Alteration of 5S RNA conformation by ribosomal proteins L18 and L25

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ABSTRACT

The effects of ribosomal proteins L18, L25 and L5 on the conformation of 5S RNA have been studied by circular dichroism and temperature dependent ultraviolet absorbance. The circular dichroism spectrum of native 5S RNA is characterized in the near ultraviolet by a large positive band at 267 nm and a small negative band at 298 nm. The greatest perturbation in the spectrum was produced by protein L18 which induced a 20% increase in the 267 nm band and no change in the 298 nm band. By contrast, protein L25 caused a small decrease in both bands. No effect was observed with protein L5. Simultaneous binding of proteins L18 and L25 resulted in CD changes equivalent to the sum of their independent effects. The UV absorbance thermal denaturation profile of the 5S RNA-L18 complex lacked the pre-melting behavior characteristic of 5S RNA. Protein L25 had no effect on the 5S RNA melting profile. We concluded that protein L18 increases the secondary, and possibly the tertiary structure of 5S RNA, and exerts a minor stabilizing effect on its conformation while protein L25 causes a small decrease in 5S RNA secondary structure. The implications of these findings for ribosome assembly and function are discussed.

INTRODUCTION

Ribosomal 5S RNA from Escherichia coli interacts directly, and strongly, with ribosomal proteins L18 and L25 (1). A third protein, L5 may bind weakly to the 5S RNA (2), and it assembles with 5S RNA in the presence of proteins L18 and L25 (3). In the presence of these three proteins, 5S RNA binds specifically to 23S RNA (4). It occurred to us that this system might serve as a microcosm for the ribosome for which such cooperative effects have been detected in 30S subunit assembly in vitro (5) and in protein binding to 16S- and 23S RNA's (6-8).

Ribonuclease digestion studies on the protein-5S RNA complexes (1) have demonstrated that one region of the RNA from nucleotides 69-110, and the stem region from nucleotides 1-10 and 110-120 are preferentially stabilized by the proteins. It was concluded that primary attachment sites of the highly
elongated proteins (9) occur within this region. In a recent fluorescence study, Feunteun et al. (10) demonstrated cooperative effects in the binding of the proteins to 5S RNA and suggested, although no direct evidence was provided, that these might result from minor structural changes in the RNA produced by protein L18. In this paper, we present direct evidence for a protein-induced conformational change in 5S RNA using circular dichroism and temperature-dependent ultraviolet absorbance.

MATERIALS AND METHODS

Buffers: Buffer A: 20 mM MgCl$_2$, 0.3 M KCl, 20 mM Na cacodylate, pH 7.4. Buffer B: 20 mM MgCl$_2$, 0.3 M KCl, 30 mM Tris·HCl, pH 7.4.

(a) Preparation of 5S RNA

5S RNA was prepared from 50 S subunits by the general procedure outlined by Monier and Feunteun (11). Ribosomes were washed in 0.5 M NH$_4$Cl to remove tRNA and the salt precipitation procedure was used to remove the majority of the 23S RNA. The 5S RNA was concentrated with either Ficoll 400 (Pharmacia) or by ultrafiltration with a UM 2 filter (Amicon) prior to Sephadex G75 gel filtration.

Two criteria were employed to test the nativity of the 5S RNA: (i) electrophoretic mobility and (ii) binding capacity. It migrated as a single band on a 12.5% polyacrylamide gel in 40 mM Tris·HCl, pH 7.4, as described by Aubert et al. (12), and it has the capacity to form specific complexes with proteins L18 and L25 (13).

(b) Preparation of ribosomal proteins

Proteins L5, L18 and L25 were fractionated either from proteins that dissociate from the 50S subunit in 2 M LiCl (4) or from total 50S subunit proteins by CM-cellulose chromatography and Sephadex G100 gel filtration (14). They were lyophilized and stored in water at -30°. L5 was relatively insoluble in water, and was therefore stored in 8M urea at -30°. Protein concentrations were determined by the method of Lowry et al. (15).
(c) **CD spectra of 5S RNA and protein-5S RNA complexes**

CD spectra were recorded using a Durrum-Jasco J-20 spectropolarimeter calibrated with camphorsulfonic acid $d_{10}$ (16). Solutions of 5S RNA were made up in sterile, Millipore filtered buffer A at a concentration of 45 µg/ml and CD spectra were recorded in a 1 cm pathlength cylindrical cell, in a volume of 0.55 ml. Maximum absorbance did not exceed 1.2. The temperature of the sample block was controlled by a circulating water bath to ± 1°C.

