Characterization and Footprint Analysis of Two 5S rRNA Binding Proteins from Spinach Chloroplast Ribosomes†

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ABSTRACT: We have characterized two ribosomal proteins (CS-L12 and CS-L13) from spinach chloroplasts that bind to the homologous 5S rRNA. Their molecular weight was determined and appears to be higher than that of the *Escherichia coli* 5S rRNA binding proteins. The footprint of both proteins on the 5S rRNA was studied by using several enzymatic and chemical probes. Unpaired nucleotides were probed with RNase T_1 and nuclease S_1 and base-paired or stacked nucleotides with RNase V_1. For the 5S rRNA/CS-L12 complex, cytosines at position N3 were probed with dimethyl sulfate and the phosphates with ethylnitrosourea. The binding site of CS-L12 has been well characterized. The protein protects large areas from RNase V_1 hydrolysis and is in close contact with residues in loop B (at phosphates 58–62) and in region E (at position N3 of cytosine 75, at phosphates 79–80 and 98–101). It is relevant that region E has been shown to contain noncanonical A–A and A–G base pairs and bulged residues, resulting in a distorted helix [Romby et al. (1988) *Biochemistry 27*, 4721–4730]. The regions protected by CS-L13 from RNase V_1 do not overlap with those protected by CS-L12. Major protections are observed in helix I (at residues 5–8), loop B (at residues 25–29), loop C (at residues 44–45), and helix III (at residues 50–54). The binding site of both proteins is discussed in the light of the three-dimensional structure model of chloroplast 5S rRNA proposed by Westhof et al. [(1989) *J. Mol. Biol.* (in press)] and is compared to the binding site of the *E. coli* 5S rRNA binding proteins.

Comprehension of the topography of the ribosomal particule requires a detailed knowledge of the spatial arrangement of the interacting components. Among the multiple RNA–protein interacting systems found in the ribosome, the 5S RNA–protein complexes are attractive models for studying RNA–protein interactions. Most of the structural studies have been performed on the prokaryotic species. A large body of work concerns the study of the structure of *Escherichia coli* 5S rRNA and its interaction with ribosomal proteins L5, L18, and L25 [e.g., Spierer and Zimmermann (1978) and Garrett et al. (1984)]. Several attempts were also made to characterize 5S rRNA–protein complexes in other organisms, e.g., in yeast (Nazar et al., 1982) and rat liver (Gross et al., 1985). In the present work, we focused on the chloroplast 5S rRNA–protein complexes. The chloroplast 5S rRNA is known as being of eubacterial type, and the chloroplast ribosomal proteins identified up to now have many similarities with *E. coli* ribosomal proteins at the sequence level (Mache et al., 1987). In a previous work, we have mapped at nucleotide resolution the conformation of the chloroplast 5S rRNA by the use of a variety of enzymatic and chemical structure probes (Romby et al., 1988). A tertiary structure model of the chloroplast 5S rRNA was constructed by computer graphic modeling integrating the above structure–probing data and stereochemical constraints (Westhof et al., 1989). From this perspective, protein footprinting experiments are of prime importance to understand the 5S rRNA–protein complex formation and to test the validity of this tertiary structure model. In the present study, we have characterized two proteins that bind to the homologous 5S rRNA. Their molecular weight has been determined as being larger than that of the *E. coli* binding proteins. The footprint of both proteins on the 5S rRNA molecule was determined by using several enzymatic and chemical probes. Results are discussed in the light of the tertiary structure model proposed Westhof et al. (1989) for the chloroplast 5S rRNA and are compared to *E. coli* 5S rRNA–protein complexes taken as reference.

Experimental Procedures

Buffers. Buffer A: Tris-HCl, 10 mM, pH 7.0; EDTA, 1 mM; bovine serum albumin, 0.02%; poly(vinylpyrrolidone), 0.02%; Ficoll, 0.02%; NaCl, 300 mM. Buffer B: Tris-HCl, 10 mM, pH 7.4; urea, 6 M; LiCl, 150 mM; 2-mercaptoethanol, 6 mM. Buffer C: Tris-HCl, 30 mM, pH 7.5; $MgCl_2$, 20 mM; KCl, 300 mM; $ZnCl_2$, 1 mM; 2-mercaptoethanol, 6 mM. Buffer D: Tris-HCl, 30 mM, pH 7.5; $MgCl_2$, 20 mM; KCl, 300 mM; 2-mercaptoethanol, 6 mM. Buffer E: Tris-HCl, 30 mM, pH 7.5; EDTA, 1 mM. Buffer F: sodium cacodylate, 50 mM, pH 7.5; $MgCl_2$, 20 mM; KCl, 300 mM; 2-mercaptoethanol, 6 mM. Buffer G: sodium cacodylate, 50 mM, pH 7.5; EDTA, 1 mM.

