Ligand-Induced Formation of Triple Helices with Antiparallel Third Strands Containing G and T†

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ABSTRACT: We have examined the effects of benzopyridoindole derivatives on triple helices with antiparallel third strands. Absorption spectroscopy, footprinting, and gel retardation experiments demonstrate that a benzopyridoindole derivative (BePI) is able to induce formation of a triple helix with an antiparallel (G,T)-containing third strand, which does not form in the absence of this ligand. This triple-helical complex is very stable with a half-dissociation temperature as high as 51 °C, and its formation is pH independent. Antiparallel oligonucleotides containing thymine and guanine bind strongly to double-helical DNA under physiological conditions in the presence of only 0.5 μM BePI. Formation of a BePI-stabilized triple helix strongly inhibits cleavage of the target duplex by DNase I.

Although triple helix formation had been discovered more than three decades ago (Felsenfeld et al., 1957), there has been a considerably renewed interest in triple-helical structures during recent years due to their potential applications as tools in molecular and cellular biology as well as in the development of therapeutic agents (Le Doan et al., 1987; Moser & Dervan, 1987; Thuong & Hélène, 1993). Triple helices are formed when an oligonucleotide binds to the major groove of double-helical DNA at an oligopurine-oligopyrimidine sequence. Two types of triple helices have been described, depending on the orientation of the third strand (Sun & Hélène, 1993). The first type involves oligopyrimidine third strands whose binding relies upon Hoogsteen hydrogen bond formation between a T·A base pair and thymine and between a C·G base pair and protonated cytosine (Figure 1) (Le Doan et al., 1987; Moser & Dervan, 1987; De Los Santos et al., 1989; Rajagopal & Feigon, 1989; Pilch et al., 1990). The (T,C)-containing oligonucleotide binds in a parallel manner to the oligopurine sequence in the so-called pyrimidine motif. Triple helices with purines in the third strand have also been described (Beal & Dervan, 1991; Pilch et al., 1991). A triple helix can be formed with third strands containing G and A by forming C·G×G and T·A×A base triplets. In this purine motif, the orientation of the third strand is antiparallel with respect to the oligopurine strand and the hydrogen-bonding scheme is called reverse Hoogsteen. (G,T)-containing oligonucleotides can also bind to double-helical DNA, in an orientation which is expected to depend on base sequence (Durland et al., 1991; Sun et al., 1991; Giovannangéli et al., 1992). Most reported examples involve antiparallel third strands (Figure 1). Their structure was extensively studied by NMR spectroscopy (Radhakrishnan & Patel, 1993).

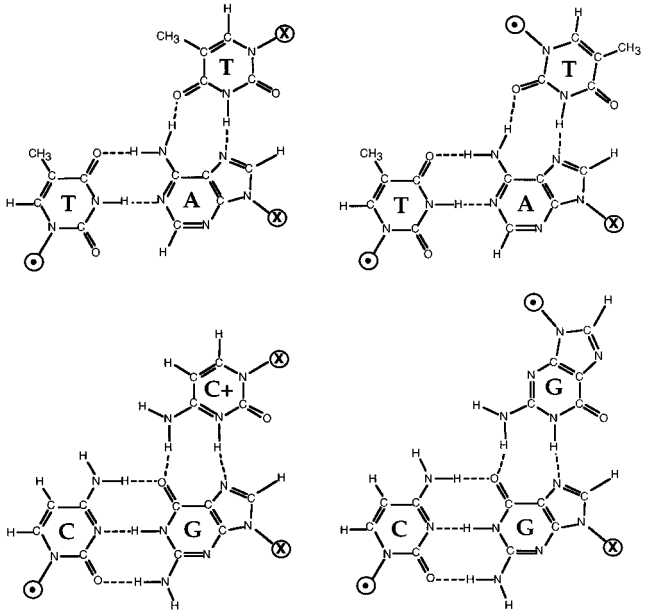

FIGURE 1: Base triplets for two triple helix motifs. (Left) T·A×T and C·G×C$^+$ base triplets with a third strand parallel to the oligopurine sequence and involving Hoogsteen hydrogen bonds. (Right) T·A×T and C·G×G triplets with a third strand antiparallel to the oligopurine target sequence and involving reverse Hoogsteen hydrogen bonds.

