The Ff Gene 5 Protein–d(pA)$_{40-60}$ Complex: ^{1}H NMR Supports a Localized Base-Binding Model†

Garry C. King and Joseph E. Coleman*

Department of Molecular Biophysics and Biochemistry, Yale University, 333 Cedar Street, P.O. Box 3333, New Haven, Connecticut 06510

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ABSTRACT: The interaction between Ff gene 5 protein (G5P) and d(pA)$_{40-60}$ serves as an improved model system for a ^{1}H NMR examination of the G5P–ssDNA interface under cooperative binding conditions. Selective deuteriation of aromatic residues enables individual Tyr (3,5)H and (2,6)H resonances to be monitored in spectra of high molecular weight nucleoprotein assemblies. Analysis of complexation-induced chemical shift changes and intermolecular NOEs indicates that Tyr 26 is the only tyrosine to interact directly with ssDNA. Tyr 41, which is immobilized upon binding, is implicated in a dimer–dimer contact role. These and other NMR data are consistent with a previously outlined model of the protein–DNA interface in which Phe 73′, Leu 28, and Tyr 26 form components of a base-binding pocket or "dynamic clamp" fringed by a cluster of positively charged residues [King, G. C., & Coleman, J. E. (1987) *Biochemistry 26*, 2929–2937]. In the present version of this model, the Phe and Leu side chains are proposed to stack on either side of a single base, while there is the possibility that Tyr 26 may H-bond to the sugar–phosphate backbone in addition to or instead of stacking. Chemical-exchange effects underscore the dynamic nature of binding at the pocket. A comparison of d(pA)$_{40-60}$ and oligo(dA)-induced chemical shift changes suggests that poly- and oligonucleotide complexes have indistinguishable base-binding loci but appear to differ in their dimer–dimer interactions. Alternative polynucleotide binding stoichiometries are explicable in terms of a single base-binding model: chemical shift data are consistent with a proposal that the common $n = 3$ and $n = 4$ modes differ basically in the extent to which the sugar–phosphate backbone is stretched between binding pockets on adjacent G5P dimers.

The first reports on the isolation of gene 5 protein (G5P) from the Ff bacteriophage family established its ssDNA-binding function and the superhelical topology of a native G5P–Ff DNA assembly (Oey & Knippers, 1972; Alberts et al., 1972). Since that time, numerous studies have been directed at uncovering the molecular basis of its preference for ssDNA and a more refined structural description of the supermolecular complex. With the advent of a revised X-ray structure for the free G5P dimer (Brayer & McPherson, 1983), these questions appeared to be close to solution. Manual model building (Brayer & McPherson, 1984) was promptly used to create a description of the isolated protein–ssDNA interaction. Helical extension of this model through the use of atomic contact analysis then produced a putative structure for the nucleoprotein assembly (Brayer & McPherson, 1985). In essence, the proposed protein–DNA interface involved electrostatic binding of the phosphodiester backbone by Arg 80, Arg 21, Arg 16, and Lys 46, while five nucleotide bases were stacked sequentially with the exposed side chains of Tyr 26, Phe 73′, Tyr 34, and Tyr 41[1] (Brayer & McPherson, 1984; McPherson & Brayer, 1985).

The concept of sequential stacking of aromatics and bases in a "ladder array" is attractive, and helped by the air of certainty that tends to surround crystallographically derived models, has become influential within the field. However, as we and others have argued previously (Gray, 1985; Kansy et al., 1986; King & Coleman, 1987), there are serious disagreements between the model-built proposals and several lines of physicochemical evidence. Most recently, a NOESY[2] study of 4- and 8-mer nucleotide binding concluded that only two aromatics were involved in stacking interactions with nucleotide bases (King & Coleman, 1987). An alternative model derived from this study suggested that Phe 73′, Tyr 26, and Leu 28, acting in concert, form a "dynamic clamp" that is the sole base-binding element. This model has attractive features of its own, but recent reports that G5P may possess different oligo- and polynucleotide binding modes (Alma et al., 1982, 1983b; Bulsink et al., 1986) posed serious questions regarding its relevance to the natural assembly. It was apparent that an experimental comparison of alternative models required a polynucleotide system which could be examined in reasonable molecular detail.

This paper describes ^{1}H NMR experiments performed on complexes between G5P and d(pA)$_{40-60}$, a system with the potential to form over one turn of supermolecular helix. Since the roles of individual tyrosines are the major points of contention between the two available models of the interaction, these residues are the main focus of our attention. Selective deuteriation of G5P is used to overcome the resolution problems presented by high molecular weight complexes. Alternative models of the G5P–ssDNA interface, questions of poly- vs oligonucleotide binding modes, and differences between

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[1] Both monomers contribute to each of the two dyad-related DNA binding sites per dimer. When a single binding site is being considered, a prime notation is used to denote a residue from the second monomer.

[2] Abbreviations: NOE, nuclear Overhauser effect; NOESY, two-dimensional NOE spectroscopy; N/P, nucleotides per protein monomer; T_1, spin–lattice relaxation time; T_{1S}, selective spin–lattice relaxation time; T_2, spin–spin relaxation time; TSP, sodium (trimethylsilyl)tetradeuteriopropionate.

