Characterization of the Pf3 Single-Strand DNA Binding Protein by Circular Dichroism Spectroscopy†

Michael D. Powell and Donald M. Gray*

Program in Molecular and Cell Biology, Mail Stop FO 31, The University of Texas at Dallas, P.O. Box 830688, Richardson, Texas 75083-0688

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ABSTRACT: We have used circular dichroism (CD) spectroscopy and gel electrophoresis to characterize the single-strand DNA binding protein (ssDBP) of the bacteriophage Pf3 and its complexes with Pf3 DNA and various DNA and RNA homopolymers. The secondary structure of Pf3 ssDBP had <1% α-helix and therefore was probably a β-sheet structure like the fd gene 5 protein (g5p). From CD titrations, the binding stoichiometry of Pf3 ssDBP was two nucleotides per protein monomer ($n = 2$) for complexes formed with all of the nucleic acids except poly[r(U)], for which $n = 3$ (in a buffer of 10 mM Tris-HCl and 70 mM NaCl, pH 8.2). Evidence of an additional binding mode of $n = 4$ for complexes formed with Pf3 DNA was found by gel electrophoresis experiments. Pf3 ssDBP showed a marked sequence dependence in binding affinities similar to that known for the fd g5p.

The gene 5 protein (g5p)[1] of bacteriophage Ff (M13,f1,fd) is a model for the single-strand DNA (ssDNA) binding proteins of filamentous phages (Kowalczykowski et al., 1981). The fd g5p plays both structural and regulatory roles during the phage life cycle. Alberts et al. (1972) showed that the g5p binds tightly and cooperatively to ssDNA in a stoichiometric manner (one protein monomer per every four DNA nucleotides, and the binding is salt-sensitive). In addition, the binding of g5p to ssDNA results in unstacking of the DNA bases (Alberts et al., 1972). Pretorius et al. (1975) and Cavalieri et al. (1976) have shown by sedimentation equilibrium that the fd g5p exists primarily as a dimer in solution. The helix-destabilizing properties of this protein help it promote the change from the synthesis of double-stranded replicative form DNA to the synthesis of single-stranded viral genome DNA (Oey & Knippers, 1972; Salstrom & Pratt, 1971). The fd g5p has been shown to be a translational repressor of multiple phage proteins (Zaman et al., 1991), and the protein plays a structural role in the prepackaging of viral DNA in preparation for the extrusion of mature phage. All of these functions are carried out by a protein of only 87 amino acids.

The crystal structure of the fd g5p without bound nucleic acid has been solved by X-ray diffraction (Brayer & McPherson, 1983; Skinner et al., 1993). The solution structure has been investigated by ^{1}H NMR (de Jong et al., 1987, 1989; King & Coleman, 1988; Folkers et al., 1991) and other techniques, such as circular dichroism (CD) spectroscopy, linear dichroism (LD) spectroscopy, electron spin resonance (ESR), and fluorescence spectroscopy [van Amerongen et al., 1990; see Kansy et al. (1986) for a review]. fd g5p is predominantly a β-sheet structure arranged into three major loops: a complex loop, a dyad loop, and a DNA binding loop. Recently, the DNA binding loop has been refined by 2D NMR studies and has been shown to be present in IKe g5p, wild-type M13, and a Y41H mutant of M13 (van Duynhoven et al., 1990; Folkers et al., 1991). Mutational analyses are being carried out on fd g5p in an effort to better understand the underlying protein–nucleic acid interactions (Stassen et al., 1992; Zabin et al., 1991). One important result from these studies is that there appear to be different residues involved in the specific binding to the 5′-untranslated region of the gene II mRNA and in the nonspecific binding to ssDNA.

The filamentous phage Pf3 is of a different structural class than the Ff phage (Day et al., 1988) and has *Pseudomonas aeruginosa* (RP1) as its host (Stanisich, 1974). The nucleotide sequence of Pf3 has been determined, and although it shares little overall sequence homology to the Ff phage, there is an apparent conservation of gene arrangement and relative size and numbers of open reading frames (Luiten et al., 1985). The coding regions for ssDNA binding proteins have been identified in both Pf3 (78 amino acids, 8907 Da; Putterman et al., 1984) and fd (87 amino acids, 9690 Da; Cuypers et al., 1974). A comparison of the amino acid sequences of fd g5p and the ssDNA binding protein (ssDBP) of Pf3 reveals little overall homology. There does, however, appear to be conservation of particular amino acids, especially in the region of the DNA binding loop of fd g5p (Peeters et al., 1983).

The *in vivo* precursor complex of Pf3 has been isolated and studied by electron microscopy, UV absorbance, CD spectroscopy, and sedimentation analysis (Casadevall & Day, 1985). In Casadevall and Day's study, the *in vivo* complex of Pf3 appeared to adopt a helical conformation similar to that seen with the fd g5p complex. In the current study, we have utilized purified protein to reconstitute complexes *in vitro*. The properties of these complexes should provide a valuable comparison with those formed by the fd g5p and aid in further elucidation of the general chemical and structural bases of the mechanisms for this entire class of proteins.

CD spectroscopy is particularly suited to the study of the aromatic residues of these proteins and the conformational changes of nucleic acids upon binding. Neither fd g5p nor Pf3 ssDBP has tryptophan residues, so that tyrosines provide the major source of optical activity in the 220–240-nm region. Of the five tyrosines present in fd g5p, three (Tyr-26, Tyr-56,

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[1] Abbreviations: ssDBP, single-strand DNA binding protein; g5p, gene 5 protein; Tris, tris(hydroxymethyl)aminomethane; CD, circular dichroism; OD, optical density; EDTA, ethylenediaminetetraacetic acid; TAE buffer, Tris–acetate (0.04 M) and EDTA (0.001 M), pH 8.0.

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and Tyr-61) are conserved in the Pf3 ssDBP (Tyr-22, Tyr-53, and Tyr-57) when the sequences are compared using the alignment of Peeters et al. (1983). The remaining two tyrosines of fd g5p (Tyr-34 and Tyr-41) are conservatively substituted by phenylalanine in the Pf3 ssDBP (Phe-28 and Phe-37). The role that the tyrosines of fd g5p play in binding has been the focus of chemical modification and NMR studies (Anderson et al., 1975; King & Coleman, 1988; Folkers et al., 1991). In these studies, Tyr-26 was shown to be the only tyrosine that directly interacts with ssDNA, while Tyr-41 was implicated in protein dimer–dimer contacts.

In this article, we describe the isolation of the Pf3 ssDBP and its characterization by use of CD spectroscopy. Stoichiometries and binding affinities of Pf3 ssDBP for the homopolymers poly[d(A)], poly[r(A)], poly[d(C)], poly[r(C)], poly[d(T)], and poly[r(U)], as well as for single-stranded Pf3 DNA, were determined by CD titrations and salt dissociation of complexes. In addition, we have used gel electrophoresis to characterize complexes of ssDBP with Pf3 ssDNA.

MATERIALS AND METHODS

Bacteriophage Isolation. Pf3 bacteriophage was obtained from L. A. Day (Public Health Research Institute, New York), and host *Ps. aeruginosa* (PA02) harboring RP1 was obtained from R. H. Olsen (University of Michigan, Ann Arbor, MI). Phages were purified from single plaque isolates and propagated in LB (10 g of tryptone, 5 g of yeast extract, and 10 g of NaCl) containing 15 μg of tetracycline (Sigma).

Protein Isolation and Purification. Pf3 ssDBP was isolated by a modification of the procedure previously described for the isolation of fd g5p (Alberts et al., 1972; Gray et al., 1982). During the treatment of lysates of infected bacterial cells with DNase, followed by 1 M NaCl treatment, significant amounts of protein were lost as insoluble precipitate (yields <0.5 mg/g of wet cells). Pf3 ssDBP was also difficult to completely redissolve after ammonium sulfate precipitation, and significant quantities of it were lost as insoluble matter. Pretorius et al. (1975) have reported solubility problems with fd g5p when disulfide bonds form between the single cysteines of fd g5p monomers. Since Pf3 ssDBP also contains a single cysteine, the same types of problems could occur with ssDBP. The buffers used in the original protocol of Alberts et al. (1972) contained 1 mM β-mercaptoethanol as a reducing agent. We tried varying the concentration of β-mercaptoethanol up to 10 mM throughout the precipitation and DNase steps with no success in eliminating the precipitation problems. Therefore, the isolation procedure was modified to exclude the 1 M NaCl treatment and the ammonium sulfate precipitation steps prior to loading on the DNA cellulose column. The buffer conditions that provided the best solubility consisted of at least 50 mM NaCl and a pH of at least 8.0, as long as the protein concentration was kept low.