The use of triple helix-forming oligonucleotides (TFOs)[1] to control gene expression is usually limited by the low stability of triple-helical complexes, compared to that of double helices, especially under physiological conditions. It has been possible to enhance the stability of triple helices formed with (T,C) oligonucleotides, e.g., by covalent attachment of an intercalating agent at the 5′-end (Sun et al., 1989). An oligonucleotide containing 5-methyl cytosines

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[1] Abbreviations: bp, base pair; TFO, triplex-forming oligonucleotide; MES, 2-(*N*-morpholino)ethanesulfonic acid; BePI, 11-[(aminopropyl)-amino]-3-methoxy-8-methyl-7*H*-benzo[*e*]pyrido[4,3-*b*]indole.

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instead of cytosines and covalently linked to the intercalating dye acridine was shown to inhibit the expression of a reporter gene in cell cultures (Grigoriev et al., 1993). Another way of stabilizing triple helices is to use triple helix-specific ligands, whose presence potentiates the inhibition of in vitro transcription (Mergny et al., 1992). Most biological effects obtained with unmodified oligonucleotides concerned triple helices in the reverse Hoogsteen configuration (Orson et al., 1991; Postel et al., 1991; McShan et al., 1992; Ing et al., 1993). One advantage of (G,T)-containing oligonucleotides is that they do not require protonation of cytosines, and thus, they can form triple helices whose stability is independent of pH. Their formation however usually requires high concentrations of magnesium or other, nonphysiologically relevant, dications (Malkov et al., 1993). In addition, they are destabilized by potassium (Cheng & Dyke, 1993). The length of G stretches and the proportion of guanines play an important role in triple helix stability (Cheng & Vandyke, 1994). Some purine oligonucleotides can bind strongly to their target sequence (Svinarchuk et al., 1994), but the stability is strongly sequence dependent (Clarenc et al., 1994). In all favorable cases, oligonucleotides contained more than 60% guanine. This high percentage of guanine can lead to the formation of structures (e.g., guanine quartets) that inhibit oligonucleotide binding to a double helix (Noonberg et al., 1995; Olivas & Maher, 1995).

Benzopyridoindole derivatives were the first triple helix specific ligands described in the literature (Mergny et al., 1992). Various derivatives were synthesized, and their effects were tested on triple helices with (T,C)-containing third strands (Escudé et al., 1995). This type of triple helix was also shown to be stabilized by coralyne (Lee et al., 1993), by unfused aromatic cations (Wilson et al., 1993), or by 2,6-disubstituted amidoanthraquinones (Fox et al., 1995). There are few reports describing ligand-induced stabilization of triple helices in the reverse Hoogsteen configuration, with the exception of reports describing the effects of monocations (Cheng & Dyke, 1993) and polycations (Malkov et al., 1993). Antiparallel triple helices were shown to be poorly stabilized by some unfused aromatic cations (Cassidy et al., 1994), but the sequences that were used ($^{5'}A_5G_5{}^{3'}$ or $^{5'}G_5A_5{}^{3'}$ in the oligopurine sequence) are not representative of typical targets for antiparallel oligonucleotides. Pilch and Breslauer (1994) showed that minor groove-binding ligands such as berenil and DAPI were able to induce formation of triple-helical polynucleotides containing a poly(dA) third strand that do not form in their absence. Here we show by thermal denaturation, gel retardation assay, and footprinting experiments that 11-[(aminopropyl)amino]-3-methoxy-8-methyl-7*H*-benzo[*e*]pyrido[4,3-*b*]indole (BePI) can induce and/or considerably stabilize triple helices with antiparallel (G,T)-containing third strands.

