DESTABILIZATION OF THE SECONDARY STRUCTURE OF RNA BY RIBOSOMAL PROTEIN S1 FROM ESCHERICHIA COLI

Wlodzimierz Szer*, José M. Hermoso**+, and Miloslav Boublik**

*Department of Biochemistry, New York University School of Medicine, 550 First Avenue, New York, N.Y. 10016
**Roche Institute of Molecular Biology, Nutley, New Jersey 07110

Received March 29,1976

SUMMARY: S1 is an acidic protein associated with the 3' end of 16S RNA; it is indispensable for ribosomal binding of natural mRNA. We find that S1 unfolds single stranded stacked or helical polynucleotides (poly rA, poly rC, poly rU). It prevents the formation of poly (rA + rU) and poly (rI + rC) duplexes at 10-25mM NaCl but not at 50-100mM NaCl. Partial, salt reversible denaturation is also seen with coliphage MS2 RNA, E. coli rRNA and tRNA. Generally, only duplex structures with a Tm greater than about 55° are formed in the presence of S1. The protein unfolds single stranded DNA but not poly d(A·T).

INTRODUCTION

We have previously shown that the presence of protein S1 on 30S ribosomes is indispensable for the binding of intact coliphage RNA during the initiation of protein synthesis (1, 2). Others have shown that S1 is associated with the 3' end of 16S RNA (3, 4) which is thought to interact with mRNA by base pairing (5, 6). It has been suggested that the function of S1 may be to hold the 3' end of 16S RNA in an unpaired conformation accessible for the binding of mRNA (4). An indication that S1 can alter the conformation of 16S RNA comes from work on thermal activation of 30S subunits (7). This process is accompanied by a decrease in the ordered structure of 16S RNA which is dependent on the presence of S1 (8). In this communication we show that protein S1 disrupts the ordered state of synthetic and natural single stranded polynucleotides.

+Present Address: University of Edinburgh, Department of Molecular Biology, Edinburgh, Scotland

Copyright © 1976 by Academic Press, Inc.
All rights of reproduction in any form reserved.

MATERIALS AND METHODS

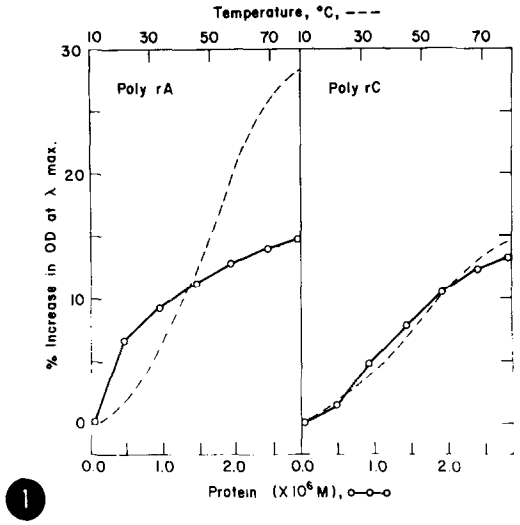
Electrophoretically homogeneous S1 was prepared as described (9). It had no nucleolytic activity toward [^{14}C]poly U, [^{14}C] poly A and [^{3}H]MS2 RNA when assayed according to Spahr (10). A molecular weight of 65,000 (11) was used to calculate the molar concentration of the protein. Synthetic polynucleotides were purchased from Miles Laboratories; their molar concentrations were determined from optical densities of mononucleotides produced by hydrolysis of the polymers with pancreatic ribonuclease or snake venom phosphodiesterase (Worthington). Phage MS2 RNA and E. coli rRNA were prepared as previously described (2, 9).

