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Accelerated Publications

fd Gene 5 Protein Binds to Double-Stranded Polydeoxyribonucleotides Poly(dA·dT) and Poly[d(A-T)·d(A-T)]†

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Received August 13, 1987; Revised Manuscript Received September 17, 1987

ABSTRACT: Circular dichroism (CD) data indicated that fd gene 5 protein (G5P) formed complexes with double-stranded poly(dA·dT) and poly[d(A-T)·d(A-T)]. CD spectra of both polymers at wavelengths above 255 nm were altered upon protein binding. These spectral changes differed from those caused by strand separation. In addition, the tyrosyl 228-nm CD band of G5P decreased more than 65% upon binding of the protein to these double-stranded polymers. This reduction was significantly greater than that observed for binding to single-stranded poly(dA), poly(dT), and poly[d(A-T)] but was similar to that observed for binding of the protein to double-stranded RNA [Gray, C. W., Page, G. A., & Gray, D. M. (1984) *J. Mol. Biol. 175*, 553–559]. The decrease in melting temperature caused by the protein was twice as great for poly[d(A-T)·d(A-T)] as for poly(dA·dT) in 5 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl), pH 7. Upon heat denaturation of the poly(dA·dT)–G5P complex, CD spectra showed that single-stranded poly(dA) and poly(dT) formed complexes with the protein. The binding of gene 5 protein lowered the melting temperature of poly(dA·dT) by 10 °C in 5 mM Tris-HCl, pH 7, but after reducing the binding to the double-stranded form of the polymer by the addition of 0.1 M Na^+, the melting temperature was lowered by approximately 30 °C. Since increasing the salt concentration decreases the affinity of G5P for the poly(dA) and poly(dT) single strands and increases the stability of the double-stranded polymer, the ability of the gene 5 protein to destabilize poly(dA·dT) appeared to be significantly affected by its binding to the double-stranded form of the polymer.

The fd gene 5 protein (G5P)[1] is one of the most extensively studied single-strand DNA binding proteins (Ray, 1978; Kowalczykowski et al., 1981). This model protein is encoded by the fd filamentous phage (Marvin & Hohn, 1969). A known biological function of this protein in the phage life cycle is to control the switch from the replication of double-stranded RF DNA to the synthesis of single-stranded viral DNA

† This work was performed by B.-C.S. in partial fulfillment of the requirements for the Ph.D. degree in the Program in Molecular Biology at the University of Texas at Dallas. Support was provided by NIH Research Grant GM 19060 from the National Institute of General Medical Sciences and by Grant AT-503 from the Robert A. Welch Foundation. A preliminary report of this work was presented at the 31st Annual Biophysical Society Meeting (Sang & Gray, 1987).
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[1] Abbreviations: CD, circular dichroism; G5P, gene 5 protein; RF, replicative form; T_m, melting temperature; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride; R, ratio of nucleotide to protein molar concentrations (R = [nucleotide]/[gene 5 protein monomer]); SDS, sodium dodecyl sulfate; dsDNA, double-stranded DNA; dsRNA, double-stranded RNA; EDTA, ethylenediaminetetraacetic acid.

0006-2960/87/0426-7210$01.50/0

molecules. G5P is thought to bind to the newly synthesized viral strand to prevent complementary strand synthesis (Pratt & Erdahl, 1968; Salstrom & Pratt, 1971).

Since G5P binds tightly and cooperatively to single-stranded DNA, the protein is a helix-destabilizing protein [reviewed by Kowalczykowski et al. (1981)]. Alberts et al. (1972) reported that the melting temperatures of T4 phage DNA, *Clostridium perfringens* DNA, and poly[d(A-T)·d(A-T)] are lowered 38.5–42.5 °C in the presence of fd G5P. Holwitt and Krasna (1982) found that G5P can lower the melting temperatures of T7 DNA, poly[d(A-T)·d(A-T)], and poly[d(A-C)·d(G-T)] by 16–38, 10–27, and 16–33 °C, respectively, depending on the salt concentration.

