Selective Stabilization of DNA Triple Helices by Benzopyridoindole Derivatives

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Abstract: A major challenge in the use of oligonucleotides in an anti-gene strategy is to stabilize triple helix formation under physiological conditions. A benzo[*e*]pyridoindole derivative was shown earlier to stabilize triple-helical better than double-helical complexes (Mergny, J. L. et al. *Science* **1992**, *256*, 1681−1684). New derivatives of the benzopyridoindole family were synthesized, and their ability to stabilize triple helices was investigated by thermal denaturation experiments using UV absorption spectroscopy. The stabilizing effects of all the available derivatives were compared and allowed us to infer some general rules regarding the role of the geometry of the molecule and of its various substituents. The melting temperature (T_m) of the triplex-to-duplex transition is increased from 18 to 49 °C (ΔT_{max} = +31 °C) upon binding of 3-methoxy-10-methyl-7-[3-(*N*-methyl-*N*-3-aminopropyl)propyl]amino-11*H*-benzo[*g*]pyrido[4,3-*b*]indole (BgPI), in a 10 mM sodium cacodylate buffer (pH 6.2) containing 0.1 M NaCl. Sequence-specific effects were also investigated. Benzo[*e*]- and benzo[*g*]pyrido[4,3-*b*]indole derivatives exhibited different properties regarding the role of the alkylamine side chain attached to the pyridine ring. Effects of these compounds on the melting of duplex DNA were also sensitive to changes in the chemical nature of the alkylamine side chain. Results are discussed in terms of respective affinities for triplex and duplex structures. A model is proposed to explain the different roles played by the alkylamine side chain for both types of molecules. For the benzo[*e*]pyridoindole derivatives, the chain is suggested to lie in the major groove of the triple helix, whereas for the benzo[*g*]pyridoindole derivatives, it lies in the minor groove. These results provide an experimental and theoretical basis for understanding intercalation of dyes in triple helices and should help to conceive more specific triple helix ligands and to design oligonucleotide-intercalator conjugates for stable triple helix formation.

Introduction

A nucleic acid triple helix was first observed with homopolyribonucleotides in 1957.[1] More recent evidence has shown that a triple helix can be formed when a short oligonucleotide binds to a DNA double helix[2] and that polypurine•polypyrimidine sequences exhibiting mirror symmetry could form intramolecular triple helices called H-DNA.[3] Triplex structures are currently the focus of important consideration because of their potential applications in the development of oligonucleotide-based therapeutic strategies as well as molecular biology tools.[4]

Triple helices are formed when an oligonucleotide binds to the major groove of double-helical DNA at an oligopurine-oligopyrimidine sequence. The first triple helical complexes which were described involved oligopyrimidine third strands whose binding rests upon Hoogsteen hydrogen bonding between a T•A Watson−Crick base pair and thymine and between a C•G base pair and protonated cytosine, leading to the formation of T•AxT and C•GxC$^+$ base-triplets (Figure 1).[2,5] They will be called the pyrimidine motif. Other types of triple helices can be formed with third strands containing G ant T or G and A, which are called the purine motif.[6] The orientation of the third strand with respect to the polypurine target depends on its sequence (for a review see ref 4).

The use of triple helix-forming oligonucleotides (TFOs) is often limited by the low stability of triple-helical complexes, compared to double helices, especially under physiological conditions. Oligopyrimidines can recognize double helical DNA specifically.[7] However, the formation of C•GxC+ triplets requires an acidic pH for optimal formation. The stability of triple helices in the pyrimidine motif can be enhanced by polycations such as spermine[8] or magnesium.[9] Chemical modification of TFOs were also shown to increase triplex stabilities. These modifications can involve non-natural bases[10] that can make pH-independent interactions with a C•G base-pair, or a non-natural sugar backbone.[11] Covalent linkage

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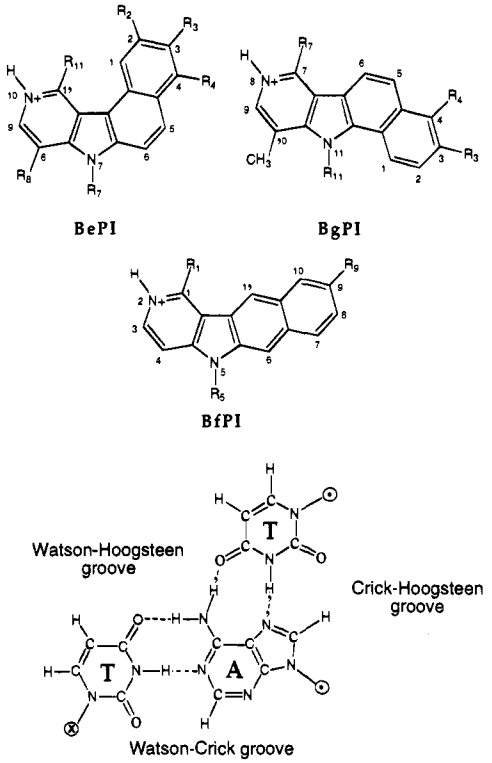

Figure 1. Structure of BPI derivatives, indicating the atomic numbering systems for each type of molecule. A base triplet is also shown for comparison, with the names given to the different grooves (see ref 25).

between an oligonucleotide and an intercalator[12] or a polyamine[13] also provides an additional binding energy.