Elimination of the 1 M NaCl treatment, ammonium sulfate precipitation, and dialysis steps from the protocol improved yields to 0.5–1.0 mg of protein/g of wet cells. Presumably the protein that bound to the DNA cellulose column was free protein in the lysate or protein that exchanged from phage ssDNA to the ssDNA of the column during loading. Passage of the lysate over the column for a second time often increased the yield.

Ps. aeruginosa (PA02) cells were grown in LB medium plus tetracycline to an OD_{600} of 0.2 (ca. 2 h), infected with Pf3 phage at a multiplicity of infection (MOI) of 10, and allowed to grow to onset of the lag phase (7–8 h total). Cells were harvested by centrifugation and washed three times in "cell" buffer (10 mM Tris and 70 mM NaCl, pH 8.2). Pellets were then stored frozen at −20 °C. Thawed pellets from 3 L of growth medium were combined and adjusted to a final volume of approximately 50 mL with cell buffer plus 1 mM β-mercaptoethanol. The cell suspension was sonicated with a 50-W sonicator, on wet ice, in alternating cycles of 15 s on and 30 s off until the lysate was nonviscous. The lysate was then clarified by centrifugation at 12000*g* in a Sorvall SS-34 rotor. The supernatants were pooled and applied directly to a DNA cellulose column (100 mL bed volume) prepared as previously described (Litman, 1968). The column was washed successively with 200-mL aliquots of cell buffer containing 0.07 M NaCl, 0.2 M NaCl, and 0.4 M NaCl. Finally, the column was washed with 100 mL of cell buffer containing 1 M NaCl. The final elution was performed in four washes of approximately 25 mL each with a 1–2-h wait between each wash. The combined 1 M washes were concentrated by ultrafiltration (Amicon YM5, >5000-Da cutoff) to a volume of approximately 5 mL. The concentrate was then placed on a Sephadex G-50 column (3 × 78 cm) calibrated with molecular weight markers (Sigma G-75-50) and eluted with cell buffer. The lagging fraction was saved and reconcentrated to give an OD_{277} of 0.5–1.0. The protein concentration was determined spectroscopically using an extinction coefficient of 4177 L mol^{-1} cm^{-1} at 277 nm, calculated from reference values for the extinction coefficients of tyrosine and phenylalanine (*CRC Handbook of Biochemistry*, 1970) and the known amino acid content of the protein. The protein thus isolated was >95% pure as determined by polyacrylamide gel electrophoresis, silver-staining, and densitometric quantitation (Figure 1). Molecular weight markers for SDS–PAGE were mixtures of bovine aprotinin (6500), cytochrome *c* (12 400), carbonic anhydrase (29 000), and bovine serum albumin (66 000), all of which were purchased from Sigma Chemical Co. (St. Louis, MO). The apparent molecular weight of the Pf3 ssDBP in SDS–PAGE gels was only about 6500, compared with the true molecular weight of 8907 (Putterman et al., 1984), but was consistent with previous observations (Casadevall & Day, 1985).

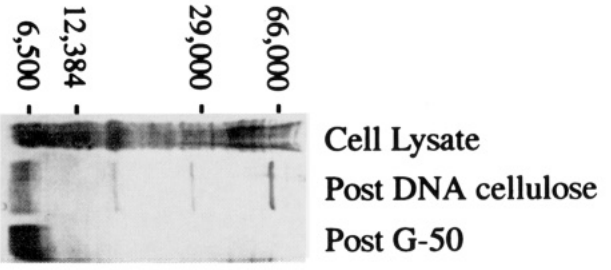

FIGURE 1: An 18% SDS–PAGE gel showing protein compositions of the cell lysate after sonication, the 1 M NaCl wash of the DNA cellulose column, and the purified protein after chromatography on a Sephadex G-50 column. Molecular weight markers were from Sigma (see Materials and Methods).

Nucleic Acids. All of the polynucleotides used in this study were purchased from Sigma. The average lengths of poly[d(A)], poly[d(T)], and poly[d(C)] were 800, 3000, and 150 nucleotides, respectively, as determined by the manufacturer. The average lengths of poly[r(A)], poly[r(U)], and poly[r(C)] were all greater than 350 nucleotides, as determined from molecular weights supplied by the manufacturer. All of the polymers were dialyzed as described by Antao (1988), and the final nucleic acid solution was dialyzed into cell buffer. The concentrations of the single-stranded forms of the

polynucleotides were determined using molar extinction coefficients (L mol^{-1} cm^{-1}) at 260 nm of 9650 for poly[d(A)] (Bollum, 1966), 8140 for poly[d(T)] (Bollum, 1966), 8015 for poly[d(C)] (Gray et al., 1992), 9200 for poly[r(U)] (Riley et al., 1966), 10 300 for poly[r(A)] (Riley et al., 1966), and 6280 for poly[r(C)] (Johnson et al., 1990). Pf3 DNA was isolated by multiple phenol extractions of whole phage and was dialyzed into cell buffer. Concentrations of Pf3 DNA were determined using an extinction coefficient of 7770 L mol^{-1} cm^{-1} at 259 nm (Clack, 1988). To check the binding of ssDBP to a shorter oligomeric DNA, a 48-nucleotide oligomer (48-mer) was used (synthesized by R. L. Ratliff, Los Alamos National Laboratory, Los Alamos, NM). The 48-mer had the following essentially random sequence: 5′-CCC ATT GTT CAC GTT AAT CCG GAA CGG CAC CTG ATA AGA ACC GGC AGG-3′. An extinction coefficient of 9416 L mol^{-1} cm^{-1} at 260 nm was calculated from a first-neighbor equation using the known extinction coefficients of monomers and dimers (Cantor et al., 1970). The 48-mer was dialyzed into cell buffer prior to use in titration experiments.

Absorption and CD Measurements. Absorption spectra were measured with a Cary Model 118 spectrophotometer, and CD spectra were obtained using a Jasco Model J500A spectropolarimeter calibrated and operated as described by Antao et al. (1988). Spectra were collected at 0.1-nm intervals and smoothed by a sliding 13-point quadratic–cubic function (Savitzky & Golay, 1964). Plotted data are shown as $\epsilon_L - \epsilon_R$ in units of L mol^{-1} cm^{-1}, per mole of amino acid or per mole of nucleotide, at nanometer intervals. Except where noted, the temperature was maintained at 20 ± 0.5 °C.

Titrations and Salt Dissociations. All titrations were performed as previously described for fd g5p (Kansy et al., 1986), except that the Pf3 ssDBP was in cell buffer (10 mM Tris-HCl and 70 mM NaCl, pH 8.2). Salt dissociation of complexes was accomplished by the addition of aliquots of 4 M NaCl. Exact concentrations were determined from the change in weight before and after the additions, taking into account the density of 4 M salt. For the dissociation experiments, each polynucleotide was saturated at a [protein monomer]/[nucleotide] molar ratio of 0.5, with the exception of poly[r(U)] which was saturated at a 0.33 molar ratio. The complexes were formed such that the total concentration of protein was 23 μM in each case. Dissociation was monitored by the change in CD at 275 nm after each addition of NaCl for all nucleic acids, except for poly[d(A)] which was monitored at 250 nm. The percent dissociation was calculated from a comparison of the CD spectrum of partially dissociated complex to the spectrum of free nucleic acid, corrected for the change in absorbance of the free nucleic acid due to the salt. The dependence of the salt dissociation values on protein concentration was determined for poly[r(A)] complexes at a [protein monomer]/[nucleotide] molar ratio of 0.5 with total protein concentrations of 20, 10, 15, and 5 μM.