Chemicals and Enzymes. DMS[1] was from Aldrich, and ENU was from Sigma. Calf intestinal phosphatase, RNases

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[1] Abbreviations: DMS, dimethyl sulfate; ENU, ethylnitrosourea; RNase V_1, ribonuclease from cobra *Naja naja oxiana* venom.

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T_1 and V_1, nuclease S_1, and T_4 RNA ligase were from P-L Biochemicals, and T_4 polynucleotide kinase, [γ-^{32}P]ATP (3200 Ci/mmol), and [5′-^{32}P]pCP (3000 Ci/mmol were from Amersham. Acrylamide and *N,N′*-methylenebis(acrylamide) were from BDH Chemicals.

Preparation of Chloroplast 5S rRNA. Spinach chloroplasts and ribosomes were isolated as described by Mache et al. (1980). The RNA was extracted from 70S ribosomes according to the method of Dyer and Bowman (1979) and was fractionated by electrophoresis on a 10% polyacrylamide (0.5% bis-)/8 M urea preparative slab gel.

Labeling of the 5S rRNA at the 5′ end was done according to the method of Silberklang et al. (1977) on RNA molecules previously dephosphorylated (Shinagawa & Padmanabhan, 1979). Labeling at the 3′ end was as described by England and Uhlenbeck (1978). The labeled RNA molecules were purified by electrophoresis on a 10% polyacrylamide/8 M urea slab gel, eluted from the gel, precipitated with ethanol, and resuspensed in the adequate buffer.

Identification and Isolation of the Chloroplast 5S rRNA Binding Proteins. The identification of the chloroplast 5S rRNA binding proteins was achieved by the protein blotting method described by Rozier and Mache (1984). In summary, the 50S ribosomal proteins were extracted with acetic acid from isolated 50S subunits by the procedure of Hardy et al. (1969) and separated by the two-dimensional polyacrylamide gel electrophoresis system of Madjar et al. (1979). The fractionated proteins were then blotted on two nitrocellulose sheets. One blot was incubated with 5′ end labeled rRNAs extracted from chloroplast 50S subunits to give a partial reference map of the ribosomal protein. The second blot was incubated with 5′ end labeled chloroplast 5S rRNA. Only the proteins still retaining the RNA after extensive washing of the nitrocellulose sheet under high ionic strength (buffer A) are considered binding proteins. A control was performed separately to verify that all proteins are eluted from the gel to the nitrocellulose sheet.

To isolate these binding proteins, total ribosomal 50S proteins were extracted with acetic acid, precipitated with acetone, solubilized in buffer B, and fractionated by reverse-phase HPLC using a Beckman ultrapore RPSC C3 silica column (Kerlavage et al., 1984). Peak fractions were collected and dried under vacuum. The protein content of each peak was identified by labeling with ^{125}I and by two-dimensional polyacrylamide gel electrophoresis in the presence of unlabeled 50S ribosomal proteins used as reference. The unlabeled proteins were visualized by Coomassie blue staining and the radioactive proteins by autoradiography. The HPLC profile and the identification of ribosomal proteins in each peak have been previously reported (Mache et al., 1987). The nomenclature used is that of Mache et al. (1980). Purity of the ribosomal proteins CS-L12 and CS-L13 was checked by SDS–polyacrylamide gel electrophoresis. The ability of the isolated proteins to bind labeled 5S rRNA was verified by nitrocellulose filter binding assays under the complex formation conditions described below.

Formation of 5S rRNA–Protein Complexes and Footprinting Experiments. Prior to the RNA–protein complex formation, RNA and proteins were renatured separately as follows. The RNA was preincubated in the appropriate buffer (buffer C, D, or F) at 50 °C for 5 min followed by a slow cooling to room temperature, and the protein was preincubated at 42 °C for 60 min and then chilled on ice according to the method of Mougel et al. (1987).

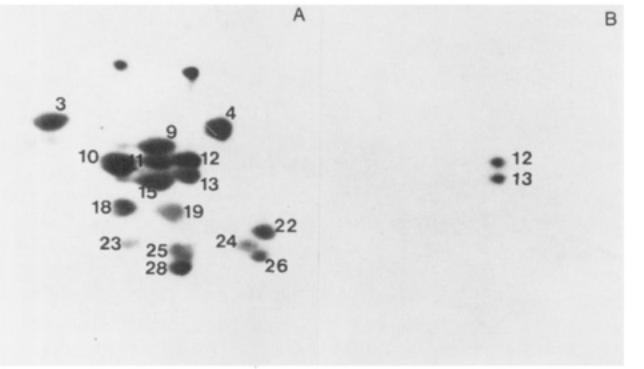

FIGURE 1: Binding of chloroplast 5S rRNA to ribosomal proteins CS-L12 and CS-L13. The proteins were separated by two-dimensional gel electrophoresis, transferred to nitrocellulose blot, and hydridized with 5′ end labeled 5S rRNA. (A) Autoradiography of a reference blot under nonstringent conditions (50 mM NaCl). (B) Autoradiography of a blot under stringent conditions (buffer A).

