Proc. Natl. Acad. Sci. USA
Vol. 74, No. 11, pp. 4786–4790, November 1977
Biochemistry

Escherichia coli ribosomal protein S1 has two polynucleotide binding sites

(protein–nucleic acid interactions/protein–nucleic acid binding constants/phage Qβ replication/protein synthesis/protein fluorescence)

DAVID E. DRAPER*, CHARLES W. PRATT†, AND PETER H. VON HIPPEL

Institute of Molecular Biology and Department of Chemistry, University of Oregon, Eugene, Oregon 97403

Communicated by V. Boekelheide, August 1, 1977

ABSTRACT **The interaction of *Escherichia coli* ribosomal protein S1 with a variety of RNA and DNA oligomers and polymers has been studied, using both a sedimentation technique and the quenching of intrinsic protein fluorescence upon nucleic acid binding to obtain equilibrium binding parameters. Two polynucleotide binding sites have been detected on S1: site I binds either single-stranded DNA or RNA and does not discriminate between adenine- and cytidine-containing polynucleotides, while site II binding is highly specific for RNA over DNA and shows a marked preference for cytidine polynucleotides over the corresponding adenine-containing species. On the basis of the binding properties of S1 to denatured DNA cellulose and poly(rC)-cellulose, it is demonstrated that every S1 molecule carries both a site I and a site II. Some possible implications of these results for mechanisms of protein synthesis and phage Qβ replication are briefly considered.**

The *Escherichia coli* ribosomal protein S1 has a number of properties which suggest that it plays a very central role in protein synthesis and RNA phage replication. Although it is a "fractional" protein [i.e., it is present in less than one copy per purified 30S subunit (1)], it is virtually required for the binding and translation of poly(rU) and some natural messenger RNAs (2). S1 has also been shown to be the "factor i" protein (3, 4) described by Revel *et al.* (5), which inhibits translation in *in vitro* systems at molar ratios of protein to ribosomes greater than one (6). This inhibition is apparently due to S1 binding the mRNA directly (7) and is most effective with polypyrimidine messengers and least effective with polypurines (8). Further, the phage Qβ codes for a replicase that complexes with S1 and two other host proteins (3). S1 is required in this complex for recognition and replication of the phage plus strand, but not the complementary minus strand (9).

All three of these activities of S1—binding mRNA to the ribosome, inhibiting translation, and aiding replication of Qβ RNA—probably depend in some way on the ability of S1 to bind polynucleotides. However, the mechanisms by which S1 functions in any of these three activities are not really understood in any detail. With regard to the influence of S1 on the binding and translation of mRNA, a number of suggestions have been made: (*i*) that S1 forms part of the mRNA binding site on the ribosome (10); (*ii*) that S1 melts a region of secondary structure in the 16S ribosomal RNA to facilitate formation of base pairs between the rRNA and mRNA in initiation of translation (11); (*iii*) that S1 acts to stabilize this same base-paired region (12); and (*iv*) that S1 affects the tertiary structure of mRNA to allow it to bind the 30S subunit (13).

Although these hypotheses predict different nucleic acid binding properties for S1, information on the interaction of this protein with nucleic acids is meager. The ability of S1 to bind RNA has been qualitatively demonstrated by filter binding assays (7, 10) and RNA-cellulose chromatography (14). Some investigation has been made of the influence of S1 on polynucleotide secondary structure (15, 16) and the possibility of sequence-specific S1 binding (17). Further understanding of the role of S1 protein in protein synthesis and in Qβ replication requires more detailed knowledge of its nucleic acid binding properties. We need to know what features of nucleic acids are recognized, whether binding is preferential for single- or double-stranded regions, whether there is any base or sequence specificity, and so forth.

To this end, we initiated a survey of S1 binding interactions, using both a sucrose gradient sedimentation technique and quenching of intrinsic protein fluorescence to measure binding constants of this protein to various oligo- and polynucleotides (18). In this communication we present results that demonstrate the existence of two independent polynucleotide binding sites on S1: one, which we call site I, binds either single-strand RNA or DNA with approximately equal affinity, while the second (site II) is highly specific for RNA and binds poly(rC) more tightly than poly(rA). This result seems to have major implications for a further definition of the roles of S1 in protein synthesis and phage replication. The detailed properties of these nucleic acid binding sites of S1 will be described elsewhere.

