The thermodynamic parameters of DNA triplex formation between oligonucleotides and double-stranded DNA segments containing adenine runs (A-tracks) were investigated to explore equilibrium structural effects exerted by flanking segments upon the A-tracks. Results obtained from isothermal titration calorimetry, temperature-dependent circular dichroism (CD), and UV melting experiments indicate that A-tracks, considered as a uniquely robust and inflexible DNA motif, can be structurally perturbed by neighboring sequences in a way that significantly affects the propensity of this motif to interact with triplex-forming oligonucleotides. These contextual equilibrium effects, which depend upon the composition and location of the flanking sequences, are likely to apply not only to the interaction of A-tracks with single-stranded DNA molecules but also to interactions with drugs and proteins. As such, the current results refine the guidelines for the design of triplex-forming oligonucleotides used for antigenic strategies. More generally, they substantiate the notion that significant data might be encoded by structural DNA parameters.

The demonstrated in vivo existence of non-B-DNA morphologies and the potential involvement of such altered structures in the regulation and fine-tuning of cellular processes (1, 2) elicited intensive studies on the factors that affect and modulate DNA conformational transitions. It is well established that the physical chemistry of these transitions is determined by an intricate array of environmental conditions and intrinsic factors, including the ionic strength, relative humidity, pH, and torsional strain. The actual structure exhibited by a given DNA segment is, however, generally considered to be dictated by the particular composition and sequential order of the base pairs within this segment. Indeed, left-handed DNA conformations, cruciforms, intramolecular tripleplexes, as well as bent or macroscopically curved motifs are detected predominantly in non-random DNA sequences such as alternating purine-pyrimidine segments, direct or inverted repeats, and purine-rich tracts (2). Several studies have indicated, however, that the kinetics of a conformational change, as well as the features of the resulting DNA structure, may be influenced or even dominated by sequences that are removed from those directly participating in the transition.

The first evidence that structural information can be transmitted along the DNA molecule was provided by the observation that the melting of a DNA segment can be strongly affected by the base composition of a contiguous region (3). The kinetics of cruciform extrusion (4), as well as of the formation of intramolecular triplex-stranded DNA motifs (5), were found to be dominated by sequences flanking the region that undergoes the transition. The effects were shown to operate over relatively long distances and to be independent of polarity. Right-to-left-handed transitions of alternating d(G-C)$_n$ and d(A-T)$_n$ segments were also found to be significantly modulated by regions flanking the alternating segment (6–8). A striking demonstration of the long range nature of contextual factors is provided by the crucial role displayed by these factors on DNA packaging processes. The propensity of DNA molecules to condense was shown to be substantially modulated by the presence of non-B-DNA motifs such as Z-DNA or telomeres within the DNA (9–11), as well as by the conformational junctions between such altered motifs and generic B-DNA (12). Notably, these observations underline the ability of DNA sequences to influence the kinetics of a structural transition that occurs at a removed site. Equilibrium structural effects of DNA context have also been observed, albeit less frequently. Salient examples are provided by structural changes induced by homopurine-homopyrimidine sequences on regions located 3’ to the G tract (13), deformations exhibited by left-handed Z-DNA segments when flanked by AT-rich sequences (6), as well as the effects exerted by phased adenine tracts on the morphology of condensed DNA structures (14).

To sustain conformational changes and to be affected by contiguous regions, DNA segments must exhibit considerable structural flexibility. In general, DNA sequences comply with this requirement, being supple, flexible, and deformable. A conspicuous exception is provided by runs of successive adenine bases, or A-tracks, which are characterized by an unusual rigidity and hence an exceptional aversion toward conformational changes (15–20). The distinctive structural features of A-tracks and the potential effects of these features upon DNA-protein interactions prompted us to examine the susceptibility of this motif to contextual effects. Toward this end, we have exploited the propensity of poly(dA)-poly(dT) segments to interact with a pyrimidine strand, thus forming intermolecular triple-stranded structures (21, 22). It has been reported recently that the ability of very short adenine tracts to form a triple-stranded motif is abolished when the A-tract is flanked by GC-rich segments (23). By using isothermal titration calorimetry (ITC),† temperature-dependent circular dichroism (CD), and UV melting techniques, we show that the formation and stability of the triplex motif dT$_n$-*dA*$_n$-$dT_n$ (where * designates third-strand binding, and $\cdot$ represents Watson-Crick base pairing) are significantly influenced by sequences that flank the

