Stoichiometry, Cooperativity, and Stability of Interactions between 5S RNA and Proteins L5, L18, and L25 from the 50S Ribosomal Subunit of *Escherichia coli*†

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ABSTRACT: Interactions of 5S RNA from *Escherichia coli* with 50S ribosomal subunit proteins L5, L18, and L25 have been evaluated by a number of criteria. From the dependence of complex formation on protein and RNA concentration in TMK buffer (50 mM Tris-HCl (pH 7.6)–20 mM $MgCl_2$–300 mM KCl), it was inferred that the three proteins differ substantially in their affinity for the nucleic acid. Measurement of the stoichiometry of association in the presence of excess protein revealed that molar protein:RNA binding ratios for L5, L18, and L25 at saturation were 0.6:1, 1.1:1, and 0.7:1, respectively. The RNA molecule therefore contains no more than one specific site of attachment for each of the proteins. Solution conditions were varied to assess the effects of pH, Mg^{2+} concentration, and K^+ concentration on the stability of the interactions. Optimal binding was observed for the L5–5S RNA complex at pH 6.5–9, [Mg^{2+}] of 10–20 mM and [K^+] of 300 to 400 mM; for the L18–5S RNA complex at pH 7.5–9, [Mg^{2+}] of 10–20 mM and [K^+] of 100–200 mM; and for the L25–5S RNA complex at pH 7.5–9, [Mg^{2+}] of 0.3–20 mM, and [K^+] of 200–300 mM. In a separate series of experiments, the association of L5 in TMK buffer was found to be cooperatively stimulated by L18 at component concentrations roughly tenfold less than were required for the association of L5 alone. The mutual influence of these two proteins upon one another was also clearly manifested in assays involving variation of pH and ionic environment. From the pattern of cooperativity, it was concluded that the binding sites for L5 and L18 in the 5S RNA are functionally related to each other, but distinct from that for protein L25.

Investigation of the reconstitution of 30S and 50S ribosomal subunits from *Escherichia coli* has demonstrated that specific interactions among their protein and RNA constituents are responsible both for the assembly and stability of the active particles (Traub & Nomura, 1968; Nierhaus & Dohme, 1974). Approximately one-third of the 21 proteins of the 30S subunit associate individually with the 16S RNA (reviewed by Zimmermann, 1974). These primary interactions lead to the organization of binding sites for most of the remaining proteins, defining a highly cooperative assembly pathway (Mizushima & Nomura, 1970; Held et al., 1974). Similar results have been obtained with components of the 50S subunit, where three out of 31 different proteins associate with the 5S RNA (Horne & Erdmann, 1972) and ten with the 23S RNA (Stöffler et al., 1971; Garrett et al., 1974). We have found that a number of the latter interactions facilitate the integration of additional large-subunit proteins which cannot themselves bind directly to the 23S RNA (Spierer & Zimmermann, 1976). Assembly of the *E. coli* 50S particle is therefore likely to proceed by a sequential pathway analogous to that for the 30S particle (Spierer & Zimmermann, 1976; Spillmann et al., 1977).

Although the 5S RNA can apparently be incorporated into the nascent 50S subunit during any one of several steps in reconstitution (Dohme & Nierhaus, 1976), its insertion is dependent upon the presence of proteins L5, L18, and L25 (Yu & Wittmann, 1973). These three proteins also bind to the 5S RNA independently, as shown by a wide variety of methods (Horne & Erdmann, 1972; Gray et al., 1973; Yu & Wittmann, 1973; Burrell & Horowitz, 1975). The 5S ribonucleoproteins are particularly advantageous for studies on protein–RNA association owing to the small size of the RNA and its high density of protein binding sites. Moreover, the sequences of all four components have been determined (Brownlee et al., 1967; Dovgas et al., 1975; Brosius et al., 1975; Chen & Ehrke, 1976) and their conformations in solution are under investigation (Österberg et al., 1976a,b). This system is also well suited to the study of cooperative interactions since L18 strongly influences the binding of L5 to the 5S RNA and the three components together can combine with the 23S RNA, even though none of them are able to do so singly (Gray et al., 1972; Spierer & Zimmermann, 1978). In many ways, the 5S ribonucleoprotein can be regarded as a discrete subassembly of the ribosome because the 5S RNA and its associated proteins may interact with the 50S subunit as a unit (Yu & Wittmann, 1973) and because they may jointly contribute to a number of functional activities of the mature particle (reviewed by Erdmann, 1976).