For filter binding assays, complex formation was done in 20 μL of the appropriate buffer containing 20 mM $MgCl_2$ and 300 mM KCl, at 33 °C for 30 min, with renatured end-labeled 5S rRNA (10^{-6} M, 40000 cpm) and increasing concentration of protein. Then 100 μL of the association buffer was added, and the samples were filtered through the nitrocellulose filters (Millipore HA, 0.45 μm) previously soaked in the buffer, washed, dried at 40 °C, and analyzed for radioactivity. Identical results were obtained when a smaller volume (30 μL) of the association buffer was added before filtering.

For footprinting experiments, complex was formed as above with a 5–10-fold molar excess of protein over the RNA. The mixture was supplemented with 1 μg of total tRNA as carrier and preincubated 10 min at 20 °C prior to the addition of the appropriate chemical or enzymatic structure probes.

Enzymatic Digestion. Digestion with nuclease S_1 (50 units) was at 20 °C in buffer C for 5 and 10 min. Digestions with RNases T_1 (10^{-3} unit) and V_1 (0.05 unit) were at 20 °C in buffer D for 5 and 10 min. The reactions were stopped by phenol extraction, and the RNA was precipitated with 2.5 volumes of ethanol in the presence of 0.3 M sodium acetate. The liberated oligonucleotides were analyzed by electrophoresis on 15% polyacrylamide (0.75% bis-)/8 M urea slab gels. The positions of the nuclease cuts were identified by running in parallel RNase T_1 and formamide ladders.

Modification with DMS. Native conditions: naked or complexed 5S rRNA was incubated in buffer D for 10 min at 20 °C in the presence of 0.5 μL of DMS. Semidenaturing conditions: the procedure was the same as for native conditions, but incubation was in buffer E. Denaturing conditions: naked 5S rRNA was incubated at 90 °C for 1 min in buffer E with 0.5 μL of DMS. The reaction was stopped by phenol extraction, and the RNA was precipitated with ethanol. The cleavage of the modified cytosines at position N3 was as described by Peattie and Gilbert (1980).

Alkylation with ENU. Phosphate alkylation was essentially as described by Vlassov et al. (1981). Native conditions: naked or complexed RNA was incubated at 20 °C for 1, 2, and 3 h in buffer F with 5 μL of a ENU-saturated ethanol solution. Denaturing conditions: naked RNA was incubated at 80 °C for 2 min in buffer G with the same amount of ENU. The reactions were stopped by phenol extraction, and the RNA was precipitated with ethanol. The alkylated RNA was cleaved at phosphotriester positions in 0.1 M Tris–HCl, pH 9.0, at 50 °C for 5 min, and the generated oligonucleotides were analyzed by gel electrophoresis. The assignments of the bands were performed by running in parallel a RNase T1