† The abbreviations used are: ITC, isothermal titration calorimetry; HPLC, high pressure liquid chromatography; PIPES, piperazine-N,N’-bis(2-ethanesulfonic acid); $T_m$, melting temperature; TFO, triplex-forming oligonucleotide.
adeno tracts. The nature of the influence depends upon the composition, location, and number of the flanking sequences, at one or both ends of the A-tracts. The results, assigned to long range equilibrium structural modifications within the A-tract, demonstrate that even a particularly robust DNA motif, generally considered to be “immune” to structural deformations, is susceptible to contextual effects in a way that markedly modulates its conformational, and hence chemical, properties. As such, the observations underline the complex interplay between conformational DNA motifs and provide an additional facet to the notion that genetic information might be encoded within DNA structure.

**EXPERIMENTAL PROCEDURES**

Oligonucleotides—Oligonucleotides containing thymines were purchased from Sigma-Genosys and purified by preparative HPLC as the 5′-dimethoxytrityl derivatives. Oligonucleotides containing adenines were purchased from Midland Certified Reagent Company (Midland, TX) and purified by ion exchange HPLC. Oligonucleotide purity was analyzed by HPLC and mass spectroscopy and found to be >95%. The concentration of the oligonucleotides was determined by UV absorption using extinction coefficient parameters from published nearest neighbor parameters (24). The precision of this determination was further confirmed by ITC experiments in which single-stranded species were titrated with their complementary strands (under conditions refractory to triplex formation) to give titration curves with a stoichiometry number of 1. The purified oligonucleotides were extensively dialyzed against 20 mM PIPES, pH 7.0. Stock solutions of the double-stranded forms were prepared by mixing equimolar amounts of complementary strands, heating to 90 °C, and cooling to room temperature at a rate of 0.5 °C/min. Triple-stranded solutions for CD and UV experiments were prepared by mixing the appropriate amounts of stock solutions containing the single-stranded and the preformed double-stranded DNA species. The resulting solutions were diluted to the required concentration in 20 mM PIPES, pH 7.0, 1.0 M KCl (buffer A) for UV measurements and in 10 mM sodium phosphate (pH 7.0), 1.0 M NaCl (buffer B) for CD studies. Triplex solutions were equilibrated at the initial temperature of the particular experiment for 15 min or at 4 °C overnight; identical results were obtained from both preparation conditions.

Isothermal Titration Calorimetry—ITC experiments were carried out on an MSC-ITC system (MicroCal Inc.). Single-stranded solution (50 μM in buffer A) was injected 22 times in 5-μl increments at 3-min intervals into the isothermal cell containing the double-stranded targets (2.5 μM in buffer A) was injected 22 times in 5-μl increments at 3-min intervals

**RESULTS**

Thermodynamic Effects of Flanking Sequences on the Formation of the Triplex Motif

Oligonucleotides represent a distinctive class of DNA-binding species that recognize the major groove of the double helix by forming Hoogsteen-type hydrogen bonds with Watson-Crick base pairs (26, 27). The process attracted considerable interest because of the potential application of triplex-forming oligonucleotides (TFOs) for targeting specific DNA sites both in vitro and in vivo (28). We studied the thermodynamic parameters of triplex formation between a pyrimidine strand and various DNA targets specified in Table I.