There are conflicting reports as to whether fd G5P binds to double-stranded DNA. Alberts et al. (1972) did not detect binding to T7 DNA by sucrose gradient sedimentation (0.05–0.15 M NaCl, pH 8); Oey and Knippers (1972) performed a similar experiment and failed to find binding to fd replicative form DNA in a low salt buffer. In addition, Day (1973) did not find any CD spectral changes upon mixing the protein and supertwisted PM2 DNA. On the other hand, Holwitt and Krasna (1982) reported that G5P formed a complex with T7 DNA in a sucrose gradient containing 0.05 M NaCl plus 0.02 M Tris-HCl, pH 8.1, although the complex was not as stable as that formed with single-stranded DNA. In a combined CD and electron microscopy study, Gray et al. (1984) determined that G5P can form a complex with double-stranded RNA and that the protein CD band at 228 nm is greatly reduced in the complex. A similar CD change was observed upon binding of the protein to double-stranded calf thymus DNA. In the present paper, we show that (1) G5P binds to two double-stranded polymers, poly(dA·dT) and poly[d(A-T)·d(A-T)], (2) the binding can be detected by changes in both the nucleic acid and protein CD components, and (3) the binding to the double-stranded form of polynucleotides appears to influence the extent of helix destabilization by the protein.

Materials and Methods

The fd G5P was isolated essentially as previously described (Gray et al., 1982). In addition, high molecular weight residual material remaining after DNA–cellulose chromatography was removed by Sephadex G-75 column chromatography (Anderson et al., 1975). G5P was eluted from the G-75 column with a buffer containing 5 mM EDTA, 20 mM Tris-HCl, 1 mM mercaptoethanol, 10% glycerol, and 0.4 M NaCl, pH 7.0. The purity of the protein from various column fractions was checked by electrophoresis in a 15% (w/v) polyacrylamide gel with 0.1% (w/v) SDS. The fractions for which a single band was observed at the molecular weight of the G5P were combined and dialyzed into 5 mM Tris-HCl, pH 7.0. The protein concentration was determined from absorption measurements and the extinction coefficient of 0.73 $mg^{-1}\cdot cm^2$ (or 7070 $L\cdot mol^{-1}\cdot cm^{-1}$) at 276 nm (Day, 1973).

Poly(dA), poly(dT), and poly[d(A-T)·d(A-T)] were purchased from Sigma Chemical Co. According to the manufacturer, these polymers were of high molecular weight and had average lengths of about 900, 3000, and 2300 nucleotides, respectively. Double-stranded poly(dA·dT) was prepared by mixing equimolar solutions of poly(dA) and poly(dT) in 0.1 M Na^+ (phosphate buffer), pH 7.0. Sharp melting transitions and a large (ca. 50%) hyperchromicity indicated essentially complete pairing of the bases in the poly(dA·dT) samples, with at most a few percent of residual single strands. All the polymers were finally dialyzed against 5 mM Tris-HCl, pH 7.0. The extinction coefficients at 260 nm used for determining concentrations of samples of poly(dA), poly(dT), and poly[d(A-T)·d(A-T)] were 9650 (Bollum, 1966), 8140 (Bollum, 1966), and 6600 (Inman & Baldwin, 1964), respectively. The extinction coefficient used for poly(dA·dT) was 6000 $L\cdot mol^{-1}\cdot cm^{-1}$ at 259 nm (Riley et al., 1966).

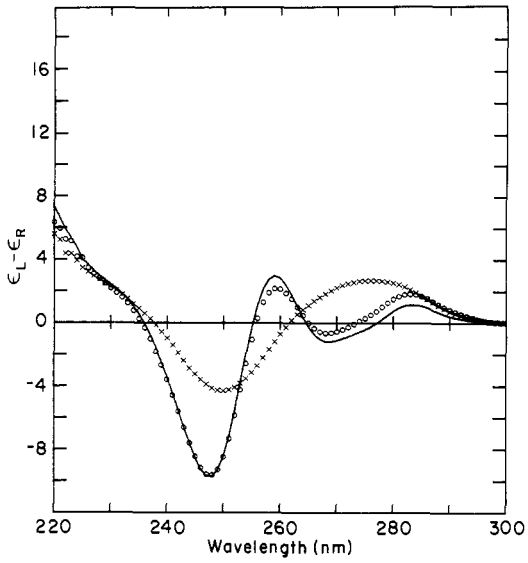

FIGURE 1: CD spectra of poly(dA·dT) at different temperatures: 20 (—), 35 (○), and 55 °C (×). The buffer used for experiments shown in this figure and the following was 5 mM Tris-HCl, pH 7.0.