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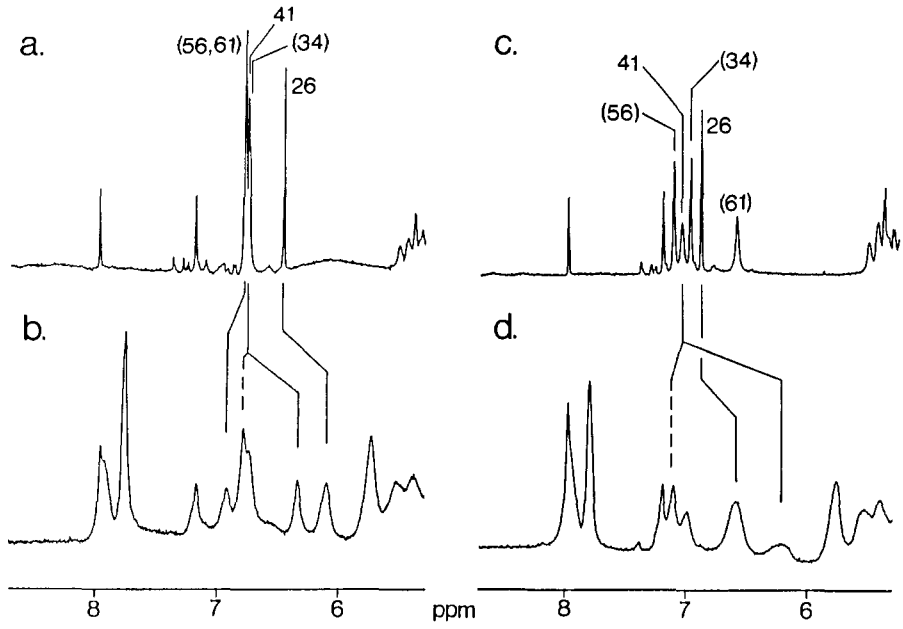

FIGURE 1: Effect of complexation with $d(pA)_{40-60}$ on the low-field resonances of selectively deuteriated G5P species (10 mM phosphate, pH 7.6, 35 °C). Spectra of (a) 0.45 mM Tyr3,5H–G5P, (b) 4.0:1 $d(pA)_{40-60}$–(Tyr3,5H–G5P) mixture, (c) 0.45 mM Tyr2,6H–G5P, (d) 4.0:1 $d(pA)_{40-60}$–(Tyr2,6H–G5P) mixture. Resonance assignments are indicated, with tentative assignments in parentheses. Singlets at 7.21 and 7.98 ppm derive from His 64.

alternative polynucleotide binding stoichiometries are examined through analysis of chemical shift changes and protein–nucleotide NOEs.

Materials and Methods

2,6,α-Trideuteriotyrosine, 3,5,α-trideuteriotyrosine, and 2,3,4,5,6,α-hexadeuteriophenylalanine were obtained by proton/deuteron exchange in sulfuric acid (Griffiths et al., 1976) and were used without further purification. An auxotrophic host for the introduction of deuteriated aromatic amino acids into G5P was constructed by P1 transduction of the aro A::Tn 10 locus from *Escherichia coli* LCB273 (CGSC 6542) into strain K37 (Hfr, su_I^+). Cultures were grown in a 10-L microfermenter (New Brunswick Scientific) in M9 salts supplemented with 10 g L^{-1} glucose, 3 g L^{-1} citrate, 100 mg L^{-1} protonated amino acids, and 50 mg L^{-1} of the required deuteriated amino acids. Bacteriophage infection was initiated at OD_{600} = 0.7–1.0, and cells were harvested 3–6 h later. Native and selectively deuteriated G5Ps were prepared by standard methods (Anderson, 1975). The deuteriated species 2,6,α-trideuteriotyrosine–2,3,4,5,6,α-hexadeuteriophenylalanine–G5P and 3,5,α-trideuteriotyrosine-2,3,4,5,6,α-hexadeuteriophenylalanine–G5P are referred to as Tyr3,5H–G5P and Tyr2,6H–G5P, respectively. Nucleotides $d(pA)_{40-60}$, $d(pA)_8$, and $d(pA)_4$ were purchsed from Pharmacia P-L Biochemicals. Concentrations on a mononucleotide basis were determined from ϵ_{260} = 9500, 10 300, and 10 700 M^{-1} cm^{-1}, respectively (Alma et al., 1982, 1983a). ^1H NMR spectra were recorded with a Bruker AM-500 spectrometer. Protein and nucleotide samples were prepared for NMR by buffer exchange on a Sephadex G-25 spun column. Typical experiments were performed on samples 0.4–0.6 mM in protein monomer. Chemical shifts are quoted relative to internal TSP.

Results

Selectively Deuteriated G5P. The aromatic regions from ^1H NMR spectra of Tyr3,5H–G5P and Tyr2,6H–G5P are shown in Figure 1, panels a and c, respectively. Both spectra appear to contain only one signal from each of the five tyrosines, suggesting that all rings flip relatively rapidly under the prevailing conditions. In addition to the spectral simplification that results from the loss of phenylalanine and vicinal tyrosine signals, multiplet collapse and a slight T_2 enhancement produce a 1.2–3-fold improvement in peak height over corresponding signals from fully protonated G5P. This enhancement is substantially greater for the (3,5)H signals, reflecting the relative proximity of the (2,6)H groups to other relaxation partners nearer the protein backbone. The His 64 signals at 7.98 and 7.21 ppm have been retained to serve as markers.