MATERIALS AND METHODS

Buffer. "Standard buffer" is 10 mM sodium phosphate at pH 7.7/1 mM Na_2EDTA/1 mM 2-mercaptoethanol/10% (vol/vol) glycerol, plus the indicated concentration of NaCl; e.g., "0.1 M standard buffer" contains 0.1 M NaCl in addition to the other buffer components.

Nucleotides. Polyribonucleotides were purchased from Miles, and polydeoxyribonucleotides and all oligonucleotides from Collaborative Research. Oligoribonucleotides of cytosine and adenosine were used in these studies; they are listed by the manufacturer as ranging in size from 18 to 22 residues, and 16 to 25 residues, respectively. For purposes of this paper, we can consider these oligonucleotides as having a length equal to the average of the stated distribution, and will refer to them as $(rC)_{\overline{20}}$ and $(rA)_{\overline{19}}$.

S1 Protein. To prepare ^3H-labeled S1, a 25-ml culture of *E. coli* B was grown for two generations in the presence of 0.5 mCi of [^3H]leucine (New England Nuclear), harvested, and lysed by lysozyme freeze-thaw in 2 ml 0.1 M Tris·HCl pH 8.0. The lysate was digested 1 hr at room temperature with DNase (100 μg/ml) in the presence of increasing concentrations of NaCl, up to 0.4 M. After cell debris and ribosomes had been pelleted

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

* Present address: Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder, CO 80302.
† Present address: Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139.

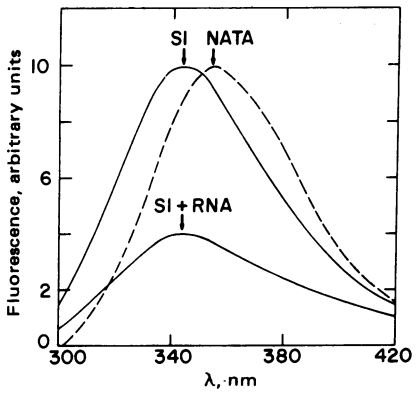

FIG. 1. Fluorescence emission spectra of S1 protein. Arrows are at the emission maxima for S1 (0.78 μM S1); *N*-acetyltryptophanamide (NATA, 2 μM); and S1 + RNA [0.78 μM S1 plus 4.8 μM $(rC)_{\overline{20}}$]. All spectra were taken in 0.1 M standard buffer.

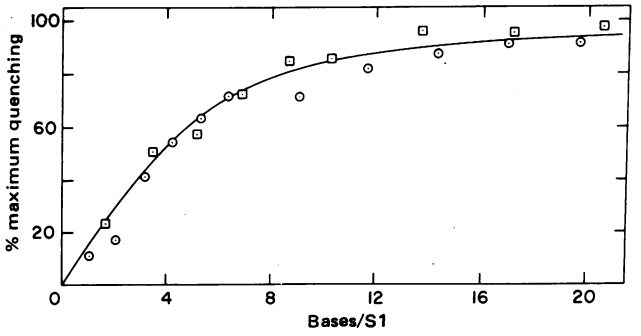

FIG. 2. Fluorescence titrations of S1 with poly(dA) (□) and poly(dC) (○). Titrations were in 0.1 M standard buffer. With poly(dA) the concentration of S1 was 0.59 μM, and the maximum quenching was 34% of the initial fluorescence. With poly(dC), [S1] = 0.52 μM, and the maximum quenching was 39%. The solid line is an "overlap" binding isotherm (23) calculated with parameters $K_a = 1.5 \times 10^6$ M^{-1} and $n = 6$ bases. (The average value of n for site I, derived from a number of independent measurements, is 5.1 ± 1.0 bases; D. E. Draper and P. H. von Hippel, unpublished data.)

in the ultracentrifuge, the lysate was dialyzed against "DNA-cellulose column buffer" containing 20 mM Tris at pH 8.0, 0.1 M NaCl, 5 mM Na_2EDTA, 1 mM 2-mercaptoethanol, and 10% glycerol. A small (0.7 × 3 cm) denatured DNA-cellulose column was prepared by the UV irradiation procedure of Litman (19), using calf thymus DNA (Worthington) denatured in a boiling-water bath for 10 min and chilled quickly on ice. After the lysate had been loaded, the column was rinsed successively with 15 ml of the column buffer, and 5 ml each of the same buffer containing 0.25, 0.5, and 1.0 M NaCl. The peak of radioactivity eluting with the 1.0 M salt wash was passed through a Bio-Gel P-30 column (1.1 × 20 cm) equilibrated with 0.1 M standard buffer. The void volume contained S1 protein that was greater than 95% pure, as judged by sodium dodecyl sulfate gel electrophoresis. The protein retains its DNA binding activity for at least several months when stored at −20°; bovine serum albumin at 0.1 mg/ml is generally added to prevent protein from sticking to the tube walls.