Absorption spectra were measured with a Cary Model 118 spectrophotomer, and CD spectra were obtained with a Jasco Model J500A calibrated as described by Kansy et al. (1986). Sample temperatures were controlled thermoelectrically to within ±0.2 °C with an accuracy of ±0.5 °C. CD spectra were smoothed by a sliding 13-point quadratic–cubic function (Savitzky & Golay, 1964) and plotted at nanometer intervals as $\epsilon_L - \epsilon_R$ in units of $L\cdot mol^{-1}\cdot cm^{-1}$.

Melting profiles show the percent hyperchromicity at 260 nm calculated as 100[OD(temperature) − OD(20 °C)]/[OD(20 °C)]. For melting profiles of polymer–G5P complexes, the absorbance of an equivalent amount of free protein (at 20 °C) was subtracted from the absorbance of the complex prior to the calculation of percent hyperchromicity.

Protein titrations of poly(dA·dT) were conducted as previously described for other polymers (Kansy et al., 1986). Typical concentrations of G5P and DNA polymer were both about 1×10^{-4} M. There was about 3–4% cumulative error in the DNA and protein concentrations calculated at the end of a titration, due to evaporation, but this did not affect the ratio of molar concentrations, R = [nucleotide]/[gene 5 protein monomer]. The salt concentration was increased by adding aliquots of a 4 M NaCl solution to samples in 5 mM Tris-HCl, pH 7.0. Complexes used for dissociation experiments had a nucleotide to protein monomer molar ratio of 3.0, with a total G5P concentration of about 25 μM. Dissociation experiments were performed at 20 °C for the poly(dA·dT)–G5P complex and at 4 °C for the poly[d(A-T)·d(A-T)]–G5P complex.

Results and Discussion

CD Spectra Showed That G5P Binds to Double-Stranded Poly(dA·dT). The CD spectra for free poly(dA·dT) at temperatures above and below its T_m of 42 °C in 5 mM Tris-HCl, pH 7.0, are shown in Figure 1. The double-stranded polymer had characteristic positive CD bands at 283 and 259 nm and negative CD bands at 269 and 248 nm. At temperatures above the T_m of 42 °C only two broad CD bands were present above 230 nm, centered at about 276 and 250 nm. A comparison