**TABLE I**

| Target duplex | Name      |
|---------------|-----------|
| 5′-A18*3′     | 18–0FE    |
| 3′-T20*5′     | 20–2(TAT)FE |
| 5′-CGCA*9CGC*3′ | 20–2(CGC)/FE |
| 3′-GGCT*9GGC*5′ | 20–2(GGC)/FE |
| 5′-CGCA*9CGC*3′ | 26–2(CGC)/FE |
| 3′-GGCT*9GGC*5′ | 20–1(FE/3′ |
| 5′-A9CGC*3′   | 18–1FE/5′ |
| 3′-T9GGC*5′   | 20–1(TAT)/FE |
| 5′-CGCA*9CGC*3′ | 20–1(FE/3′ |
| 3′-GGCT*9GGC*5′ | 20–1(TAT)/FE |

Fig. 1a depicts a typical isothermal titration profile obtained by 22 5-μl injections of the 18-mer TFO dT18 into the ITC cell containing the double-stranded open-ended DNA target 18–0FE (Table I) at 20 °C. An exothermic heat pulse is detected following each injection, whose magnitude progressively decreases until a plateau, corresponding to the heat of dilution of the single-stranded species in the buffer and indicating saturation, is reached. The heat evolved in each injection was corrected for the heat of dilution, which was determined separately by injecting the single-stranded oligonucleotide into the buffer, and divided by the number of moles injected. The resulting values were plotted as a function of the molar ratio between the single-stranded species and the double-stranded segments and fitted to a sigmoidal curve by using a nonlinear least squares method (25). The equilibrium association constant (K2) and the enthalpy change (∆H) that characterize the formation of the triplex-stranded structure are directly obtained from the titration curve; the Gibbs free energy change (∆G) and the entropy change (∆S) of the process are then calculated from the equation ∆G = −RTln K2 = ∆H − ∆S.

Titrations curves obtained from the interaction between the oligonucleotide dT18 and four double-stranded DNA targets, 18–0FE, 20–2(CGC)/FE, 20–1(FE/3′, and 20–1(FE/5′, are shown in Fig. 1b. In all cases, titration reaches half-saturation near the equimolar ratio, indicating a one-to-one binding to form the triplex structures. The thermodynamic parameters derived from the curves are summarized in Table II. Also included in the table are the parameters exhibited by the interaction between dT18 and 20–2(TAT)/FE. As previously reported (22, 29, 30), triplex formation reveals negative ∆S and ∆H values, indicating that the process is characterized by an unfavorable entropy change and is, as such, driven by a large negative enthalpy change. Conspicuous differences are, however, observed in the magnitude of the thermodynamic parameters exhibited by the various processes. Specifically, the interaction of the TFO with either 20–2(CGC)/FE or 20–2(TAT)/FE, which contain flanking sequences at both ends of the A-tract, is associated with significantly smaller enthalpy changes than those exhibited by the interaction between dT18 and the double-stranded DNA target 18–0FE, in which no flanking ends are present. The equilibrium association constants K2 for the formation of the triplexes dT18–20–2(CGC)/FE and dT18*20–2(TAT)/FE are 54 and 34 times smaller, respectively, than the K2 of dT18*18–18–0FE formation. Whereas the thermodynamic parameters of triplex formation are found to be sensitive to the presence and base pair composition of the flanking sequences (which are GC- or AT-rich), they are unaffected by their sequential order. Specifically, we find that the formation of
The context of equilibrium effects in DNA molecules is critical for interpreting the thermodynamic parameters determined by isothermal titration calorimetry (ITC). The ITC technique is particularly useful for assessing the stability of triplex structures, which can be formed by the interaction between single-stranded oligonucleotides and double-stranded target DNA.

**Effects of Flanking Sequences on the Thermal Stability of the Triplex Motif**

**Circular Dichroism** — The ITC technique was used to obtain the thermodynamic parameters that characterize the formation of various triplex species. We have conducted CD melting studies on preformed triplex forms to assess their relative stability. The ellipticities revealed by double-stranded and triplexed DNA motifs are conspicuously different, providing a direct indication that under experimental conditions similar to those used in the ITC experiments, a mixture of dT18 with either 18–0FE or 20–2(CGC)FE at an equimolar ratio reveals the formation of the triplex forms as predominant products.

