RNA OF THE SMALL SUBUNIT OF THE *ESCHERICHIA COLI* RIBOSOME WITH ADDITIONAL PROTEIN BINDING SITES

Characterization of the structure by electron microscopy

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1. Introduction

A procedure has previously been described which preserves additional protein binding sites on the RNA of the small ribosomal subunit of *Escherichia coli* [1–3]. Here we refer to this RNA as 16 S RNA**; in contrast to RNA prepared by the traditional phenol–SDS method, which we refer to as 16 S RNA*. The 16 S RNA** has an altered physical configuration as revealed by a reduced mobility on polyacrylamide–agarose electrophoresis [2,3].

Electron microscopy has been used extensively to map the secondary structure of 28 S ribosomal RNA in eukaryotes [4–10] as well as that of the ribosomal RNA precursor [4–8]. In addition, electron microscopy has revealed some secondary structure in the precursor to bacterial ribosomal RNA, but no structure was apparent in the mature 16 S and 23 S molecules [9]. It therefore seemed of interest to determine whether the 16 S RNA** retained any structural features which could be revealed directly by electron microscopy. We found that the 16 S RNA* is a predominantly linear molecule. In dramatic contrast 16 S RNA** forms a more condensed structure with frequent loops. This observation is the subject of this communication.

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2. Materials and methods

Ribosomal subunits from *Escherichia coli* were prepared according to Gordon and Ramjoué [10]. The procedure of Traub et al. [11] was used to prepare 16 S RNA*, and that of Hochkeppel et al. [1,2] for the 16 S RNA**. Isokinetic 5.1–30.2% sucrose gradients in reconstitution buffer (0.03 M tricine, 0.4 M potassium chloride, 0.02 M magnesium acetate, pH 7.4) were prepared for RNA sedimentation analysis. RNA Samples (100 µl) containing 0.2 A_{260} units were loaded on a gradient and centrifuged for 140 min at 56 000 rev/min in an SW56 Beckman rotor at 4°C. The 16 S RNA was spread for electron microscopy by the following modification of the method of Wellauer and Dawid [4]. Samples were incubated in 90% formamide, 1.5 M urea, 5 mM Tris, 1 mM EDTA, pH 8.5, at room temperature for 3 h. After addition of cytochrome *c* (0.1 mg/ml) the samples were spread onto a hypophase of distilled H_2O, picked up on thin carbon films, stained [12] with alcoholic uranyl acetate and rotary shadowed with platinum at an angle of 6°. For denaturation, the RNA were first heated to 63°C, then formaldehyde was added to a final concentration of 3%, and the incubation was continued for 20 min at 63°C. Pictures were taken in a Philips 301 electron microscope. The magnification of the instrument was calibrated with the carbon grating replica (Fullam, 28 000 lines/in).

3. Results

Wellauer and Dawid [4] have described a procedure which reproducibly reveals secondary structure in the 28 S RNA of eukaryotes. The structures from the *E. coli* ribosome which we are seeking is not completely stable [2]. We have therefore used a procedure modified from that of Wellauer and Dawid, giving less denaturation (see Materials and methods). Figure 1a shows a typical field of 16 S RNA prepared by conventional phenol extraction, 16 S RNA*, and fig.1b the acetic acid–urea procedure, 16 S RNA**. The insets show individual molecules at higher magnification.

It can be seen that the 16 S RNA** has a considerable more compact structure than the 16 S RNA*. The statistical significance of this observed difference is confirmed in the histograms of fig.2 (solid bars) and table 1. As the 16 S RNA** showed considerable structure, the exact molecular length was difficult to determine. We therefore adopted the empirical procedure of measurement of end-to-end lengths. The resultant distribution showed that the average length of the 16 S RNA** (fig.2a) was about half that of the 16 S RNA* (fig.2b). The significance was further confirmed by the histogram of a mixture which yielded a bimodal distribution identical to the sum of the separate distributions (inset in fig.2).

That the striking difference in length was not a result of fragmentation or aggregation was demonstrated by the following controls. Both RNA preparations yielded indistinguishable length distributions when fully denatured in the presence of formaldehyde prior to electron microscopy (fig.2, open bars). The length of the denatured molecules (table 1) was consistent with the length determination of Nikolaev et al. [9]. Further, the two preparations yielded the same sedimentation profile under 'reconsitution buffer' conditions (fig.3) confirming earlier results that the sedimentation was

