CONFORMATIONAL CHANGES IN ESCHERICHIA COLI RIBOSOMAL RIBONUCLEIC ACID

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SUMMARY. The effects of isolated 30S ribosomal proteins on the tertiary structure of Escherichia coli 16S ribosomal RNA were investigated using light scattering, ultraviolet difference spectroscopy, and sedimentation velocity techniques. Ribosomal proteins S4, S7, S8 and S20, which bound to the RNA, also caused a conformational change which resulted in a decreased light scattering and reduced absorption at 270 nm by 16S RNA. In contrast to these results, little or no difference was detected in the sedimentation velocity of 16S RNA-protein complexes compared to the sedimentation of protein-free RNA.

The importance of the secondary and tertiary structure of ribosomal RNA in both the biosynthesis and functioning of the ribosome has been recently emphasized (1). The direct interaction of certain ribosomal proteins with ribosomal RNA has been demonstrated to be critical in the biogenesis of the subunits (2). At least seven different proteins from the 30S subunit have been identified as binding specifically to 16S ribosomal RNA in stoichiometric amounts (3). Complex formation between proteins and RNA has been detected by sedimentation, gel filtration and electrophoretic methods (4,5,6). We have investigated the methods of light scattering and ultraviolet difference spectroscopy and have shown that these can be used as sensitive assays to examine 30S ribosomal protein-mediated changes in the structure of 16S ribosomal RNA.

MATERIALS AND METHODS

Preparation of Ribosomal Subunits and RNA. Ribosomes were isolated as previously described (7) from E. coli D10 cells (8) grown in nutrient broth and harvested at 2/3 log phase. Ribosomal subunits were separated by zonal centrifugation through a 7.4-34% sucrose gradient (9). Ribosomal RNA was extracted from cell lysates with phenol and sodium dodecyl sulfate as described (10). RNA species were fractionated on sucrose gradients (10) and were examined for homogeniety by analytical sucrose gradient centrifugation (11) and by polyacrylamide gel electrophoresis (6).

Ribosomal Protein Preparation. Ribosomal proteins were extracted from 30S subunits by the LiCl-urea procedure and were fractionated by the phosphocellulose column method of Hardy et al. (12). The 30S proteins were identified by polyacrylamide gel electrophoresis of column fractions (10% gel run at pH 4.5) in a 6M urea buffer (12).

Light Scattering. Light scattering measurements were conducted in a Farrand Spectrofluorometer equipped with a Heathkit linear recorder. The procedure followed was a modification of the method of Zitromer and Flaks (13). The excitation and emission monochromators were set to 436 nm. A sample volume of 2 ml was used in a 4 ml cuvette and readings were made at an angle of 90° with all slits set at 10 nm. The temperature was regulated to ± 1° by an electrical resistor on the base of the cuvette holder.

For all experiments, 16S ribosomal RNA was dissolved in TMK buffer (0.01 M Tris-HCl, pH 7.6, 0.02 M $MgCl_2$, 0.35 M KCl). After several minutes at 40° for equilibration, ribosomal protein was generally added in a two- to three-fold molar excess. Between 2 and 7 determinations were made with each protein. The initial light scattering was assigned a relative value of 10, and subsequent measurements were normalized to the initial condition and corrected for the amount of RNA used in the experiment. All buffer solutions were sterilized and filtered through a 0.45 μ Millipore filter (HAWP) to reduce nuclease contamination and spurious scattering from dust particles.

Ultraviolet Difference Spectroscopy. A Beckman model 25 recording spectrophotometer was used at 270 nm. In all ultraviolet difference absorption experiments, the sample cuvette received the protein and the reference cuvette an equal volume of buffer. Absorbance changes were followed during an incubation at 37°, on a recorder attached to the spectrophotometer.

Sedimentation Measurements. The Beckman model E Analytical Ultracentrifuge was equipped with a 4 cell mask and ultraviolet absorption optics. Boundary sedimentation was performed at 48,000 rpm in a AN-H rotor at 20°. In sedimentation determinations, the RNA-protein mixture was allowed to incubate for 45 minutes at 40° prior to centrifugation in the analytical ultracentrifuge at 20°. For these studies a protein-free sample of 16S RNA was incubated and sedimented with each set of three experimental samples. Sedimentation coefficients were calculated according to the method outlined by Chervenka (14).

Detection of Binding. RNA-protein complex formation was determined by the gel electrophoresis method of Garrett et al (6).