MATERIALS AND METHODS

Chemicals and Oligonucleotides. Oligonucleotides were purchased from Eurogentec (Seraing, Belgium). They were precipitated with ethanol, and their concentration was calculated using a nearest-neighbor model (Cantor & Warshaw, 1970). BePI was synthesized according to previouslypublished procedures (Nguyen et al., 1990). It has been referred to as compound **9e** in a previous study regarding triple helices in the pyrimidine motif (Escudé et al., 1995).

Thermal Denaturation Experiments. Triple helix stability was measured using UV spectroscopy. All thermal denaturation studies were carried out on a Uvikon 940 spectrophotometer, interfaced to an IBM-AT personal computer for data collection and analysis. Temperature control of the cell holder was achieved by a Haake P2 circulating water bath. The temperature of the water bath was decreased from 80 to 0 °C and then increased until 80 °C at a rate of 0.1 °C/min using a Haake PG 20 thermoprogrammer, and the absorbance at 260 nm was recorded every 10 min. The formation of the triple helix is associated with a hypochromism at this wavelength. Absorbance of the duplex was subtracted from that of the triplex. When a triple helix was formed, the resulting difference curve showed a single transition which permitted the determination of the half-dissociation temperature (T_m) of the third strand from the duplex.

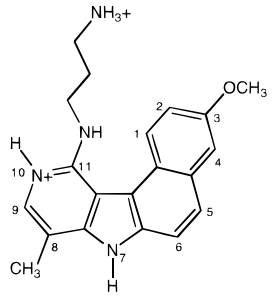
Gel Retardation Experiments. Gel electrophoresis was run on a 10% polyacrylamide/bisacrylamide (19:1) nondenaturing gel in a 50 mM MES buffer (pH 6) containing 10 mM $MgCl_2$. Each sample (2 μM each strand) was incubated for 1 night at 4 °C in a 50 mM MES buffer (pH 6) containing 10 mM $MgCl_2$, 50 mM NaCl, 10% sucrose, and 5 μg of tRNA and then loaded onto the gel and allowed to migrate for 3 h at 5 W.

Footprinting Experiments. Oligonucleotides were labeled at their 5′-end with T4 polynucleotide kinase. Samples were prepared by mixing 20 nM labeled strand (purine- or pyrimidine-rich), 100 nM Watson−Crick complementary strand, the appropriate concentration of the third strand, and finally the triplex specific ligand in a 20 mM cacodylate buffer containing 0.1 M NaCl, 5 mM $MgCl_2$, and 0.5 mM $CaCl_2$. The mixture was allowed to equilibrate for 1 night at room temperature before digestion with DNase I. The reaction was stopped after 1 min by cooling at −80 °C. Then samples were dried, resuspended in formamide, and loaded on a 20% denaturing gel.

RESULTS

Observation of Triplex Melting by UV Absorption Spectroscopy. Thermal denaturation experiments were used to monitor triplex formation between a 36 bp target and various 14-mer oligonucleotides, in the absence and presence of BePI. Absorbance at 260 nm was measured versus temperature. The triplex was formed by mixing both strands of the duplex (1 μM) with 1.5 μM third strand in a pH 6.5 cacodylate buffer (10 mM) containing 0.1 M NaCl and 5 mM $MgCl_2$. All oligonucleotides containing T and C, T and G, or A and G were investigated, with their sequence oriented parallel (p) or antiparallel (a) to the oligopurine target sequence (Figure 2). In the absence of ligand, two transitions could be detected in the presence of the TC_p oligonucleotide at pH 6.5. The one at the highest temperature can be observed with the duplex alone and is due to duplex melting. The one at the lower temperature was attributed to the dissociation of the third strand from the duplex. This second transition could not be observed with CT_a, TG_p, GT_a, AG_p, or GA_a oligonucleotides. Upon addition of 10 μM BePI (Figure 2), the melting temperature of the triple helix formed with the TC_p oligonucleotide was slightly shifted toward higher temperatures (from 24 to 26 °C), as previously reported (Mergny et al., 1992). Addition of 10 μM BePI in