Resonance assignments are indicated with the free protein spectra (Figure 1a,c). Assignments for the Tyr 26 and Tyr 41 signals have been unequivocally determined from comparison with spectra of the Y26F and Y41F site-directed mutants (G. C. King, T. C. Terwilliger, and J. E. Coleman, unpublished data). Others come from a previous NOESY study of protonated G5P and should be regarded as reasonably firm if not certain (King & Coleman, 1987). Wild-type G5P has a tendency to aggregate at NMR concentrations (Garssen et al., 1978), causing some aromatic resonances to shift according to sample conditions. The Tyr 41 (2,6)H signal is particularly sensitive in this respect. Details of mutant species, native spectral characteristics, and their implications for resonance assignments will be presented elsewhere.

The G5P–$d(pA)_{40-60}$ Complex: Protein Resonances. Traditionally, ^1H NMR studies of ssDNA binding proteins have used complexation-induced upfield shifts of protein signals as an indication of stacking interactions with nucleotide bases (Coleman et al., 1976; Garssen et al., 1977). Shift data can also report on the disruption of base–base stacking in purine polynucleotides.

Chemical shift changes that are observed to accompany cooperative complexation of G5P with $d(pA)_{40-60}$ are summarized in Table I. Figure 1, panels b and d, displays the low-field regions from spectra of $d(pA)_{40-60}$ mixed with Tyr3,5H–G5P and Tyr2,6H–G5P, respectively. Both mixtures have a nucleotide-to-protein (N/P) ratio of 4.0 (±0.2), which corresponds to the standard site size of G5P (Kansy et al., 1986; Alma et al., 1983a). Complexes formed in the presence and absence of 200 mM NaCl yield effectively identical spectra. Protein resonances of the mixtures have line widths

Table I: Chemical Shift Changes That Accompany Formation of G5P–d(pA)$_{40-60}$ Complexes at an N/P Ratio of 4.0[a]

signal	Δδ (ppm)	signal	Δδ (ppm)
Tyr 26 (3,5)H	−0.34	Phe 73 (ring)H	−0.76
Tyr 26 (2,6)H	−0.30	Ade H2	0.41
Tyr 41 (3,5)H	−0.41 (0.02)	Ade H8	0.05
Tyr 41 (2,6)H	−0.85 (0.04)	Ade H1′	0.17
Tyr (56/61) (3,5)H	0.14	Ade H4′	−0.08
Leu 28 methyls	−0.50		

[a] Conditions: 0.45 mM G5P in 10 mM phosphate, pH 7.5, 35 °C. Values for the Leu 28 methyl and Phe 73 (ring)H signals are averaged. Values in parentheses are tentative. Shift changes for other tyrosine ring proton resonances and bulk nucleotide signals are <0.03 ppm in magnitude.

in the range 25–100 Hz, reflecting the high molecular weights expected for the resulting assemblies. Spectral changes induced by polynucleotide binding are considered below, with an emphasis on the higher quality Tyr3,5H–G5P spectra.

Three Tyr (3,5)H signals are significantly perturbed by complex formation, while the remaining two appear to be unaffected (Figure 1b; Table I). The Tyr 26 (3,5)H resonance of bound G5P appears 0.34 ppm upfield of its free position at an N/P ratio of 4.0, as would be expected from the results of previous oligonucleotide binding studies. A 50-ms irradiation of the bound Tyr 26 signal produces small NOEs on the nucleotide sugar H1′, H4′, and possibly H3′ resonances (Figure 2a), consistent with proximity to nucleotide. A protein signal at 0.43 ppm also receives an NOE. This resonance is expected to derive from the nearby Leu 28 methyl groups (King & Coleman, 1987), placing their signals approximately 0.50 ppm upfield of their free positions. Lengthening the irradiation period to 500 ms induces difference peaks for all nucleotide signals through the effects of spin diffusion (Figure 2b). In addition to the effect on the Tyr 26 signal, binding causes the relative diminution of the major Tyr (3,5)H peak near 6.8 ppm (Figure 1a), accompanied by the rise of signals near 6.3 and 6.9 ppm (Figure 1b). The first of these perturbed resonances lies 0.41 ppm upfield of its original position and is assigned to Tyr 41. Cut-and-weigh comparison of this signal with the His 64 C-4H marker yields 1.0 ± 0.1 protons intensity, consistent with immobilization of the ring upon binding. In contrast to the behavior of the Tyr 26 resonance, a 500-ms irradiation of the resolved Tyr 41 signal produces no detectable NOEs on nucleotide resonances (Figure 2c). The difference peak at 6.81 ppm, which has approximately 20% intensity at mixing times down to 50 ms, may be due to saturation transfer to the symmetrically related (5)H signal of the immobilized ring or to an NOE with Tyr 34. The complementary effect is observed when the overlapped peak at 6.8 ppm is irradiated (Figure 2d), but no NOEs on nucleotide signals are apparent. The final perturbed (3,5)H signal from Figure 1b appears 0.14 ppm *down*field of its free position. Again, long-term irradiation of this peak (not shown) produces no observable NOEs on any nucleotide resonances.