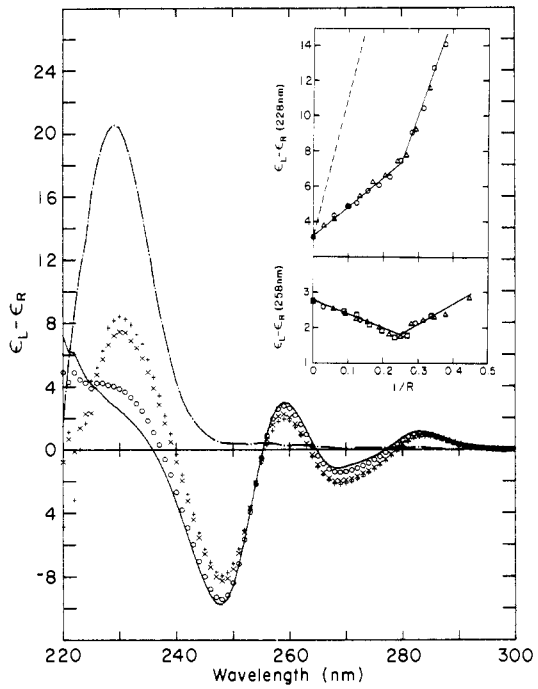

FIGURE 2: CD spectra at 20 °C during a representative titration of double-stranded poly(dA·dT) with fd gene 5 protein (G5P): free G5P (-·-), free poly(dA·dT) (—), and poly(dA·dT) with added G5P to give $1/R$ ratios of 0.06 (○), 0.20 (×), and 0.25 (+). In all figures, CD spectra for DNA–protein complexes are in units of L·(mol of nucleotide)$^{-1}$·cm^{-1}. The protein spectrum in this figure is 1/4 of its molar magnitude for comparison with the spectrum of the complex at $1/R = 0.25$. The inset shows the CD at 228 (top) and 258 nm (bottom) vs $1/R$ during repeat titrations (with different symbols). The top panel also shows the CD at 228 nm that would result from the additions of unperturbed gene 5 protein to the solution (---).

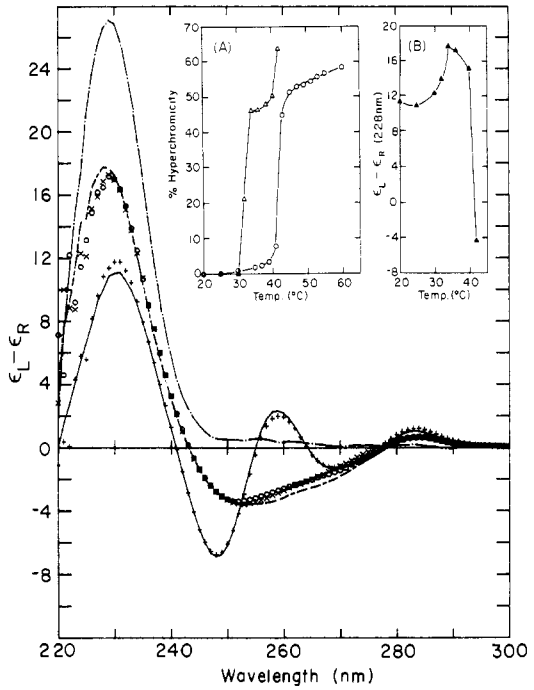

FIGURE 3: CD spectra of the poly(dA·dT)–G5P complex ($1/R = 0.33$) at increasing temperatures: 20 (—), 30 (+), and 35 °C (×) and after cooling back to 20 °C (○). Also shown are the spectrum of free G5P (molar magntiude × 1/3) at 20 °C (-·-) and the average of the spectra at 20 °C of the separate poly(dA)–G5P and poly(dT)–G5P complexes, both at $1/R = 0.33$ (---). The two insets show (A) the absorbance melting profiles at 260 nm of the poly(dA·dT) duplex (○) and the poly(dA·dT)–G5P complex ($1/R = 0.33$) (△) and (B) the CD at 228 nm vs temperature for the poly(dA·dT)–G5P complex (▲).

of spectra at 20 and 35 °C showed that there were premelting changes in the three long-wavelength bands, the first two bands at 283 and 269 nm becoming more positive as the polymer approached its T_m. The CD characteristics at long wavelengths could be used to monitor the state of the polymer in the presence of G5P, which has negligible intrinsic CD above 250 nm.

A titration experiment of double-stranded poly(dA·dT) with added G5P is shown in Figure 2. As the molar ratio of [protein monomer]/[nucleotide] ($=1/R$) increased from 0 to 0.25, the 283-, 269-, and 259-nm CD bands all became more negative. By comparison with Figure 1, it is seen that these CD changes were not the same as the premelting changes in the CD spectrum of the polymer. The absorbance of the polymer at 260 nm also did not increase, but actually decreased by 8.8% when G5P was added to give a ratio of $1/R = 0.25$. Thus, the CD changes above 250 nm in Figure 2 reflected some aspect of an interaction between the protein and the double-stranded polymer. These CD changes probably resulted from a perturbation of the duplex structure of poly(dA·dT). It seemed unlikely that more than a minor contribution to the CD changes arose from an induced optical activity in the long-wavelength bands of Tyr-26 and/or Phe-73 aromatic residues of G5P, since aromatic amino acids do not appear to contribute significantly to the long-wavelength CD changes observed upon binding of G5P to single-stranded DNA polymers (Kansy et al., 1986) even though these residues do interact significantly with single-stranded DNA (King & Coleman, 1987).