Despite the very considerable interest in the properties of the 5S RNA, basic information on the nature of its association with L5, L18, and L25 is surprisingly meager. In the present communication, we have attempted to fill some of the gaps through a systematic investigation of protein–5S RNA interactions. Specifically, we have examined the dependence of binding on protein concentration as well as the effects of pH and ionic environment on the stability of the complexes. In addition, we have determined the stoichiometry of association in each case in order to assess the specificity of complex formation. Finally, we have documented the cooperative influence of L18 on the binding of L5 both qualitatively and quantitatively under conditions in which L5 cannot bind independently to the 5S RNA.

Materials and Methods

Preparation of Ribosomal Proteins. Ribosomal subunits, either unlabeled or labeled with ^3H-labeled amino acids, were prepared from *E. coli* MRE600 according to Muto et al.

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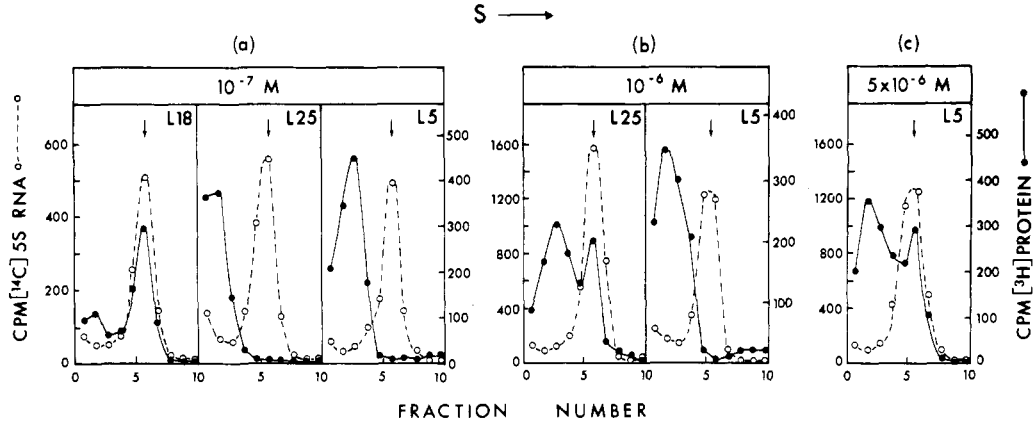

FIGURE 1: Binding of proteins of L18, L25, and L5 to the 5S RNA. ^{14}C-labeled 5S RNA was mixed with two molar equivalents of ^{3}H-labeled L18, L25, or L5 in 100 μL of TMK buffer and incubated for 15 min at 30 °C. Protein-RNA complexes were separated from unbound protein by sucrose gradient centrifugation as described in Materials and Methods. Concentration of 5S RNA in the incubation mixtures was (a) 10^{-7}, (b) 10^{-6}, and (c) 5×10^{-6} M. Specific activity of the 5S RNA was 2000, 800, and 200 counts per min per μg, and that of the proteins was 2450, 800, and 150 counts per min per μg, in a, b, and c, respectively. (●—●) ^{3}H-labeled protein; (O- - -O) ^{14}C-labeled 5S RNA.

(1974). Proteins were extracted from purified 50S subunits with 67% acetic acid and separated by chromatography on phosphocellulose and carboxymethylcellulose (Zimmermann & Stöffler, 1976).