**UV Melting Studies** — UV melting curves of the preformed triplex forms dT18*18–0FE and dT18*20–2(CGC)FE (Table I) exhibit identical thermodynamic features.

**Reliance of Isothermal Titration Measurements**

The accuracy of the ITC measurements critically depends upon the precise identification of all species that are present in the calorimeter cell and of all processes that may occur as titration proceeds. The evaluation of the thermodynamic parameters exhibited by triplex formation can be hampered by the presence of single-stranded DNA species in the ITC cell. These species may result from either dissociation or disproportionation of the double-stranded targets (22, 31). The presence of single-stranded DNA molecules may lead to an erroneous interpretation of the ITC results, because the titration curves would represent, in such a case, the sum of both duplex and triplex formation. On the basis of previously reported observations (22), we submit that under the experimental conditions used in the current study, the probability of dissociation or disproportionation processes is low enough to render the concentration of single-stranded species insignificant. It has indeed been shown that for dA19dT19 virtually all dA19 is in the duplex form below 300 K at 1.015 M NaCl (22). Notably, the tendency of double-stranded molecules containing flanking sequences at the 3' or 5' end of the A-tract to undergo melting and disproportion reactions would be even smaller. The absence of single-stranded species is further implied by the observation that the titration of dT18 into the ITC cell containing the various duplex targets results in a monophasic titration curve. The presence of single-stranded molecules would have resulted in the initial formation of double-stranded species. Because the enthalpy change associated with the formation of duplex forms is significantly larger than that exhibited by triplex formation (22), a biphasic titration curve would have been observed.

**Fig. 1.** a, isothermal titration calorimetry profile for triplex formation between the single-stranded oligonucleotide dT18 and the double-stranded target 18–0FE at 20 °C. b, titration curves corrected for heat of dilution and shown as a function of the molar ratio between the injected oligonucleotide dT18 and the following targets: 18–0FE (□), 20–1FE/5′ (×), 20–1FE/3′ (▲), and 20–2(CGC)FE (○). Data were fitted by a nonlinear least squares method. The concentration of dT18 was 50 μM; the concentration of the double-stranded targets was 2.5 μM in buffer A.

**dT18*20–2(CGC)FE** and of dT18*20–2(GCG)FE (Table I) exhibit identical thermodynamic features.

**Formation of triple-stranded structures containing one flanking sequence at either the 3' or 5' end of the A-tract is characterized by ΔH and Kₜ values that are smaller than those revealed by the interaction between dT18 and 18–0FE but larger than those associated with the formation of dT18*20–2(CGC)FE.**

Notably, the thermodynamic parameters revealed by the interaction of dT18 with the targets dT18*20–2(CGC)FE or d(GCGT₂₀GCG) dA₁₈ (duplexes containing single-stranded, as opposed to double-stranded, flanking ends) are identical to those exhibited by the interaction of dT18 with the open-ended double-stranded target 18–0FE (data not shown).

**Reliance of Isothermal Titration Measurements**

The accuracy of the ITC measurements critically depends upon the precise identification of all species that are present in the calorimeter cell and of all processes that may occur as titration proceeds. The evaluation of the thermodynamic parameters exhibited by triplex formation can be hampered by the presence of single-stranded DNA species in the ITC cell. These species may result from either dissociation or disproportionation of the double-stranded targets (22, 31). The presence of single-stranded DNA molecules may lead to an erroneous interpretation of the ITC results, because the titration curves would represent, in such a case, the sum of both duplex and triplex formation. On the basis of previously reported observations (22), we submit that under the experimental conditions used in the current study, the probability of dissociation or disproportionation processes is low enough to render the concentration of single-stranded species insignificant. It has indeed been shown that for dA₁₉dT₁₉ virtually all dA₁₉ is in the duplex form below 300 K at 1.015 M NaCl (22). Notably, the tendency of double-stranded molecules containing flanking sequences at the 3' or 5' end of the A-tract to undergo melting and disproportion reactions would be even smaller. The absence of single-stranded species is further implied by the observation that the titration of dT₁₈ into the ITC cell containing the various duplex targets results in a monophasic titration curve. The presence of single-stranded molecules would have resulted in the initial formation of double-stranded species. Because the enthalpy change associated with the formation of duplex forms is significantly larger than that exhibited by triplex formation (22), a biphasic titration curve would have been observed.