Protein complexes with the RNA were formed by pipetting a 3-fold molar excess of the particular protein from concentrated stock solutions (5-10 mg/ml) directly into the cell. CD spectra were recorded after equilibration to the temperature of the sample holder. In certain experiments, the protein-nucleic acid mixture was then incubated, in parallel with a control sample of 5S RNA for 20 minutes at 33°C as described by Feunteun et al. (10). The samples were allowed to re-equilibrate to the experimental temperature and CD and UV spectra were again recorded.

In order to control for possible aggregation of the complexes, gel filtration under the ionic and concentration conditions of the experiments was performed. Samples containing 0.6 mg of each complex in 1 ml of buffer A were run on a 1 x 80 cm Sephadex G-100 column. In each case, only a single peak eluted corresponding to the monomeric protein-nucleic complex and we concluded that the complexes did not aggregate. It has previously been shown that free native 5S RNA does not aggregate under these conditions (17, 18).

CD melting experiments with 5S RNA and 5S RNA·L18 complexes were carried out by preparing solutions of 5S RNA and protein-RNA complexes as indicated above, but without incubation at 33°C. The temperature was monitored in a cuvette filled with buffer placed directly behind the sample cell to avoid contamination as well as evaporation of the sample at high temperatures. Once the sample had reached the desired temperature, the spectra were recorded and the temperature re-checked.
No protein optical activity contributions were observed at wavelengths greater than 250 nm. All spectra were recorded at least twice. The mean molar residue ellipticities were calculated assuming a mean residue weight of 321 (based on 120 residues per 5S RNA chain).

(d) Hyperchromicity measurements

Native 5S RNA (48 μg) was dissolved in 0.2 ml buffer A, and a 3-fold molar excess of the appropriate protein was added. The solution was incubated for 30 min at 33°, cooled slowly and diluted to 3 ml. Ultraviolet absorbance measurements were made on a Beckman ACTA V spectrophotometer equipped with an Auto sampler accessory. Temperature was monitored by a platinum resistance thermometer inserted into a dummy cuvette. Absorbance temperature measurements were made every 25 sec. The rate of heating was 0.02°C per sec.

RESULTS

(a) CD spectral changes in 5S RNA

The CD spectra of native 5S RNA at increasing temperatures is shown in Fig. 1. At 5°C 5S RNA exhibits a band of positive rotational strength at 267 nm, and two negative bands, at 235 nm and 298 nm, respectively. CD bands close to these wavelengths are characteristic of most ribosomal RNAs (19) and many t-RNAs (20). As the temperature is increased from 9° to 68° there is a regular decrease in the absolute magnitude of the 298 nm band (Fig. 1). In contrast, little change was seen in the 267 nm band below temperatures of about 50°. In all cases, changes in the ellipticity were accompanied by a red shift.

Quick cooling of the sample to 22°C after heating to 68°C produced a CD spectrum corresponding to that of the stable denatured form generated by urea-EDTA treatment (21). Although the conformation of the RNA prepared by both of these procedures is assumed to be approximately the same, slight topographical differences have been detected (12). In this spectrum (not shown) the 298 nm band is absent and the molar ellipticity of the 267 nm band is increased by 13 per cent over that observed for the native structure at 22°. Slow
Figure 1. The effect of temperature on the CD spectrum of 5S RNA. Spectra were recorded using a single sample of 5S RNA in buffer A at the indicated temperatures as described in Materials and Methods. [θ] is the mean molar residue ellipticity. Relative uncertainty is indicated by a bar at the right.

cooling (68° to 22° in 2 hours) of the RNA resulted in a CD spectrum identical to that of the native form.