A second type of CD change confirmed that the protein interacted with the double-stranded form of poly(dA·dT). Free G5P has a large intrinsic CD band at 228 nm from one or more tyrosyl residues (Day, 1973). The 228-nm CD band of the sample increased during the titration due to the large positive CD of the added G5P at this wavelength. The CD band at 228 nm of the protein was greatly reduced on a molar basis, however, by binding to the polymer. Figure 2 shows one-fourth the molar CD spectrum of free G5P for comparison with the spectrum of the sample for which $1/R = 0.25$. With the assumption that the CD of the DNA at 228 nm did not change significantly upon binding of the protein, the 228-nm band of the protein decreased in molar magnitude by 67–68% upon formation of the poly(dA·dT)–G5P complex. This was more than 1.6 times the decrease in this band expected for the binding of the protein to a mixture of the individual poly(dA) and poly(dT) single strands (for which the decreases are 39% and 43%, respectively; Kansy et al., 1986) and was comparable to the 62% decrease in the magnitude of this band seen upon binding of G5P to double-stranded RNA (Gray et al., 1984).

Finally, the titration plots of CD changes at 258 and 228 nm as a function of $1/R$, shown as insets in Figure 2, provided evidence that a complex of defined stoichiometry was formed between double-stranded poly(dA·dT) and G5P. The titrations were linear and had break points at $1/R = 0.25$, as if G5P bound to the double-stranded polymer with the same stoichiometry as in its strongest binding mode for single-stranded DNAs (Kansy et al., 1986). The actual stoichiometry of binding to double-stranded poly(dA·dT) could not be determined from these experiments, however, since CD changes continued to occur at higher protein concentrations, possibly due to the CD contribution from a second binding mode.

Solution Conditions Significantly Affect the Destabilization of Poly(dA·dT) by G5P. CD spectra of poly(dA·dT) in the presence of G5P at different temperatures in 5 mM Tris-HCl, pH 7.0, are presented in Figure 3. The 259-nm positive CD band became negative and the 248-nm negative CD band

decreased significantly as the sample temperature was raised to 35 °C. Minor premelting CD changes mimicked those shown in Figure 1 for the free polymer. The CD spectrum of the complex at 35 °C matched the average of the spectra of the individual poly(dA)–G5P and poly(dT)–G5P complexes (Figure 3). Also, at 35 °C the nucleic acid absorbance at 260 nm was 45% hyperchromic relative to the absorbance at 20 °C. These changes showed that there was separation of the strands of the poly(dA·dT) polymer. The spectra in Figure 3 show that the tyrosyl 228-nm CD band of the protein increased during strand separation but was still 48% less than that of the free protein. Thus, the protein 228-nm CD band was reduced in magnitude upon binding of the protein to the single-stranded polymers, as expected (Day, 1973; Kansy et al., 1986), but it was not reduced as much as upon binding to the poly(dA·dT) duplex.

Melting profiles of the poly(dA·dT)–G5P complex ($1/R$ = 0.33) and of free poly(dA·dT) are shown in inset A of Figure 3. The T_m of double-stranded poly(dA·dT) in the presence of G5P was 32 °C, a decrease of only 10 °C from the T_m of 42 °C for the free polymer. At temperatures close to 40 °C, the protein in the complex denatured, the absorbance increased due to aggregation (Figure 3, inset A), and the CD at 228 nm became negative (Figure 3, inset B). (The CD spectrum of heat-denatured G5P was much different from that shown for the native protein and had only a broad negative band down to 210 nm.)