The Tyr (2,6)H signals behave analogously (Figure 1c,d). A Tyr 26 resonance of approximately two-proton intensity appears upfield of its free position upon binding, to overlap the Tyr (61) resonance (Figure 1d). Irradiation of the overlapped Tyr 26/(61) peak for 50 ms produces NOEs at the positions of nucleotide sugar signals (Figure 3a). Increasing the irradiation period results in the development of nucleotide difference peaks, though spin diffusion from the underlying Tyr (61) resonance is severe (Figure 3b). Disappearance of the resolved Tyr 41 (2,6)H signal from the free G5P spectrum is accompanied by the rise of a one-proton resonance which receives the largest complexation-induced shift yet observed

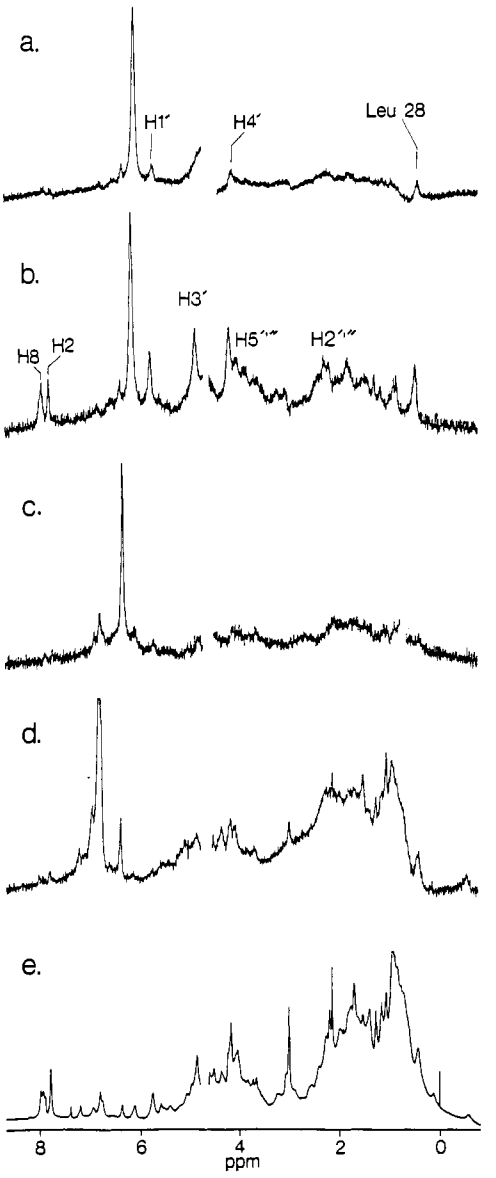

FIGURE 2: Magnetization transfer experiments on a (Tyr3,5H–G5P)–d(pA)$_{40-60}$ mixture (10 mM phosphate, pH 7.6, 35 °C, N/P = 5.0). (a–d) Difference spectra following irradiation of (a) Tyr 26 signal at 6.13 ppm for 50 ms, (b) Tyr 26 signal for 500 ms, (c) Tyr 41 (3)H signal at 6.36 ppm for 500 ms, (d) the overlapped signal at 6.81 ppm for 500 ms. (e) Reference spectrum.

for a G5P resonance (Figure 1d). However, irradiation of this (2)H signal for 50 or 500 ms does not result in NOEs on nucleotide resonances (Figure 3c). The same is true for the remaining unshifted Tyr (2,6)H signals (not shown). In sum, the Tyr (3,5)H and (2,6)H resonance data point clearly to a nucleotide binding role for Tyr 26 and a change in the environment of Tyr 41 upon binding.

An average chemical shift change of −0.76 ppm is observed for the Phe 73 signals of a partially deuteriated G5P sample on binding to d(pA)$_{40-60}$ at an N/P ratio of 4.0 (not shown). This value serves as a benchmark for the tyrosine chemical shift data.

Nucleotide Resonances. Two of the seven bulk nucleotide resonances receive substantial shifts upon complexation, while two others are slightly perturbed (Table I). At an N/P ratio of 4.0, the bound Ade H2, Ade H8, and sugar H1′ signals lie 0.41, 0.05, and 0.17 ppm downfield of their free positions, respectively. The observed Ade H2 shift change compares to that caused by thermally induced unwinding in the vicinity of 75 °C.