**Effects of Flanking Sequences on the Thermal Stability of the Triplex Motif**

Circular Dichroism — The ITC technique was used to obtain the thermodynamic parameters that characterize the formation of various triplex species. We have conducted CD melting studies on preformed triplex forms to assess their relative stability. Because the ellipticities exhibited by double-stranded and triplexed DNA motifs are conspicuously different (22), the spectra depicted in Fig. 2, a and b provide a direct indication that under experimental conditions similar to those used in the ITC experiments, a mixture of dT₁₈ with either 18–0FE or 20–2(CGC)FE at an equimolar ratio reveals the formation of the triplex forms as predominant products. The CD spectra of the triplexed complexes dT₁₈*18–0FE and dT₁₈*20–2(CGC)FE were recorded at different temperatures, ranging between 10 and 50 °C (Fig. 2, c and d, respectively). As temperature increases, both triplex species undergo dissociation, gradually assuming ellipticity features characteristic of the duplex form. The temperature-dependent patterns revealed by the two complexes are, however, markedly different. Whereas the mixture containing dT₁₈ and 18–0FE still exhibits a substantial contribution of a triplexed motif at 40 °C, the ellipticities revealed by the mixture of dT₁₈ and 20–2(CGC)FE indicate that already at 30 °C the duplex DNA form predominates and that at 40 °C the triplex is completely dissociated.

UV Melting Studies — UV melting curves of the preformed triplex forms dT₁₈*18–0FE and dT₁₈*20–2(CGC)FE are shown in Fig. 3. The denaturation profile is biphasic, as clearly indicated by the derivative plot (Fig. 3, inset). The transition occurring at the lower temperature results from the dissociation of the triplexed complexes into a duplex and a single-stranded species, whereas the high temperature transition corresponds to the melting of the DNA duplexes. As expected, the 20–2(CGC)FE duplex, which is stabilized by two CG-rich flanking ends, melts into single strands at a higher temperature than that revealed by the corresponding transition of 18–0FE duplex. In sharp contrast, the dissociation of the dT₁₈*20–2(CGC)FE into double- and single-stranded species occurs at a significantly lower temperature (27 °C) than the corresponding transition of dT₁₈*18–0FE (38 °C). Thus, both the CD and UV melting profiles indicate that the thermal stability of dT₁₈*20–2(CGC)FE is substantially lower than that of dT₁₈*18–0FE.

We have carried out UV melting experiments on triplex structures obtained from the interaction between dT₁₈ and double-stranded moieties containing one flanking sequence located at either the 3' or 5' end of the adenine tract (Table III). In both cases, the triplex motif melts into double- and single-
stranded species at temperatures that are lower than the $T_m$ of $d\text{T}_{18}^{*18–0\text{FE}}$ and higher than the $T_m$ of $d\text{T}_{18}^{*20–2(\text{CGC})\text{FE}}$, in which two flanking ends are present. The position of the flanking sequence relative to the A-tract appears to significantly affect the $T_m$. $d\text{T}_{18}^{*20–1\text{FE/5}}$ undergoes melting transition at a lower temperature than $d\text{T}_{18}^{*20–1\text{FE/3}}$, thus indicating that a flanking sequence located at the 5' end of the A-tract destabilizes the triplex motif to a higher extent than does that positioned at the 3' end.