(b) CD spectral changes in 5S RNA induced by L18

Addition of ribosomal protein L18 to 5S RNA in 3-fold molar excess at temperatures between 4°C and 22°C produces a 20 percent increase in the 267 nm band but only marginal changes in the 298 nm band (Fig. 2). Titration of 5S RNA with protein L18, monitored by the change in ellipticity at 267 nm (T = 6°C), is shown in Fig. 3. The binding isotherm is linear, and saturation occurs at a protein:RNA ratio of approximately 3.

UV absorption spectra of the 5S RNA before and after protein binding showed little change, in agreement with earlier
Figure 2. The effect of ribosomal protein L18 on the CD spectrum of 5S RNA. A 3-fold molar ratio of protein was added at 4-6°C to obtain the 5S RNA + L18 spectrum in buffer A. Experimental details are described in Materials and Methods.

work (10). Addition of protein L18 induced a very slight increase in the absorbance (2.8% or less) and a shift in the maximum wavelength from 256.5 and 257.5 nm. We conclude that the perturbations in the CD spectrum generated by L18 cannot be attributed to changes in optical density, but must be due to a conformational rearrangement of the 5S RNA.

Alteration of the CD spectrum of the 5S•L18 complex as a function of temperature is shown in Fig. 4. Differences in the temperature-dependent perturbation of 5S RNA and the 5S RNA•L18 complex are most clearly seen in Fig. 5 where the ellipticity values from Figs. 1 and 4 are shown as a function of temperature for both the 267 and 298 nm maxima. The effect of L18 binding can be summarized as follows (cf. Fig. 5).
Figure 3. The saturation binding of L18 to 5S RNA. The near UV maximum mean molar residue ellipticity is shown as a function of moles of protein L18 added per mole of 5S RNA. Conditions are the same as in Fig. 2.

(i) In the absence of L18, the 298 nm band is strongly diminished at temperatures 30° to 40° lower than observed for the disappearance of the 267 nm band. (ii) Binding of L18 to 5S RNA significantly raises the temperature of disappearance of the 298 nm band. (iii) In the absence of protein L18, there is little change in the 267 nm band below temperatures of 50°C. (iv) In contrast, the ellipticity of the 267 nm band of the 5S RNA·L18 complex at 49° is decreased to a value close to that of free 5S RNA. (v) Cooperativity in the temperature-dependence curves appears more pronounced in the presence of L18.

The 70° spectrum of the 5S RNA·L18 complex must be interpreted with caution, due to the formation of a fine precipitate at temperatures above 65°. Absorbance measurements at 320 nm indicate that significant light scattering occurs under these conditions.

(c) The effect of protein L25 on the CD spectrum of 5S RNA

When CD measurements of the 5S RNA·L25 complex were performed as described for the 5S RNA·L18 complex, variable, time-
dependent changes were observed. We attribute this to the fact that L25 binds less strongly to 5S RNA than does L18 (R. Zimmermann, personal communication; we have confirmed this finding, using the electrophoretic binding assay). Under our binding conditions, 5S RNA•L25 complex formation was found to be highly concentration dependent. Formation of the 5S RNA•L25 complex was therefore carried out at high concentrations of RNA and protein, followed by brief centrifugation to remove any precipitated material, and dilution to standard concentrations (Materials and Methods) immediately before the spectra were taken. Under these conditions, spectra of the 5S RNA•L25 complex were no longer time-dependent.

An example of a CD spectrum of the 5S RNA•L25 complex, obtained in this manner, is shown in Fig. 6. In contrast to
Figure 5. The change in maximum mean molar residue ellipticity for 5S RNA and the 5S RNA±L18 complex as a function of temperature at (a) ca. 267 nm and (b) ca. 298 nm. Ellipticity values are from the experiments shown in Figs. 1 and 4. Note the differences in sign between ellipticity values in (a) and (b).

L18, L25 produced a 4% decrease in the 267 nm band, as well as a significant decrease in the 298 nm band.

(d) The effect of protein L5 on the CD spectrum of 5S RNA
The study of protein L5 was difficult because of its very low solubility in the buffer. Addition to a solution of 5S RNA invariably produced a fine precipitate. Upon centrifugation of the L5-5S RNA solution the CD and UV spectra were taken of the supernatant and were identical to 5S RNA spectra. While this could be interpreted as either a lack of binding or a lack of conformational change, electrophoretic binding studies did show that some binding occurs. The technique of concentrated mixing used for the L25 experiments did not make any difference.