Gel Electrophoresis. Gel electrophoresis of complexes was carried out in 1% agarose (SeaKem) in 0.04 M Tris–acetate and 0.001 M EDTA (pH 8.0; TAE) buffer for 1.5–2 h at 5 V/cm. Gels were then stained in ethidium bromide solution (0.5 μg/mL, Sigma) and destained in distilled water. After photodocumentation, the same gels were counterstained with 0.1% Coomassie blue in 40% methanol and 10% acetic acid, and were destained in 40% methanol and 10% acetic acid.

RESULTS

Molecular Weight of Pf3 ssDBP in Solution. The molecular weight of Pf3 ssDBP in solution as determined by gel filtration on a Sephadex G-50 column (see Materials and Methods) was 19 000 ± 2000. This was close to the predicted weight of a dimer (17 814) based on the known weight of the monomer (8907; Putterman et al., 1984).

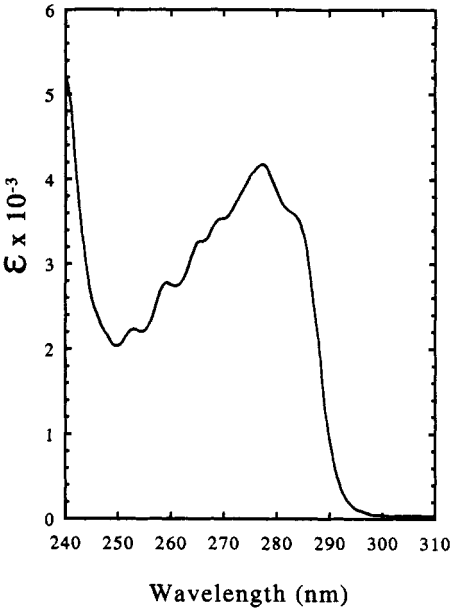

FIGURE 2: Molar absorption spectrum of Pf3 ssDBP. The measured spectrum in this figure and the spectral data in Figures 3–9 and 11 were taken with samples in 10 mM Tris-HCl and 70 mM NaCl, at pH 8.2 and 20 °C, except as indicated.

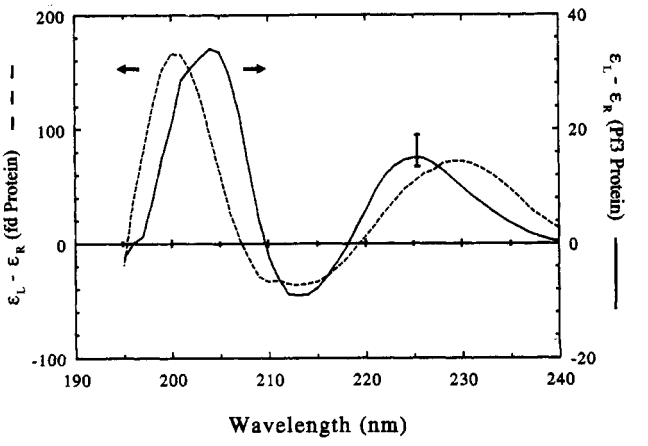

FIGURE 3: CD spectra of Pf3 ssDBP (—) and fd g5p (---). Note that the scale for the Pf3 protein (right axis, solid line) is one-fifth the scale of the fd protein (left axis, dashed line). The vertical bar represents the range of magnitudes in the 226-nm CD band of the five protein preparations used in this study. The buffer used for Pf3 ssDBP was 10 mM Tris-HCl and 70 mM NaCl (pH 8.2). The buffer used for the fd g5p spectrum was 50 mM Tris-HCl (pH 8). Spectra were taken at 20 °C. $\epsilon_L - \epsilon_R$ values are per mole of protein monomer.

Spectroscopy of the Free Pf3 ssDBP. A typical absorption spectrum of free Pf3 ssDBP is shown in Figure 2. This spectrum is similar to published spectra of fd g5p (Day, 1973), with the exception of the better resolution of bands between 250 and 270 nm due to phenylalanine. Pf3 ssDBP contains eight phenylalanines and three tyrosines, as compared with three phenylalanines and five tyrosines in fd g5p. The CD spectra of free ssDBP and fd g5p are shown in Figure 3. The scale for the Pf3 ssDBP is one-fifth that for the fd g5p. The CD band centered at approximately 229 nm for fd g5p is due primarily to the contributions of the tyrosines in this protein (Day, 1973). A similar CD band centered at approximately 226 nm is present in the spectrum of Pf3 ssDBP. The

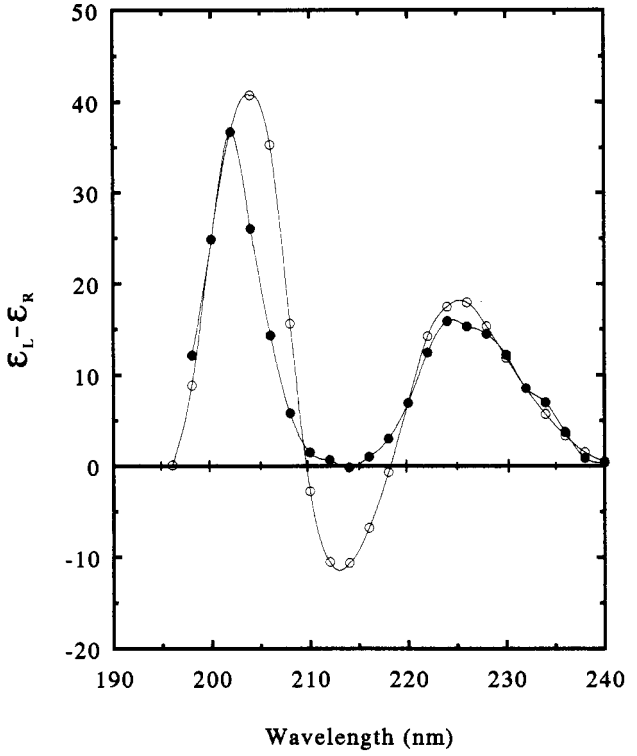

FIGURE 4: Measured CD spectrum of Pf3 ssDBP (○) and a spectral fit (●) to the reference spectra from Chang et al. (1978) plus reference spectra for tyrosine and phenylalanine. One of the best fits (shown here) included only the reference spectrum of *N*-acetyl-L-tyrosinamide plus 5% of the random secondary structure component. $\epsilon_L - \epsilon_R$ values are per mole of protein monomer; the spectral fit included 3.3 mol of *N*-acetyl-L-tyrosinamide.

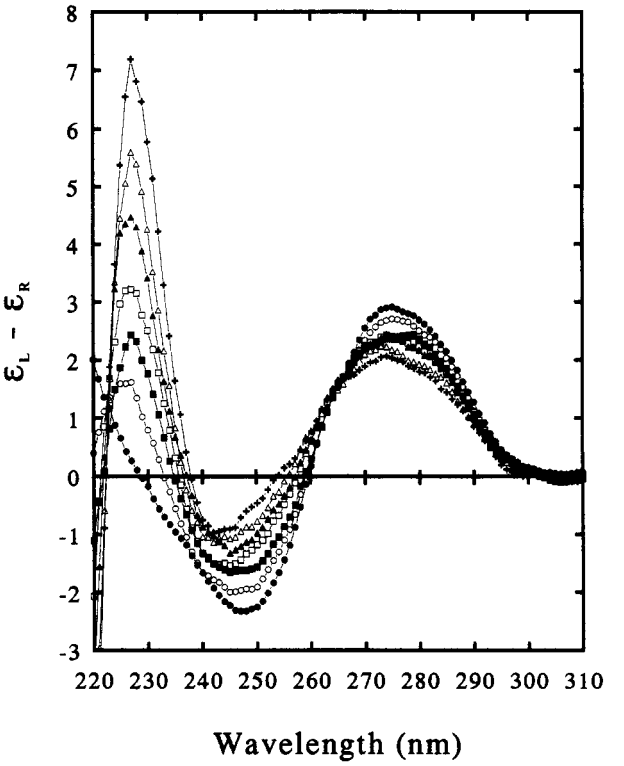

FIGURE 5: Titration of fd ssDNA with Pf3 ssDBP. Spectra are for free DNA (●) and DNA-protein complexes at [protein monomer]/[nucleotide] molar ratios of 0.12 (○), 0.16 (■); 0.22 (□); 0.30 (▲); 0.40 (△), and 0.51 (+). $\epsilon_L - \epsilon_R$ values are per mole of nucleotide.

magnitude of this CD band for the Pf3 ssDBP, as determined from the average of five preparations of protein used in this study, was (16 ± 3.0) $\epsilon_L - \epsilon_R$ in units of L mol^{-1} cm^{-1}, per mole of protein monomer. This was only about one-fifth the magnitude of the same band for the fd g5p, although ssDBP contains three of the five tyrosines present in fd g5p.