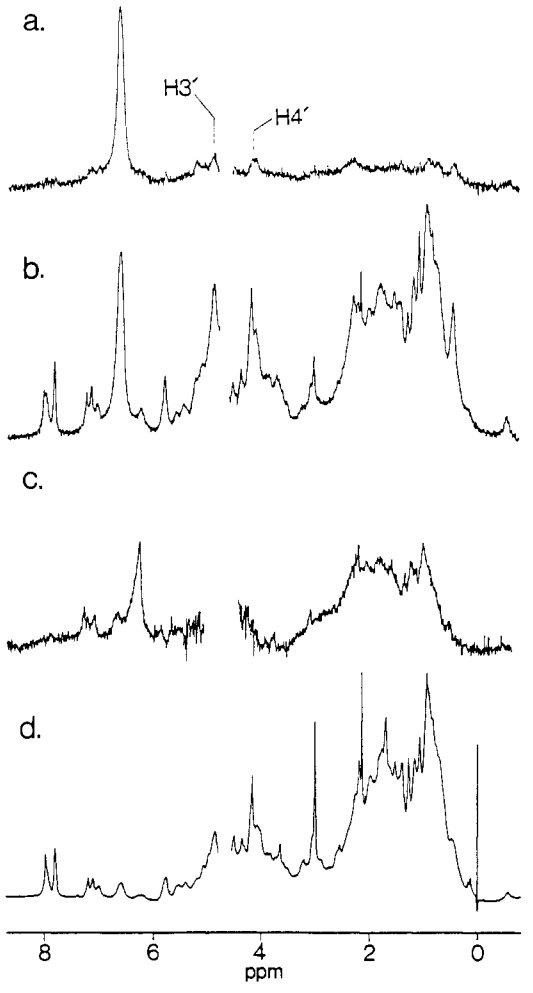

FIGURE 3: Magnetization transfer experiments on a (Tyr2,6H–G5P)–d(pA)$_{40-60}$ mixture (10 mM phosphate, pH 7.6, 35 °C, N/P = 4.2). (a–c) Difference spectra following irradiation of (a) overlapped Tyr 26/(61) resonance at 6.59 ppm for 50 ms, (b) Tyr 26/(61) signal for 500 ms, (c) Tyr 41 (2)H resonance at 6.19 ppm for 500 ms. (d) Reference spectrum.

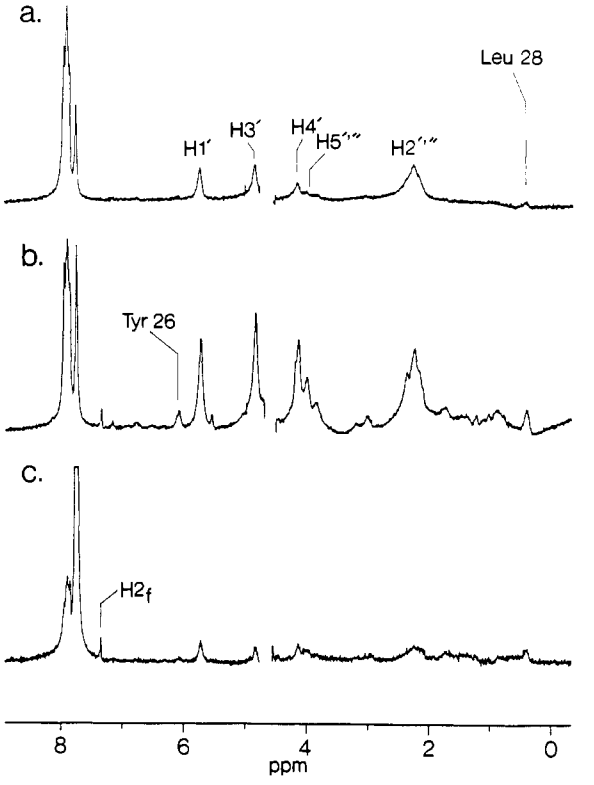

FIGURE 4: Magnetization transfer experiments on the nucleotide resonances from Figure 2e. Difference spectra following irradiation of (a) overlapped Ade H8 (80% bound/20% free) signals at 7.93 and 7.88 ppm for 75 ms, (b) Ade H8 signals for 500 ms, (c) Ade H2 bound signal at 7.78 ppm for 100 ms.

As shown in Figure 4a, irradiation of the Ade H8 signals from a Tyr3,5H–G5P–d(pA)$_{40-60}$ mixture for 75 ms produces NOEs on the sugar H2′,″, H3′, and H1′ signals. A small intermolecular NOE on the Leu 28 methyl signals is also evident. When the irradiation period is increased to 500 ms, spin diffusion produces intense difference peaks for all nucleotide resonances (Figure 4b), but the number of protein peaks is still quite limited. When excess polynucleotide (N/P = 5.0) is present, irradiation of the bound Ade H2 signal results in saturation transfer to the free Ade H2 signal at 7.37 ppm in addition to small difference peaks for other sugar signals and the Leu 28 methyl resonance (Figure 4c). Complementary irradiation at the Leu 28 position produces, in addition to NOEs from overlapping methyl signals, intermolecular NOEs at the Ade H2 and Ade H8 positions (not shown).

Evidence for Conformational Dynamics. Features of the ^{1}H NMR spectra suggest the presence of mobility within the G5P–d(pA)$_{40-60}$ complex. For example, the bound Tyr 26 (3,5)H signal of Figure 1b has 1.5 ± 0.1 protons intensity rather than an integral value, and its apparent intensity varies with conditions. At temperatures below 25 °C, two signals separated by approximately 0.05 ppm can be observed under the Tyr 26 (3,5)H envelope. Such an observation is consistent with immobilization of a flipping ring at lower temperatures or the manifestation of two slowly interconverting conformations which differ in some other manner. Additional evidence for mobility within the complex comes from the behavior of the Ade H2 and Ade H8 signals of bound nucleotide (Figures 1b,d and 5), which show no evidence of heterogeneity [the Ade H8 resonance overlaps with the His 64 C-2H signal, causing an apparent peak multiplicity which is unrelated to genuine signal heterogeneity]. Clearly, the average environments of all lattice sites are equivalent, one reason for which may be rapid exchange between sites.