A



B

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NS   5'TTCCCCCTTTTTTT  3'
TCp  5'TTCTTCTCCTTTCT  3'
CTa  3'TTCTTCTCCTTTCT  5'
TGp  5'TTGTTGTGGTTTGT  3'
GTa  3'TTGTTGTGGTTTGT  5'
AGp  5'AAGAAGAGGAAAGA  3'
GAa  3'AAGAAGAGGAAAGA  5'

5'  GATACGAGTTAAGAAGAGGAAAGATTGAGCTGATAG  3'
3'  CTATGCTCAATTCTTCTCCTTTCTAACTCGACTATC  5'
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FIGURE 2: Chemical structure of the BePI derivative (A) and sequences of the oligonucleotides (B) used in the present study.

the presence of the GT_a oligonucleotide led to the observation of a transition that was not observed in the absence of BePI. Derivatives of the melting curves showed that this transition occurred at a much higher temperature than that observed with the TC_p oligonucleotide (Figure 2B). Subtracting the absorbance of the duplex from that of the triplex showed a single transition permitting us to measure a T_m of 51 °C (Figure 3C). When the experiments were performed in a buffer containing 0.1 M NaCl, but no $MgCl_2$, a triple helix also formed with GT_a as the third strand. Its melting temperature was 45 °C. The pH dependency of triplex melting was investigated for both TC_p and GT_a oligonucleotides. At pH 7, in the presence of 5 mM $MgCl_2$ and 10 μM BePI, the GT_a oligonucleotide still dissociated at 51 °C, whereas the melting temperature obtained with the TC_p oligonucleotide decreased from 26 to 18 °C. Addition of BePI to the CT_a and TG_p oligonucleotides also induced a transition of low amplitude much below the melting of the duplex. The melting temperature of these structures occurred at 8 °C for CT_a and at 25 °C for TG_p, at pH 6.5 in the presence of 0.1 M NaCl and 5 mM $MgCl_2$. For both (G,A)-containing oligonucleotides, no difference between the melting profile of the duplex alone and in the presence of the third strand could be detected, either in the absence or in the presence of BePI.

Gel Retardation Experiments. Another technique to detect triple helix formation involves gel retardation. The labeled duplex (2 μM each strand) was incubated in the presence of 2 μM of third strand and various amounts of BePI and then loaded onto a nondenaturing gel. In the absence of ligand, only the TC_p oligonucleotide produced a band shift. No triple helix could be detected either with CT_a, TG_p, GT_a, AG_p, or GA_a, even if the duplex was incubated in the presence of up to 40 μM third strand. However, when increasing amounts of BePI were added before electrophoresis to the mixture of duplex with GT_a oligonucleotide, a complex that migrated more slowly than the duplex appeared. The migration was similar to that of the triple helix formed with the TC_p third strand (Figure 4). No effect of BePI was

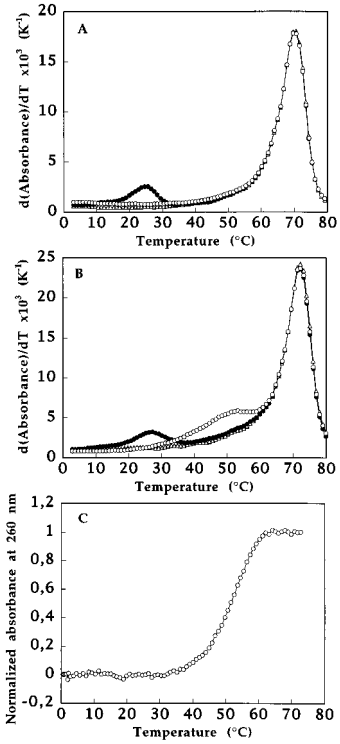

FIGURE 3: Thermal denaturation profile of the 36 bp duplex (△) and the mixture of the 36 bp duplex and the TC_p (●) and GT_a (○) oligonucleotides, in the absence (A) or in the presence (B) of 10 μM BePI. (C) Normalized difference in absorbance between the mixture of the 36 bp duplex with oligonucleotide GT_a and the duplex alone, in the presence of 10 μM BePI. This curve allowed us to determine a half-dissociation temperature of 51 °C for the triple helix formed with the GT_a oligonucleotide in the presence of 10 μM BePI.

