –) [^{14}C] protein.

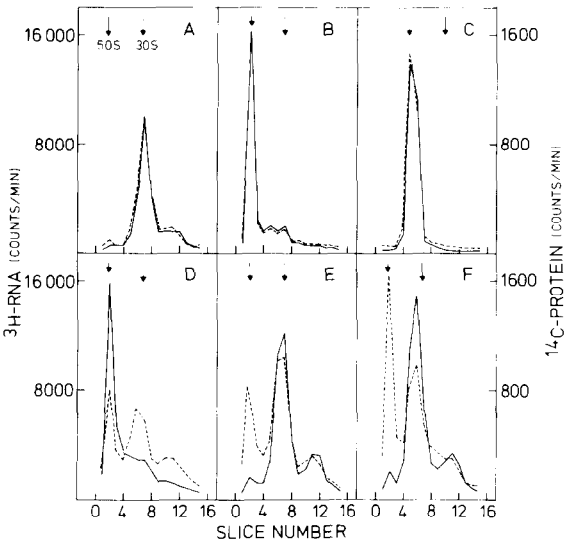

Fig.2. Gel electrophoresis patterns of labelled and unlabelled sub-particle mixtures. Sub-particles were incubated under reconstitution conditions (see text and [10,11]), and then dialysed into standard buffer at concentrations of 30–40 A_{260} units/ml. 10μl aliquots of labelled sub-particle were mixed with varying excess amounts of the complementary unlabelled sub-particle, with or without EDTA, before loading on the gel. A) Labelled 30S alone, plus EDTA (5 mM). B) Labelled 50S alone, plus EDTA. C) Labelled 50S with unlabellled 30S (in 3-fold excess), minus EDTA. D) Labelled 50S with unlabelled 30S (in 20-fold excess), plus EDTA. E) Labelled 30S with unlabelled 50S (in 2-fold excess), plus EDTA. F) Labelled 30S with unlabelled 50S (in 10-fold excess), plus EDTA. (———) [^{3}H] RNA; (– – – –) [^{14}C] protein. In each diagram the two arrows mark the respective positions in the gel (found by staining with methylene blue) of unlabelled 30S and 50S sub-particles, with or without EDTA, as appropriate.

protein transfer was observed when labelled 50S particles were mixed with 16S RNA in EDTA. It should be noted that in the absence of EDTA (fig.1C) the 30S particle has a compact conformation and can therefore be readily separated from 16S RNA (cf. [9]). In this case it was possible to show that (in the absence of EDTA) no significant transfer of protein took place between 30S sub-particles and isolated 16S RNA, (data not shown).

The result of fig.1D shows clearly that in EDTA the proteins are free to move from one RNA strand to another, which in turn implies that they can move freely within the confines of their own RNA strand. This would be proven if it were shown that the proteins could not only move to a heterologous RNA strand, but could also displace (and be displaced by) proteins from another ribonucleoprotein particle in the presence of EDTA. Accordingly, we mixed together labelled 50S particles with unlabelled 30S particles (and vica versa) under various conditions, separated the two components on a gel as in fig.1, and looked for the appearance of labelled protein in the unlabelled sub-particle. Examples from a typical series of experiments are shown in fig.2. In this case both 30S and 50S particles were subjected to a reconstitution incubation (see [10] for 30S, and [11] for 50S) to ensure that the proteins were in their specific locations at the beginning of the experiment. The sub-particles were then dialysed into 'standard buffer' (see Materials and methods) and mixed together in various proportions in the presence or absence of EDTA, before applying to the gel.