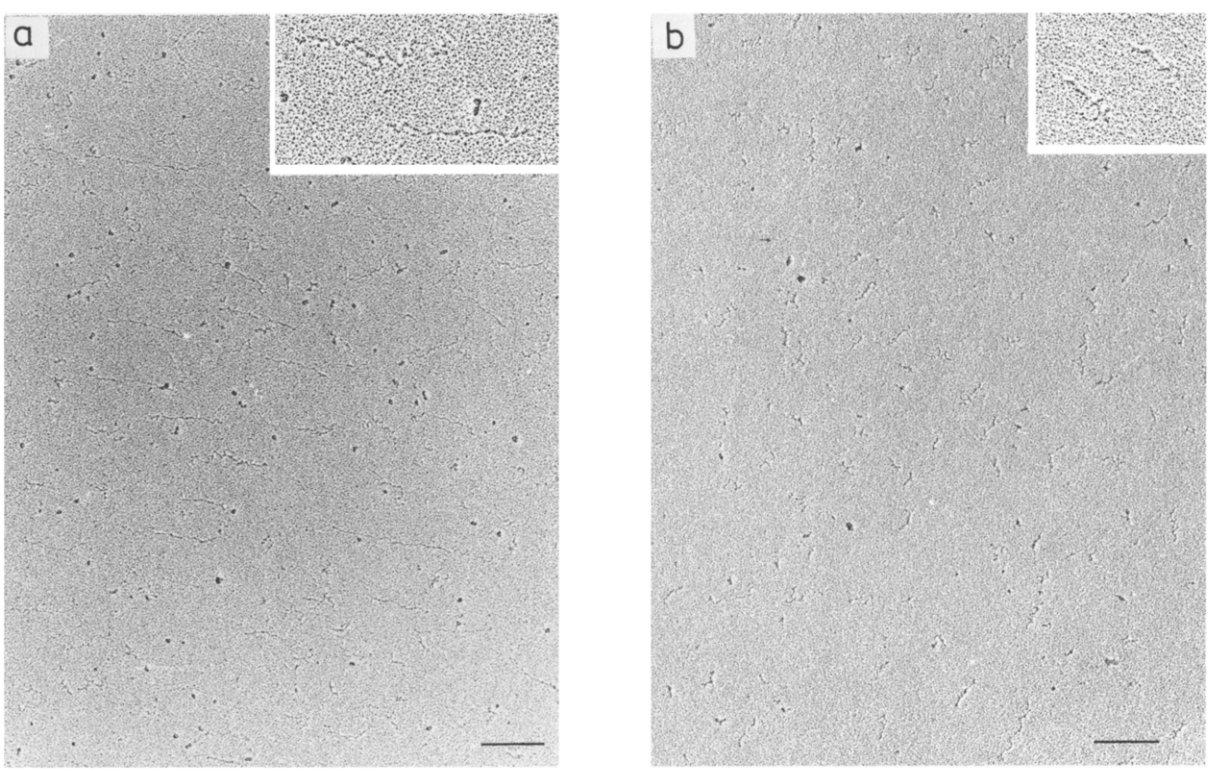

Fig.1. Electron micrograph of (a) 16 S RNA* and (b) 16 S RNA** spread from formamide/urea. Bars indicate the scale and are 0.25 μm. Insets: individual molecules enlarged a further two times.

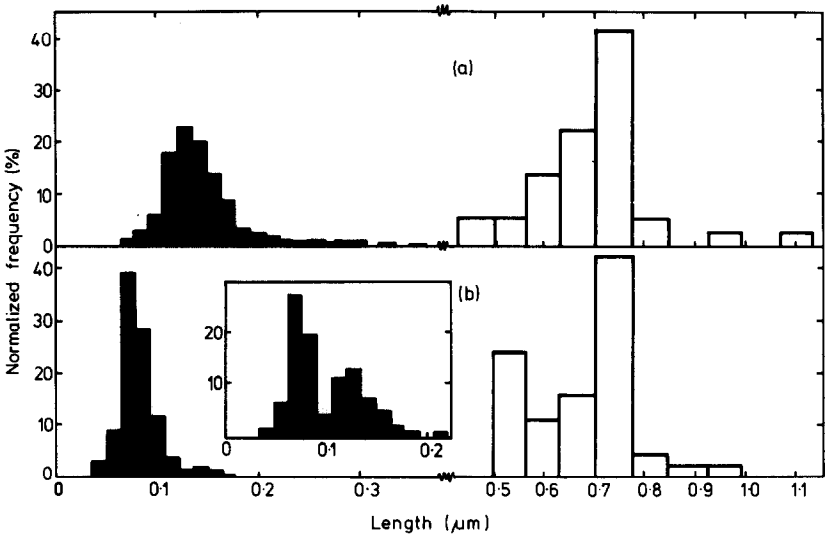

Fig.2. Histograms of the length distributions of various RNA preparations. Length was taken as linear distance from one end of a molecule to the other. In the case of ambiguity due to branching, the longest value was taken. (a) 16 S RNA*, (b) 16 S RNA**. Insert (one): one mixture of 16 S RNA* and 16 S RNA**. Solid bars: standard spreading. Open bars: first denatured in formaldehyde. Frequency was expressed as a percent of the total number of molecules measured. Measured were: 325 molecules for the 16 S RNA*, 309 for the 16 S RNA**, 370 for the mixture, 36 for the denatured 16 S RNA* and 45 for the denatured 16 S RNA**.

not greatly changed [1]. This was also found under denaturing conditions in the presence of SDS (data not shown).