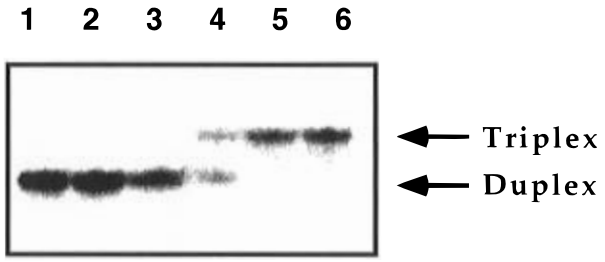

FIGURE 4: Gel retardation experiments at pH 6 and 4 °C in a buffer containing 20 mM MES and 10 mM $MgCl_2$. The labeled duplex (2 μM of each strand) was incubated in the presence of 2 μM oligonucleotide NS (lane 1), GT_a (lanes 2−5), or TC_p (lane 6), in the absence (lanes 1, 2, and 6) or in the presence of BePI (lane 3, 0.5 μM; lane 4, 2 μM; lane 5, 5 μM). See Figure 2 for oligonucleotide sequences.

observed with CT_a, TG_p, AG_p, and GA_a oligonucleotides under these conditions.

Footprinting Experiments. DNase I footprinting was carried out on the 36 bp duplex containing the putative target for the GT_a oligonucleotide. BePI was previously shown to bind to duplex DNA. Thus, the effects of BePI on the digestion pattern of the duplex alone were first investigated. It was shown that, at BePI concentrations above 1 μM, DNA cleavage by DNase I was inhibited in a sequence independent manner, suggesting an effect of BePI on DNase I itself. At concentrations below 1 μM, the cleavage pattern was not affected by BePI. Thus, all experiments with triple helices were carried out in the presence of 0.5 μM BePI. In the absence of BePI ligand, the target sequence was protected