Preparation of 5S RNA. Cultures of *E. coli* MRE600 were grown, labeled with [^{14}C]uracil when appropriate, and harvested as previously described (Muto et al., 1974). All subsequent steps were carried out between 0 and 4 °C. Cells were broken by grinding with alumina and the cell paste was extracted with 1 to 3 vol of buffer I (10 mM Tris-HCl (pH 7.6)–1 mM $MgCl_2$–100 mM KCl) containing 5 μg of DNase (Worthington) per mL. The slurry was centrifuged for 15 min at 10 000 revolutions/min in a Sorvall SS-34 rotor and the resulting supernatant was centrifuged again for 30 min at 16 000 revolutions/min in the same rotor. The second supernatant was layered over an equal volume of 30% sucrose in buffer I and ribosomal particles were sedimented at 37 000 revolutions/min for 16 h in a Spinco 50.2 Ti rotor. This procedure was found to free the subunits of tRNA. After the supernatant was removed, the pellets were rinsed and resuspended in buffer II (10 mM Tris-HCl (pH 7.6)–1 mM $MgCl_2$), and sodium dodecyl sulfate was added to a final concentration of 0.2%. Ribosomal RNA was extracted from the subunits with an equal volume of 90% phenol. Following low-speed centrifugation, the phenol phase was reextracted with 1 vol of buffer II and the aqueous phases were combined and twice reextracted with phenol. The RNA was precipitated from the final aqueous phase with 2 vol of absolute ethanol, recovered by sedimentation, and resuspended in buffer I. This solution was dialyzed for 2 h against 200 vol of buffer I and layered on 5–20% linear sucrose gradients in the same buffer which were then centrifuged for 24 h at 26 000 revolutions/min in a Spinco SW27 rotor. The contents of each tube were monitored continuously for absorbance at 260 nm with the aid of a flow cuvette, fractions containing 5S RNA were pooled, and the RNA was precipitated with ethanol. Each batch of RNA was checked for purity by electrophoresis in 10% polyacrylamide gels at pH 7.2 (Bishop et al., 1967) or pH 8.3 (Peacock & Dingman, 1967). Only those preparations in which 5S RNA comprised at least 95% of the total RNA were used in binding experiments.

Preparation of Protein–RNA Complexes. From 0.1 to 50 μg (10 to 2500 pmol) of ^{3}H-labeled proteins L5, L18, or L25 were incubated with 0.4 to 20 μg (10 to 500 pmol) of ^{14}C-labeled 5S RNA for 15 min at 30 °C in TMK buffer (50 mM Tris-HCl (pH 7.6)–20 mM $MgCl_2$–300 mM KCl), or an appropriate variant, and chilled on ice. Alterations in buffer composition for specific experiments are indicated in the text. Since proteins were stored in 6 M urea and since this substance was found to impede complex formation at concentrations in excess of 0.2 M, protein–RNA mixtures were generally dialyzed against the incubation buffer for 30 min at 0 °C prior to heating.

Analysis of Protein–RNA Complexes. Chilled reaction mixtures were sedimented through 4-mL 3–15% sucrose gradients, made up in the same buffer that was used for incubation, at 42 000 revolutions/min for 16 h in a Spinco SW60 rotor. After centrifugation, the gradients were fractionated and the radioactivity in each tube was measured (Muto et al., 1974). The stoichiometry of protein and RNA in the complexes was computed from the amount of ^{3}H and ^{14}C radioactivity in the 5S peak and from the specific activities of the components, which varied from 80 to 2450 counts per min per μg for the ^{3}H-labeled proteins and from 100–2000 counts per min per μg for the ^{14}C-labeled 5S RNA in different experiments. These values were converted to molar quantities using molecular weights of 20 172 for L5 (Chen & Ehrke, 1976), 12 770 for L18 (Brosius et al., 1975), 10 912 for L25 (Dovgas et al., 1975) and 38 853 for the 5S RNA (Brownlee et al., 1967).

Results

Interaction of Proteins L18, L25, and L5 with 5S RNA in TMK Buffer. Proteins L18, L25, and L5 from the 50S ribosomal subunit of *E. coli* were incubated individually with 5S RNA in TMK buffer and the reaction mixtures were fractionated by sucrose gradient centrifugation. Figure 1a shows that L18 interacts with 5S RNA when the concentration of components in the incubation is 10^{-7} M, but that neither L25 nor L5 forms a stable complex under these conditions. If the concentrations of protein and RNA are raised by an order of magnitude, however, the binding of L25 can be detected (Figure 1b) and a further fivefold increase in component concentration results in the stable attachment of L5 (Figure 1c). The use of still higher concentrations of L5 and L25 did not notably enhance their interaction with the RNA. The results suggest that the three proteins differ markedly in their affinities for the 5S RNA. This conclusion has been confirmed by measurement of the association constants for each of the complexes with a membrane filter assay (Spierer et al., 1978).