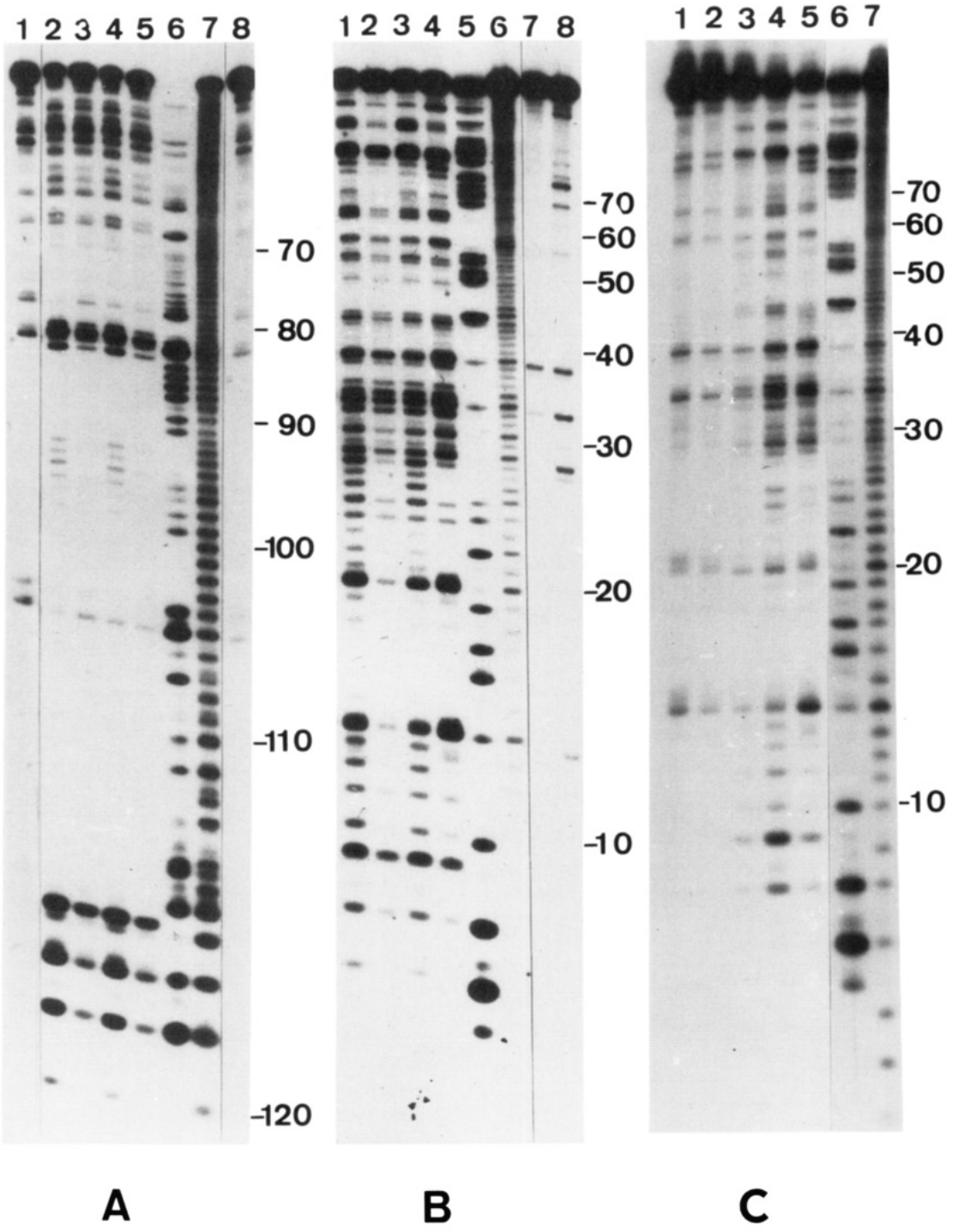

FIGURE 2: Gel electrophoresis fractionation of products resulting from RNase V_1 hydrolysis of naked 5S rRNA or of 5S rRNA complexed with proteins CS-L12 (A, B) and CS-L13 (C). (A) 3′ end labeled 5S rRNA: (lanes 1, 8) incubation controls of 5S rRNA complexed with CS-L12 and of naked 5S rRNA, respectively; (lanes 2, 4) RNase V_1 digestion of naked 5S rRNA for 5 and 10 min at 20 °C, respectively; (lanes 3, 5) RNase V_1 digestion of 5S rRNA complexed with CS-L12 for 5 and 10 min, respectively; (lane 6) RNase T_1 ladder; (lane 7) formamide ladder. (B) 5′ end labeled 5S rRNA: (lanes 1,3) RNase V_1 digestion of naked 5S rRNA for 5 and 10 min at 20 °C, respectively; (lanes 2, 4) RNase V_1 digestion of 5S rRNA complexed with CS-L12 for 5 and 10 min, respectively; (lane 5) RNase T_1 ladder; (lane 6) formamide ladder; (lanes 7, 8) incubation controls of naked 5S rRNA and 5S rRNA complexed with CS-L12, respectively. (C) 5′ end labeled 5S rRNA: (lanes 1, 2) incubation controls of complexed 5S rRNA with CS-L13 and of naked 5S rRNA, respectively; (lanes 3, 5) RNase V_1 digestion of 5S rRNA complexed with CS-L13 for 5 and 10 min, respectively; (lane 4) RNase V_1 digestion of naked 5S rRNA for 10 min; (lane 6) RNase T_1 ladder; (lane 7) formamide ladder.

ladder. The extent of phosphate alkylation was measured by densitometry of the autoradiographs.

Results and Discussion

The validity of the protein blotting method used to identify the 5S rRNA binding proteins has been previously discussed by Rozier and Mache (1984). Figure 1 shows that two proteins designated CS-L12 and CS-L13 [see the nomenclature of Mache et al. (1980)] retained the 5′ end labeled 5S rRNA on nitrocellulose filters under ionic conditions (300 mM NaCl). These proteins did not bind to isolated chloroplast 16S rRNA (not shown). These two proteins could be obtained in a pure form by fractionation on HPLC. Their purity and their molecular weight were estimated by SDS–polyacrylamide gel