To better understand the mechanism by which flanking sequences affect the stability of the triplex motif, we have conducted UV melting studies on triple-stranded structures in which the TFO strand is further removed from the junction between the A-tract and the flanking ends. We find that although the $T_m$ of the triplex $d\text{T}_{18}^{*20–2(\text{CGC})\text{FE}}$ is higher than that of $d\text{T}_{18}^{*20–2(\text{CGC})\text{FE}}$, it is still substantially lower than the temperature at which dissociation of $d\text{T}_{18}^{*18–0\text{FE}}$ occurs (Table III). To further assess the range of the effects exerted by flanking sequences, melting profiles exhibited by triplex structures derived from the interaction of a shorter TFO, $d\text{T}_{14}$, with the various double-stranded targets were obtained. As expected, the dissociation of double-stranded structures contain-

### Table II

| Triplex                  | $K_a$ | $\Delta G$ | $\Delta H$ | $\Delta S$ |
|--------------------------|-------|------------|------------|------------|
| $d\text{T}_{18}^{*18–0\text{FE}}$ | $(1.19 \pm 0.23) \times 10^6$ | $-10.84 \pm 0.11$ | $-73.8 \pm 0.7$ | $-214.9 \pm 2.0$ |
| $d\text{T}_{18}^{*20–1\text{FE/3}}$ | $(2.91 \pm 0.23) \times 10^5$ | $-10.02 \pm 0.05$ | $-59.4 \pm 0.5$ | $-168.5 \pm 1.5$ |
| $d\text{T}_{18}^{*20–1\text{FE/5}}$ | $(1.18 \pm 0.23) \times 10^5$ | $-9.49 \pm 0.02$ | $-65.0 \pm 0.4$ | $-189.4 \pm 1.3$ |
| $d\text{T}_{18}^{*20–2(\text{CGC})\text{FE}}$ | $(2.19 \pm 0.17) \times 10^5$ | $-8.51 \pm 0.04$ | $-56.8 \pm 1.6$ | $-164.9 \pm 5.3$ |
| $d\text{T}_{18}^{*20–2(\text{TAT})\text{FE}}$ | $(3.48 \pm 0.55) \times 10^5$ | $-8.78 \pm 0.09$ | $-66.7 \pm 2.9$ | $-197.7 \pm 9.5$ |

FIG. 3. UV ($A_{260}$) melting curves of the triple-stranded species $d\text{T}_{18}^{*18–0\text{FE}}$ (○) and $d\text{T}_{18}^{*20–2(\text{CGC})\text{FE}}$ (○). Inset, corresponding derivative curves $dA_{260}/dT$. The DNA concentration was 1.6 $\mu$M in buffer A.
ing dT$_{14}$ occurs at a substantially lower temperature than that of the corresponding triplex forms with dT$_{18}$ as TFO (Table III). Yet, although the dT$_{14}$-containing triplex motifs are located further away from the junction sites between the A-tracts and the flanking sequences than are the dT$_{18}$ triplexes, the destabilizing effects exerted by these flanking sequences remain. Thus, a decrease of 11 °C is detected in the $T_m$ of both dT$_{18}$*20–2(CGCA)FE and dT$_{14}$*20–2(CGCA)FE relative to dT$_{18}$*18–0FE and dT$_{14}$*18–0FE, respectively. Notably, the destabilizing effect of a flanking sequence located at the 5’ end of the A-tract in the dT$_{14}$ triplexes is substantially larger than that exerted by a 3’ flanking end, as was found to be the case for the dT$_{18}$-containing triplexes. $T_m$ values exhibited by triplex species containing single-stranded sequences at either one or two ends of the A-tract (i.e. dT$_{18}$*d(CGCA$_{20}$)dT$_{18}$ or dT$_{18}$*d(CGCA$_{20}$CGC)dT$_{18}$) are identical to the $T_m$ of dT$_{18}$*18–0FE. Notably, the UV melting profiles obtained either by heating or cooling the samples were identical, thus indicating the reversibility of the process.