RESULTS

Ribosomal Protein Mediated Conformational Changes. Purified 30S ribosomal proteins were examined for their effect on the tertiary structure of 16S ribosomal RNA by the light scattering method. Ribosomal proteins which interacted directly with the RNA produced significant changes in the RNA structure upon binding. A typical light scattering recording of a 16S RNA-30S ribosomal protein (S4) binding experiment is indicated in Figure 1A. The incubation period (50 minutes) was marked by a substantial decrease in RNA light scattering.

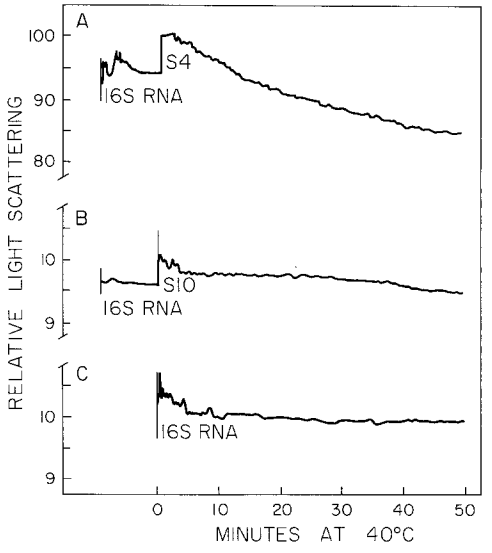

Figure 1. Relative light scattering recording of 16S RNA incubated at 40° with and without ribosomal proteins. A sample of 7.5 A_{260} units/ml of RNA (0.63 nmoles) was dissolved in TMK buffer in the spectrofluorometer cuvette. At time 0 an excess of ribosomal protein was added and change in relative scattering was monitored for the time indicated on the recorder tracing.

The incubation of a non-binding protein (S10) with 16S RNA (Figure 1B) gave a very small change in light scattering. Incubation of RNA alone (Figure 1C) produced no significant change in light scattering over the period observed.

The effect of nine different 30S ribosomal proteins on the structure of 16S RNA was examined by this method. Several determinations were made with each protein at molar ratios of protein to RNA between 0.5 and 8.0. Representative results of these determinations are listed in Table 1. The largest structural changes in the RNA were mediated by the ribosomal RNA-binding proteins S4, S7 and S20. Protein S8 also bound to 16S RNA but promoted a minimal light scattering change. Relatively limited conformational changes were also produced by five additional proteins which demonstrated no RNA binding. For the binding proteins, the magnitude of the light scattering change was dependent upon the ratio of protein to RNA. For the four binding proteins the half-maximal change in scattering occurred in about 15 minutes at 40°.

TABLE I: Summary of 16S TNA-30S Ribosomal Protein Binding Experiments

30S Ribosomal Protein	Protein Binding by Polyacrylamide Gel Analysis	Relative RNA Light Scattering Change per hour	Ultraviolet Absorption Change at 270 nm per 10 min	Sedimentation Constant ($S_{20,w}$)
S4*	+	15.0 (2.4)†	0.040 (0.3)†	23.2 (2.4)†
S20*	+	4.0 (2.7)	0.060 (0.4)	23.5 (1.9)
S7*	+	3.8 (1.0)	0.050 (2.0)	21.8 (1.1)
S8*	+	0.8 (2.9)	0.010(0.95)	20.7 (3.3)
S9 + S11	-	1.3 (1.3)	0.025 (1.8)	20.4 (1.0)
S10	-	1.0 (3.9)	-	22.2 (1.9)
S6	-	0.8 (2.6)	0.025 (8.2)	21.5 (2.9)
S1	-	0.1 (0.7)	0.000 (0.4)	20.9 (0.4)
RNA alone	-	0.4	0.005	21.4

*16S RNA binding proteins identified by Schaup et al. (5) and Garrett et al. (6).

†Molar ratio of protein to RNA in parentheses.

The rate of conformational alteration may not be the same as the rate of protein binding (see Discussion).

Ultraviolet difference spectroscopy also proved to be a sensitive technique for monitoring RNA-protein interactions. A ultraviolet difference recording is shown in Figure 2A for an RNA sample incubated with a binding protein (S4) and in Figure 2B with a non-binding protein (S6). Figure 2C shows the control recording with RNA incubated alone. The protein-mediated binding resulted in a decrease in the absorbance at 270 nm of the RNA sample. Table 1 indicates the magnitude of the absorbance changes for the binding and non-binding proteins.