UV-melting profiles of polymers and their UV spectra in the presence of S1 were taken in a Beckman or Gilford spectrophotometer equipped with jacketed thermostated compartments (10mm cuvets). The protein was added directly to the cuvet in 5µl portions; optical density readings were corrected for the residual contribution of S1 in the 260nm region. Circular dichroic spectra were recorded with a Cary 61 spectropolarimeter using 5mm jacketed thermostated cells. Data are expressed as degree of ellipticity, $\theta°$, at the particular wavelength and are not corrected for the solvent refractive index.

RESULTS

Synthetic polyribonucleotides. At neutral pH poly rA and poly rC form single stranded helices with stacked bases which exhibit a non-cooperative thermal denaturation (for review, see ref. 12). It is known that S1 binds to single stranded polyribonucleotides, both synthetic and natural, since labeled polymers are retained on Millipore filters in the presence of the protein (9, 11, 13). We observed that addition of S1 to solutions of poly rA or poly rC at 10-20° induced a hyperchromic effect in UV or a decrease in the positive extremum in CD, both accompanied by a red shift in λ_{max}. A typical hyperchromicity experiment is shown in Fig. 1. The increases in optical density on addition of S1 are faster than can be determined in this experiment and do not change with time. At concentrations of S1 greater than about 3.0×10^{-6}M, measurements at 320nm indicate the appearance of a precipitate with both polymers.

Poly rU represents a different structural model since it forms a helical intramolecular hairpin (14) which undergoes a cooperative helix-coil transition. with a Tm of 28° in the presence of spermine (15). As seen from Fig. 2, S1 denatures the helix at 10°, the transition to the coil form being nearly complete



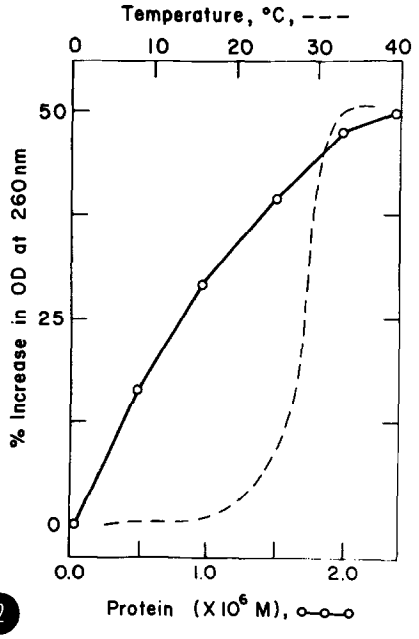


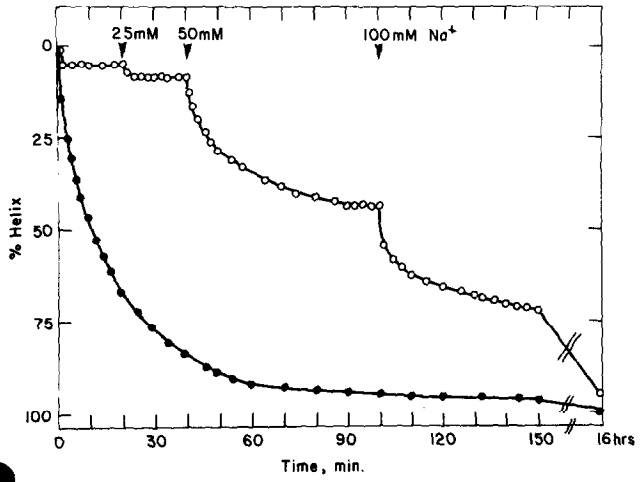
FIG. 1. Denaturation of poly rA and poly rC by S1 at 18°. The protein was added to a solution of poly rA (3.3×10^{-5}M) or poly rC (3.4×10^{-5}M) in a buffer containing 5mM Tris-HCl, pH 7.4, 10mM NaCl, o-o-o. The UV-melting profiles in the same buffer in the absence of S1 are shown by the dashed lines (refer to upper abcissa).