4. Discussion

The pictures shown here demonstrate that 16 S RNA** has a more compact structure than 16 S RNA*. Elsewhere, it had been reported that the 16 S RNA** had a more extended structure, based on the reduced electrophoretic mobility [2,3]. We propose that the explanation of this apparent inconsistency lies in the rigidity of the structure: the 16 S RNA* exists in a more flexible form which extends readily under the denaturing conditions of the spreading technique, but is in a form closer to a compact sphere in solution. In fact, electron microscopy of 16 S RNA with other techniques has shown the presence of such balls [13]. The 16 S RNA**, on the other hand, may be more rigid due to more extensive hydrogen bonding.

It should be pointed out that we do not see a unique pattern of secondary or tertiary structure in the 16 S RNA**. There seem to be a finite number of possible

Table 1

Preparation	Mean length (μm)		Extension factor
	− HCHO	+ HCHO	
16 S RNA*	0.153 ± 0.042	0.70 ± 0.12	4.6:1
16 S RNA**	0.082 ± 0.021	0.68 ± 0.11	8.3:1

Average end-to-end lengths of different preparations. The data is derived from the histograms of fig.1 and is expressed as mean ± *SD*. The extension factor is defined as the ratio of the mean length after formaldehyde treatment to that with no treatment.

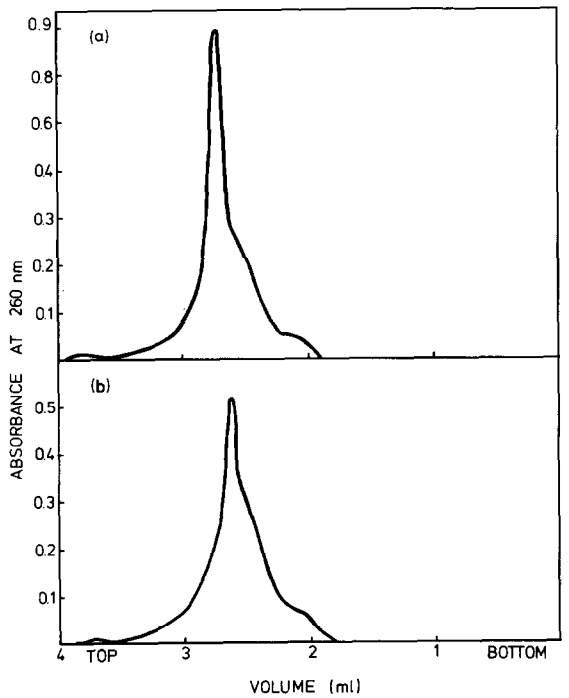

Fig.3. Isokinetic sucrose gradients of (a) 16 S RNA*, (b) 16 S RNA**. Conditions are as described in Materials and methods.

arrangements. We have not yet attempted to catalogue all possible structures, especially as this is on the limit of the resolution.

The increase in end-to-end length due to denaturation seen in fig.2 and table 1 can be used as an empirical index of the amount of secondary and tertiary structure. This index is referred to as the 'extension factor' in the table. One can in fact compute the increase in length expected from the secondary structure predicted from the primary structure by Ehresmann et al. [14]. The theoretical extension factor of 6.0 : 1 thus obtained is intermediate between our values given in table 1. This leads one to speculate that a high proportion of the secondary structure predicted from the sequence remains in the 16 S RNA* under the spreading conditions, while these and additional longer range tertiary interactions, not predicted from the primary structure, exist in the 16 S RNA**. Most of the secondary structure loops would be below the limit of resolution of the spreading technique. The secondary structure interaction would still result in the physical shortening of the molecules seen in the 16 S RNA*, and tertiary interactions would result in the even further shortening seen in the 16 S RNA**.

In summary, the electron microscopy confirms the existence of an altered physical arrangement in the 16 S RNA** which correlates with the additional protein binding sites. The procedure also shows a previously unrecognized potential for the study of structure which is on or below the limit the resolution of the electron microscopy technique.

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