Milligram quantities of S1 were also prepared using DNA-cellulose chromatography. A crude S1 fraction was prepared from either *E. coli* B (Grain Processing, Muscatine, IA) or *E. coli* MRE 600 (Medical Research Establishment, Salisbury, England) by pelleting ribosomes from a crude lysate, resuspending in buffer [20 mM Tris at pH 8.0/0.1 M NH_4Cl/5 mM $Mg(OAc)_2$/3 mM 2-mercaptoethanol] and dialyzing versus 1 mM Tris at pH 7.7 to release S1 (10). After the ribosomes had been repelleted, the supernatant was dialyzed against DNA-cellulose column buffer and chromatographed by the same procedure used for [^{3}H]S1, scaled up to accommodate a column containing 4.5 g of cellulose and 50–60 mg of denatured DNA. For a final purification step, the protein eluting with 1.0 M salt was sedimented on sucrose gradients in 2 M NH_4Cl, and the S1 peak was located by reading gradient fractions in a fluorimeter. Protein purified by this procedure is better than 95% pure by sodium dodecyl sulfate gel electrophoresis, and comigrates with the S1 band of purified 30S subunits electrophoresed in either sodium dodecyl sulfate at pH 8.5 or 6 M urea at pH 4.5. The A_{280}/A_{260} ratio is 1.65, and Lowry protein analysis (20) gives an extinction coefficient at 280 nm of 0.60 ml mg^{-1} cm^{-1}.

Fluorescence Measurements. Uncorrected fluorescence emission spectra were measured with a Schoeffel spectrofluorimeter as described by Kelly and von Hippel (21). Titrations of S1 protein were performed in a Hitachi–Perkin Elmer MPF-2A fluorimeter at 25°, using a cuvette of inside dimensions 3 mm × 3 mm. The exciting wavelength was 294 nm, and emission was read at 340 nm, relative to a standard of *N*-acetyltryptophanamide (21). All titrations reported here were performed in 0.1 M standard buffer at 26°. Oligomer binding constants and fluorescence quenching maxima were calculated from double reciprocal plots; binding constants are reported on a per mol oligomer basis, and the reproducibility of the measured oligomer binding constants is approximately ±10% (22). Fluorescence quenching titrations with nucleic acid polymers were plotted as Scatchard plots and fitted to a non-cooperative binding equation for overlapping potential binding sites as described by McGhee and von Hippel (23). Intrinsic (per mole site) binding constants (to ±20%) and binding site sizes (to ±30%) were obtained by this technique.

RESULTS AND DISCUSSION

Fluorescence properties of S1

Protein fluorescence is generally due entirely to tryptophan and tyrosine residues. Using the spectral method of Edelhoch (24), we have determined that S1 has 9 ± 1 tyrosines and 5 ± 1 tryptophans. The tyrosine value is in good agreement with amino acid analyses of S1 (10); the tryptophan content had not been previously determined. The fluorescence emission spectrum of S1 is shown in Fig. 1. The emission maximum (344 nm) is blue-shifted from the maximum of the compound *N*-acetyltryptophanamide (355 nm), a model for a protein tryptophan completely exposed to solvent. Thus the S1 tryptophans are (on the average) in a somewhat hydrophobic environment. No separate contribution of tyrosine (emission maximum 310 nm) to the spectrum could be detected (25), indicating that all the tyrosine fluorescence is emitted via energy transfer to neighboring tryptophans.

Fig. 1 also shows the decrease in fluorescence intensity that occurs upon addition of RNA to S1. Maximal quenchings of about 65% are observed with RNA, accompanied by a slight shift in the emission maximum to 342–343 nm. With DNA, the maximum quenching is substantially less (see below).

Binding of S1 to DNA involves one binding site

Fluorescence Titrations. By following the decrease in fluorescence intensity at the emission maximum as nucleic acids are added, equilibrium binding curves can be obtained. Such titrations of S1 with DNA homopolymers poly(dC) and poly(dA) are shown in Fig. 2. The quenchings have been plotted as percent of maximum quenching to emphasize the similarity between the two titrations; we find that poly(dC) consistently gives a slightly higher maximum quenching (average value

about 35%) than poly(dA) (average 31%). These values are roughly half the maximum quenching seen with RNA polymers.