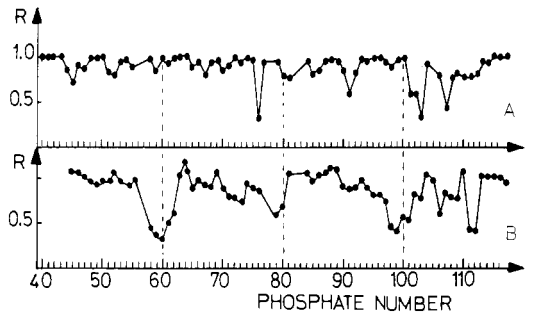

FIGURE 3: Reactivities of the phosphate of naked 5S rRNA (A) or of naked 5S rRNA complexed with CS-L12 (B). (A) Pattern of phosphate reactivities toward ENU in native 5S rRNA as compared to those of the denatured molecule. *R* is the ratio of the intensity of the corresponding electrophoretic bands of the alkylated native RNA over the denatured molecule. The intensities were measured as peak heights on the densitogram of the gel. (B) Pattern of phosphate reactivities of native 5S rRNA complexed with protein CS-L12 as compared to those of the naked RNA. The compression of bands 15–45 on the gel did not allow scanning of this region; nevertheless, the reactivity of phosphates could be estimated by visual inspection. Nucleotides 1–15 were not tested.

electrophoresis at 26000 for CS-L12 and 24000 for CS-L13 (results not shown). Note that the gene coding for protein CS-L13 has been recently identified (Zhou et al., 1989) and that the molecular weight of the gene product has been determined as 23245, which is close to the above experimental value. The sizes of proteins CS-L12 and CS-L13 differ significantly from the molecular weight of the *E. coli* 5S rRNA binding proteins L5 (20200), L18 (12800), and L25 (10700) (Wittmann, 1986).

Nitrocellulose filter binding assays showed that saturation of the labeled 5S rRNA could be obtained by using a 3–5-fold molar excess of CS-L12 and 6–10-fold molar excess of CS-L13 (results not shown). These conditions were used for the following footprinting experiments. Single-stranded regions were probed with RNase T_1 and nuclease S_1 and double-stranded or stacked regions with RNase V_1. Typical experiments are shown in Figure 2. For the 5S rRNA/CS-L12 complex, cytosines at position N3 were probed with DMS and phosphates with ENU. A quantitative measure of the phosphate reactivity of naked RNA over the RNA complexes with CS-L12, as deduced from polyacrylamide gel electrophoresis, is represented in Figure 3.

The footprint of both proteins is reported on the secondary structure model of the chloroplast 5S rRNA (see Figure 4) derived from sequence comparison (Delihas & Andersen, 1982) and modified according to the structure probing experiments of Romby et al. (1988). In the presence of protein CS-L12, phosphate 58–60 in the 3′ strand of the internal loop B exhibit strong protection toward ENU as well as phosphates 98–99 at the junction between region E and helix IV. Weak protection is detected at phosphates 61, 62, 79, 80, 111, and 112. Phosphates 100 and 101, whose reactivity is weak in the naked RNA molecule (Romby et al., 1988), are further reduced by the presence of the protein. Binding of CS-L12 causes a decreased reactivity of cytosine 75 at position N3 toward DMS, whereas all other cytosines reactive in naked RNA [see Romby et al. (1988)] exhibit the same reactivity. Due to the small size of ENU and DMS, the decrease of reactivity strongly suggests a close contact between these residues and the protein. The interaction of the protein with region E and helix IV is further confirmed by the observed protection from RNase V_1 at positions 93–98. Strong protection from RNase V_1 is also detected in loop A (at positions 13–16) and in helix II (at positions 18–23 and 64–66), whereas weak protection from RNase V_1 is found in loop B (at positions 24–25), in helix II (at positions 63–65), and at the 5′ and 3′ ends of helix I (at positions 9, 10, and 116–120). Nuclease T_1 and S_1 cleavages in loop C and D as well as the two RNase V_1 cuts in loop C still occur in the 5S rRNA/CS-L12 complex. Also, position N3 of cytosines in loop C remains reactive upon protein binding. These observations suggest that the two external loops C and D are not in contact with the protein. No enhanced reactivity or new reactivity was induced upon protein binding as it was also observed for the *E. coli* 5S rRNA/L18 and /L25 complexes (Douthwaite et al., 1982) and for several *E. coli* 16S rRNA–protein complexes [e.g., Mougel et al. (1987, 1988) and Swensson et al. (1989)]. This suggests that no major conformational rearrangements of the 5S rRNA occur upon protein binding.

Information regarding the 5S rRNA/CS-L13 complex is more limited as compared to that on the 5S rRNA/CS-L12 complex, since no footprint could be obtained with chemical probes. Nevertheless, a footprint of the protein could be obtained with RNase V_1. In that case, it is difficult to discriminate close contact and steric hindrance effect. Strong protection is found in the 5′ strand of helix I (at residues 5–8), in loop B (at residues 25–29), in helix II (at residues 50–53), and in loop C (at residues 44–45). Note that the two RNase V_1 cuts in the external loop C reflect the existence of a tertiary interaction between U37 and G45 in the naked RNA (Westhof et al., 1989). Again, no increased reactivity induced by CS-L13 protein is observed in 5S rRNA.