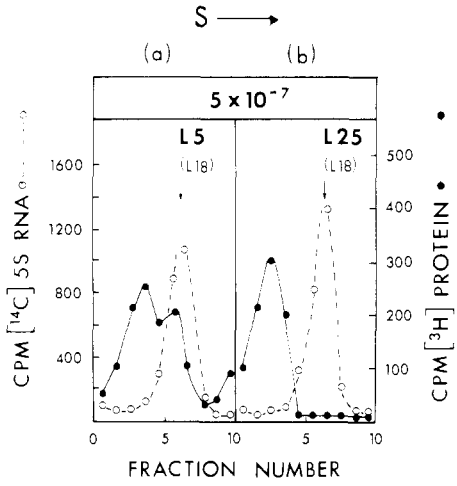

FIGURE 2: Influence of L18 on the interaction of L25 and L5 with the 5S RNA. Two μg of ^{14}C-labeled 5S RNA (specific activity, 2000 counts per min per μg) was incubated with an equimolar amount of either (a) ^{3}H-labeled L5 or (b) ^{3}H-labeled L25 (specific activity, 1200 counts per min per μg) and a twofold molar excess of unlabeled L18 in 100 μL of TMK buffer. Reaction mixtures were fractionated by sucrose gradient centrifugation in the usual way. Concentration of 5S RNA in the incubation was 5×10^{-7} M. (●—●) ^{3}H-labeled protein; (O- - -O) ^{14}C-labeled 5S RNA.

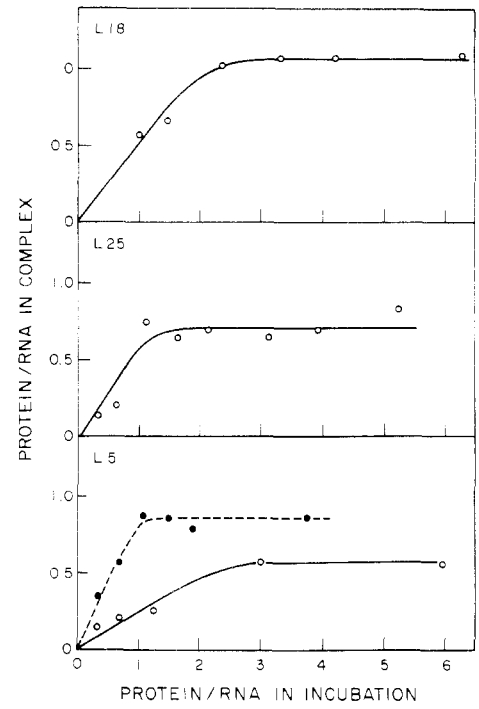

FIGURE 3: Saturation curves for the interaction of L18, L25, and L5 with 5S RNA; saturation binding of L5 to 5S RNA in the presence of L18. Increasing quantities of ^{3}H-labeled L18, L25, or L5 were added to a fixed quantity of ^{14}C-labeled 5S RNA (corresponding to 10^{-7} M for L18, 10^{-6} M for L25, and 5×10^{-6} M for L5) in 100 μL of TMK buffer and incubated under standard conditions. To obtain molar L5:5S RNA input ratios of 3:1 and 6:1, reaction mixtures of 150 μL and 300 μL, respectively, were used in order to ensure that the protein did not precipitate. Following analysis of the complexes by sucrose gradient centrifugation, the molar ratios of protein and RNA sedimenting in the 5S peak were calculated from the specific activities and molecular weights of the components as described in Materials and Methods. For experiments in which L5 binding was measured in the presence of L18, increasing amounts of ^{3}H-labeled L5 were incubated with ^{14}C-labeled 5S RNA at a final concentration of 5×10^{-7} M and 2 molar equiv of unlabeled L18. (O—O) Molar protein:5S RNA ratio; (●- - -●) molar L5:5S RNA ratio in the presence of L18

Cooperative Binding of L5 to the 5S RNA in the Presence of L18. The next set of experiments was designed to determine whether the association of any one of the three proteins with the 5S RNA can stimulate the binding of another. Specifically, we investigated the interval of component concentrations from 10^{-7} to 10^{-6} in which only the L18–5S RNA interaction was detectable by our methods when each protein was tested individually. Radioactively labeled L5 or L25 was incubated with an equimolar amount of 5S RNA and 2 molar equiv of unlabeled L18 and binding was measured by sucrose gradient centrifugation. L5 was found to associate with the 5S complex at a minimum concentration of 5×10^{-7} M (Figure 2a). This assay demonstrates that the affinity of L5 for the L18–5S RNA complex is about ten times higher than its affinity for the 5S RNA alone. By contrast, L18 did not promote the binding of L25 (Figure 2b). Moreover, protein L25 was found to have no influence on associations involving L5 and L18 (data not shown).