The solid line in Fig. 2 has been generated using the "overlap" equation of McGhee and von Hippel (23). This binding equation takes into account the fact that the binding of (protein) ligands to a homogeneous lattice so as to cover more than one lattice unit (i.e., more than one nucleotide residue in single-stranded binding) is intrinsically anticooperative, simply because the number of potential binding sites covered per ligand is not a linear function of the number of ligands bound. (On a long lattice every nucleotide residue represents the beginning of a potential binding site, and more potential sites are occluded per ligand bound at low than at high ligand binding density.) If binding is otherwise noncooperative, two binding parameters, K_a (the association constant, in units of M^{-1}) and n (the site size, expressed as nucleotide residues covered per ligand), describe the binding isotherm and can be systematically varied by computer iteration to provide a least-squares best fit to the binding data.

Both the poly(dA) and poly(dC) titrations are well fit with a binding constant of 1.5×10^6 M^{-1} and a site size of six nucleotide residues. This experiment suggests that S1 binding to DNA shows little base specificity, and the shape of the binding isotherm also confirms that this binding is noncooperative.

Sedimentation Analysis. The fluorescence titrations would not detect DNA binding that does not result in quenching of intrinsic protein fluorescence. Also, this method does not distinguish between (e.g.) one DNA binding site giving 30–35% quenching, and two sites of identical affinity, each responsible for one-half the observed quenching. To examine these possibilities, the affinity of S1 for denatured DNA was also measured using a sucrose gradient band sedimentation technique.

This method provides a rapid and accurate measurement of association constants for a variety of interactions and will be described in detail elsewhere. ^{3}H-labeled binding protein and a rapidly sedimenting nucleic acid are mixed at very small protein to nucleic acid ratios (the number of potential protein binding sites on the nucleic acid is thus essentially equivalent to the number of nucleotide residues), layered onto a sucrose gradient, and centrifuged. For binding constants smaller than $\sim 10^{10}$ M^{-1} (assuming a diffusion-controlled association reaction), the rate of dissociation of the complex is fast compared to the rate of sedimentation; as a consequence the protein and nucleic acid in the band are in equilibrium throughout the run. As the nucleic band moves through the gradient, dissociated protein is left behind and new equilibria are continuously reestablished between the nucleic acid and the protein remaining in the band. A simple mathematical analysis of the fraction of protein still migrating with the nucleic acid after the band has traversed a given distance predicts that this fraction will be a steep function of the binding constant and the initial nucleic acid concentration, making an accurate estimation of the protein–nucleic acid binding constant possible.‡

Fig. 3 shows the sedimentation of [^{3}H]S1 protein with denatured λ phage DNA. The *left* panel demonstrates that at sufficiently high DNA concentrations all the label migrates with the DNA; thus every S1 molecule is capable of binding DNA. Upon lowering of the DNA concentration (*right*), some of the labeled protein remains behind the DNA peak. On the basis of determination of the fraction of protein remaining bound to DNA after sedimentation as a function of component concentration, it is shown that single-stranded DNA binds noncooperatively to S1 and a binding constant of 3.0×10^6 M^{-1} is estimated, in good agreement with the binding constants for homopolymer DNA determined by fluorescence. Because the fluorescence titrations monitor occupancy of (quenchable) binding *sites*, and the sedimentation method monitors protein *molecules* bound, we conclude from these results that S1 has a single binding site for DNA.

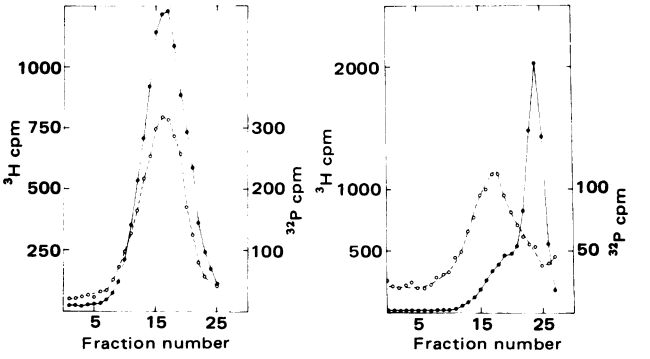

FIG. 3. Sucrose gradient sedimentation of [^{3}H]S1 (●—●) with denatured λ [^{32}P]DNA (O- - -O). Samples (0.2 ml) of protein and denatured DNA were layered on 5.0-ml 5–20% sucrose gradients in 0.1 M standard buffer, and centrifuged 4 hr at 35,000 rpm in a Beckman SW 50.1 rotor at 5°. (*Left*) [DNA] = 4.8 μM; (*Right*) [DNA] = 1.0 μM.