In 5 mM Tris-HCl, pH 7.0, with no added salt, the single-strand poly(dA)–G5P and poly(dT)–G5P complexes that were formed by heating the duplex to 35 °C in the presence of G5P did not reanneal upon a reduction in temperature to 20 °C (Figure 3). A melted poly[d(A-T)·d(A-T)]–G5P complex, with a self-complementary DNA sequence, could be readily reannealed in 5 mM Tris-HCl, pH 7.0 (see below). Therefore, it seemed unlikely that poly(dA) and poly(dT) strands from a melted poly(dA·dT)–G5P complex were being held in juxtaposition within the same molecular complex by G5P dimers; rather the poly(dA) and poly(dT) strands were probably separated into physically distinct complexes during melting.

The binding of G5P to double-stranded poly(dA·dT) was sensitive to NaCl concentration, with about 20% of the protein being dissociated from a poly(dA·dT)–G5P complex ($1/R$ = 0.33) by the addition of 0.1 M NaCl, as monitored by the increase in the 228-nm CD band (not shown). Melting profiles for the poly(dA·dT) duplex and the poly(dA·dT)–G5P complex were obtained at 0.1 M NaCl plus 5 mM Tris-HCl, pH 7. The polymer alone had a T_m of 67.5 °C in this buffer, an increase of 25.5 °C from its T_m in the absence of added NaCl. The T_m of the poly(dA·dT)–G5P complex, however, only increased from 32 to ~38 °C following the addition of 0.1 M NaCl (data not shown). Thus, in the presence of added 0.1 M NaCl, the T_m depression of the polymer caused by G5P increased from 10 to ~30 °C.

G5P Also Binds to Double-Stranded Poly[d(A-T)·d(A-T)]. The decrease in the T_m of poly[d(A-T)·d(A-T)] caused by G5P was determined for comparison with that found for poly(dA·dT). Figure 4 shows the CD spectrum of poly[d(A-T)·d(A-T)] and the CD changes upon adding protein to the polymer at 0 °C in 5 mM Tris-HCl, pH 7.0 ($1/R$ = 0.33). As was the case for poly(dA·dT), the spectral changes at wavelengths above 250 nm caused by adding G5P to poly[d(A-T)·d(A-T)] differed from the premelting CD changes observed upon raising the temperature of this polymer (Greve et al., 1977). In fact, magnitudes of the long-wavelength CD

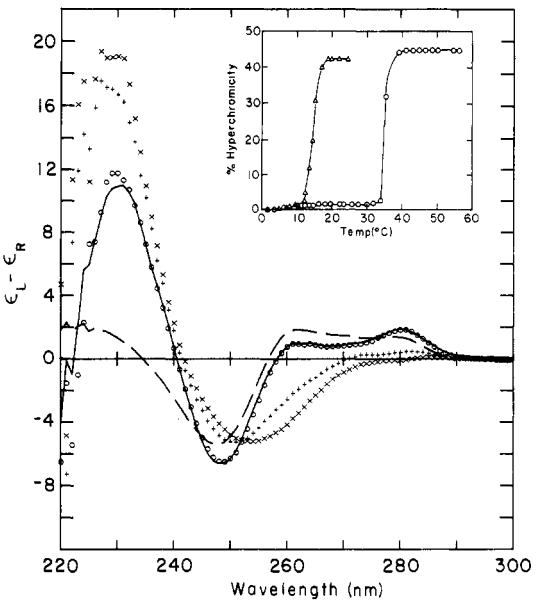

FIGURE 4: CD spectra of the poly[d(A-T)·d(A-T)]–G5P complex ($1/R$ = 0.33) at increasing temperatures: 0 (—), 15 (+), 20 (×), and 0 °C after heating to 20 °C (○). The spectrum of free poly[d(A-T)·d(A-T)] is shown at 0 °C (– –). The inset shows absorbance melting profiles at 260 nm of the poly[d(A-T)·d(A-T)] duplex (○) and the poly[d(A-T)·d(A-T)]–G5P complex (△) ($1/R$ = 0.33).

bands changed in the same direction upon adding G5P as upon lowering the temperature of the duplex polymer (Greve et al., 1977). A decrease of about 69% in the 228-nm tyrosyl band of the protein confirmed that G5P bound to the double-stranded form of poly[d(A-T)·d(A-T)]. Poly[d(A-T)·d(A-T)] was not hypochromic in its complex with G5P.