**DISCUSSION**

Adenine tracts reveal unique structural features that differentiate this motif from all other DNA sequences. Specifically, base pairs within A-tracts are characterized by an unusually large propeller twist, which acts to substantially enhance purine-purine stacking interactions, to stabilize a hydration network along a particularly narrow minor groove, and to enable cross-strand bifurcated hydrogen bonds in the major groove (19). Altogether, these particular properties confer upon A-tracts an unusual rigidity that is manifested by the monomorphic structure exhibited by poly(dA)·poly(dT) fibers (15) as well as by the very limited conformational variation that is revealed by A-tracts in different crystalline structures (17). An extensively studied property of adenine runs is their ability to generate a permanent bend in the helical DNA trajectory (32), a structural feature considered to represent an important factor in the regulation of cellular processes in both prokaryotic and eukaryotic systems. Most of the models suggested to account for A-tract-dependent bends are based on the highly limited deformability of this motif and its unique structure that significantly differs from that revealed by generic B-type DNA segments (19). According to these models, DNA bending results from structural perturbations that are effected by the A-tracts upon flanking sequences that are manifested, in particular, in the junction sites between A-tracts and flanking generic B-DNA segments.

The observations reported in this study indicate that A-tracts are capable not only of producing contextual effects on flanking sequences but also of being significantly influenced by such sequences. This influence is manifested by the ability of flanking segments to modulate the interaction between adenine runs and single-stranded DNA molecules. Specifically, the presence of CG- or AT-rich flanking segments located at either one end or both ends of the A-tract is shown by isothermal titration calorimetry to attenuate the association constants of triplex formation, relative to the $K_r$ revealed by the corresponding process involving an open-ended A-tract. Temperature-dependent CD and UV studies complement this observation by demonstrating that the thermal stability of the triplex motif is modulated by sequences that flank the A-tract target.

The thermodynamic parameters presented in Table II, along with the $T_m$ values summarized in Table III, indicate that the open-ended 18–0FE segment is characterized by structural features that are optimally suited for the interaction with a TFO. This optimal conformation is clearly perturbed by the presence of one conformational junction at either end of the A-tract and even more so by the presence of junctions at both ends. The notion that the conformational perturbation within the A-tract motif is causally related to junctions between this motif and generic B-DNA helices is substantiated by the observation that single-stranded segments, which are located at either one end or two ends of the A-tract but do not form a structural junction, do not affect triplex formation or stability. Results obtained with a long A-tract target (26–2(CGCA)FE) and a short TFO (dT$_{14}$) clearly indicate that the structural perturbation induced by the junctions is not localized but rather transmitted over the adenine runs.

Although the nature of the structural effects exerted by the junctions and flanking segments upon the A-tracts cannot be directly elucidated from the thermodynamic parameters reported here, some clues can, however, be derived. Interactions of the TFO with A-tract targets containing two flanking ends that are either CG- or AT-rich (20–2(CGCA)FE and 20–2(TAT)FE, respectively) exhibit very similar $K_r$ values. Yet, the $\Delta H$ and $\Delta S$ values of these two processes differ (Table II). Specifically, the formation of dT$_{18}$*20–2(CGCA)FE is associated with a less favorable enthalpy change and a less unfavorable entropy change than those that accompany the formation of dT$_{18}$*20–2(TAT)FE.

We propose that the apparent enthalpy-entropy compensation that leads to similar $K_r$ values for the two processes implies a different mode of contextual effects. The relatively rigid CG-rich flanking ends enforce a particular conformation upon the junctions, which is propagated along the A-tract. Presumably, this perturbation affects the structural features of the major groove of the adenine run in such a way that renders the configuration along this groove less favorable for the formation of hydrogen bonds between the TFO and the target duplex. The structural perturbation exerted upon the A-tract by the flexible AT-rich flanking ends is relatively smaller, allowing for a more optimal interaction of the A-tract with the TFO. This difference should be, and indeed is, reflected by a less favorable $\Delta H$ value for dT$_{18}$*20–2(CGCA)FE formation than that accompanying the formation of dT$_{18}$*20–2(TAT)FE.