Existence of a second polynucleotide binding site

Having established the existence of a single DNA binding site, we now ask whether further titration with RNA might reveal another binding site. Using the fluorescence assay to detect binding, we first titrated S1 with the DNA oligomer $(dA)_4$. As expected, this oligomer shows binding to a single site; the measured affinity constant for this oligomer to S1 is 4.4×10^6 M^{-1} (ref. 22; D. E. Draper and R. H. von Hippel, unpublished data). After addition of a 20- to 25-molar excess of $(dA)_4$ over S1 to ensure saturation of this binding site, S1 was titrated further with either $(rA)_{\overline{19}}$ or $(rC)_{\overline{20}}$ (Fig. 4, curves a and b). Binding of the RNA oligomers to a second site on S1 is indeed observed. The rC oligomer titrates S1 nearly stoichiometrically, to a binding density of *two S1 proteins per oligomer*. Thus, n (the binding site size) for this second site must be ~10 nucleotide residues. The corresponding rA oligomer has a much weaker affinity for S1 under these conditions, but also binds to this second site.

The binding site detected with DNA polymers and oligomers we call site I. We have now shown that when this site is saturated with DNA there remains a second population of binding sites that can be titrated with RNA, which we term site II. Two deductions about the binding specificity of site II can be made from the data presented in Fig. 4: it must be highly specific for RNA over DNA; and, in contrast to site 1, it has a substantial preference for binding oligo(rC) over oligo(rA). This latter binding preference has been shown to be due to the fact that oligo- and poly(rC) bind cooperatively to site II, while oligo and poly(rA) do not (ref. 22; D. E. Draper and P. H. von Hippel, unpublished data). This difference in the *net* binding affinity of site II for poly(rC) and poly(rA) may be the basis for the pyrimidine specificity of the S1 "factor i" activity (18).

A comparison of titrations in the presence and absence of $(dA)_4$ shows that site I is capable of binding RNA as well as DNA. Curve c of Fig. 4 is a titration of S1 with $(rC)_{\overline{20}}$ in the absence of any competing DNA oligomer; the titration curve shows a sharp break indicative of two populations of binding

‡ Using this method, we have measured binding affinities of ribonuclease for native and denatured DNA, and have reproduced within a factor of two the values determined by a completely independent technique (26, 27).

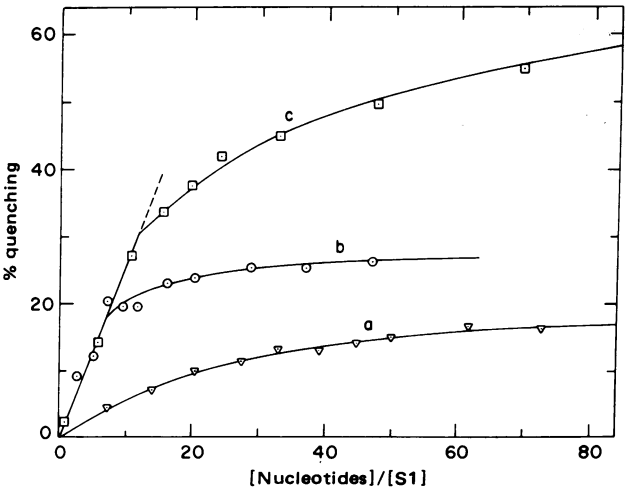

FIG. 4. Fluorescence titrations of S1 with RNA and DNA oligomers. All titrations are in 0.1 M standard buffer, concentrations of S1 are curve a, 0.65 μM; curve b, 0.74 μM; curve c, 0.76 μM. Titrations a and b are in the presence of 16 μM $(dA)_4$; the indicated quenching is *in addition* to the 43% quenching from the $(dA)_4$. Titration a is with $(rA)_{\overline{19}}$; the solid line is calculated for a binding isotherm with $K_a = 3.7 \times 10^6\ M^{-1}$ and a maximum quenching of 18.1%. Titrations b and c are with $(rC)_{\overline{20}}$. The solid line in b is calculated for two cooperatively interacting proteins (ref. 22; D. E. Draper and P. H. von Hippel, unpublished data). In c, the solid line above 30% quenching is calculated for a binding constant of $1.5 \times 10^6\ M^{-1}$, and a maximum quenching of 32%.