FIG. 2. Denaturation of helical poly rU by S1 at 10°. The protein was added to a solution of poly rU (2.1×10^{-5}M) in the buffer of Fig. 1 supplemented with 2×10^{-5}M spermine, o-o-o. The UV-melting profile of poly rU in the same buffer in the absence of S1 is shown by the dashed line (refers to upper abcissa).

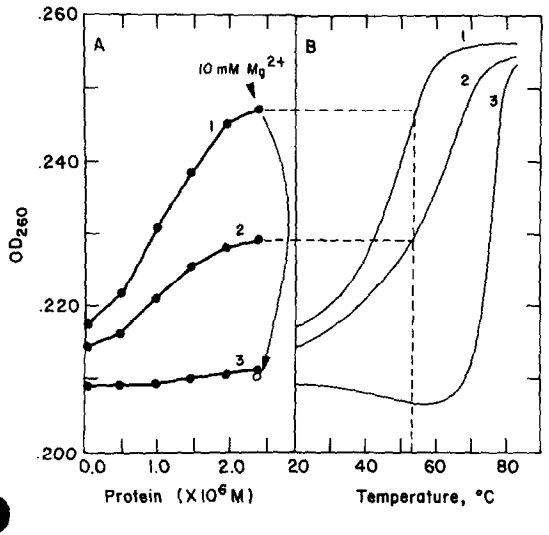
---

when the molar ratio of S1 to nucleotide approaches 1:10. In the absence of spermine, poly rU does not form a helix above 10°, and is almost fully hyperchromic. Mixing of S1 with poly rU at 10° in the absence of spermine produced no changes in UV or CD but prevented the formation of the helix on subsequent addition of spermine.

Fig. 3 shows that S1 prevents the formation of the poly (rA + rU) duplex in the presence of 10-25mM NaCl at 20°. In 50mM NaCl about 50% duplex is formed. This corresponds to a decrease in the Tm of about 26°, the Tm of poly (rA + rU) in 50mM NaCl being 46-47° (16). A further increase in NaCl to 100mM allows almost 100% duplex formation. In the presence of S1, duplex formation



3



4

FIG. 3. Effect of S1 on the formation of poly (rA + rU) at 20°. Poly rA and poly rU (2.4×10^{-5}M each) were mixed in a 10mM sodium phosphate buffer, pH 7.2 without S1, •-•-•, or in the presence of S1 (2.4×10^{-6}M), o-o-o. Arrows show additions of NaCl from a 4.0 M stock solution to the polymer solution containing S1. Zero time values were calculated from known optical densities of polymers.

FIG. 4. Denaturation of coliphage *MS2* RNA by S1 at 20°. A. Changes in OD_{260} were followed in a 5mM Tris-HCl buffer, pH 7.4, containing 10mM NaCl (curve 1); 100mM NaCl (curve 2); and 10mM NaCl, 10mM $MgCl_2$ (curve 3). Addition of $MgCl_2$ to a solution the RNA previously denatured by S1 (arrow) produces a drop in OD_{260}, O. B. UV-melting profiles are shown for comparison (salt conditions of plate A). Dashed lines show that the hyperchromic effect of S1 (2.4×10^{-6}M) at 10mM or at 100mM NaCl corresponds to that produced by heating of either polymer solution to 53-54°.

is slow, whereas in its absence poly (rA + rU) is formed almost instantly at 100 mM NaCl and 20°. This suggests that the dissociation of the protein from the polynucleotides may be the rate-limiting step. Analogous results were obtained with S1 and the poly (rI + rC) pair (not shown).

