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Alteration of polynucleotide secondary structure by ribosomal protein S1

(RNA–protein interactions/aurintricarboxylic acid)

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ABSTRACT **Ribosomal 30S protein S1 causes disruption of the secondary structure of certain pyrimidine-containing polynucleotides. Helical poly(U), poly(C,U), and neutral and acidic poly(C) are stoichiometrically converted by S1 to structures indistinguishable from their partially or completely thermally denatured forms, as revealed by circular dichroism. Of the several double- and triple-stranded helical polynucleotides tested that contain one polypurine strand and at least one polypyrimidine strand, only the conformation of the DNA·RNA hybrid, poly(A)·poly(dT), is perturbed. In the presence of S1, this hybrid undergoes a transition to a new structure that has a circular dichroism spectrum unlike either the native or thermally denatured forms. Intercalated ethidium bromide is released from poly(A)·poly(dT) by S1, confirming the occurrence of a conformational rearrangement. The translation inhibitor, aurintricarboxylic acid, completely inhibits the action of S1 on polypyrimidines, but has no effect on the conformational perturbation of poly(A)·poly(dT). The possible relation between these observations and the biological function of protein S1 is discussed.**

Assignment of specific functional roles to the macromolecular components of the ribosome remains one of the most challenging problems of molecular biology. It can be said that none of the 57 ribosomal components may unambiguously be assigned a particular function in protein synthesis. The reason for this uncertainty is that the structural and functional properties of the ribosome depend to a high degree on cooperative interactions between the various constituent molecules. It would be of great utility in studies of this kind to be able to observe the functional properties of a single ribosomal macromolecule in the presence of all the others. Although such an experiment is impossible in principle, it might be reasonable to approach the situation with model systems containing, for example, a single ribosomal component and one other molecular species of defined structure and properties. Such a model system would be analogous to enzyme–substrate systems in which complex macromolecular substrates are replaced by simple molecules with predictable properties. Any model system suffers from the limitation of having a significantly different structure from the authentic biological system, but nevertheless may yield important clues about the function of a molecular structure that is otherwise too complex to study.

We have chosen to study as a model system the interaction of ribosomal protein S1 with polynucleotides. Protein S1 is of considerable interest since it has been found to be part of the replicase enzyme of Qβ bacteriophage (1), and has been shown to be identical to a protein that selectively inhibits translation *in vitro* (2–4), in addition to its presence in 30S subunits of ribosomes. Although S1 has been reported to reside in only a small fraction of 30S ribosomes (5, 6), recent studies have shown that it is present in all 70S ribosomes actively engaged in the polymerization process (7, 8). Detailed structure-function studies have suggested the involvement of S1 in the binding of messenger RNA (9, 10), and there are indications that it is important in a ribosomal function involving 30S-50S subunit interaction (11). Previous studies have implied that S1 binds to RNA molecules containing polypyrimidine sequences (12, 13), and this property may be related to its ability to bind to the 3′ terminus of 16S RNA (14, 15). The choice of model systems used in these studies reflects the observed interactions between S1 and pyrimidine-containing polynucleotides.

We have examined the interaction of S1 with a variety of polynucleotides of known structure by means of circular dichroism (CD). These studies reveal a dramatic disruption of the secondary structure of certain polynucleotide double helices by protein S1. These results may provide valuable insight into the role of S1 in protein synthesis and in viral RNA replication.

MATERIALS AND METHODS

Solutions. Buffer I contained 0.1 M NaCl, 0.01 M Na cacodylate, 0.01 M $MgCl_2$ at pH 7.0. Buffer II contained 0.1 M NaCl, 0.01 M Na cacodylate at pH 7.0. All measurements were taken on samples dissolved in buffer I except for poly(A)·poly(U).

Polynucleotides. Polynucleotides were obtained from P-L Biochemicals, and were used without further purification. Concentrations of homoribopolynucleotides were determined spectrophotometrically after alkaline hydrolysis. Double- and triple-stranded polynucleotides were either obtained commercially or generated from homopolymer constituents as described previously (16–18). Because of the results obtained in experiments, the stoichiometry of poly(A)·poly(dT) in our buffer system was checked by the method of continuous variations (16). Poly(dA)·2 poly(U) was generated by mixing equal A_{260} amounts of poly(dA) and poly(U), heating to 60°, and incubating at 4° overnight. The base composition of poly(C,U) was found to be C:U (0.93:1), as determined by the method of Katz and Comb (19).