Stoichiometry of Interaction of L18, L25, and L5 with the 5S RNA. One of the chief criteria used to verify the existence of a specific protein binding site in a molecule of RNA requires that no more than 1 mol of protein bind per mol of RNA even when the protein is present in excess (Schaup et al., 1970; Garrett et al., 1971). In order to assess the specificity of interaction of L18, L25, and L5 with the 5S RNA, we have determined the molar ratio of components in all three complexes at saturation. Increasing amounts of each protein were incubated with fixed amounts of 5S RNA in the range of concentrations found to yield quantitative binding (see above). The mixtures were resolved on sucrose gradients and molar protein:RNA ratios were calculated from the quantities of ^{3}H-labeled protein and ^{14}C-labeled RNA in the 5S peak.

The data presented in Figure 3 show that L18, L25, and L5 saturate the 5S RNA at molar binding ratios of 1.1:1, 0.7:1, and 0.6:1, respectively (solid lines). It is of particular interest that the L18–5S RNA complex contains only one molecule of protein and not two as previously suggested (Gray et al., 1973; Feunteun et al., 1975). The relatively low plateau value and high protein:RNA input ratio necessary for saturation in the case of L5 most likely result from partial dissociation of the components during centrifugation (see Figure 1). Attempts to enhance the stability of the complex by working at higher protein and RNA concentrations were unsuccessful because L5 was found to be insoluble in TMK buffer at concentrations greater than 10^{-5} M.

A saturation curve was also constructed for the binding of L5 to the 5S RNA in the presence of L18 (Figure 3, dashed line). In this instance, L18 promotes stoichiometric binding of L5 at a molar input ratio of 1:1. These observations demonstrate that the L5 used in the present experiments was nearly all in active form and, more importantly, that L5 associates with a single specific site in the 5S RNA when cooperatively stimulated by L18 as well as when tested alone.

Effect of pH on the Stability of the Protein–5S RNA Complexes. Proteins L18, L25, and L5 were incubated with equimolar amounts of 5S RNA in buffers containing standard concentrations of $MgCl_2$ and KCl at pH values from 6 to 10. Complex formation was analyzed in buffers identical with those used for the incubations. Figure 4 shows that both L18 and L25 bind at their maximum levels between pH 7.5 and 9.0, whereas the lower limit for optimal association of L5 extends to pH 6.5 (solid lines). The investigation of complexes containing L5 and L18 together revealed that these two proteins exert a strong mutual influence on one another (Figure 4,

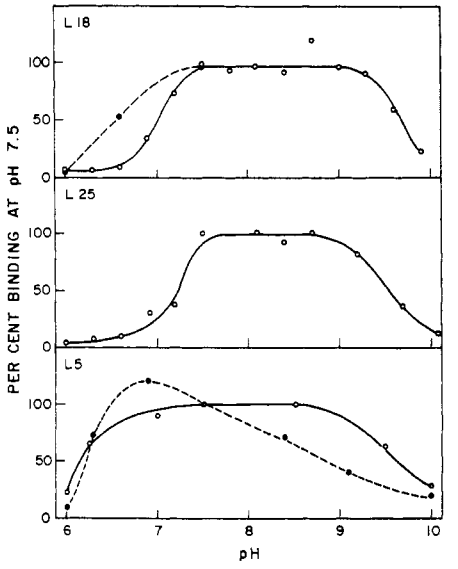

FIGURE 4: Effect of pH on the stability of protein-5S RNA complexes. Equimolar amounts of proteins and 5S RNA were incubated in 100 μL of 50 mM Tris-maleate (titrated with NaOH for pH 6 to 9) or 50 mM glycine-50 mM NaCl (titrated with NaOH for pH 9 or above), 20 mM $MgCl_2$, and 300 mM KCl. Protein-RNA complexes were analyzed by sucrose gradient centrifugation. The amount of protein bound to the RNA at pH 7.5 was taken as 100%. Concentrations of proteins and RNA in the incubation mixtures were 10^{-7} M for L18, 10^{-6} M for L25, 5×10^{-6} M for L5, and 5×10^{-7} M for labeled L18 or L5 in the presence of unlabeled L5 or L18, respectively. (O—O) Individual protein-5S RNA complexes; (●- - -●) L18-5S RNA complex in the presence of L5 (upper panel) or L5-5S RNA complex in the presence of L18 (lower panel).