Curve Fitting of ssDBP CD Spectra. We fit the CD spectra of Pf3 ssDBP with the protein reference spectra derived by Chang et al. (1978), plus the reference spectra of *N*-acetyl-L-tyrosinamide and *N*-acetyl-L-phenylalaninamide (Adler et al., 1973). Fractions of various secondary structure components cannot be determined from a CD spectral analysis above 190 nm, with the exception of the α-helical secondary structure (Hennessey & Johnson, 1981; Woody, 1985; Yang et al., 1986). Analysis of the Pf3 ssDBP gave less than 1% α-helix, which was similar to the analysis results previously published for the fd g5p and IKe g5p (Sang & Gray, 1989). The crystallographic structure of fd g5p is largely composed of β-structures, with only eight residues in 3_{10}-helices (Skinner et al., 1993). Like the g5p proteins from class I phages, the Pf3 ssDBP is probably a predominantly β-sheet structure. As shown in Figure 4, the CD spectrum of Pf3 ssDBP above 200 nm is dominated by tyrosine. The magnitude of the 226-nm band was equivalent to 3 mol of *N*-acetyl-L-tyrosinamide, each having 1.1 times the magnitude of the isolated compound.

CD Spectroscopy of Complexed Nucleic Acid. Figure 5 shows a typical titration of fd ssDNA with Pf3 ssDBP. On this scale, the protein had no significant CD in the region of 240–310 nm. We therefore interpreted CD changes in this region as being due to changes in the nucleic acid structure upon binding. In titrations of fd DNA with ssDBP (Figure 5), the DNA CD band centered at approximately 275 nm became less positive upon binding ssDBP and the CD band at 248 nm became less negative. The crossover point near 260 nm was shifted toward shorter wavelengths upon binding ssDBP. The titration of Pf3 ssDNA with ssDBP resulted in very similar CD changes. In contrast, titrations of fd ssDNA with fd g5p show a crossover point that moves toward longer wavelengths and a DNA CD band at 250 nm that is unchanged, although the CD band at 275 nm does become less positive (Day, 1973; Kansy et al., 1986). The percent hyperchromicities of the ssDNA were similar upon binding the two proteins (Table I). Thus, the nucleic acids in the fd and Pf3 complexes are probably in conformations that differ slightly in their residual base–sugar or base–base interactions. Each nucleic acid used in this study had characteristic changes, but in general, the positive and negative bands above 240 nm became less intense and the crossover point near 260 nm moved toward shorter wavelengths as more Pf3 ssDBP was added. The *in vitro* complex formed with Pf3 ssDNA had CD spectra similar to that of the *in vivo* complex reported by Casadevall and Day (1985). The spectrum of the *in vivo* complex showed a crossover point that was shifted toward shorter wavelengths and a decreased CD band at 275 nm compared with the spectrum of free DNA. The magnitudes of the protein CD band at 226 nm and the nucleic acid CD band at 275 nm in the *in vivo* complex resemble those of *in vitro* complexes formed at a [protein monomer]/[nucleotide] ratio of approximately 0.25.

Titration curves were constructed by plotting the changes in the nucleic acid CD above 240 nm upon binding of ssDBP. Typically, the 275-nm CD band showed the greatest change upon binding, and this wavelength was used for most of the plots. Figure 6 shows the changes in the nucleic acid CD with increasing ratios of protein to nucleic acid for the DNA polynucleotides poly[d(A)], poly[d(T)], and poly[d(C)], as well as for Pf3 ssDNA and 48-mer DNA. In each case, the change in CD was linear up to the end point, indicating that

Table I: Comparison of Pf3 ssDBP and fd g5p Binding Characteristics

	Pf3 ssDBP[a]			fd g5p[b]		
nucleic acid	absorbance % change (260 nm)	binding modes (n)	[NaCl] (M) at 50% dissociation	absorbance % change (260 nm)	binding modes (n)	[NaCl] (M) at 50% dissociation
Pf3/fd DNA[c]	+12.5[d]	2, 4[e]	0.52[f]	+11.7	4, 3[g]	0.54
poly[d(A)]	+21.9	2	0.33	+21.7	4, 3	0.28
poly[r(A)]	+23.5	2	0.51		4	0.28
poly[d(C)]	-5.9	2	0.12	-10.8	4	>1.0
poly[r(C)]	+6.9	2	0.21		4	0.27
poly[d(T)]	-9.9	2	>4.0	-15.4	4, 3	>2.0
poly[r(U)]	-7.3	3	1.28		4	0.58
48-mer	+4.2	2	0.61			

[a] All Pf3 ssDBP titrations were performed in 10 mM Tris-HCl (pH 8.2) and 70 mM NaCl at 20 °C. Salt dissociations were begun with complexes in the same buffer, and NaCl was added to bring the [NaCl] to the indicated value for 50% dissociation. Figures represent an average of three or more experiments. [b] Binding modes for the DNA polymers are from Kansy et al. (1986). Salt dissociation values and percent hyperchromicities for the DNA polymers are from Sang and Gray (1989). All values for RNA polymers are from Bulsink et al. (1985). All of the salt dissociation values have been interpolated to 23 μM total protein concentration from the slopes of the plots of log [$K\omega$] vs log [NaCl] from Bulsink et al. (1985). [c] fd and Pf3 DNA gave equivalent results with Pf3 ssDBP. Only fd DNA was used in the fd g5p experiments. [d] Percent absorbance changes are for the first binding mode when two end points were observed. Errors are ±1.5% or less. [e] Values shown for n are rounded to the nearest whole number. Errors are ±0.3 based on ranges of the end points of two or more linear regressions. [f] Errors are ±0.05 or less based on ranges of two or more regressions. [g] The end points of titrations with fd ssDNA, poly[d(A)], and poly[d(T)] are dependent on the salt concentration. The $n = 3$ mode is seen in low ionic strength conditions (5 mM Tris-HCl), while the $n = 4$ mode is seen in higher ionic strength conditions (5 mM Tris-HCl and 0.2 M NaCl). See Kansy et al. (1986) for details.

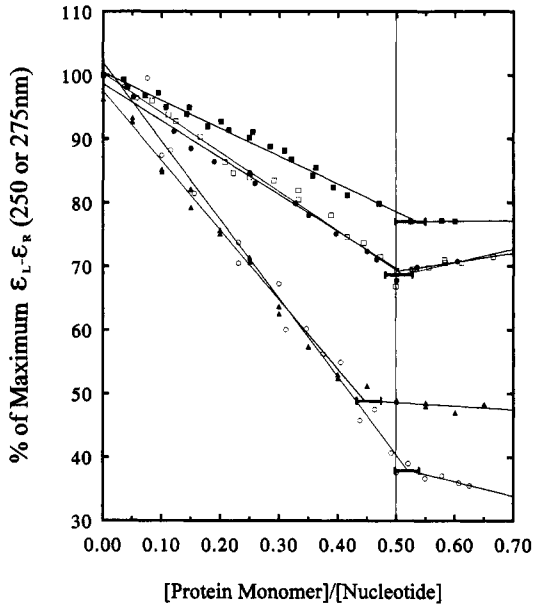

FIGURE 6: Titration plots for DNAs: Pf3 ssDNA (●); poly[d(A)] (○); poly[d(T)] (□); poly[d(C)] (■) and 48-mer DNA (▲). With the exception of poly[d(A)], all of the plots represent the change in molar CD at 275 nm upon binding of Pf3 ssDBP expressed as a percentage of the CD of the free nucleic acid. Because the change in CD for poly[d(A)] at 275 nm was more of a peak shift than a simple reduction of this band upon binding ssDBP, the change in the 250-nm band was used. The vertical line is at a [protein monomer]/[nucleotide] ratio of 0.5 or two bases per protein monomer. In Figures 6 and 7, the horizontal error bars represent the errors in the end points as determined from linear regressions of the combined data from duplicate or triplicate titrations.