MS2, Ribosomal and Transfer RNA. Since single stranded RNA in solution also contains hairpin helices of varying lengths as well as single stranded stacked regions (12), we investigated the effect of S1 at 20° on coliphage MS2 RNA, rRNA and tRNA from E. coli. The effect of S1 on the structure of MS2 RNA (Fig. 4) is more pronounced at lower concentrations of NaCl. When stabilized by Mg^{2+} ions, the ordered structure of MS2 RNA is hardly affected by the protein. At a nearly saturing concentration of 2.4×10^{-6}M S1, denaturation of MS2 RNA corresponds to optical density changes obtained by heating the RNA to 52-54° whether in 10 or 100mM NaCl. When Mg^{2+} is added to a solution of the RNA previously denatured by S1 the original optical density is regained (Fig. 4). This appears to be analogous to the effect of increased NaCl on the formation of the poly (rA + rU) complex (Fig. 3) and it again shows that S1-induced denaturation can be reversed by counterions. It is known that the effect of DNA unwinding proteins is also reversed by cations (17).

Nearly identical results were obtained when experiments of Fig. 4 were carried out with 16S and 23S rRNA and with tRNA. In all cases denaturation by a nearly saturating concentration of S1 at 20° corresponds to the hyperchromic effect obtained by heating the RNA to 52-55° in the absence of S1, and can be reversed by Mg^{2+} or Na^{+}. Fig. 5 shows the effect of S1 on CD spectra of 23S RNA.

DNA and Synthetic Polydeoxyribonucleotides. Double-stranded DNA from E. coli is not denatured by protein S1 even at 0.1-1.0 mM NaCl; single stranded DNA (i.e. preparations heated above Tm and quenched at 0°) is unfolded by S1

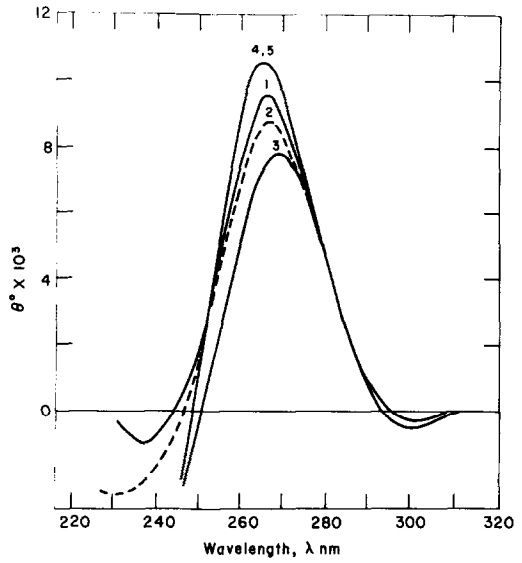


FIG. 5. Effect of S1 on circular dichroic spectra of 23S rRNA at 25°. Ellipticity of the RNA (0.812 A_{260} unit/ml in the buffer of Fig. 1) at 0, 3.0×10^{-6} and 4.5×10^{-6}M S1 is shown by curves 1, 2, and 3, respectively. Curve 4, reversal by Mg^{2+} (solution represented by curve 3 was made 10mM in $MgCl_2$). Curve 5, solution represented by curve 1 (no S1), made 10mM in $MgCl_2$. Curves 4 and 5 are superimposable in the 250-280 nm region.

as are single stranded RNAs (see preceding section). The poly d(A·T) copolymer is not affected under conditions where it is unfolded by DNA unwinding proteins (18, 19). No spectral changes are seen when S1 is mixed with poly dA or with poly dT. The result with poly dT was expected since poly dT, in contrast to poly rT (20) and poly rU (15), is a fully hyperchromic random coil under any ionic conditions (16). The rate of formation of the poly (dA + dT) duplex was markedly decreased by S1 ($t_{1/2}$ increased from 11 to about 90 min at 16mM NaCl and 20°). This suggests that S1 binds to poly dT rather than to poly dA since binding to poly dA should have been detected by optical methods. A more detailed account of the effect of S1 on polydeoxynucleotides will appear elsewhere.