Relaxation measurements were undertaken to further investigate the nature of the inferred mobility. Nonselective T_1 measurements indicate the presence of severe spin diffusion, as expected from the high molecular weights of the complexes. All signals relax with approximate exponentiality, yielding apparent T_1's in the range of 1–2 s. However, the NOE spectra of Figures 2–4 suggest that the protein and nucleotide signals form separate spin pools, with relatively inefficient cross-diffusion. This may arise from two sources: few intermolecular proton–proton interactions (apparent from the NOE data) and/or uncorrelated motions of the two species (a possible cause of Ade H2 and H8 signal averaging). In favorable instances, divergent correlation times may be detected from an examination of selective T_1/T_2 ratios (Jardetzky, 1979). T_{1S} values of 15–35 ms were measured for the G5P tyrosine signals and the polynucleotide H8 and H1′ resonances, while the Ade H2 value was 140 ms. The tyrosine, H8 and H1′ signals possess T_{1S}/T_2 ratios in the range of 3.0–5.0, while the ratio for the Ade H_2 resonance is larger at 9.3. These relaxation time data provide no clear evidence for substantially greater motional freedom of one species with respect to the other.

Oligonucleotide Binding. There have been suggestions that G5P may utilize different poly- and oligonucleotide binding modes (Alma et al., 1982, 1983b; Bulsink et al., 1986). With

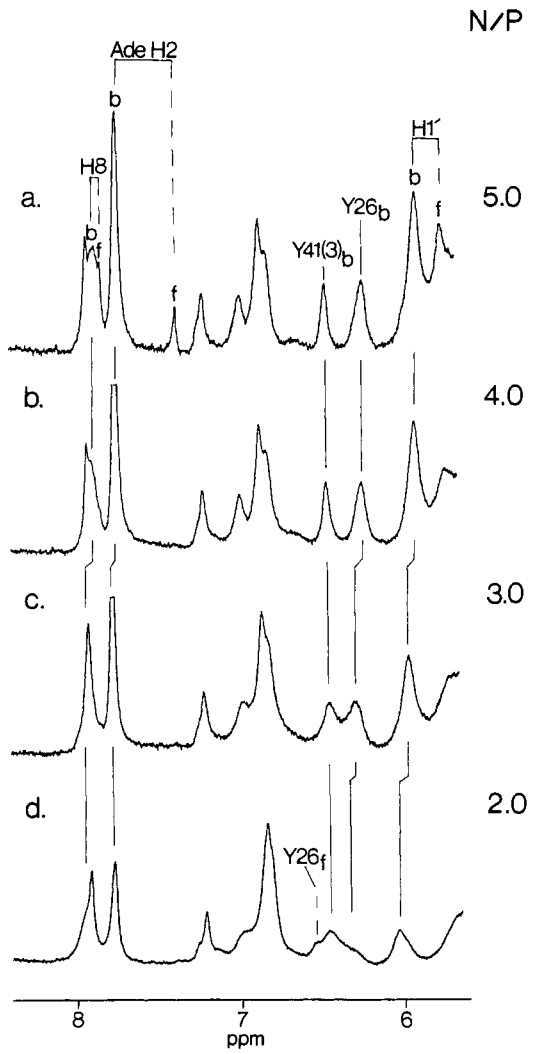

FIGURE 5: Effect of N/P ratio on low-field resonances of (Tyr3,5H–G5P)–d(pA)$_{40-60}$ mixtures. Resonances that derive from free and bound species are denoted f and b, respectively.

this in mind, oligonucleotide binding to selectively deuteriated G5P was examined to check for consistency with previous studies. At an N/P ratio of 4.0, d(pA)$_4$ and d(pA)$_8$ binding caused the Tyr 26 (3,5)H resonance to shift significantly, by −0.18 and −0.27 ppm, respectively. These species caused shifts of −0.06 and −0.08 ppm on the Tyr 41 (3,5)H signal. Both resonances exhibit fast exchange between free and bound species. The Tyr 26 (3,5)H resonance shifts are very close to those reported in our NOESY study of oligonucleotide binding to fully protonated G5P (King & Coleman, 1987). The small Tyr 41 (3,5)H shift changes are identical with those that we previously ascribed to a Tyr 34 (6)H resonance. Details of the revised assignment will be reported in connection with studies of mutant G5Ps.

Alternative Polynucleotide Stoichiometries. There is convincing evidence that G5P can utilize both $n = 4$ and $n = 3$ binding stoichiometries on polynucleotide lattices (Kansy et al., 1986; Gray, 1985). Chemical shifts of binding-perturbed resonances may provide a convenient comparison of different stoichiometric modes. In the presence of excess nucleotide (Figure 5a), both the G5P and bound-nucleotide resonances appear at chemical shifts characteristic of the standard $n = 4$ mode (Table I). Signals from free nucleotide, somewhat broadened by chemical-exchange effects, are also evident. When the N/P ratio of the mixture is reduced to 4.0, all observed signals derive from bound species and appear at their characteristic $n = 4$ positions (Figure 5b). Significant changes can be observed when the N/P ratio is lowered further. At an N/P ratio of 3.0 (Figure 5c), the bound Ade H2, H8, and H1′ signals move 0.04, 0.05, and 0.06 ppm *further downfield* of their free positions. In contrast, the Tyr 26 (3,5)H signal appears 0.06 ppm *closer* to its free position. The Tyr 41 (3)H signal, which receives such a large complexation-induced shift, is unmoved by the change in stoichiometry. This trend continues at an N/P ratio of 2.0, where the presence of the (exchange-broadened) free Tyr 26 (3,5)H resonance at 6.47 ppm indicates that protein is in excess (Figure 5d). Now the Ade H2, H8, and H1′ resonances appear a total of 0.46, 0.09, and 0.14 ppm downfield of their free positions, while the complexation-induced shift of the Tyr 26 signal is reduced to −0.23 ppm. As before, the bound Tyr 41 (3)H signal remains unmoved.