sites with widely different binding affinities. [Binding constants and cooperativity parameters for both sites have been measured for various oligo- and polynucleotides as a function of environmental conditions (ref. 22; D. E. Draper and P. H. von Hippel, unpublished data).] The first part of this titration is nearly identical to the titration of S1 with $rC_{\overline{20}}$ in the presence of $(dA)_4$ (curve *b*), and can be ascribed to site II binding. The second portion of curve *c*, which is eliminated by addition of excess $(dA)_4$, must be due to site I binding. The break in curve c is sharp enough so that, to a good approximation, the quenching up to the break (~30% quenching) can be considered entirely due to site II binding, and the remainder of the quenching ascribed entirely to site I. From these data an intrinsic association constant of $\sim 1.5 \times 10^6\ M^{-1}$ for site I binding to single-stranded poly(ribocytidylic acid) may be estimated for these conditions.[§] We note that this value is very close to the binding constants determined for polydeoxyribonucleotides to site I (above). We showed previously that titration of S1 with $(rA)_{\overline{19}}$ alone gives a titration curve analyzable in terms of a two-site binding isotherm (18), though in that case the difference in binding affinity between the two populations of sites is only about a factor of five. We conclude, therefore, that site I is capable of binding either DNA or RNA oligomers.

All S1 molecules contain both a site I and a site II

Finally, we ask whether the two binding sites observed are both present in every protein molecule, or whether some portion of the protein carries a site I, and the remainder a site II. A report

[§] Estimation of binding constants from titrations with oligomers must take into account explicitly the statistical factor due to the fact that, in principle, the protein can bind to the oligomer lattice in a number of different ways (e.g., see ref. 28). Sometimes, as a result of special modes of binding, this factor must be modified (28). This situation, as it applies to the binding sites of S1 protein, will be discussed in detail elsewhere. The binding constants presented in this paper have been corrected for this factor as necessary, and so are directly comparable.

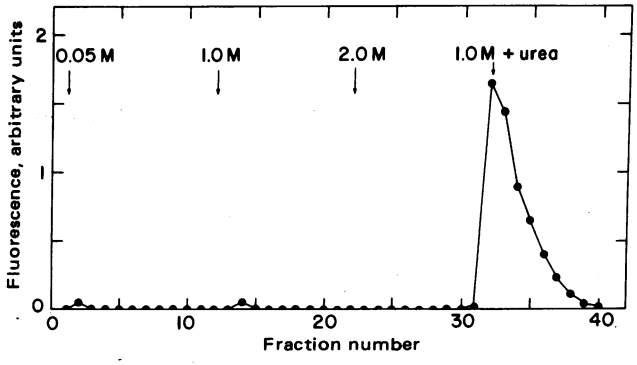

FIG. 5. Elution of S1 from poly(rC)-cellulose. Twenty-eight micrograms of S1 (purified by DNA-cellulose chromatography) were loaded onto a poly(rC)-cellulose column (0.7 × 3.0 cm) in 0.05 M standard buffer, and eluted successively with 5.0 ml each of 0.05, 1.0, and 2.0 M standard buffer, followed by 1.0 M standard buffer plus 6 M urea. Fractions (0.5 ml) were assayed for S1 fluorescence.

that S1 is separable into two components under some conditions (29) raises the possibility that there could be two different conformations of S1, or that modified and unmodified forms of S1 with different nucleic acid binding properties could coexist. The fluorescence titrations we have presented support the presence of both sites on every molecule; for example, $(rA)_{\overline{19}}$ binds in a stoichiometry of *two oligomers per protein* (18), i.e., a *total* of two binding sites per protein; while $(dA)_4$ binds with a maximum stoichiometry of *one oligomer per protein*, i.e., one site I per protein. In addition, the following results provide further evidence that *each* S1 molecule contains both a site I and a site II:

(*i*) Our purification procedure for S1, using DNA-cellulose, ensures that every protein has a DNA binding site. This is supported by the sucrose gradient shown in Fig. 3, which demonstrates that every S1 protein is capable of DNA binding. Even if site II had a weak affinity for DNA (not detectable by fluorescence measurements) that might enable it to bind DNA-cellulose, two peaks of S1 protein should be found during chromatography because site I binding to polynucleotides is very salt dependent (ref. 22; D. E. Draper and P. H. von Hippel, unpublished data). In fact, only one sharp peak of S1 is seen (at 0.65 M NaCl) in the elution of S1 from a DNA-cellulose column with a salt gradient (data not shown).