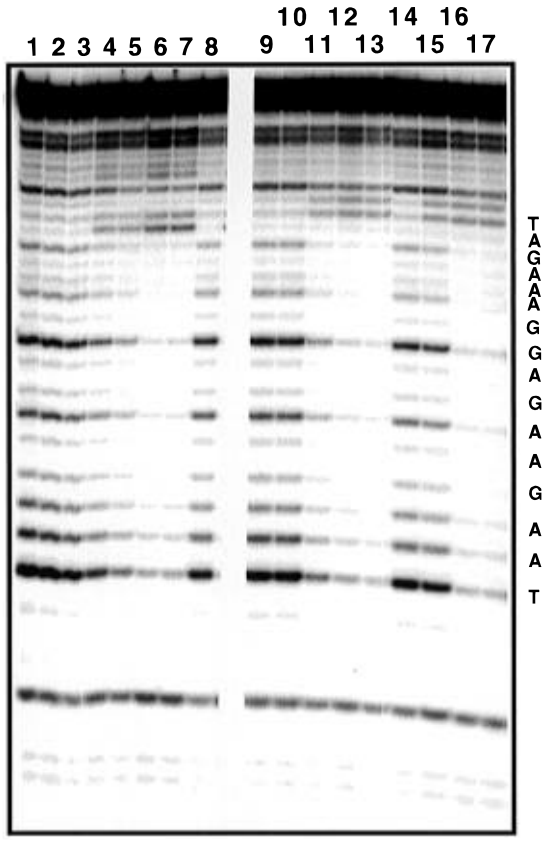

FIGURE 5: DNase I footprinting experiments carried out at pH 6.5 in a buffer containing 20 mM cacodylate, 5 mM $MgCl_2$, and 0.5 mM $CaCl_2$. Duplex labeled on the purine strand was incubated in the presence of various concentrations of third strand, in the absence (lanes 8−13) or in the presence (lanes 1−7 and 14−17) of 0.5 μM BePI. The third strands were NS (2 μM, lanes 1 and 9), GT_a (lane 2, 0.02 μM; lane 3, 0.05 μM; lane 4, 0.2 μM; lane 5, 0.5 μM; lane 6, 2 μM; lane 7, 5 μM; lane 8, 20 μM without BePI), or TC_p (lanes 10 and 14, 0.2 μM; lanes 11 and 15, 0.5 μM; lanes 12 and 16, 2 μM; lanes 13 and 17, 5 μM).

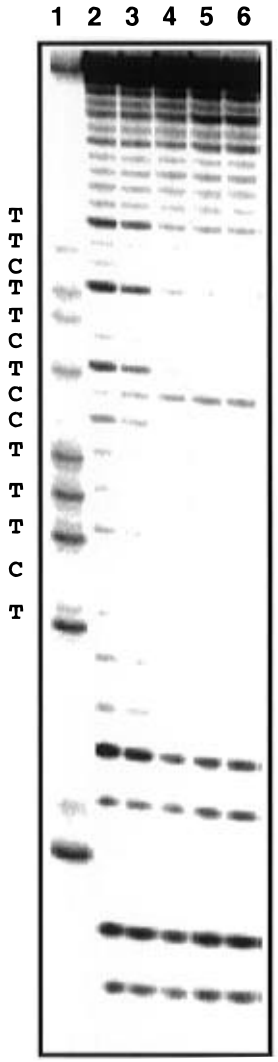

FIGURE 6: DNase I footprinting experiments carried out at pH 6.5 in a buffer containing 20 mM cacodylate, 5 mM $MgCl_2$, and 0.5 mM $CaCl_2$. Duplex labeled on the pyrimidine strand was incubated in the presence of various concentrations of third strand and 0.5 μM BePI: lane 1, sequence of T; lane 2, 2 μM NS; lane 3, 0.5 μM GT_a; lane 4, 2 μM GT_a; lane 5, 5 μM GT_a; lane 6, 20 μM GT_a.

from DNase I cleavage by the TC_p oligonucleotide (2 μM) at 25 °C. However, it was not protected by any of the other oligonucleotides (GT_a, TG_p, CT_a, AG_p, and GA_a) at concentrations up to 60 μM, neither at 4 °C nor at 25 °C. In contrast, in the presence of 0.5 μM BePI, a clear protection was obtained with the GT_a oligonucleotide (Figure 5), but not with TG_p, CT_a, GA_a, or AG_p. Both TC_p and GT_a oligonucleotides produced an increased cleavage on the 3′-side of the oligopurine sequence, whereas cleavage within the oligopurine sequence was inhibited. However, the site of enhanced cleavage observed with the antiparallel GT_a oligonucleotide was different from that observed with the parallel TC_p oligonucleotide. The increased band obtained with the antiparallel oligonucleotide was shifted by one nucleotide closer to the 5′-side of the oligopurine sequence when compared with the increased band obtained with the parallel oligonucleotide.

A footprint could also be seen on the pyrimidine-rich strand of the target duplex. This footprint required higher concentrations of third strand than those required for the purine strand. Change in the footprinting pattern appeared at 0.5 μM, and maximum protection was obtained at 5 μM; whereas these two values were 0.2 and 2 μM for the purine strand. No enhanced cleavage was detected at the 3′- or 5′-end of the oligopyrimidine sequence. However, an enhanced cleavage appeared within the oligopyrimidine sequence (Figure 6). Neither the TG_p nor the CT_a oligonucleotides were able to produce any footprint, nor did the parallel and antiparallel (G,A)-containing oligonucleotides, even in the presence of BePI.