The relatively weak interaction between the TFO and the duplex 20–2(CGCA)FE results in a smaller attenuation of the conformational freedom of the interacting species, which is, in turn, associated with a less unfavorable entropy change. Notably, such a mode of enthalpy-entropy compensation, where the experimental conditions are fixed while the structure of the interacting molecules varies (congener series), has been previously discussed and demonstrated (33, 34). The particularly large unfavorable entropy change revealed by the formation of dT$_{18}$*20–2(TAT)FE may be interpreted in terms of previous findings, according to which triplex formation results in an enhanced base pair stacking at the 5’ junction between the triplex and the duplex (35). If such a pronounced stacking is propagated, it would influence a flexible AT-rich flanking sequence to a higher extent than it would affect the robust GC-
rich end, leading to an attenuated conformational freedom within the AT-rich segments. We thus propose that in double-stranded targets flanked by CG-rich segments, the dominating contextual effects are those exerted upon the A-tract by the junctions between the A-tract and the flanking segments. In contrast, contextual effects in duplexes flanked by AT-rich segments are mainly exerted upon these flanking sequences by the junctions between the triplex and duplex motifs, with a minimal structural perturbation within the target A-tract. This interpretation is clearly consistent with the relatively high melting temperature of dT_{18}*-20–2(TAT)FE (Table III).

The open-ended A-tract target and the A-tract targets flanked by one or two CG-rich segments provide an example of a partial enthalpy-entropy compensation within a congener series. As a general trend, going from the 18–0FE through A-tracts that contain a single flanking end to the target with two junction sites is associated with a significant decrease of the negative \( \Delta H \) values that is only partially compensated by a decrease of unfavorable \( \Delta S \) contributions. The thermodynamic parameters associated with the binding of a TFO to adenine runs flanked by one junction at either the 5' or 3' end of the A-tract are intriguing. Although the \( \Delta H \) revealed by the interaction between dT_{18} and 20–1FE/5' is more favorable than that characterizing the binding of dT_{18} to 20–1FE/3', the association constant of the former process is slightly smaller. The difference in the \( K_a \) values derives from an unfavorable entropic contribution that is more pronounced in dT_{18}*-20–1FE/5'. We propose that the interpretation suggested for the difference between the parameters revealed by the formation of dT_{18}*-20–2(CG)FE and dT_{18}*-20–2(TAT)FE also applies to the interaction of the TFO with 20–1FE/5' and 20–1FE/3'. Specifically, the junction between the A-tract and the 5' flanking sequence is influenced by the 5' triplex-duplex junction to a larger extent than the 3' junction, because of the enhanced base pair stacking at the 5' end of the triplex-duplex motif. This influence and the resulting perturbation at the 5' flanking sequence results in a larger negative entropy contribution, leading to a slight destabilization of 20–1FE/5' relative to 20–1FE/3'.

In this study we show that even a particularly inflexible DNA motif such as A-tracts may be structurally perturbed by neighboring sequences in such a way that will affect the propensity of this motif to interact with other molecules. The transmitted equilibrium structural effects are finely tuned by the composition of the flanking sequences as well as by their location relative to the A-tract. Because the unique properties of A-tracts derive from the intrinsic rigidity and invariance of this motif, even subtle structural perturbations sustained by the A-tracts are likely to be consequential, as indeed is demonstrated here. Moreover, the particularly strong stacking interactions between the base pairs along the A-tract allow the structural perturbation to be propagated over this motif. The contextual effects reported here were studied through the interaction of adenine runs with a single-stranded DNA ligand, thus providing potentially important insights into a more refined choice of TFOs applied for the antigene strategy. Such contextual effects are, however, likely to apply to the interaction of A-tracts with other DNA-binding species such as drugs and proteins. As such, the current results highlight the structural complexity of DNA molecules and provide a new dimension to the notion that significant information might be encoded by DNA structural parameters.

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