Preparation of Protein S1. Protein S1 was isolated from 30S ribosomal subunits prepared from *Escherichia coli* strain Q13 and tested for translational activity as described previously (20). Sodium dodecyl sulfate gel electrophoresis of the purified protein revealed a single band.

Physical Measurements. Ultraviolet absorbance measurements were made on a Beckman ACTA V spectrophotometer equipped with an Auto-Sampler accessory. Transition temperatures were calculated as previously described (21).

CD spectra were recorded using a Durrum-JASCO J-20 spectropolarimeter calibrated with camphorsulfonic acid-d_{10} (22).

Abbreviations: CD, circular dichroism; t_m, melting temperature; BSA, bovine serum albumin; ATA, aurintricarboxylic acid.

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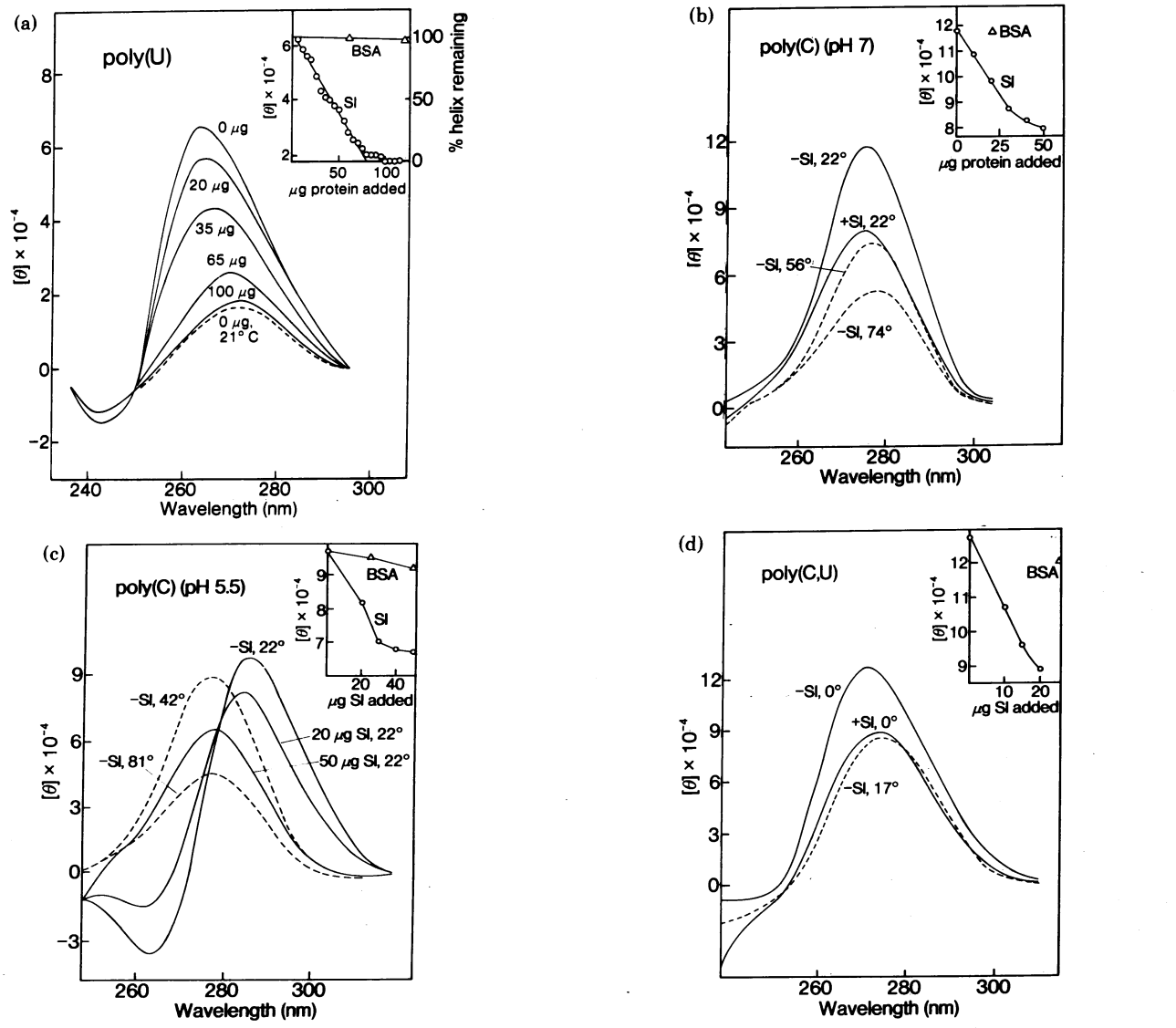