DISCUSSION

The results presented in this paper clearly demonstrate that BePI induces the formation of triple helices that otherwise would not form with a (G,T)-containing antiparallel third strand. Evidence comes from DNase footprinting experiments which show that no footprint can be observed in the absence of BePI, whereas adding only 0.5 μM BePI is sufficient to observe a footprint at concentrations as low as 0.5 μM for the third strand. The target sequence contains five C·G out of 14 base pairs and no more than three contiguous T·A base pairs. Thermal denaturation experiments showed that binding of a TC_p oligonucleotide to this sequence was only poorly enhanced by 10 μM BePI. This is probably due to electrostatic repulsions between BePI andprotonated cytosines, as previously observed with such a $C{\cdot}G{\times}C^+$-rich sequence (Mergny et al., 1992). Binding

of the TC_p oligonucleotide decreases when pH increases and becomes marginal at physiological pH. A triple helix with a (G,T)-containing third strand can form independently of pH but requires particular conditions in term of cations, and sequence effects in both affinity and orientation of the third strand are not completely understood. In all reported examples, antiparallel triple helices form in the presence of divalent cations. Here we have shown by thermal denaturation experiments that a triple helix could be formed in the presence of BePI and NaCl but in the absence of any divalent cation. Despite the large number of reports on the binding of (G,T)-containing oligonucleotides to double helices, absorption spectroscopy was almost never used to characterize the stability of antiparallel triple helices. Many attempts made in our laboratory failed to detect triple helix formation by this method. This could be due to different factors. First, triple helices could form without any effect on absorption spectra as observed with oligonucleotides containing 8-oxoadenines (Jetter & Hobbs, 1993). Second, purine-rich oligonucleotides often self-associate, and this association could compete with triplex formation, depending on temperature and strand concentration (Noonberg et al., 1995; Olivas & Maher, 1995). In the range of concentrations used for spectroscopic experiments, all the TFOs could be involved in self-associated structures. It has already been shown that the amplitude of a triplex-to-duplex transition was increased when BePI binds to parallel triple helices (Mergny et al., 1992). The hyperchromism that we observed with the GT_a oligonucleotide in the presence of BePI could be partly due to the change in absorbance of BePI when the triplex dissociates. We could not observe any transition using absorbance spectroscopy in the absence of BePI, but DNase I footprinting showed that no triple helix was formed under these conditions. Thus, we do not have any information on the spectroscopic properties of the triple helix formed with the GT_a third strand in the absence of BePI.

Gel retardation experiments performed at 4 °C confirmed the formation of a complex that migrated to the position expected for a triple helix. Since no BePI was present either in the electrophoresis buffer or in the gel, this result indicates that the triplex does not dissociate during migration. It seems likely that BePI remains associated to the triplex, since the complex has been shown to be very stable by spectroscopic experiments.

Cleavage products from DNase I digestion were analyzed on a denaturing gel and compared to the Maxam−Gilbert sequence. There is a difference in migration of the chemically and enzymatically cleaved products (the former leaves a 3′-phosphate while the latter leaves a 3′-OH terminus). Thus, a band corresponding to a G in the chemical sequence for example migrates slightly faster than the product of an enzymatic cleavage that occurs on the 5′-side of this G. The increased cleavage sites that occurred on the 3′-side of the oligopurine sequence and within the oligopyrimidine sequence are presented in Figure 7 for both the TC_p and GT_a oligonucleotides. Footprinting of parallel and antiparallel triple helices was clearly different on the 3′-side of the oligopurine sequence. The increased band obtained with the antiparallel oligonucleotide was shifted by one nucleotide closer to the 5′-side of the oligopurine sequence when compared with the increased band obtained with the parallel oligonucleotide. The last base pair of the target sequence is cleaved in the presence of GT_a, but not in the presence of

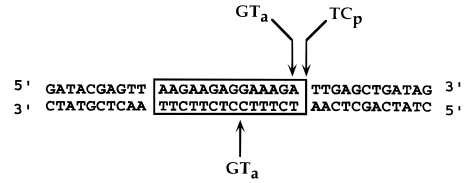

FIGURE 7: Summary of the hypersensitive sites of cleavage (arrows) induced by triple helix formation on both strands of the DNA duplex.