Chemical exchange behavior during these titrations is complicated. As indicated in Figure 5, panels a and d, the significantly perturbed signals manifest slow exchange between free and bound states, as may be expected for a cooperative binding process. Slow exchange has been observed over the temperature range 5–35 °C in the presence of 0–200 mM NaCl. However, shift changes that occur between N/P ratios of 4.0 and 2.0 are continuous, indicating fast exchange between different stoichiometric modes. The increased line widths evident in progressing from Figure 5a to Figure 5d are consistent with the formation of higher molecular weight complexes at lower stoichiometries, though a contribution from exchange broadening is present in each case.

The Tyr (2,6)H resonances behave in a similar manner to the (3,5)H signals (not shown). The bound Tyr 26 (2,6)H resonance shifts partially back toward its free position as the N/P ratio is lowered, while the Tyr 41 (2)H signal appears to maintain its large upfield shift within this range.

Discussion

Poly- versus Oligonucleotide Binding. The major aim of this paper has been to examine whether an alternative view of the G5P–ssDNA interaction derived from oligonucleotide binding studies (King & Coleman, 1987) is relevant to cooperative polynucleotide binding. As shown in Figure 5, the d(pA)$_{40-60}$ system has the required characteristics of a "normal" polynucleotide mode: a preferred $n = 4$ stoichiometry (Alma et al., 1983a; Kansy et al., 1986) and slow exchange between free and bound species (Alma et al., 1983b). The resulting short nucleoprotein assemblies may contain two DNA strands in antiparallel or one strand bound as a hairpin, with the latter considered to be the most likely arrangement.

Distinct modes of oligo- and polynucleotide interaction were originally proposed from observations that binding to these lattices exhibited different stoichiometries, chemical-exchange behaviors, and salt dependences (Alma et al., 1982, 1983a,b). This study provides a clear indication that these effects do not arise from differences in base-binding loci. Interaction with both types of lattice causes significant and selective perturbation of resonances from Phe 73, Leu 28, and Tyr 26 (this work; King & Coleman, 1987), signifying the existence of a common base-binding pocket (and by implication, phosphodiester-binding arrangement). Of course, the possibility of small structural differences is apparent, but there is no ready means of discerning these at present. There are some differences in the magnitudes of chemical shift changes induced by oligo- and polynucleotides (this work; King & Coleman, 1987), but their significance is uncertain due to the fact that similar differences exist between oligonucleotide-induced shift changes reported by different authors [see Alma et al. (1981,

1982, 1983b), O'Connor and Coleman (1983), and King and Coleman (1987)].

The striking difference that we observe between poly- and oligonucleotide binding lies in the behavior of the Tyr 41 resonances. Interaction with polynucleotide causes these signals to shift markedly and display exchange characteristics consistent with immobilization of the ring (Figure 1), but oligonucleotide binding causes only a slight perturbation (King & Coleman, 1987). Such observations are consistent with a recognition role for Tyr 41 at the dimer–dimer interface of a cooperative assembly. Supporting this view, preliminary characterization of Y41F–G5P (G. C. King, T. C. Terwilliger, and J. E. Coleman, unpublished data) indicates that this mutant protein possesses a reduced cooperativity parameter for poly(ethenoadenosine) binding. The Tyr 41 chemical shift data would thus suggest that G5P is unable to form "proper" dimer–dimer contacts on oligonucleotide lattices. There is independent evidence for this proposal. Alma et al. (1982, 1983a) have reported that oligonucleotide binding is associated with a much lower cooperativity parameter, supported by the fluorescence depolarization studies of Bulsink et al. (1986). Kansy et al. (1986) have shown that binding of 7- and 20-mers causes a significantly smaller decrease in the intensity of the 228-nm tyrosyl CD band of G5P than homologous polynucleotides.

The G5P–ssDNA Interface. The results of this study do not support a ladder array model of the G5P–ssDNA interaction in which nucleotide bases are sequentially stacked against four aromatic side chains (Brayer & McPherson, 1984). Instead, they are consistent with a view of the protein–DNA interface in which the side chains of Phe 73′, Tyr 26, and Leu 28 form a localized pocket or "clamp" which interacts with only one or two of the (four) bases nominally within the DNA binding region (King & Coleman, 1987).