FIG. 1. CD spectra of helical polypyrimidines in the presence and absence of protein S1. The effect of S1 (○) and BSA (△) on the ellipticity maxima is shown in the *inserts*. Thermally perturbed spectra are indicated by *broken lines*, and S1-perturbed or unperturbed spectra are indicated by *solid lines*. Measurements were taken at indicated temperatures in buffer I (final volumes = 2.7 ml for panel a and 0.5 ml for panels b–d) as described in *Materials and Methods*. Addition of ATA (2×10^{-3} μmol/μg of S1) to S1 solution before it was mixed with polynucleotide gave spectra superimposable with the unperturbed spectra for all four polynucleotides. (a) Poly(U) (5.6×10^{-2} μmol of UMP, pH 7.0). Spectra were taken at 0° (solid lines) or 21° (broken lines). (b) Poly(C) (1.3×10^{-2} μmol of CMP, pH 7.0). The spectrum labeled +S1, 22° was taken in the presence of 50 μg of S1. (c) Poly(C) (1.3×10^{-2} μmol of CMP, pH 5.5). (d) Poly(C,U) (1.3×10^{-2} μmol of CMP + UMP, pH 5.5). The spectrum labeled +S1, 0° was taken after addition of 20 μg of S1.

Ethidium bromide steady state fluorescence measurements were taken with a Hitachi-Perkin-Elmer MPF-2A fluorescence spectrometer, using polynucleotide concentrations similar to those used in the CD experiments. Lifetime measurements were determined using an apparatus built by Dr. David S. Kliger. A pulsed coumarin 102 dye laser tuned to 490 nm served as the light source. The pulse-width at half-height was 7.5 nsec.

RESULTS

At low temperatures, poly(U) attains a stable secondary structure, thought to be a series of hairpin loops, and exhibits a transition to a disordered form with a melting temperature (t_m) of 4° in 0.01 M Mg^{2+} (23). This structural transition can be monitored by observation of the 262 nm circular dichroism (CD) band, which undergoes a loss of ellipticity accompanied by a small red shift upon melting of the poly(U) structure (ref. 23 and Fig. 1a). Addition of ribosomal protein S1 to helical poly(U) at 0° results in a CD spectrum indistinguishable from that of the thermally denatured form in the region between 245 and 300 nm (Fig. 1a). Below this region, differences in ellipticity attributable to protein may be seen. The effect of S1 is stoichiometric, rather than catalytic, requiring one molecule of S1 for the denaturation of about 48 nucleotide residues in poly(U) (Fig. 1a, insert). No change in the CD spectrum is seen upon addition of comparable concentrations of bovine-serum albumin (BSA).

Aurintricarboxylic acid (ATA), a known inhibitor of protein synthesis, completely inhibits the denaturation of helical poly(U) by S1 at a concentration of 2×10^{-3} mmol of ATA per mg of S1. At comparable concentrations, ATA is known to inhibit binding of mRNA to ribosomes and binding of poly(U) to S1 (12, 24). The inhibitory effect of ATA is observed whether it is added to the protein or the polynucleotide solution prior