(e) The 5S RNA±L18±L25 complex
Fig. 6 shows the effect of addition of L18 to the 5S RNA±L25 complex. As in the case of the 5S RNA±L18 complex, L18 induces an increase in the ellipticity at 267 nm, resulting in a spectrum which corresponds roughly to a linear combination of the effects of L18 and L25 alone. The magnitude of the 298 nm band of the 5S RNA±L18±L25 complex is midway between that of the 5S RNA±L18 and 5S RNA±L25 complexes (Fig. 6).
Figure 6. CD spectra of 5S RNA and its complexes with proteins L18 and L25. L25 was pre-incubated with 5S RNA (10:1 molar ratio) at 33°C in buffer B and diluted prior to taking the spectrum as described in the text. Subsequently, L18 was added (3:1 molar ratio). A spectrum of 5S RNA•L18 complex at identical concentrations is shown for comparison.

(f) CD spectra of denatured 5S RNA in the presence of ribosomal proteins

The denatured form of 5S RNA was prepared according to Scott et al. (21). Addition of the ribosomal proteins in the order L18, L25, L5, each in 3-fold molar excess, with a spectrum taken after each addition, and centrifugation after the addition of L5, showed no change in the CD spectrum relative to free 5S RNA.

(g) UV thermal denaturation profile of 5S RNA and 5S RNA-ribosomal protein complexes

The thermal denaturation profile of 5S RNA under our buffer conditions shows a pre-melting absorbance change and
possibly a biphasic profile with the first phase occurring between 35 and about 50° (see Fig. 7). A similar result was obtained by Scott et al. (21) in the absence of magnesium ions. The thermal denaturation profile of the 5S RNA•L18 complex lacks any premelting behavior (Fig. 7). Assuming an approximate hyperchromicity for both 5S RNA (21, 22) and 5S RNA•L18 complex of 20 percent, we obtain $T_m$ values of 69°C and 72°C respectively. The 5S RNA•L25 complex gave a thermal denaturation profile that was superimposable upon the 5S RNA profile. The 5S RNA•L18•L25 complex melted the same as 5S RNA•L18 (not shown). It was impossible to measure the entire thermal

![Figure 7. The thermal denaturation profile of 5S RNA and 5S RNA + L18. The absorbance at 260 nm of 5S RNA and its complex with L18 were measured in buffer A as described in Materials and Methods.](image-url)
denaturation profile due to the high $T_m$ of the 5S RNA in our buffer system, and the fact that L18 and L5 start to precipitate above 65°.

**DISCUSSION**

One of the most intriguing questions confronting workers in this field is why the ribosome is composed of RNA and proteins, both of which are apparently essential for structure and function. Underlying this question is the possibility that proper functioning of the ribosome depends on cooperative interactions between ribosomal proteins and rRNA in addition to the conventional notion that these two moieties function independently in various aspects of the translational process (23). A further possibility is that protein-induced conformational changes of the rRNA are important in ribosome assembly. It is difficult to test these ideas in the complete ribosomal system, because (i) little net difference in secondary structure has been detected between free rRNA and rRNA assembled in the ribosome (24) and (ii) the effect of a single protein on total rRNA conformation is probably not easily measurable. Fortunately, the 5S RNA•L18•L25 complex provides a model system for the study of ribosomal protein-rRNA interactions. The net perturbation of secondary structure of 5S RNA by L18 and L25 can be readily detected because of the relatively small size of 5S RNA.

We have presented here direct evidence for a conformational change in 5S ribosomal RNA caused by the binding of a ribosomal protein. The most dramatic effect is due to protein L18, which causes a large increase in the 267 nm circular dichroism band. The magnitude of the molar ellipticity of a nucleic acid in the near UV is usually a good measure of the extent of secondary structure. We interpret the effect of L18 as an increase in the secondary, and possibly the tertiary structure of 5S RNA. We cannot exclude the possibility that part of the observed increase originates from electronic effects due to interactions between bases and amino acid side chains. The 5S RNA molecule appears to be slightly stabilized against thermal denaturation by bound L18 (Fig. 7). In the absence of protein L18, 5S RNA exhibits a pre-melting behavior.
below 50°. This pre-melting is absent in the 5S RNA–L18 complex, suggesting that some heat-labile feature of the 5S RNA structure is stabilized by protein L18. The fact that L18 does not decrease the CD of 5S RNA implies the stabilization of nucleic acid structure responsible for the pre-melting behavior rather than the destruction of such structure.