An analysis of complex formation between various proteins and the 16S RNA was also attempted by looking for alterations in sedimentation values of RNA containing bound protein, relative to an unreacted control. Numerous analyses of RNA samples in the analytical ultracentrifuge showed little or no detectable

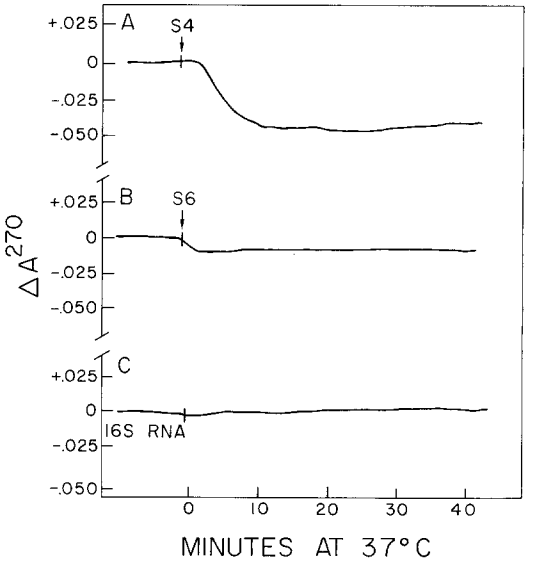

Figure 2. Ultraviolet difference spectroscopy of 16S RNA incubated at 37° with and without ribosomal proteins. A sample of RNA (1.1 A_{260} units/ml 0.09 nmole) in TMK buffer was divided between the reference and sample cuvettes of the spectrophotometer. At time 0 equivalent volumes of buffer and ribosomal protein solution were added to the reference and sample cuvettes respectively, and the change in absorbance at 270 nm was recorded.

change in the sedimentation values observed, and with the exceptions of proteins S4 and S20, the differences observed are within the experimental error (+5%) for the determination of the sedimentation coefficient. Protein-free 16S RNA in TMK buffer had a sedimentation coefficient of 21.4S in very good agreement with previous studies (15).

DISCUSSION

Several previous studies have indicated that ribosomal proteins were capable of altering ribosomal RNA structure. Miall and Walker (16) calculated that the effective hydrodynamic volume of protein-free 16S RNA was 2.1 times the volume occupied by the RNA within the 30S subunit. They proposed that ribosomal proteins folded the RNA into its more compact structure during ribomaturation. Studies by Sypherd (11) showed that heat-denatured 16S RNA could be renatured by incubation with unfractionated 30S ribosomal proteins. More recently, Schulte et al. (15,17), have examined the binding requirements of three ribosomal proteins to the RNA and have concluded that conformational changes in the RNA (and possibly in the proteins) occurred as a result of the binding interaction. Their work also indicated that bound ribosomal proteins conferred only negligible changes in the sedimentation velocity of 16S RNA.

Those proteins previously identified as forming site-specific complexes with 16S RNA (4,5,6) were capable of mediating the greatest conformational changes in the RNA. Proteins S4, S7 and S20 consistently produced the largest changes in the 16S RNA tertiary structure. Proteins S15 and S17, which have also been identified as RNA binding (18) were not examined. The greatest folding of the RNA as determined by the reduction in light scattering was caused by protein S4. This protein has been identified as interacting with as much as 1/3 of the structure of the 16S ribosomal RNA (19) and might be expected to produce a maximal folding of the RNA. Conversely, a consistently small change in light scattering was produced by protein S8 whose RNA binding site may consist of only about 40 nucleotides (19).

The kinetics of the light scattering change for the binding proteins

indicate a half-time for the conformational alteration of about 15 minutes. This time may be compared with a period of 7-8 minutes for half-maximal binding of protein S8 to the RNA under the same conditions, as estimated from the data of Schulte and Garrett (17). It is likely that binding of ribosomal proteins to the RNA is a more rapid process than the overall tertiary structural changes which need not occur simultaneously with binding.

The data presented indicates that certain ribosomal proteins mediated a change in the conformation of a 16S ribosomal RNA molecule. This change is significant in that it may mimic the tertiary structure modifications of 16S RNA during ribosome biosynthesis. Zimmermann (19) and Kurland (20) have recently suggested that the process of subunit assembly may rely heavily upon these specific interactions to promote the formation of a functional ribosome.

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