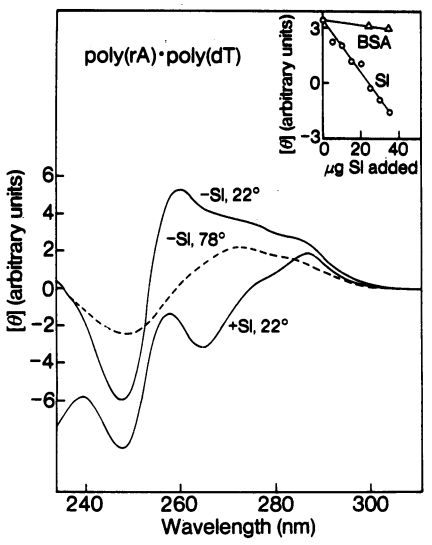

FIG. 2. CD spectra of poly(A)·poly(dT) in the presence and absence of S1. Spectra were taken with 0.1 A_{260} unit of poly(A)·poly(dT) and, where indicated, 35 μg of S1. The specific ellipticity [θ], is given in arbitrary units due to the uncertainty in calculating the extinction coefficient for this polymer. Measurements were made at the indicated temperatures in buffer I, pH 7.0 (final volume = 0.5 ml). The *insert* shows the effect of increasing amounts of S1 (○) or BSA (△) on the magnitude of the ellipticity maximum near 260 nm.

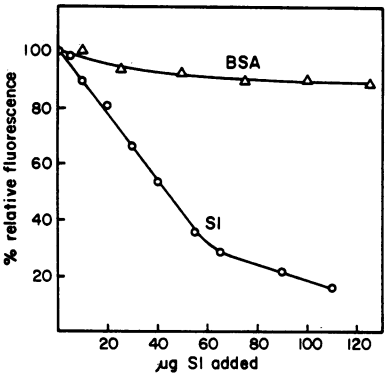

FIG. 3. Displacement of ethidium bromide from poly(A)·poly(dT) double helix by protein S1. Increasing amounts of S1 (○) or BSA (△) were added to 0.1 A_{260} unit of poly(A)·poly(dT) in 2.0 ml of buffer I, pH 7.0, 22°, containing 5×10^{-6} M ethidium bromide. Excitation was at 490 nm, with a spectral band width of 12 nm. After each protein addition, the fluorescence was scanned from 520 to 660 nm, using an emission band width of 14 nm. The intensity of the emission at the maximum (582 nm) relative to that of the ethidium bromide–polynucleotide complex in the absence of S1 (100%) is plotted as a function of protein concentration. Identical lifetimes (23.8 ± 0.5 nsec) were obtained for ethidium bromide bound to polynucleotide in the presence and absence of S1 protein. A small amount of quenching (about 6%) was observed at very high protein/polynucleotide ratios (>200 μg of added protein).

to mixing, but is not observed if ATA is added after addition of S1 to poly(U), thus suggesting that the binding of S1 to poly(U) is very strong. This effect is consistent with the binding studies of Tal *et al.* (12).

The CD spectrum of poly(C) at pH 7 [whose structure is thought to be a single-stranded helix (25, 26)] was found to be significantly perturbed upon the addition of S1 (Fig. 1b). At 22°, S1 induces a decrease in ellipticity equivalent to that caused by thermal denaturation between 50° and 60°. When the pH is changed to 5.5 poly(C) attains a double-helical conformation (27–29) with a t_m of 31° under our buffer conditions. Addition of S1 at 22° also changes the CD spectrum of this structure to that seen between 50° and 60° (Fig. 1c). However, when the temperature of this acidified polynucleotide is lowered to 5°, perturbation by S1 cannot be induced. The more stable acid poly(C) at pH 4.2 (t_m = 67°) can also have its CD spectrum perturbed by S1, but curiously, BSA also causes this change, although to a lesser extent (data not shown). Due to temperature-dependent CD changes in both acidic and neutral poly(C) at high temperatures (>70°), the spectra of the completely thermally denatured forms cannot be uniquely defined. Hence, it is impossible to determine the exact number of bases unstacked per S1 molecule. However, if the spectra taken at 81° for acidic poly(C) and at 74° for neutral poly(C) are assumed to approximate the thermally denatured structures, and the amounts of protein necessary to produce these spectra are known, we calculate that one protein molecule unstacks no more than 15 CMP residues. This may be a reflection of the greater conformational stability of poly(C). Inhibition of the action of S1 on both forms of poly(C) is seen at ATA concentrations identical to that which inhibits the poly(U)–S1 interaction.