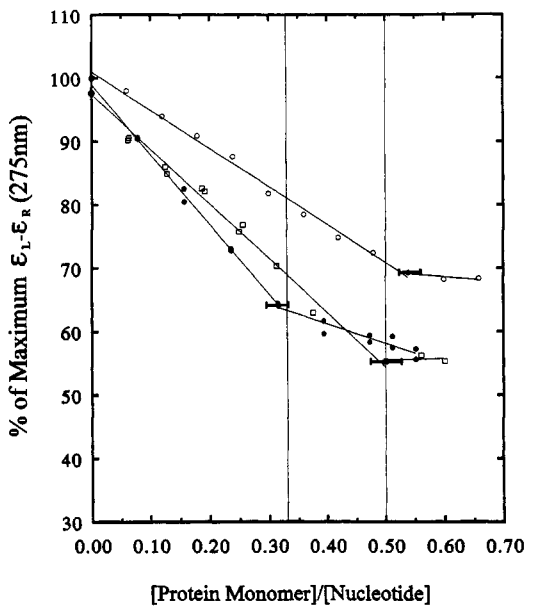

FIGURE 7: Titration plots for RNAs: poly[r(U)] (●); poly[r(C)] (○); and poly[r(A)] (□). All of the plots represent the change in molar CD at 275 nm expressed as a percentage of the CD of the free nucleic acid. The vertical lines represent [protein monomer]/[nucleotide] ratios of 0.33 (one monomer per three nucleotides) and 0.5 (one monomer per two nucleotides).

binding was stoichiometric, and reached an end point at a [protein monomer]/[nucleotide] molar ratio of 0.5 ± 0.03. Thus, the Pf3 ssDBP appeared to cover only two nucleotides per protein monomer, as compared with the three or four nucleotides per protein monomer for fd g5p (Kansy et al., 1986). A similar plot is shown in Figure 7 for the RNA polynucleotides poly[r(U)], poly[r(C)], and poly[r(A)]. Poly[r(A)] and poly[r(C)] showed titration end points at a [protein monomer]/[nucleotide] molar ratio of 0.5 ± 0.04, but poly[r(U)] reached an end point at approximately 0.33 ± 0.03, which corresponded to a binding site size of three nucleotides per protein monomer. Binding modes for Pf3 ssDBP and fd g5p for these nucleic acids are summarized and compared in Table I.

CD Spectroscopy of Complexed Protein. Titration curves were plotted using the CD data at 226 nm to determine whether any observable changes were occurring in the CD of the tyrosine band of ssDBP itself during each titration. At this wavelength, the CD of the ssDBP had a large positive value, while many nucleic acids had little or no CD (see Figures 4 and 5). In all cases, the contribution of the nucleic acid was subtracted to obtain the CD of the protein alone, with the assumption that the nucleic acid CD did not change significantly at this wavelength on protein binding. Plots of the 226-nm band for all of the titrations revealed no breakpoints and were approximately the same magnitudes at all [protein]/[nucleic acid] ratios as the calculated values of the CD expected from addition of the ssDBP alone. Figure 8 shows a typical example of the CD at 226 nm upon the addition of ssDBP to

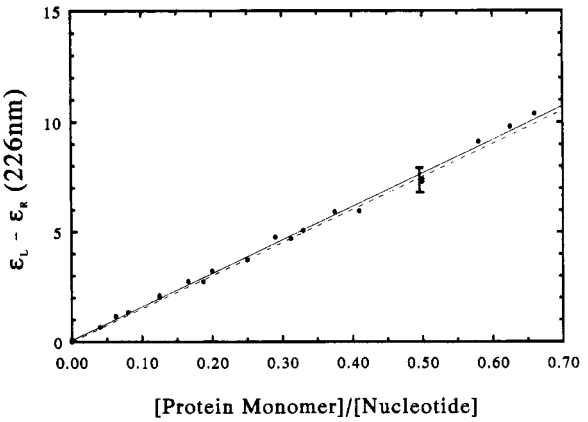

FIGURE 8: Change in CD at 226 nm upon titration of fd ssDNA with Pf3 ssDBP. Shown is a plot of the average protein CD (228 nm) during triplicate titrations of fd ssDNA (●). The dashed line represents the CD expected from additions of protein to a buffer solution in the absence of nucleic acid. In all of the titrations in this study, there were no breakpoints in titration plots of the protein CD at 226 nm, and the absolute values were close to those expected from the addition of protein alone. The vertical bar represents the range of variation in the slopes of the triplicate plots. $\epsilon_L - \epsilon_R$ values are per mole of nucleotide.

fd ssDNA and its comparison with the CD expected from the addition of protein at the same dilution in the absence of DNA. The closeness of the comparison justified the assumption that the nucleic acid CD did not significantly change at 226 nm upon binding, since exactly compensating changes in the nucleic acid and protein CDs would be unlikely. The lack of significant change in the CD at 226 nm of the ssDBP upon binding any of the DNAs or RNAs was in contrast to the tyrosine band of g5p, which shows a decrease in the CD at 229 nm, the magnitude of which is dependent on the nucleic acid being titrated (Kansy et al., 1986).

Absorbance Spectroscopy of Complexed Nucleic Acids. The percent hyperchromicity (%H) at 260 nm of the nucleic acid upon binding of ssDBP was determined by the following formula:

$$\%H = [\{A_{260,\text{free nucleic acid}}\} - \{(A_{260,\text{complex}}) - (A_{260,\text{free protein}})(\text{protein dilution factor})\} \times \{1/(\text{nucleic acid dilution factor})\}]100/[A_{260,\text{free nucleic acid}}]$$

That is, the protein absorbance was subtracted from the absorbance of the complex, with the assumption that the protein absorbance was unchanged upon forming a complex. The results for each nucleic acid titrated with the Pf3 ssDBP are summarized in Table I along with the values from the literature for the same nucleic acids titrated with fd g5p. In general, nucleic acids that were more stacked in solution such as poly[r(A)] and poly[d(A)] became unstacked upon binding Pf3 ssDBP, and nucleic acids that were less stacked in solution such as poly[d(T)] and poly[r(U)] became stacked upon binding. These changes were very similar to those reported for nucleic acids complexed with fd g5p and suggested that the structure of the nucleic acid in the Pf3 complex was relatively unstacked, although some residual base stacking was present as evidenced by the hypochromic changes of poly[d(T)], poly[d(C)], and poly[r(U)]. Both fd and Pf3 phage DNAs undergo similar hyperchromic shifts when complexed with their respective binding proteins. Clack and Gray (1992) have reported that the ssDNA inside the intact Pf3 and fd phages is actually hypochromic to a very similar extent (41% for fd and 39% for Pf3). Together, these observations suggest that the change in base-stacking of the DNA during the transition from precursor complex to phage is similar during the morphogenesis of both phages.

Salt Dissociation of Complexes. To determine the relative binding affinities for the nucleic acids used in this study, salt dissociation experiments were performed on the complexes formed. It has been shown that the product of the intrinsic binding constant (K) multiplied by the cooperativity factor (ω) is equal to the reciprocal of the free protein concentration (L) at the midpoint of the binding isotherm (McGhee & von Hippel, 1974; Alma et al., 1983), with the assumption that $\omega >> n$ (the binding site size). That is, $K\omega = 1/L$, at 50% dissociation.