DISCUSSION

This paper shows that homogeneous protein S1 unfolds a variety of stacked or helical single stranded polynucleotides. As in the case with DNA unwinding

proteins (17-19), there is no sharp specificity in the binding of polynucleotide substrates to S1, e.g. single stranded natural RNA and DNA are unfolded, but certain preferences can be discerned. Polypyrimidines appear to be a preferred target, e.g. poly rA is much less affected than are poly rC and poly rU. Tracts of pyrimidine nucleotides appear to be essential, e.g. the hairpin helix of poly rU is disrupted but the analogous poly d (A·T) structure is not.

Since S1 is naturally associated with the 3' end of 16S rRNA, it may function to hold this region in an unpaired conformation. This could be essential for allowing base pairing between a pyrimidine-rich sequence near the 3' end of 16S rRNA and a complementary purine-rich sequence present in the cistron initiation region of many natural mRNAs (5, 6). Our data indicate that while S1 will prevent the formation of relatively unstable helical regions, it will allow more stable structures, viz, those with a Tm greater than about 55°. S1 is the α subunit of phage Q_β replicase and is required for in vitro transcription of Q_β RNA (+) strands or poly rC (21). It is conceivable that its role in replication is related to the unwinding properties described here. S1 is the first protein involved in the unwinding of RNA. It is not clear whether the unfolding of single stranded DNA by S1 is of any physiological significance.

Acknowledgements

We are indebted to Drs. J. Thomas and M. Rush for comments on the manuscript. We thank Miss M. DiPiazza for excellent technical assistance. This work was aided by Grants AI-11517 and CA-16239 from the National Institutes of Health.

References

1. Szer, W., and Leffler, S. (1974) Proc. Natl. Acad. Sci. USA, 71, 3611-3615.
2. Szer, W., Hermoso, J.M., and Leffler, S. (1975) Proc. Natl. Acad. Sci. USA, 72, 2325-2329.
3. Dahlberg, A.E. (1974) J. Biol. Chem., 249, 7673-7678.
4. Dahlberg, A.E., and Dahlberg, J.E. (1975) Proc. Natl. Acad. Sci. USA, 72, 2940-2944.

5. Shine, J., and Dalgarno, L. (1975) Nature, 254, 34-38.
6. Steitz, J.A., and Jakes, K. (1975) Proc. Natl. Acad. Sci. USA, 72, 4734-4738.
7. Zamir, A., Miskin, R., and Elson, D. (1971) J. Mol. Biol., 60, 347-364.
8. Hermoso, J.M., Boublik, M., and Szer, W. (1976) Arch. Biochem. Biophys., in press.
9. Hermoso, J.M., and Szer, W. (1974) Proc. Natl. Acad. Sci. USA, 71, 4708-4712.
10. Spahr, P.F. (1964) J. Biol. Chem., 239, 3716-3726.
11. Inouye, N., Pollack, Y., and Petre, J. (1974) Eur. J. Biochem., 45, 100-117.
12. Felsenfeld, G., and Miles, H.T. (1967) in Ann. Rev. Biochem., 36, 407-448.
13. Miller, M.J., Niveleau, A., and Wahba, A.J. (1974) J. Biol. Chem., 249, 3803-3807.
14. Thrierr, J.C., Dourlent, M., and Leng, M. (1971) J. Mol. Biol., 58, 815-830.
15. Szer, W. (1966) J. Mol. Biol., 16, 585-588.
16. Riley, M., Maling, B., and Chamberlain, M.J. (1966) J. Mol. Biol., 20, 359-389.
17. Anderson, R.A., and Coleman, J.E. (1975) Biochemistry, 14, 5485-5491.
18. Alberts, B.M., and Frey, L. (1970) Nature, 227, 1313-1318.
19. Weiner, J.H., Bertsch, L.L., and Kornberg, A. (1975) J. Biol. Chem., 250, 1972-1980.
20. Shugar, D., and Szer, W. (1962) J. Mol. Biol., 5, 580-582.
21. Kamen, R., Kondo, M., Romer, W., and Weissmann, C. (1972) Eur. J. Biochem., 31, 44-51.