Perturbation of the random copolymer poly(C,U) (1:1) by protein S1 is shown in Fig. 1d. This polynucleotide was found to have a hyperchromic thermal transition of 12° (pH 5.5). The transition is accompanied by a decrease in intensity and a shift in the ellipticity from 271 to 276 nm. The same transition is effected at 0° by the addition of one molecule of S1 per 42 nucleotide residues. Addition of S1 to poly(U) at 22° or to poly(dT) at any temperature above 0° does not induce CD changes in these polynucleotides, which are believed to assume unstacked random coil conformations under our conditions (16, 30, 31). We find no alteration at 22° of the CD spectrum of poly(A) (pH 7.0), a single-stranded stacked helical polynucleotide (32–34) which melts noncooperatively over a large temperature range. However, at pH 5.5 poly(A) forms a double-stranded helix (34–37) with a t_m of 56°. Both S1 and BSA slightly decrease the positive ellipticity of the acidic poly(A) at 265 nm, indicating the occurrence of nonspecific protein–nucleic acid interactions.

Several double- and triple-stranded helical polynucleotides containing one polypurine strand and at least one polypyrimidine strand were tested for susceptibility to structural perturbation by S1. Alteration of secondary structure was observed only with the RNA·DNA hybrid, poly(A)·poly(dT), a double-helical structure (t_m = 63°). S1 induces a transition to a new structure exhibiting a CD spectrum unlike either the native or thermally denatured forms (Fig. 2). Addition of S1 to the intercalated, ethidium bromide-poly(A)·poly(dT) complex results in the decrease of ethidium fluorescence without a concomitant change in the fluorescence lifetime of the remaining bound dye (see Fig. 3). No new lifetime species are generated other than the very short one associated with free ethidium. We interpret these observations to show that ethidium is released from the poly(A)·poly(dT) complex by S1. The CD spectrum of the ethidium-poly(A)·poly(dT) S1 mixture is identical to that without ethidium, suggesting that ethidium displacement is accompanied by a conformational change in the polynucleotide.

The unexpected CD spectrum of the S1–poly(A)·poly(dT) mixture might be attributed to a structure arising from the interaction of S1 with one denatured, separated polynucleotide strand. To test this possibility, we carried out an experiment using tandem CD cells, each containing one of the two constituent polynucleotides. The CD spectrum of the S1–poly(A)·poly(dT) mixture could not be mimicked by adding S1 to the

poly(A) cell, the poly(dT) cell or both cells. Mixing of the contents of the two cells, after addition of S1 to one or the other of the two strands, however, resulted in a spectrum identical to that obtained by addition of S1 to poly(A)·poly(dT). Thus, the observed CD spectrum depends in some way on the interaction of the two polynucleotide strands. It is interesting that the conformational perturbation of poly(A)·poly(dT) by S1 is not affected by ATA at concentrations up to 10-fold higher than that used in the polypyrimidine experiment.

It would seem plausible to test for partial strand separation by a determination of the UV thermal denaturation profile. The melting profiles in the presence and absence of S1 were found to be identical at a protein concentration that induces large changes in the CD spectrum of the polynucleotide. However, a CD spectrum of the poly(A)·poly(dT)–S1 complex taken at 60° (5° below the t_m) reveals that the spectrum reverts back to that of poly(A)·poly(dT) in the absence of S1. Thus, thermal denaturation studies are not useful in this instance because S1 dissociates from the polymer before the t_m is reached.

The CD spectra of the double-helical polynucleotides, poly(A)·poly(U) (in buffer II), poly(dA)·poly(dT), poly(I)·poly(C), and poly(G)·poly(dC), and the triple-helical polynucleotides, poly(A)·2 poly(U) and poly(dA)·2 poly(U), are not perturbed by S1. Nor was any CD change detected with the helical "hairpin" oligoribonucleotide $A_8U\text{-}G\text{-}U_6$.

DISCUSSION

The simplest structural interpretation of the CD measurements is that alteration of the polypyrimidine conformation by protein S1 is similar to that resulting from thermal denaturation, whereas the structure of poly(A)·poly(dT) induced by S1 is significantly different from the thermally denatured form (Figs. 1 and 2). In the presence of ethidium bromide, the latter structural change is accompanied by release of intercalated dye, supporting the interpretation that the S1-induced structure of poly(A)·poly(dT), although different from the thermally denatured form, is also significantly different from the original helical form (Fig. 3). Furthermore, CD measurements performed with tandem cells containing separate complementary strands show that the structural change in poly(A)·poly(dT) induced by S1 is not accompanied by complete strand separation.