To determine whether the salt dissociations using Pf3 ssDBP complexes were dependent on the total protein concentration in a manner similar to those of fd g5p complexes, salt dissociations of Pf3 ssDBP complexes with poly[r(A)] were carried out at four different total protein concentrations (see Materials and Methods). A plot of log [$K\omega$] against log [NaCl] resulted in a linear plot. The slope of this line as determined by linear regression was -2.5 ± 0.1, which was different from the value of -4.0 ± 0.8 determined for M13 g5p by Bulsink et al. (1985). However, these slopes are related to the number of counterions released from the protein and the nucleic acid (Record et al., 1978) and were consistent with the different stoichiometries of binding ($n = 4$ for fd g5p and $n = 2$ for Pf3 ssDBP). That is, the difference in the number of counterions released from the nucleic acid should be approximately $(4 - 2)\,\theta$, where θ is the average number of cations bound per phosphate. Taking 2.82 Å as the axial charge spacing for poly[r(A)] (Saenger, 1984), θ is about 0.61 [see Record et al. (1978)] and $2\theta = 1.2$. This difference in the calculated number of counterions released is quite close to the difference in the slopes of log [$K\omega$] *vs* log [Na^+] for the two proteins: $4.0 - 2.5 = 1.5$.

Since the concentration of salt needed for the dissociation of DNA-protein complexes is dependent on the total concentration of protein present (McGhee & von Hippel, 1974), salt dissociations were performed on complexes of Pf3 ssDBP with different nucleic acids, all at the same total ssDBP concentration of 23 μM (Figure 9) (see Materials and Methods). The concentration of NaCl needed to dissociate the complexes by 50% was used to rank the binding affinities of the Pf3 ssDBP to various substrates, with the assumption that they all had approximately the same dependence of $K\omega$ on salt concentration. The results of the salt dissociations are summarized in Table I. The relative order of binding of ssDBP was [d(T)] >> [r(U)] >> 48-mer > Pf3/fd DNA, [r(A)] > [d(A)] > [r(C)] > [d(C)]. This is similar to the order of fd g5p binding to these nucleic acids, with the exception of poly[d(C)]. Poly[d(C)] ranked lowest in binding affinity to Pf3 ssDBP, while it ranked next to poly[d(T)] in order of binding to fd g5p. This may have been partially the result of the short lengths (150 nucleotides) of poly[d(C)] used in the present experiments; however, this was not the major factor in the low affinity observed, since the 48-mer DNA had a salt dissociation value even higher than that of intact molecules of Pf3 ssDNA. There was a larger difference in the binding affinities of Pf3 ssDBP for poly[d(A)], poly[r(A)], and poly[r(C)], such that ssDBP was more salt-stable when complexed with the RNAs poly[r(A)] and poly[r(C)] than with the DNA analogs poly[d(A)] and poly[d(C)]. fd g5p binds poly[r(A)], poly[d(A)], and poly[r(C)] with similar affinities.

Gel Electrophoresis. As an alternate means of monitoring the binding of ssDBP to viral DNA, gel electrophoresis band

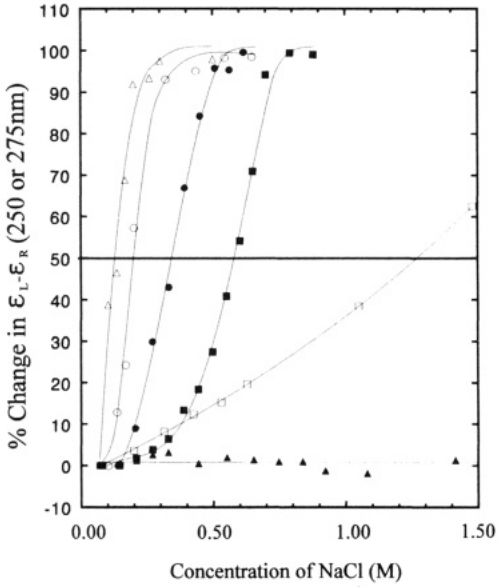

FIGURE 9: NaCl dissociation of Pf3 ssDBP complexes with poly-[d(T)] (▲); poly[r(U)] (□); poly[r(A)] (■); poly[d(A)] (●); poly-[r(C)] (○); and poly[d(C)] (△). The CD of the nucleic acid was monitored at 250 or 275 nm (see Materials and Methods). The horizontal line represents 50% dissociation, which is the point of comparison for relative binding affinities. In each case, the total protein concentration was 23 μM. Complexes formed with poly-[d(T)] were not dissociable up to 4 M NaCl. Curves shown are third-order polynomial fits to the data, with the exception of the data for poly[d(T)] which was fit with a linear regression. Salt concentrations were increased by the addition of aliquots of NaCl (4 M) to complexes formed in 10 mM Tris-HCl and 70 mM NaCl (pH 8.2).

shift experiments were performed. Fixed concentrations of Pf3 or fd DNA were titrated with increasing amounts of ssDBP. The total volume of each sample was loaded onto a 1% agarose gel, electrophoresed, and stained with ethidium bromide (see Materials and Methods). Figure 10A shows the results of such a titration of Pf3 ssDNA with ssDBP. Titrations of fd ssDNA gave identical results. With increasing amounts of ssDBP, the entire DNA band shifted to a decreased mobility. This suggested that all of the ssDNA strands were partially covered by ssDBP at each titration point. When the [protein monomer]/[nucleotide] ratio reached 0.25, a breakpoint in the change in mobility relative to free DNA could be seen. This breakpoint is shown clearly in the plot (Figure 10) of the relative mobilities of Pf3 ssDNA at each titration point. Addition of more ssDBP only slightly affected the mobility after reaching a ratio of 0.25, but the fluorescence from the ethidium bromide-stained ssDNA in the complex became increasingly quenched. Coomassie-stained gels showed that all of the protein was in the band that was stained by ethidium bromide up to a [protein monomer]/[nucleotide] ratio of 0.50. After a ssDBP monomer to nucleotide ratio of 0.50 was reached, free protein was visualized in Coomassie counter stained gels.

The same type of band shift experiment was repeated using an oligomer of 48 nucleotides with a nucleotide composition similar to that of Pf3 ssDNA (Figure 10B). In this case, the mobility was decreased in an all-or-none fashion. As increasing amounts of ssDBP were added, increasing fractions of the ssDNA sample were maximally shifted in mobility. The DNA sample was saturated at a [protein monomer]/[nucleotide] molar ratio of 0.5. A CD titration of the same oligomer with ssDBP (Figure 6) showed that the end point of this titration was close to that seen in the band shift experiment.

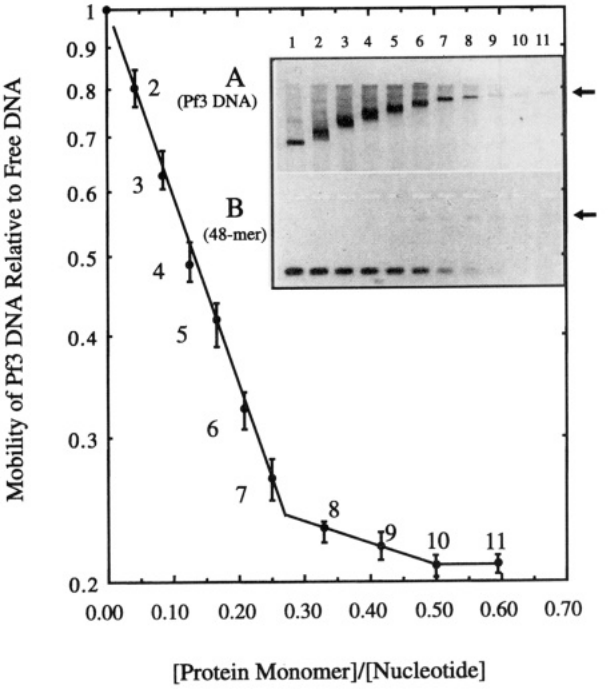

FIGURE 10: Relative electrophoretic mobility of Pf3 ssDNA with increasing amounts of Pf3 ssDBP. The lines represent linear regressions through the data points shown for the experiment in inset A. The vertical bars represent the range of three experiments done at the same ratios of monomer/nucleotide. The breakpoint was at a [protein monomer]/[nucleotide] ratio of approximately 0.25, which corresponded to one monomer covering four nucleotides. Inset A: Gel electrophoresis of complexes formed with Pf3 ssDNA. Shown is a digitized negative image of a 1% agarose gel in TAE buffer, stained with ethidium bromide. Lane 1 contained free Pf3 ssDNA, and lanes 2–11 had [protein monomer]/[nucleotide] ratios of 0.04, 0.08, 0.13, 0.17, 0.21, 0.25, 0.33, 0.42, 0.50, and 0.58, respectively. All lanes had the same 25 μL of DNA at a concentration of 80 μM (which is roughly the same concentration of DNA used in the CD titrations). Inset B: The results of a similar experiment using 48-mer DNA at the same [protein monomer]/[nucleotide] ratios and under identical conditions are shown. The arrows in insets A and B mark the positions of the fully saturated complexes.