The roles of the individual residues will be considered in turn. Stacking of the phenylalanine side chain is well established from earlier NMR studies of oligonucleotide binding. Complexation causes substantial upfield shifts of its ring proton resonances [e.g., Alma et al. (1982), O'Connor and Coleman (1983), and King and Coleman (1987)] that are base-dependent (Coleman & Armitage, 1977; King & Coleman, 1987), and NOEs with base proton signals can be observed (Alma et al., 1981, 1983b; King & Coleman, 1987). Until recently the number of tyrosine residues involved in the protein–nucleotide interaction has been subject to disagreement. However, the two most recent reports on Ff G5P and its homologous counterpart from the IKe bacteriophage agree that oligonucleotide binding significantly perturbs the signals of only one tyrosine (King & Coleman, 1987; De Jong et al., 1987). It is clear that this is also the case for polynucleotides: only the Tyr 26 signals exhibit the chemical shift changes and intermolecular NOEs diagnostic of a direct role in the interaction. However, there is some evidence that this role may involve more than stacking. While the induced upfield shifts of the Tyr 26 signals are base-dependent in oligonucleotide systems (Coleman & Armitage, 1977; King & Coleman, 1987) hinting at stacking, they tend to be less than those of the Phe 73 signals. More importantly, reported intermolecular NOEs reveal a greater proximity of the Tyr 26 ring protons to sugar groups (Figures 2 and 3 of this work; Alma et al., 1981, 1983b), suggesting the possibility that the hydroxyl group may hydrogen bond to the sugar–phosphate backbone in addition to or instead of stacking. This possibility will be the subject of further investigation. A stacking role proposed for Leu 28 (King & Coleman, 1987) is strengthened by this study: chemical shift and NOE data suggest a close interaction between this side chain and nucleotide base/s. Aromatics need not be the only types of residue involved in base binding.

At present, the structural view of the protein–ssDNA interface remains largely schematic. NOE data for the G5P–$d(pA)_4$ complex have been interpreted in terms of the enclosure of two bases by the "clamp" (King & Coleman, 1987) but could be equally consistent with a single-base enclosure. Support for a one-base site comes from ESR studies of G5P binding to spin-labeled poly(dT), which concluded that only one of four bases within the DNA binding channel was strongly immobilized (Kao et al., 1985). The Ade H2 chemical shift behavior (Table I) suggests that substantial destacking of adenine bases occurs upon binding. At the same time, the NOE data of Figure 4a are consistent with the maintenance of an anti glycosidic bond angle, probably reflecting the conformation of bases outside the pocket, but which may apply to the clamped base as well. One difficulty in improving the structural detail of the model lies in the evident need for a substantial movement of the flexible "DNA binding loop" (Brayer & McPherson, 1983) from its crystallographic position. The available NMR data do not allow various possible positions of this loop and its attendant side chains to be distinguished.

Dynamic processes are difficult to characterize but appear to be an essential element of the interaction that should not be overlooked. Alma et al. (1983b) first noted that the Ade H2 groups of a G5P–$d(pA)_{25-30}$ mixture have the same magnetic environment, which they interpreted to indicate a minimal interaction of H2 groups with protein. The present relaxation data, where the bound Ade H2 signal has a higher T_{1S}/T_2 ratio than any other resonance in the low-field region, support this view. However, we also find no evidence for heterogeneity of the Ade H8 signal, suggesting an alternative explanation. The base in the pocket may be free to exchange with its neighbours, making the clamp aspect of the interaction one of form rather than literal effect. The simultaneous presence of fast exchange between different stoichiometric modes and slow exchange between free and bound species supports this proposal. Rapid exchange of bases in and out of the pocket would not be unexpected, since the binding energetics are dominated by electrostatic interactions (Alma et al., 1983a).

Alternative Polynucleotide Binding Stoichiometries. The existence of an $n = 3$ polynucleotide binding mode as an alternative to the standard $n = 4$ mode has been firmly established from CD (Kansy et al., 1986) and EM (Gray, 1985) studies. Chemical shift data from Figure 5 suggests a structural rationalization of these modes that arises as a consequence of single-base binding. The increasing chemical shift perturbations observed for the H2, H8, and H1′ nucleotide resonances as the N/P ratio is reduced from 4.0 to 2.0 are consistent with a model in which the $n = 3$ mode is achieved simply by greater extension of the sugar–phosphate backbone, so that every third base is bound by adjacent pockets rather than every fourth. Maintenance of a normal dimer–dimer interface at lower stoichiometries is suggested by the immobility of the highly perturbed Tyr 41 signals, though there is a possibility that changes could occur in other regions of the dimer–dimer interface without influencing Tyr 41. The behavior of the Tyr 26 signal upon lowering the N/P ratio suggests an alteration of interactions at the base-binding pocket, the nature of which is unclear.

The chain-stretching proposal, while attractively simple, is subject to several uncertainties. In the first instance, the Tyr

26 resonance behavior could also be rationalized in terms of bypassing a certain proportion of the available base-binding pockets, so that some individual G5P–ssDNA interactions are entirely electrostatic. At the limit, this type of interaction may come to resemble the dsDNA binding reported by Sang and Gray (1987). ESR experiments of the type performed by Kao et al. (1985) could help resolve this question. Secondly, EM studies on G5P–fdDNA complexes (Gray, 1985) suggest that an $n = 3$ complex has approximately 15% more superhelical turns and approximately 15% more nucleotides per turn than an $n = 4$ complex, rather than the 30% more turns predicted. Further studies are necessary to determine whether this discrepancy is significant.

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