It cannot be ruled out that these two kinds of conformational change occur by independent mechanisms, possibly at two different sites within the S1 molecule. Such a possibility is also suggested from the lack of inhibition by ATA of the poly(A)·poly(dT)–S1 interaction.

Because of the ability of S1 to bind tightly to pyrimidine-rich single-stranded polynucleotides (12, 13), a reasonable mechanism for the observed conformational changes would be the displacement of an equilibrium between double- and single-stranded forms by the removal of single-stranded molecules from the equilibrium process. This mechanism has been suggested for several DNA denaturing proteins (38). However, in the case of the protonated double helixes at pH 4.2, particularly poly(C), the acidic protein could act to change the pK_a of the polynucleotide in addition to the specific interaction postulated above. The evidence for this is the fact that BSA causes a smaller, but still significant, CD change in the double-helical poly(C) at 4.2. The protons responsible for the base pairing in double-helical poly(C) could act to promote the interaction of the protein with the nucleic acid, or the protein could simply remove the protons from the helix, causing some denaturation. The specific binding by S1 to the resulting denatured regions could account for its increased effect relative to BSA.

The above mechanisms, however, fail to account for the interaction of S1 with poly(A)·poly(dT). This molecule is very similar in terms of thermal stability to several of the polypyrimidines that contain double and triple helixes and whose CD spectra are not perturbed by S1. Perhaps poly(A)·poly(dT) has a conformation that is susceptible to perturbation by the protein while still in the double-stranded form.

Evidence that S1 interacts with the 3′ terminus of 16S RNA has recently been presented (14, 15). It is thought that this end, unusually rich in pyrimidine residues (39–42), forms a base-paired complex with mRNA during initiation of protein synthesis; such a complex has been shown to exist with R17 RNA (43). A necessary step in the formation of the complex is the disruption of the 16S RNA secondary structure at the 3′ terminus, which has been proposed to contain a hairpin loop (44). The responsibility of S1 for this disruption has been suggested (15) and certainly is not unreasonable in light of our results.

An analogous situation is the role that S1 may play in the function of Qβ replicase during initiation of RNA synthesis (45) from the (+) strand of Qβ RNA. The 3′ terminus of this RNA also contains a region rich in pyrimidine residues, most of which are thought to be base-paired (46). Again, the function of S1 may be to interact with the double-stranded region of the RNA, resulting in disruption of the secondary structure, and thereby facilitating initiation of viral RNA replication.

It is important to consider how closely the conformations of the synthetic polynucleotides used in this study resemble those of natural RNAs with which S1 may interact. In addition to double-stranded regions, mRNA and rRNA contain noncomplementary single-stranded domains, hairpin loops, and bulges. The secondary structure of a binding site on the nucleic acid that contains both single-strand and base-paired pyrimidine residues might be easier to perturb than one containing only double helix. Indeed, both of the pyrimidine-rich regions of the 3′ ends of 16S and Qβ RNAs contain a single-stranded stretch of pyrimidine residues adjacent to the base-paired sequences. Single-stranded regions, hairpin loops, and bulges are undoubtedly more susceptible to conformational perturbation than most of the polynucleotide structures used in this study. However, we have shown that S1 is capable of altering the conformation of the relatively stable double helical polynucleotide, poly(A)·poly(dT), which most likely adopts a different conformation than either RNA or DNA. Unusual geometries could exist in rRNA complexed with different ribosomal proteins. It must be emphasized that S1 has been shown to be complexed with or in the vicinity of other nucleic acid binding proteins, namely, elongation factors Ts and Tu and host factor I in Qβ replicase, and initiation factor 3 (47), as well as particular ribosomal proteins. These proteins could augment the effect of S1 on nucleic acid secondary structures. Further studies of S1-mediated conformational alterations of naturally occurring RNA fragments in the presence and absence of other nucleic acid binding proteins are required.

Note Added in Proof. D. Draper and P. H. von Hippel have reported (*ICN-UCLA Sym. Mol. Cell. Biol.*, in press) that S1 protein contains two polynucleotide binding sites.

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