The above footprinting results are reported on the three-dimensional structure model (see Figure 5) proposed by Westhof et al. (1989). In this model, the RNA is divided in three arms: 1 (helix I), 2 (helix II, loop B, helix III, loop C), and 3 (helix V, region E, helix IV, loop D), with loop A as the hinge region. The RNA adopts a Y-shape structure in which helices II and V are stacked and not far from collinearity, as well as helix II, loop B, and helix III. The Y-shape originates in part from the conformation of loop A and from the peculiar structure of the internal region E. As described by Romby et al. (1988), the unusual structure of region E is based on several noncanonical A–A and A–G base pairs and contains three bulged-out (C75, U78, and U105) and one bulged-in (A101) residues. This peculiar structure is stabilized by magnesium ions (Romby et al., 1988). By use of the chemical probes, it appears that protein CS-L12 is centered in the fork of the molecule between arms 1 and 3 (Figure 5A) and suggests a close contact of the protein with region E and with the 3′ strand of loop B. The protections from RNase V_1 may partly result from a steric hindrance effect. The absence of any protection in helix III and loop C argues for the absence of any tertiary interaction between loop C and region E. Protections induced by protein CS-L12 are well explained by the proposed model in which helices II and V are not far from collinearity. However, the weak protection from RNase V_1 located in the 3′ end of helix I cannot easily be rationalized. One explanation would be that the binding of protein CS-L12 induces some change in the orientation of helix I leading to a decrease of accessibility in that region. The protections induced by CS-L13 from RNase V_1 are essentially located in helix I, loop B, helix III, and loop C. On the tertiary structure model, these protections are located on one face of the molecule comprising arms 1 and 2 (Figure 5B). The protections by the protein of residues 25–29 and 50–53 can be paralleled with the stacking between A27 and the bulged out G54 proposed in the three-dimensional model (Westhof et al., 1989). Note that RNase V_1 cleavages at positions 33–35, located on the