DISCUSSION AND CONCLUSIONS

Spectroscopy of the Free Pf3 ssDBP. The CD spectrum of free Pf3 ssDBP (Figure 3) was similar to that of fd g5p, which is a predominantly β-sheet protein (Brayer & McPherson, 1983; Skinner et al., 1993). The spectra of both proteins are dominated by the optical activity of their tyrosines. The magnitudes of the tyrosine CD bands for fd g5p and Pf3 ssDBP are not simply related to the number of tyrosines present. Pf3 ssDBP contains three of five tyrosines present in fd g5p, and if all of the contributions of the tyrosines to the CD signal were equally positive, the total tyrosine CD at 226–229 nm for ssDBP should be three-fifths that of fd g5p. However, the actual CD of the Pf3 ssDBP was only one-fifth that of fd g5p (Figure 3). Thus, the average tyrosyl CD at 226 –229 nm was less for Pf3 ssDBP than for fd g5p.

In contrast to the CD of fd g5p (Kansy et al., 1986), the CD band at 226 nm of the Pf3 ssDBP did not decrease upon the binding of ssDBP to any of the nucleic acids used in this study. Since ssDBP lacks the equivalent tyrosines 34 and 41 of fd g5p, it is possible that one of these tyrosines is responsible for the change in this band of fd g5p upon binding. Alternately, more than one tyrosine may be involved in compensating changes. A mutant of fd g5p that has a phenylalanine substituted for the tyrosine at position 34 of fd g5p (Y34F) lacks a change in the tyrosyl CD band upon binding nucleic

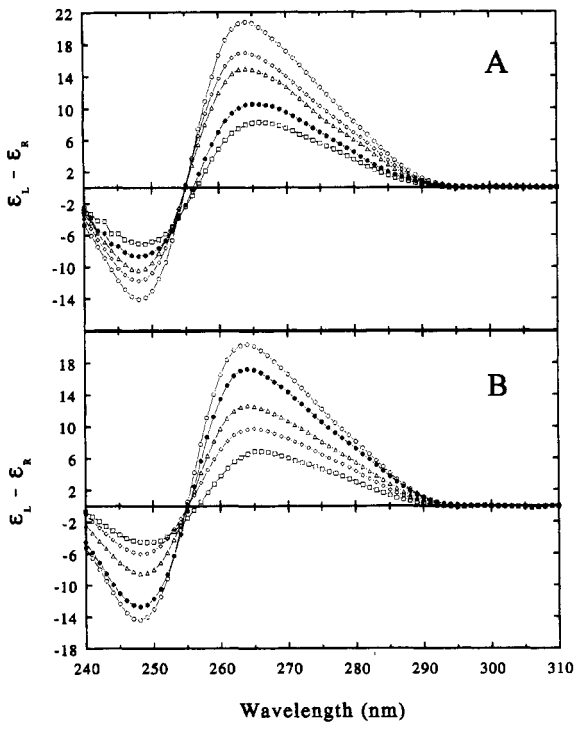

FIGURE 11: (A) CD spectra of poly[r(A)] at 20 (○) and 80 °C (□) and at [protein monomer]/[nucleotide] ratios of 0.18 (◇), 0.30 (△), and 0.5 (●), with Pf3 ssDBP at 20 °C. The spectrum at 80 °C has been corrected for sample volume expansion. (B) CD spectra of free poly[r(A)] in NaCl concentrations of 0.07 (○) and 1 M (●). Also shown are poly[r(A)]·Pf3 ssDBP complexes at a [protein monomer]/[nucleotide] ratio of 0.5 in NaCl concentrations of 0.07 (□), 0.43 (◇), and 0.53 M (△).

acids (B. L. Mark, unpublished results), so that it is indeed possible that the two wild-type proteins have differing CD changes on binding only because the fd g5p has a tyrosine (Tyr-34) that is not present at an equivalent position in the Pf3 ssDBP.

Nucleic Acid CD Spectroscopy. Since ssDBP has only a small CD signal in the longer wavelength region (above 245 nm), the CD changes seen in this region when ssDBP was added to polynucleotides were considered to be primarily due to changes in the nucleic acid upon binding ssDBP. The changes in the CD spectra of all of the nucleic acids used in this study upon forming complexes with ssDBP were similar, at least in part, to the CD changes upon heating the nucleic acid. Figure 11A shows the CD spectra of poly[r(A)] free, complexed with Pf3 ssDBP, and heated. For comparison, Figure 11B shows spectra of the poly[r(A)]·Pf3 ssDBP complex, poly[r(A)] in 0.07 M NaCl, poly[r(A)] in 1 M NaCl, and partially dissociated complexes. The shape and magnitude of the CD bands of the poly[r(A)] complexed with Pf3 ssDBP were almost identical to the values for the same bands in the spectrum of free poly[r(A)] when heated to 80 °C. These CD changes represent the simple unstacking of the bases of poly[r(A)] upon binding to Pf3 ssDBP, as has been described in previous work with fd g5p (Sang & Gray, 1989). As illustrated in Figure 11B, salt dissociation essentially reversed the CD changes of poly[r(A)] found upon binding ssDBP.

Changes seen in the nucleic acid CD spectra of fd ssDNA and Pf3 ssDNA upon binding Pf3 ssDBP were similar in that both had reduced CD magnitudes of the positive and negative bands above 240 nm. In addition, the absorption spectra showed that poly[r(A)], fd ssDNA, and Pf3 ssDNA were hyperchromic when complexed with ssDBP (see Table I), further suggesting that these nucleic acids were unstacked upon binding Pf3 ssDBP. The changes in the CD spectra of the other polynucleotides upon binding were not consistent with simple unstacking. In fact, several polypyrimidines were hypochromic on binding Pf3 ssDBP and fd g5p (Table I).

Binding Stoichiometry. The binding stoichiometries as determined from breakpoints in titration curves with the Pf3 ssDBP (Figures 6 and 7) revealed a binding site size of close to two nucleotides per protein monomer ($n = 2 \pm 0.3$) for all of the nucleic acids used in this study, with the exception of poly[r(U)] where $n = 3 \pm 0.2$. These values were in contrast to the apparent value of $n = 6 \pm 0.5$ determined by Casadevall and Day (1985) for *in vivo* complexes of Pf3. However, there is precedence for *in vitro* and *in vivo* complexes having different [nucleotide]/[protein monomer] ratios. Pretorius et al. (1975) observed that *in vivo* complexes of fd g5p had an apparent stoichiometry of $n = 4.70 \pm 0.13$, compared with $n = 4.05 \pm 0.17$ for *in vitro* complexes. Pratt et al. (1974) showed that *in vivo* complexes contained 20% less protein per DNA than *in vitro* complexes. If a similar situation holds true for Pf3 complexes, *in vitro* complexes of Pf3 ssDBP might be formed with a lower [protein monomer]/[nucleotide] ratio than that found for *in vivo* complexes. Our values of $n = 2$ or $n = 4$ for *in vitro* complexes formed with the Pf3 ssDBP imply that the *in vivo* complexes isolated by Casadevall and Day (1985) may not have been fully saturated with protein.