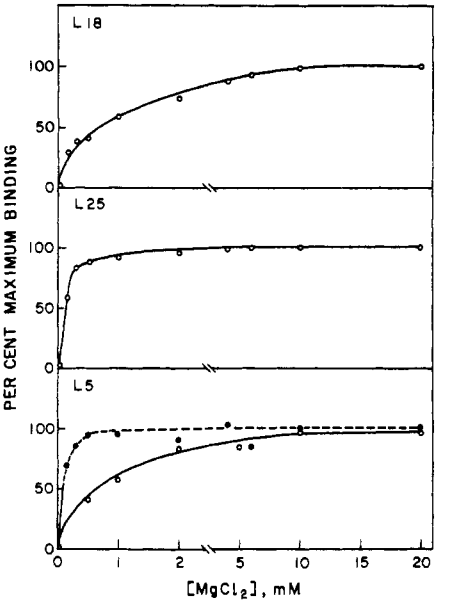

FIGURE 5: Effect of Mg^{2+} concentration on the stability of protein-5S RNA complexes. Equimolar amounts of proteins and 5S RNA were incubated in 100 μL of 50 mM Tris-HCl, pH 7.6, 0-20 mM $MgCl_2$, and 300 mM KCl. Protein-RNA complexes were analyzed by sucrose gradient centrifugation. The maximum protein binding observed was taken as 100%. Concentrations of proteins and RNA in the incubation mixtures were as in Figure 4. (O—O) Individual protein-5S RNA complexes; (●- - -●) L5-5S RNA complex in the presence of L18.

dashed lines). In particular, the presence of L5 increases the stability of the L18-5S RNa interaction below pH 7.5 (upper panel) while L18 appears to produce a gradual decline in L5 binding at higher pH values (lower panel). Neither of these proteins affected the association of L25, however. The above considerations provide further evidence of the cooperativity inherent in L5-L18-5S RNA interactions and underscore their independence from associations involving L25.

Effect of Mg^{2+} Concentration on the Stability of the Protein-5S RNA Complexes. Complexes of L18, L25, and L5 with the 5S RNA were formed in 50 mM Tris-HCl (pH 7.6)-300 mM KCl containing $MgCl_2$ at concentrations between 0 and 20 mM. The mixtures were centrifuged through sucrose gradients made up in the same buffers as the incubations and the amount of protein sedimenting with the 5S peak was used to evaluate the stability of the protein-RNA interactions. As illustrated in Figure 5, none of the proteins was able to associate with the 5S RNA in the absence of Mg^{2+}. The L25-5S RNA complex was found to be very resistant to low concentrations of Mg^{2+}, however, since maximum binding was attained at about 0.3 mM. The L5- and L18-5S RNA complexes, in contrast, exhibited reduced stability when the $MgCl_2$ concentration fell below 5-10 mM. The dashed line in Figure 5 demonstrates that L18 greatly stimulates the interaction of L5 at low Mg^{2+}. We infer that L18 also associates with the complex in these circumstances since L5 binding is dependent upon its presence at the protein concentrations utilized in this assay.

Effect of K^+ Concentration on the Stability of the Protein-5S RNA Complexes. In this series of experiments, proteins were mixed with 5S RNA in buffers of the usual pH and Mg^{2+} concentration, but which varied in KCl concentration from 0 to 400 mM. Incubations and sedimentation were both carried out in the same buffer and the relative amounts of

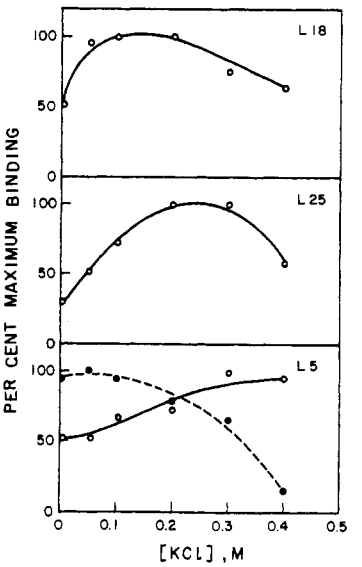

FIGURE 6: Effect of K^+ concentration on the stability of protein-5S RNA complexes. Equimolar amounts of proteins and 5S RNA were incubated in 100 μL of 50 mM Tris-HCl (pH 7.6), 20 mM $MgCl_2$, and 0-400 mM KCl. Protein-RNA complexes were analyzed by sucrose gradient centrifugation. The maximum protein binding observed was taken as 100%. Concentrations of proteins and RNA in the incubation mixtures were as in Figure 4. (O—O) Individual protein-5S RNA complexes; (●- - -●) L5-5S RNA complex in the presence of L18.