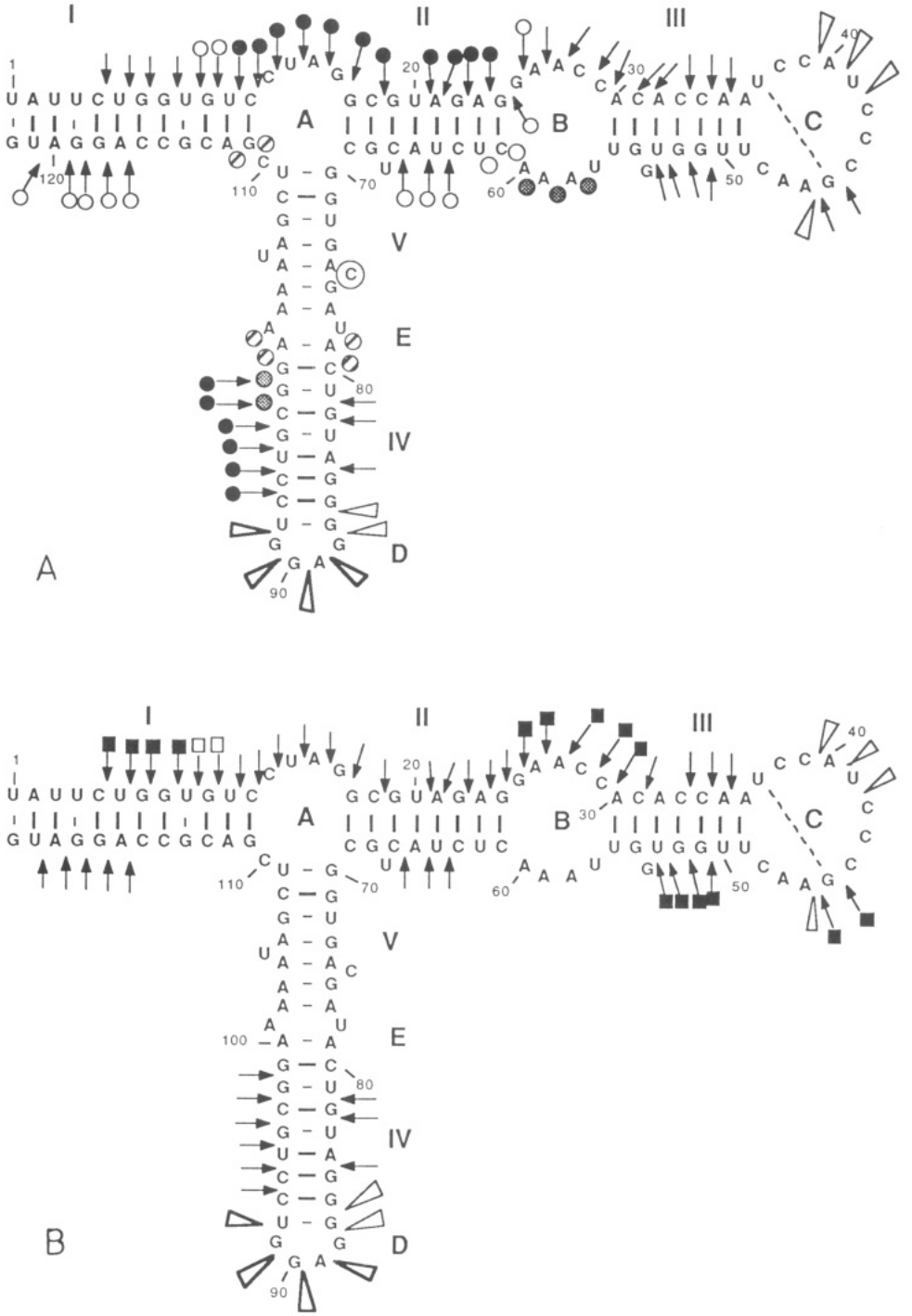

FIGURE 4: Secondary structure model of chloroplast 5S rRNA with the regions protected by CS-L12 (A) and CS-L13 (B). The secondary structure is from Delihas and Andersen (1982) with the modifications of Romby et al. (1988). RNase V_1 cleavages in free 5S rRNA are represented by arrows and single-stranded nuclease cleavages by an elongated triangle. The position of the nuclease cleavage is located at the 3′ phosphate of the cleaved nucleotide. Protections induced by CS-L12 and CS-L13 are represented by ○ and □, respectively. Phosphates protected by CS-L12 are shown by a slashed circle. The intensity of the symbols is proportional to the intensity of the protection. Encircled nucleotide represents the cytosine protected at position N3.

other side, still occur in the presence of the protein. The absence of protection in helix IV suggests again the absence of a close proximity of loop C with arm 3.

Conclusion

The present study permits characterization of proteins CS-L12 and CS-L13 that bind specifically and independently to the 5S rRNA in spinach chloroplast ribosomes. Our results show that the two proteins bind preferentially to helical and irregular regions that contain noncanonical base pairs and that their binding sites do not overlap. Taking into account several types of RNA–protein interaction in the ribosomal field, Garrett et al. (1984) have proposed two types of models for RNA–protein interaction sites. The first one is based on regular helices containing bulged nucleotides. The importance of bulged residues for protein recognition has been evidenced in the case of *E. coli* 5S rRNA/L18 complex (Peattie et al., 1981; Christiansen et al., 1985). Also, this has been particularly well studied for the R17-coat protein binding site by Wu and Uhlenbeck (1987), who stressed the role of a bulged-out adenine and of the nature of the surrounding base pairs in the specific RNA–protein interaction. In this view,