Protein L25 has a more subtle effect on the structure of 5S RNA. The CD spectrum shows a small decrease at 267 nm, and a decrease in the 298 nm band. Thus, the effect of L25 is to cause a small decrease in the 5S RNA secondary structure, in contrast to the effect of L18. High concentrations of protein and RNA were required to observe this effect. This is probably due to the lower binding constant of protein L25 (R. A. Zimmermann, personal communication). The effect of simultaneously binding of L18 and L25 gave an additive, rather than a cooperative result. We conclude that proteins L18 and L25 exert their effects on the 5S RNA structure independent of each other. Tritton and Crothers (25), in a CD study on the binding of protein L24 to its 23S RNA binding site, observed a small decrease in both the 265 nm and 295 nm ellipticities, similar to the effect we have observed for L25 and 5S RNA. Although protein L5 has also been observed to bind to complexes involving 5S RNA (2), we did not observe any effect of L5 on the CD spectrum of 5S RNA.

Bands of negative rotational strength in the 290-300 nm region have been observed in 5S and other ribosomal RNAs (19, 21, 26) and in tRNAs (20). As far as we are aware, the origin of these bands is unknown. The results presented here show that changes in the 267 nm band can be produced without concomitant alteration of the 298 nm band, demonstrating that these two bands originate from independent structural features. It is also interesting that protein L25 causes a perturbation of the 298 nm band, while protein L18 does not. Elucidation of the structural basis of the 298 nm CD band would be of significant help in the correlation of CD spectra with specific conformational features of RNA molecules.

No perturbations of the CD spectrum of denatured 5S RNA were observed upon addition of ribosomal proteins. This result correlates well with the observation that specific protein
binding is absolutely dependent on the native conformation of 5S RNA (12).

Our findings correlate well with much of the work of Feunteun et al. (10). In their study they showed that while L18 decreases the number of ethidium bromide binding sites on 5S RNA from 5 to 2, L25 does not appear to have any effect on ethidium bromide binding. One could conclude from their study that either L18 binds at the same sites as ethidium bromide, causing direct displacement, or that L18 perturbs the structure, causing the release of ethidium by binding at some region of the 5S RNA other than the ethidium binding sites. Our study does not conclusively support either of these possibilities, but does tell us that an increase in structure probably accompanies the L18 binding. Unless the ethidium sites are disordered at the same time that the L18 site is ordered, direct displacement by L18 seems a more favorable model. While L25 by itself does not decrease the number of ethidium bromide molecules bound to 5S RNA, the number of sites for 5S RNA·L18·L25 is reported as 1.5 rather than 2 as for 5S RNA·L18 (10). Depending on whether one considers this change significant, cooperativity between L18 and L25 may be inferred. Our study does not show any cooperative effect between L18 and L25 on the CD spectrum of 5S RNA.

The biological significance of these findings is not yet clear. Alterations of rRNA structure by ribosomal proteins may be an essential feature of ribosome assembly, in which an RNA molecule may be held in a metastable morphopoietic conformation to facilitate its interaction with another ribosomal component. Indirect evidence for such a mechanism in the assembly of the 30S subunit has been presented (27). Another way in which one might interpret these findings is that ribosomal protein-induced conformational changes in rRNA may be an essential feature of ribosome function. For example, it has previously been suggested that ribosomal protein S1, a protein capable of perturbation of nucleic acid secondary structure, may function by altering the conformation of 16S RNA (28-30). Weidner et al. (31) have recently suggested that 5S RNA may exist in different conformational states at different stages of the translational process. Transitions between these conforma-
tional states might then be mediated by protein-5S RNA interactions. The ability of a given protein to exert an effect on 5S RNA conformation might, in turn, be modulated by its interaction with other ligands.

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