Figs.2A and 2B show the positions in the gel of labelled 30S and 50S sub-particles in the presence of EDTA. Fig.2C shows the effect of mixing labelled

50S with unlabelled 30S, in the absence of EDTA. In this case, the sub-particles are not unfolded, and therefore run further into the gel (cf. fig.1C and [9]). No labelled protein was observed in the region of the 30S peak (fig.2C), although the 30S was present in a three-fold excess on an optical density basis; in fact this excess could be increased to twenty-fold without any protein exchange occurring. The converse experiment (labelled 30S with excess unlabelled 50S) showed a similar result. If on the other hand the sub-particles were mixed in the presence of EDTA, with an excess of unlabelled sub-particle anywhere in the range from two- to twenty-fold, an immediate exchange of protein occurred (figs.2D,E,F). In fig.2D, radioactive 50S was mixed with a twenty-fold excess of unlabelled 30S, and here a distinct peak of radioactive protein can be seen, moving in the 30S position (cf. fig.2A). In figs.2E and 2F the converse experiment is shown, with a two-fold and ten-fold excess respectively of unlabelled 50S; this shows that as the proportion of unlabelled 50S is increased, the radioactive 30S proteins are progressively 'chased' onto the unlabelled 50S sub-particle. In all cases (cf. fig.1D), the protein exchange is accompanied by a lowering of the protein: RNA radioactivity ratio in the labelled sub-particle. The effect is difficult to quantitate from these experiments, due to the small amount of breakdown and/or sub-particle interaction which usually occurs. However, it is clear from fig.2 that in the presence of EDTA a substantial interchange of proteins has taken place between the labelled and unlabelled sub-particles.

In order to determine whether some proteins remain specifically bound to the RNA in EDTA, the experiment of fig.2 was repeated (using a five-fold excess of unlabelled sub-particle in each case), and the radioactive proteins in each peak were analysed both on two-dimensional gels [12] and Sarkosyl gels [7] using the methods we have described [13]. Obviously, a 50S protein that remained specifically bound would not be found in the 30S peak (cf. fig.2D), nor a specifically bound 30S protein in the 50S peak (cf. figs. 2E,F). Preliminary results showed that, although there seemed to be some variation in the ease with which the various proteins migrated to the opposite particle, all of the proteins (except for a few minor proteins which were not tested) were able to partake in the exchange. In particular there seemed to be no correlation between ease of exchange and strength of binding to the RNA; e.g. proteins S4, S7 and S20, all of which are RNA-binding proteins [14–16], and similarly proteins L6, L16, L19 and L24 [16], were among those proteins most extensively exchanged.

Further experiments under various conditions of low ionic strength, using the system of analysis of fig.2, showed that the protein exchange occurred concomitantly with unfolding of the sub-particles, as judged by mobility in the gel. The phenomenon is not an artefact of gel electrophoresis, since precisely analogous results were found when the sub-particle mixtures in EDTA were separated in sucrose gradients in 10 mM Tris–HCl pH 7.8, 50 mM KCl, 0.3 mM magnesium acetate (a condition which we routinely use for the separartion of pure ribosomal sub-particles [7]).

Several points can be made concerning these experiments. Firstly, the demonstration of free protein exchange in EDTA inevitably means that some experiments which have been made with EDTA particles lose much of their significance. Secondly, our results can be used to explain some anomalies; e.g. the fact that mamalian ribosomal sub-particles obtained after dissociation by puromycin treatment show distinct protein patterns on two-dimensional gel electrophoresis [17], whereas those prepared by dissociation with EDTA always showed cross-contamination [18]. Thirdly, the protein exchange test as exemplified by fig.2 provides a simple method of determining whether or not a particular experimental condition or separation system is viable for experiments relating to ribosomal topography. Finally, the demonstration of protein exchange in unfolded sub-particles has a bearing on the nature of ribosomal protein-RNA interaction. We have not determined at which stage of unfolding the exchange first occurs (see e.g. [2]), but it is nevertheless already known that, in unfolded particles in EDTA, the secondary structure of the RNA is still largely intact [19,20]. In agreement with this, addition of EDTA to either sub-particle under our experimental conditions produced only a very small hyperchromic effect (0–4%) at 260 nm. Since protein exchange occurs under these conditions, this suggests strongly that the site specificity of the RNA-protein binding is determined mainly by the tertiary structure of the RNA.

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