As the temperature was increased to 20 °C, the long-wavelength positive bands of the poly[d(A-T)·d(A-T)]–G5P complex disappeared, the 228-nm CD band increased in magnitude, and the CD spectrum changed to that previously reported for a complex between the protein and single-stranded poly[d(A-T)] (Kansy et al., 1986); see Figure 4. Absorbance melting profiles for the poly[d(A-T)·d(A-T)]–G5P complex and free poly[d(A-T)·d(A-T)] are shown in the inset of Figure 4. We found that the T_m of the polymer was decreased by 20.5 °C, from 35 to 14.5 °C, in the presence of G5P in 5 mM Tris-HCl, pH 7.0. Complete renaturation of the complex occurred upon lowering the temperature back to 0 °C (Figure 4), with no evidence of a kinetic block for reannealing of this self-complementary sequence.

CONCLUSIONS

G5P Binds to Double-Stranded Nucleic Acids. Upon mixing G5P with double-stranded poly(dA·dT) and poly[d(A-T)·d(A-T)], we observed CD changes in the long-wavelength region above 250 nm, where the CD bands of nucleic acids dominate, and at 228 nm, where G5P has a tyrosyl band. The changes at long wavelengths were not like those due to strand separation, and there was no associated hyperchromicity at 260 nm. Specific dsDNA–G5P complexes may be formed similar to the dsRNA–G5P complex that has been visualized by electron microscopy (Gray et al., 1984). The situation contrasts with that for the T4 gene 32 protein, which binds very weakly to double-stranded poly[d(A-T)·d(A-T)] (Jensen et al., 1976) and which causes no detectable CD changes when added to either double-stranded poly(dA·dT) or poly[d(A-T)·d(A-T)] (Greve et al., 1978).

The affinity of G5P for double-stranded nucleic acids depends on the nature of the double-stranded structure. The binding was slightly stronger to poly(dA·dT) than to poly[d-

(A-T)·d(A-T)], with 50% dissociation at 0.14 and 0.12 M NaCl, respectively (data not shown), while complexes with double-stranded RNA are largely dissociated at 0.05 M NaCl (Gray et al., 1984). It was interesting that the greater binding affinity of G5P to double-stranded poly(dA·dT) appeared to be correlated with a smaller melting point depression for this polymer (10 °C) than for poly[d(A-T)·d(A-T)] (20.5 °C) under the same conditions.

The Melting Point Depression of Poly(dA·dT) by G5P. G5P binds to poly(dT) with an exceptionally high affinity. This interaction is 2 orders of magnitude stronger than that with other single-stranded polymers, including poly(dA) and fd DNA (Porschke & Rauh, 1983; Bulsink et al., 1985). We had expected that the high binding affinity of G5P for poly(dT) would result in a facilitated melting of double-stranded poly(dA·dT) with a decrease in T_m no less than that observed for other DNAs in the presence of G5P.

We found that the T_m of the poly(dA·dT)–G5P complex was 30 °C less than that of the free polymer when 0.1 M NaCl was added to the buffer. However, without 0.1 M NaCl the T_m of the polymer–G5P complex was lowered by only 10 °C from the T_m of the free polymer. This seemed surprising since, as the salt concentration is lowered, the double-stranded polymer becomes less stable and the protein binds stronger to the poly(dA) and poly(dT) single strands (Bulsink et al., 1985). Holwitt and Krasna (1982) also previously found that the T_m depressions of T7 DNA and two DNA polymers caused by G5P were not as great when the salt concentration was lowered from 10 to 1 mM. Our CD measurements have now shown that G5P can bind to double-stranded poly(dA·dT) and that this binding is salt-sensitive. Thus, an enhanced binding of G5P to the double-stranded form of a polymer at low salt concentrations might reduce the destabilization that would otherwise occur in the presence of this protein.

Registry No. Poly(dA·dT), 24939-09-1; poly[d(A-T)·d(A-T)], 26966-61-0.

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