(*ii*) Chromatography of S1 on poly(rC)-cellulose demonstrates that every S1 protein carries a site II. We have shown that all the S1 purified by DNA-cellulose chromatography binds to poly(rC) at high salt concentrations. Furthermore, as noted above, one of the characteristics of site II is a very high (cooperative binding) affinity for poly(rC). In addition, we have shown that site II binding of polyribonucleotides is virtually salt independent; in fact, binding becomes somewhat *tighter* at high salt concentrations (ref. 22; D. E. Draper and P. H. von Hippel, unpublished data). Also Carmichael (14) has shown that S1 cannot be eluted from poly(rC)-cellulose with high concentrations of salt, but requires urea for removal.

Fig. 5 confirms this finding with S1 purified by our DNA-cellulose procedure, and shows that NaCl concentrations of up to 2 M are insufficient to remove S1 from poly(rC)-cellulose. The binding of poly(rC) to site I is certainly no stronger than the binding to denatured DNA; the binding constant for this material derived from the data of Fig. 4 is comparable to that obtained for poly(dC) and denatured DNA from Figs. 2 and 3. Thus, any S1 proteins containing only site I should elute from the poly(rC)-cellulose column at approximately the same concentration of salt required to elute them from denatured DNA-cellulose; no such protein can be detected in our experi-

ment. S1 does not form dimers or other multimers, even at very high concentrations (3); hence any S1 molecules retained by a polynucleotide affinity column must be interacting *via* a polynucleotide binding site.

We therefore conclude that every S1 protein has both a site I and a site II. Besides demonstrating this fact, this set of experiments is also a rigorous demonstration of the purity and full activity of the protein preparation with respect to its nucleic acid binding properties.

Implications of two S1 binding sites

The different specificities of site I and site II for RNA and DNA provide a convenient method for distinguishing between polynucleotides binding in the two sites. Titrations with DNA oligomers and polymers can be used to investigate site I binding, while titrations with RNA in the presence of saturating DNA oligomer probe site II. Using this approach, we have determined that the two sites have very different binding properties: site I recognizes the sugar-phosphate backbone of single-stranded DNA or RNA with little base discrimination, while site II binds the base and sugar of RNA and shows little or no interaction with backbone phosphates. The apparent tight binding of site II to oligo- and poly(rC) is due to cooperative binding with polypyrimidines (ref. 22; D. E. Draper and P. H. von Hippel, unpublished data).

The presence of these two very different binding sites on S1 will require a re-examination of physical chemical data on S1 already in the literature. For instance, in studies on the effects of S1 on polynucleotide conformation as detected by circular dichroism (15) and UV absorption (16), it is not clear in all cases which of the two binding sites is responsible for the effects seen, or whether an effect is due to both sites interacting simultaneously with a polynucleotide. The RNA–DNA specificities of the two sites can of course be used to resolve these questions.

The identification and characterization of two S1 binding sites has important implications for the function of this protein in both protein synthesis and Qβ phage replication. The Qβ replicase complex is known to bind two specific widely separated sequences on the Qβ RNA simultaneously (30, 31). Because S1 protein is an essential part of the complex, one or both of the S1 binding sites could therefore be involved in facilitating recognition of the appropriate RNA sequences by this system.

Both S1 binding sites may be involved in protein synthesis as well. It has been suggested that S1 binds to a specific region of 16S ribosomal RNA (32) and facilitates initiation of translation by perturbing the secondary structure of the 16S rRNA (11). Others have suggested that S1 binds directly to the mRNA (2, 10) and may be required to melt out the secondary or tertiary structure of the message (13). With two sites available S1 could, of course, bind both 16S rRNA (perhaps *via* site II) and mRNA (*via* site I), either simultaneously or sequentially, and thus facilitate proper binding of mRNA to the ribosome in translation. Further characterization of the binding properties of the two sites should aid in establishing the biological functions of each.

We are very grateful to Mrs. Ruth Draper for her excellent technical assistance in many phases of this work. This research was supported in part by U.S. Public Health Service Research Grants GM-15792 and GM-15423, as well as by a Predoctoral Traineeship (to D.E.D.) from U.S. Public Health Service Training Grant GM-00444, and U.S. Public Health Service Postdoctoral Fellowship GM-51288 (to C.W.P.). This work has been submitted (by D.E.D.) to the University of Oregon Graduate School in partial fulfillment of the requirements for the Ph.D degree.