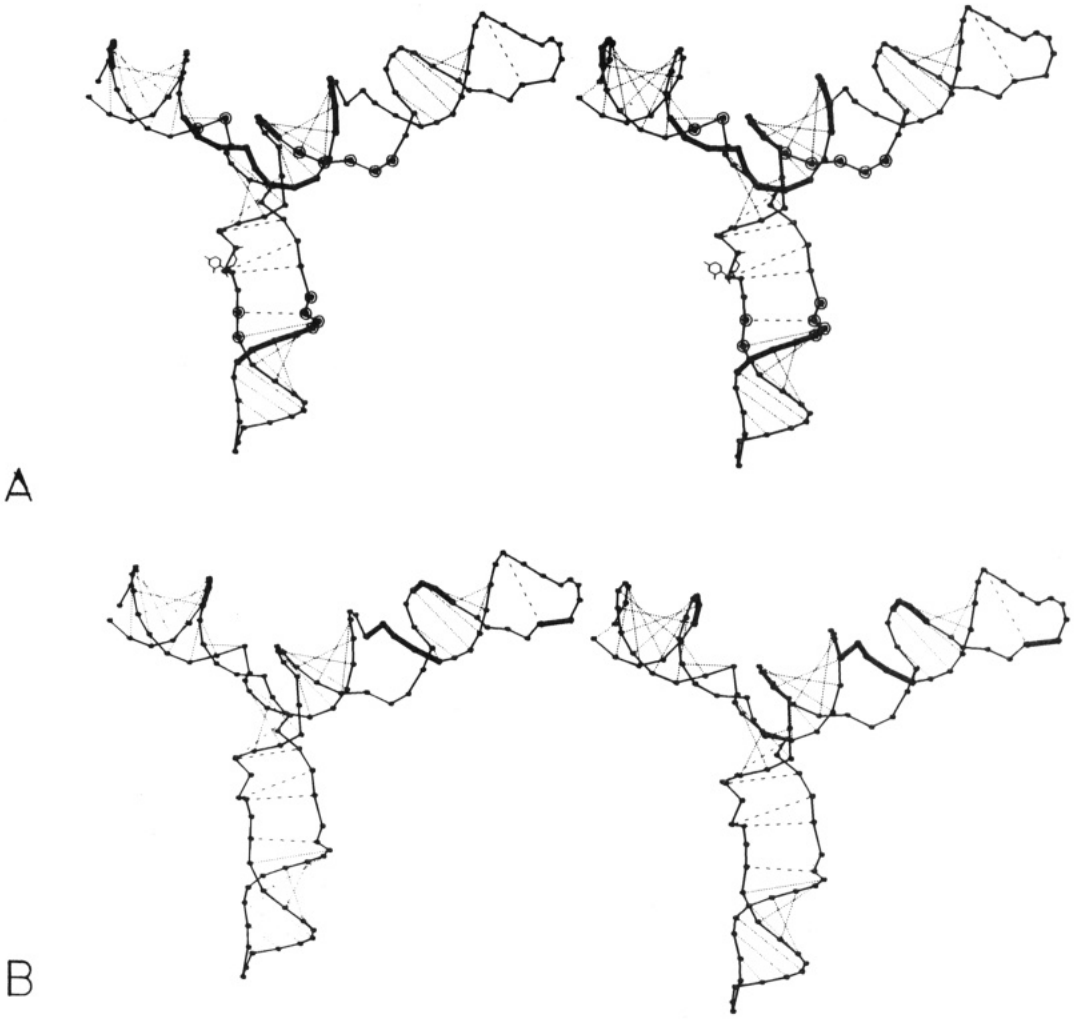

FIGURE 5: Phosphate backbone of the three-dimensional model of chloroplast 5S rRNA with the binding sites of proteins CS-L12 (A) and CS-L13 (B) proteins. The model is from Westhof et al. (1989). Stereoscopic views were drawn with the program PLUTO (S. Motherwell and P. Evans, MRC Cambridge). Phosphates protected by CS-L12 are represented by spheres; cytosine 75 protected by CS-L12 is drawn; RNase V_1 cleavages protected by CS-L12 and CS-L13 are represented by heavy lines, respectively.

protein CS-L13 essentially interacts with the 3′ strand of helix III, which contains a bulged nucleotide (G54), and with the 5′ part of loop B. The second type of RNA–protein interaction is characterized by the existence of irregular helical structures containing noncanonical base pairs. This type of recognition is probably used by protein CS-L12, which appears to be in close contact with region 70–106. Indeed, this domain contains two unusual G–U pairs in helix IV and several G–U, A–G, and A–A pairs in region E. The presence of bulged-out C75 shown to be in close contact with protein CS-L12 would lead to an intermediate type between the two types proposed by Garrett et al. (1984). Such a specific structure for an RNA–protein binding site has already been described in other ribosomal systems as the 16S rRNA binding site of *E. coli* ribosomal protein S8, which contains three bulged adenines and a noncanonical U–U pairing (Mougel et al., 1987), and that of protein S15 which contains G–U and G–A pairs and bulged-out purines (Mougel et al., 1988).

Compared to the *E. coli* system, several interesting points should be mentioned. In *E. coli*, three proteins (L5, L18, and L25) bind individually to the 5S rRNA. In the chloroplast system, only two 5S rRNA binding proteins could be identified, and their molecular weights are significantly higher than that of the *E. coli* proteins. In the case of protein CS-L13, its gene has been recently identified (Zhou et al., 1989): no sequence homology could be detected with the *E. coli* 5S rRNA binding proteins. However, surprisingly the central part of CS-L13 was found to be 35% homologous to the *E. coli* protein L22. The binding sites of all three *E. coli* proteins has been characterized by footprinting experiments [e.g., Peattie et al. (1981), Douthwaite et al. (1982), Huber and Wool (1984), and Christiansen et al. (1985)]. There are striking similarities between the binding domain of CS-L12 and that of *E. coli* protein L25 which protects domain 70–109 of the *E. coli* 5S rRNA (Douthwaite et al., 1979, 1982; Huber & Wool, 1984). For protein CS-L13, similarities with the *E. coli* binding proteins are less evident, except perhaps for *E. coli* L18. *E. coli* protein L18 has its primary attachment in helix II in which A66 has been found important for protein recognition (Christiansen et al., 1985). However, extending protections have been observed in helix III and loop C [e.g., Douthwaite et al. (1982) and Huber and Wool (1984)]. CS-L13 essentially protects loop C and helix III, but no protection from RNase V_1 could be detected in helix II. However, it must be emphasized that information is limited as compared to that for the CS-L12/5S rRNA complex since no chemical probes could be used.

In conclusion, we have defined the RNA sites of two primary binding proteins on 5S rRNA from spinach chloroplasts. They have separate domains that are consistent with the three-dimensional model proposed for the chloroplastic 5S rRNA. However, further experimental evidence is necessary

to define more precisely the primary attachment of these two proteins such as site-directed mutagenesis.

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