protein bound are plotted in Figure 6. The results obtained in the absence of KCl should be regarded with caution because the proteins were poorly soluble under these conditions. This was a particular problem in the case of L5 where protein concentrations of 5×10^{-6} M and above were used. Although L5 and L25 associate with the 5S RNA optimally at about 300 mM KCl and L18 at about 100 mM KCl, it is apparent that the stability of the complexes is not drastically influenced by

salt concentrations between 100 and 400 mM. The effects of increasing KCl on L5 in the presence of L18 run counter to those on L5 alone and result in a steady decrease of L5 binding which roughly parallels the stability of the L18–5S RNA complex (Figure 6, dashed lines).

Discussion

The 5S RNA from *E. coli* has been shown to selectively bind L5, L18, and L25 from an unfractionated mixture of 50S subunit proteins (Horne & Erdmann, 1972; Burrell & Horowitz, 1975) and to associate independently with each of these components in pure form (Yu & Wittmann, 1973; Gray et al., 1973; Feunteun et al., 1975). The stoichiometry of the interactions has not been studied in a systematic fashion, however, although it has been argued on the basis of indirect evidence that the RNA molecule can combine with two molecules of L18 (Gray et al., 1973; Feunteun et al., 1975). We have demonstrated here that the 5S RNA associates with no more than one copy of L5, L18, or L25 at saturation and we conclude that there is a single specific binding site for each protein in the nucleic acid molecule. Furthermore, we have found complex formation to be strongly concentration-dependent, indicating that the three proteins differ appreciably in their affinities for the 5S RNA. Because the sucrose gradient technique employed in these experiments requires recourse to nonequilibrium conditions, it cannot be used to accurately quantitate the strength of the protein–RNA interactions. Nonetheless, we have recently been able to determine association constants for these complexes by means of a membrane filter assay (Spierer et al., 1978). They demonstrate that L25 binds to the 5S RNA roughly five times more tightly, and L18 nearly 100 times more tightly, than L5. The relatively low affinity of L5 and the correspondingly high component concentrations needed for detection of complex formation may help to explain earlier confusion over the capacity of this protein to associate with the 5S RNA (see Erdmann, 1976).

The individual protein–5S RNA complexes differ markedly in their response to changes in pH and in Mg^{2+} and K^+ concentrations. The sharp decrease in the association of L18 and L25 below pH 7.5 may reflect the titration of functional groups which are important in binding. Although the instability of all three complexes above pH 9 could result from partial hydrolysis of the RNA, no salient changes in the mobility of the 5S peak on sucrose gradients were evident at pH values less than 10. The pH optima for association of L5, L18, and L25 with the 5S RNA are in general narrower than those for the interaction of S4 and S8 with the 16S RNA and of L24 with the 23S RNA (Schulte & Garrett, 1972; Schulte et al., 1974).

The dependence of specific protein–5S RNA interaction upon Mg^{2+} at concentrations below 10 mM is much less than for ribonucleoprotein complexes containing 16S or 23S RNA. The large ribosomal RNAs undergo a precipitous decline in sedimentation rate as well as in binding capacity between 10 and 1 mM Mg^{2+}, implying that their ability to associate with ribosomal proteins is closely correlated with their conformation (Schulte et al., 1974). The relative insensitivity of the 5S complexes to reduced Mg^{2+} could therefore mean that RNA tertiary structure does not play as important a role in such interactions. Alternatively, the 5S RNA may simply remain in its active configuration at lower Mg^{2+} concentrations than the larger RNAs. This appears to be true for tRNA, which is similar in size to the 5S RNA, where maximum stabilization of tertiary structure occurs between 1 and 3 mM Mg^{2+} (Stein & Crothers, 1976). In any case, it is clear from the present results that L5, L18, and L25 are all capable of binding to the 5S RNA during the first step of 50S subunit reconstitution in vitro which is carried out at 4 mM Mg^{2+} (Nierhaus & Dohme, 1974). This lends credence to the notion that the 5S ribonucleoprotein is assembled independently and then inserted into the nascent subunit intact (Yu & Wittman, 1973).