TC_p (figure 7). Thus, the T·A×T base triplet at the 3′-side of the oligopurine sequence might not be formed. This result is consistent with experiments of Cheng and Van Dyke showing that modifications (flanking sequence or mismatch) on the 3′-side of the oligopurine sequence have little influence on triplex stability (Cheng & Vandyke, 1994). On the 5′-side, the poor activity of DNase I did not allow us to observe differences between the two motifs. Cleavage protection seems to be extended by two bases away from the 5′-side of the target sequence. For the strand containing the oligopyrimidine sequence, no enhanced cleavage appeared at either end of the sequence, but a hypersensitive cleavage site appeared within the oligopyrimidine sequence after triple helix formation. It corresponds to a cleavage between the two contiguous cytosines as shown by comparison with the Maxam−Gilbert sequence (Figure 6). This enhanced cleavage could be due to a particular structure of the triple helix itself but also to the deformation induced by intercalation of a BePI molecule. The pyrimidine-rich strand is cleaved on the 3′-side just at the end of the triplex sequence but is protected three bases after the 5′-end.

From the results of spectroscopic experiments, it seems that BePI can induce binding of the TG_p oligonucleotide to its target double helix. This could not be confirmed by gel retardation or footprinting experiments. The stability of the induced triplex could be too low to be stable during migration in the band shift assay and may require higher concentrations of BePI than those used for footprinting experiments. Further experiments will be necessary to investigate the effects of BePI on triple helices with (G,T) parallel third strands.

The results presented in this report show that BePI has a dramatic effect on the formation of antiparallel triplexes containing T·A×T and C·G×G triplets. BePI does not change the footprinting pattern of parallel triple helices. Differences between the footprints generated by the TC_p and the GT_a oligonucleotides may result from changes in the structure of the triple helix or from interactions with BePI. It is likely that BePI also binds at the duplex−triplex junction for antiparallel triple helices, as previously described for other intercalating agents (Perrouault et al., 1990). It was previously shown that covalent attachment of an oxazolopyridocarbazole moiety to the 5′-end of purine oligonucleotides considerably enhances the stability of the triple helix (Mouscadet et al., 1994). With the sequence investigated, no triple helix could be observed with (G,A) oligonucleotides expected to form T·A×A and C·G×G triplets. In triple helices involving antiparallel (G,T) oligonucleotides, BePI could intercalate between a T·A×T and a C·G×G triplet or between two T·A×T triplets. T·A×T and C·G×G triplets are not isomorphous (Sun & Hélène, 1993). NMR studies have shown that TpG and GpT steps in triple helices have a distorted conformation (Radhakrishnan & Patel, 1993). Intercalation of a BePI molecule could make these steps

energetically much more favorable by providing an additionnal binding energy and relieving part of the stress due to the distortion at TpG or GpT steps.

CONCLUSION

This study has shown that BePI can induce formation of triple helices that would otherwise not form with (G,T) antiparallel third strands. Triple helices can thus be formed with short oligonucleotides containing fewer guanines than those previously studied. Ligand-induced formation of triplexes has important potential applications. Triplex formation at specific sequences could interfere with gene expression. The use of shorter oligonucleotides with only few guanines should avoid quadruplex formation by the oligonucleotide and provide more potential targets for triplex formation. Furthermore, alternate strand triple helix formation has been described which combines parallel and antiparallel structures for the binding of a single (T,G)-containing oligonucleotide to alternating oligopurine/oligopyrimidine sequences (Sun et al., 1991). BePI also enhances binding of these oligonucleotides (De Bizemont et al., 1996). The intramolecular triplex structure called H-DNA was studied in sequences containing G and A or T and C in the third strand. Potential H-DNA structures could be found for an (A,G)-rich sequence adjacent to a mirror sequence in which A's are replaced by T's. The length of the asymmetric mirror repeat sequence necessary to adopt this structure could be shorter in the presence of BePI.

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