1. Voynow, P. & Kurland, C. G. (1971) *Biochemistry* **10,** 517–524.
2. Van Duin, J. & Van Knippenberg, P. H. (1974) *J. Mol. Biol.* **84,** 185–195.
3. Groner, Y., Scheps, R., Kamen, R., Kolakovsky, D. & Revel, M. (1972) *Nature New Biol.* **239,** 19–20.
4. Wahba, A. J., Miller, M. J., Niveleau, A., Landers, T. A., Carmichael, G. C., Weber, K., Hawley, D. A. & Slobin, L. I. (1974) *J. Biol. Chem.* **249,** 3314–3316.
5. Revel, M., Pollack, Y., Groner, Y., Scheps, R., Inouye, H., Berissi, H. & Zeller, H. (1973) *Biochimie* **55,** 41–51.
6. Van Dieijen, G., Van der Laken, C. J., Van Knippenberg, P. H. & Van Duin, J. (1975) *J. Mol. Biol.* **93,** 351–365.
7. Jay, G. & Kaempfer, R. (1974) *J. Mol. Biol.* **82,** 193–212.
8. Miller, M. J. & Wahba, A. J. (1974) *J. Biol. Chem.* **249,** 3808–3813.
9. Kamen, R., Kondo, M., Roemer, W. & Weissman, C. (1972) *Eur. J. Biochem.* **31,** 44–51.
10. Tal, M., Aviram, M., Kanarek, A. & Weiss, A. (1972) *Biochim. Biophys. Acta* **281,** 381–392.
11. Dahlberg, A. E. & Dahlberg, J. E. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 2940–2944.
12. Steitz, J. A., Wahba, A. J., Laughrea, M. & Moore, P. B. (1977) *Nucleic Acids Res.* **4,** 1–5.
13. Van Dieijen, G., Van Knippenberg, P. H. & Van Duin, J. (1976) *Eur. J. Biochem.* **64,** 511–518.
14. Carmichael, G. G. (1975) *J. Biol. Chem.* **250,** 6160–6167.
15. Bear, E. G., Ng, R., Van Derveer, D., Johnson, N. P., Thomas, G., Schleich, T. & Noller, H. F. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 1824–1828.
16. Szer, W., Hermoso, J. & Boublik, M. (1976) *Biochem. Biophys. Res. Commun.* **70,** 957–964.
17. Senear, A. W. & Steitz, J. A. (1976) *J. Biol. Chem.* **251,** 1902–1912.
18. Draper, D. E. & von Hippel, P. H. (1976) in *Molecular Mechanisms in Gene Expression,* eds. Nierlich, D. P., Rutter, W. J. & Fox, C. F. (Academic Press, New York), pp. 421–425.
19. Litman, R. M. (1968) *J. Biol. Chem.* **243,** 6222–6233.
20. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193,** 265–275.
21. Kelly, R. C. & von Hippel, P. H. (1976) *J. Biol. Chem.* **251,** 7229–7239.
22. Draper, D. E. (1977) Ph.D. Dissertation (University of Oregon, Eugene, OR).
23. McGhee, J. D. & von Hippel, P. H. (1974) *J. Mol. Biol.* **86,** 469–489.
24. Edelhoch, H. (1967) *Biochemistry* **6,** 1948–1954.
25. Eisinger, J. (1969) *Biochemistry* **8,** 3902–3908.
26. Jensen, D. E. & von Hippel, P. H. (1976) *J. Biol. Chem.* **251,** 7198–7214.
27. Jensen, D. E. & von Hippel, P. H. (1977) *Anal. Biochem.* **80,** 267–281.
28. Kelly, R. C., Jensen, D. E. & von Hippel, P. H. (1976) *J. Biol. Chem.* **251,** 7240–7250.
29. Lee-Huang, S. & Ochoa, S. (1972) *Biochem. Biophys. Res. Commun.* **49,** 371–376.
30. Wollenweider, H. J., Koller, T., Weber, H. & Weissman, C. (1976) *J. Mol. Biol.* **101,** 367–377.
31. Kamen, R. I. (1975) in *RNA Phages,* ed. Zinder, N. D. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 203–234.
32. Dahlberg, A. E. (1974) *J. Biol. Chem.* **249,** 7673–7678.