The titrations of Pf3 or fd ssDNA monitored by CD showed an end point of $n = 2$. However, from gel electrophoresis we also found evidence for an $n = 4$ mode of binding to fd and Pf3 ssDNA. We conclude that there were two modes of binding, but that only one was detected by CD. Indeed, we have recently isolated a mutant (Y22F) of Pf3 ssDBP that shows evidence in CD titrations of a $n = 4$ binding mode (M. D. Powell and D. M. Gray, manuscript in preparation). A somewhat similar observation has been made for Pf1 g5p (Carpenter & Kneale, 1991), where an $n = 2$ binding mode was found by fluorescence spectroscopy and a single $n = 4$ binding mode was found by CD spectroscopy.

In the case of fd g5p, two modes of binding ($n = 3$ and 4) are detected by CD titrations with some polynucleotides but not others (Kansy et al., 1986). That is, different stoichiometric binding modes are not always distinguishable for these single-strand DNA binding proteins using a single technique. The model proposed by Carpenter and Kneale (1991) to explain their results involves the binding of four nucleotides by each half of a Pf1 g5p dimer to give the $n = 4$ mode, with a shift to the binding of four nucleotides to only one monomer of each dimer in the $n = 2$ mode (see Figure 12, $n = 2$ mode, A). They proposed that the nucleic acid structure remained the same during the transition from $n = 4$ to $n = 2$, so that the switch from $n = 4$ to $n = 2$ binding modes was not detected by nucleic acid CD measurements.

In the case of Pf3 ssDBP, it is also possible that two modes of binding exist in which full and half-occupied dimers are present. However, in this case, the nucleic acid CD must continue to change in the same manner during the transition from the $n = 4$ mode to the $n = 2$ mode as it does for the transition from free nucleic acid to the $n = 4$ mode, since there is no breakpoint in the CD titrations at $n = 4$. Salt-dissociation experiments also did not resolve two modes of binding with any of the nucleic acids in this study, suggesting that the binding affinities of both modes were more similar than in the case of fd g5p.

Alternatively, there could be an $n = 2$ binding mode in which the number of nucleotides covered by each monomer of the dimer shifts from four to two (see Figure 12, $n = 2$

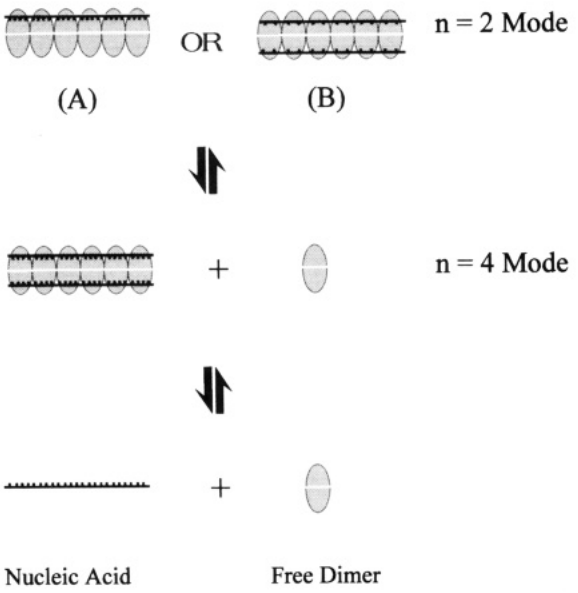

FIGURE 12: A model of possible binding modes of Pf3 ssDBP. Two models are shown for an $n = 2$ binding stoichiometry. The first model (A) has only one of the two monomer binding sites of a dimer in contact with the nucleic acid: the one monomer binding four nucleotides, as proposed by Carpenter and Kneale (1991) for the Pf1 binding protein. The second model (B) has nucleic acid strands bound to each of the monomers with the nucleic acid being stretched and/or the protein dimers being compressed, so that there are only two nucleotides bound per each protein monomer.

mode, B). In either model, the change in the nucleic acid structure would have to be continuously perturbed in the same manner during both transitions to explain the lack of a breakpoint in the CD titrations. Moreover, the $n = 4$ and $n = 2$ modes resulted in complexes that were not readily distinguished by their electrophoretic mobilities in gels under our conditions.

Binding of Oligomeric DNA. In bandshift experiments with the 48-mer DNA, the mobility shift was quite different. As each aliquot of protein was added, a fraction of the free DNA was completely bound, resulting in two distinct bands. One band contained fully free DNA, and the other contained completely saturated DNA. The mobility and degree of quenching of the protein-saturated DNA remained the same throughout the titration. When a [protein monomer]/[nucleic acid] ratio of 0.5 was reached, all of the free DNA was saturated. It is possible that in the longer Pf3 ssDNA there are regions of high affinity, such as tracts of [d(T)], that were saturated before regions with less affinity became saturated. This would account for the partial shift of all the strands in the band shift experiments with Pf3 ssDNA. In the shorter 48-mer DNA, the differences in binding site affinities may be small compared with the cooperativity of binding, and thus the 48-mer was saturated in an all-or-none fashion. There were no differences seen in the mobility of complexes formed with the 48-mer DNA. It seems that binding to the 48-mer occurred directly in the $n = 2$ mode without an intervening $n = 4$ transition or that any differences in the mode of binding were not resolved by these experiments.

Specificity of Binding to Nucleic Acids. fd g5p shows a significant difference in its binding affinities for polynucleotides of different base compositions (Sang & Gray, 1989). Pf3 ssDBP shows similar differences in binding affinities for different homopolymers. It has been proposed that nucleotide stacking in the free polymer is inversely related to the binding affinity of fd g5p for the polymer (Sang & Gray, 1989). Both poly[d(T)] and poly[r(U)] are unstacked in solution relative to poly[d(A)] and poly[r(A)], and both bind with high affinity to fd g5p and Pf3 ssDBP (Table I). Also, both poly[d(T)] and poly[r(U)] show a hypochromic change in absorbance at 260 nm upon binding, compared with hyperchromic changes for poly[d(A)] and poly[r(A)]. Poly[d(C)] is also unstacked in solution and shows a hypochromic change upon binding to fd g5p and Pf3 ssDBP. However, while poly[d(C)] binds to fd g5p very tightly (>1.0 M NaCl), it has the lowest affinity of all of the polynucleotides tested for Pf3 ssDBP (Table I). Other than this exception, the relatively simple rule seems to hold for both proteins.

fd g5p generally binds to DNA homopolymers with greater affinity than to RNA homopolymers with the same base composition. However, Pf3 ssDBP binds some RNA homopolymers with greater affinity than the corresponding DNA homopolymer. Poly[r(A)] and poly[r(C)] bind substantially tighter than poly[d(A)] and poly[d(C)], respectively, although poly[r(U)] is less tightly bound than poly[d(T)]. The sugar–phosphate backbone appears to play an important role in the binding interaction, but a role that is specific for the particular protein.

It is interesting to note that, despite the differences between Pf3 ssDBP and fd g5p in their binding affinities for the various homopolymers, the amount of NaCl needed to dissociate complexes of fd g5p with fd ssDNA and Pf3 ssDBP with Pf3 ssDNA (at the same protein concentrations) is almost identical (see Table I). The $K\omega$ values for fd g5p and Pf3 ssDBP at physiological salt concentration (0.2 M NaCl) are also similar. Assuming that the slope of log [$K\omega$] *vs* log [NaCl] is similar for Pf3 ssDBP binding to poly[r(A)] and Pf3 ssDNA, we estimate that Pf3 ssDBP has a $K\omega$ of $\sim 0.9 \times 10^6$ for binding to its DNA at 0.2 M NaCl. This $K\omega$ may be compared with the value of 1.3×10^6 for fd g5p binding to its DNA, also at 0.2 M NaCl (Bulsink et al., 1985). Likewise, Pf3 and fd ssDNA undergo hyperchromic changes of similar magnitude upon complexation with their respective binding proteins. Finally, both proteins exhibit two modes of binding, at least under some conditions. These results reflect the similar basic functions of these proteins, even though there are differences in the protein sequences and in the details of the binding mechanisms. This lends hope to the idea that the conserved elements between these two proteins are important in their basic function, and further study of Pf3 ssDBP may aid in the understanding of how this entire class of proteins functions.

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