Within fairly broad limits, variation of K^+ and temperature do not strongly influence the interaction of the 5S RNA with L5, L18, and L25. Binding takes place efficiently at KCl concentrations of 100–400 mM and at temperatures between 0 and 40 °C. Moreover, there is only a small difference in the association constants at the temperature extrema (Spierer et al., 1978).

The criteria used to characterize individual protein–5S RNA interactions were also applied to complexes formed in the presence of both L5 and L18. Cooperativity in the association of these two proteins with the 5S RNA was evident in all cases. The mutual influence of L5 and L18 was particularly apparent in the altered stability of the ternary complex to variations in pH and Mg^{2+}. These results suggest that such effects entail some degree of protein–protein interaction. Cooperativity was also manifest in the dependence of binding on component concentration. Under our conditions, L18 stimulates the stoichiometric attachment of L5 at concentrations of RNA and protein approximately tenfold less than those which permit formation of the L5–5S RNA complex. In addition, L5, L18, and 5S RNA together have been found to interact with the 23S RNA at component concentrations fivefold lower than those required for the binding of L5 to the L18–5S RNA complex (Spierer & Zimmermann, 1978). Such cooperative phenomena are of great interest because they are believed to play a major role in the assembly of both 30S and 50S ribosomal subunits (Mizushima & Nomura, 1970; Held et al., 1974; Spierer & Zimmermann, 1976).

Our understanding of the molecular nature of cooperativity in the 5S ribonucleoprotein complex is still very rudimentary. L18 binding could elicit shifts in the secondary or tertiary structure of the 5S RNA which improve or augment its stereochemical complementarity to L5. Indeed, circular dichroism studies indicate that one or more helical regions within the RNA molecule are substantially altered by the attachment of L18 (Bear et al., 1977; Spierer et al., 1978). The response of the L5, L18–5S RNA complex to variations in ionic conditions nonetheless suggests that the two proteins are in close functional contact, whether through protein-mediated changes in the 5S RNA or through direct protein–protein interaction. Surprisingly, we have obtained no evidence for cooperative interaction between L18 and L25 as earlier reported (Feunteun et al., 1975). Although segments of the RNA that are protected from RNase digestion by the two proteins appear to be structurally contiguous (Gray et al., 1973; Erdmann, 1976), we propose that they are not functionally coupled under our conditions.

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Effect of Specific Lysine Modification on the Reduction of Cytochrome *c* by Succinate–Cytochrome *c* Reductase†

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ABSTRACT: The reduction of cytochrome *c* by succinate–cytochrome *c* reductase was studied at very low cytochrome *c* concentrations where the reaction between cytochrome c_1 and cytochrome *c* was rate limiting. The rate constant for the reaction was found to be independent of ionic strength up to 0.1 M chloride, and to decrease rapidly at higher ionic strength, suggesting that the interaction between cytochrome c_1 and cytochrome *c* was primarily electrostatic. The reaction rates of cytochrome *c* derivatives modified at single lysine residues to form trifluoroacetylated or trifluoromethylphenylcarbamylated cytochromes *c* were studied to determine the role of individual lysines in the reaction. None of the modifications affected the reaction at low ionic strength, but at higher ionic strength the reaction rate was substantially decreased by modification of those lysines surrounding the heme crevice, lysine-8, -13, -27, -72, and -79. Modification of lysine-22, -25, -55, -99, and -100 had no effect on the rate. These results indicate that the binding site on cytochrome *c* for cytochrome c_1 overlaps considerably with that for cytochrome oxidase, suggesting that cytochrome *c* might undergo some type of rotational diffusion during the electron-transport process.

The mechanism by which cytochrome *c* transfers electrons from cytochrome c_1 to cytochrome oxidase is not well understood. Several lines of evidence suggest that there is some difference between the reaction sites on cytochrome *c* for cytochrome c_1 and cytochrome oxidase. Smith et al. (1976) have compared the reactivity of several cytochromes *c* with several different oxidases and reductases and have concluded that the two reaction sites are different but both close to the heme crevice. Smith et al. (1973) found that a purified cytochrome *c* specific antibody completely inhibited the cytochrome oxidase activity of cytochrome *c*. The antibody significantly decreased the rate of reduction of cytochrome *c* by succinate–cytochrome *c* reductase but did not completely prevent it.

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