A wide variety of DNA intercalators and minor groove binders have been shown to stabilize double-helical structures. Fewer compounds have been found to interact with triple-helical structures. Ethidium bromide was shown to intercalate in a triplex.[14] This intercalation can stabilize the triple-helical polynucleotide polydT·polydAxpolydT,[14b] but ethidium bromide binds to triple helices containing both T·AxT and C·GxC+ triplets with a lower affinity than to a duplex.[14a] Some minor groove binding ligands interact with triple helices but destabilize them.[15] Benzopyridoindoles (BPIs) were the first molecules reported to bind to a triplex more tightly than to a duplex, thus providing a strong stabilization of triple helices involving the pyrimidine motif.[16] Then, it was reported that coralyne could also stabilize C·GxC+ containing triple helices,[17] and that unfused aromatic cations were able to stabilize T·AxT triplexes.[18] Physical studies using spectroscopic and hydrodynamic experiments have shown that BPIs bind both double and triple helices by intercalation.[19] Linear dichroism studies have provided evidence for an intercalated structure.[20] However, NMR studies have not yet elucidated the precise structure of the intercalation complex. Several benzopyridoindole derivatives were synthesized in order to improve our understanding of the effects of various substituents on interactions with nucleic acid helices. Their structure is shown on Figure 1 and Tables 1−3. Their ability to stabilize triple helices was studied by absorption spectroscopy. Some of them appear to be very efficient to discriminate between triple and double helices. They could be interesting candidates for enhancing intermolecular triple helix formation, but they could also interact with H-DNA structures and thus have effects on gene expression.

Results

Influence on Triple Helix Stability. One of the simplest methods to analyze triplex stability consists in using thermal denaturation experiments. Thus, a triple helix-specific ligand should increase the melting temperature of the triplex-to-duplex transition, displacing the equilibrium

$$D + M \leftrightarrow T$$

to the right (D is duplex, M the third strand oligonucleotide, and T is triplex). Binding of a 14-mer oligonucleotide to a 26-bp target was followed by absorption spectroscopy. The lengths for the target duplex and the oligopurine-oligopyrimidine sequence were chosen in such a way as to allow us to detect two transitions: the one at low temperature corresponds to the dissociation of the third strand from the duplex (3 → 2 transition), whereas the one at higher temperatures is due to melting of the duplex (2 → 1 transition). Triple helix formation was studied for the two sequences described in Figure 2. Both triple helices contain 14 base triplets. The first one (called C3 triplex) contains three C·GxC+ and 11 T·AxT triplets (among which six are contiguous). The second triple helix (C5 triplex) differs by the replacement of two T·AxT by two C·GxC+ base triplets. Triplexes were formed by first mixing both strands of the duplex at 1.5 μM concentration of each strand and then adding 1.8 μM of the third strand in a pH 6.2 cacodylate buffer (10 mM) containing 0.1 M NaCl. In the absence of any ligand, the thermal denaturation profile for the triplex was not reversible, due to slow kinetics of triplex formation.[21] The apparent half-dissociation temperature obtained upon increasing temperature was 18 °C for the C3 triplex and 26 °C for the C5 triplex. Most of the ligands investigated in this study not only stabilized the triple helix but also eliminated the hysteresis phenomenon. Moreover, the hypochromism observed during triple helix formation was more important in the presence of the ligand, probably because of the hyperchromism of the ligand itself induced by intercalation and stacking between base pairs. A

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Table 1. Structure and Results of T_m Measurements for Benzo[*e*]pyridoindoles[a]

art	R11	R8	R7	R4	R3	R2	ΔT_{max}^{3-2} (°C)	ref
1e	-H	CH_3			OCH_3		+21	N
2e	$-NH_2$	CH_3			OH		+20	N
3e	$-NH_2$	CH_3			OCH_3		+28	N
4e	$-NH(CH_2)Ph$	CH_3			OH		+13	N
5e	$-NH(CH_2)Ph$	CH_3			OCH_3		+21	N
6e	$-NH(CH_2)_2N(CH_3)_2$	CH_3			OCH_3		+8	ref 29c
7e	$-NH(CH_2)_2CH(CH_3)_2$	CH_3			OCH_3		+22	N
8e	$-NH(CH_2)_3NH_2$	CH_3			OH		+13	ref 29c
9e	$-NH(CH_2)_3NH_2$	CH_3			OCH_3		+20	ref 29c
10e	$-NH(CH_2)_3NH(CH_3)$	CH_3			OH		+10	N
11e	$-NH(CH_2)_3NH(CH_3)$	CH_3			OCH_3		+18	N
12e	$-NH(CH_2)_3N(CH_3)_2$				OH		+6	ref 29c
13e	$-NH(CH_2)_3N(CH_3)_2$				OCH_3		+13	ref 29c
14e	$-NH(CH_2)_3N(CH_3)_2$	CH_3					+3	ref 29b
15e	$-NH(CH_2)_3N(CH_3)_2$	CH_3			OH		+10	ref 29b
16e	$-NH(CH_2)_3N(CH_3)_2$	CH_3			OCH_3		+17	ref 29b
17e	$-NH(CH_2)_3N(CH_3)_2$	CH_3				OH	+7	ref 29c
18e	$-NH(CH_2)_3N(CH_3)_2$	CH_3				OCH_3	+9	ref 29c
19e	$-NH(CH_2)_3N(CH_3)_2$	CH_3		OCH_3			+4	N
20e	$-NH(CH_2)_3N(CH_3)_2$	CH_3	CH_3		OH		+10	ref 29b
21e	$-NH(CH_2)_3N(CH_3)_2$	C_2H_5			OH		+10	ref 29c
22e	$-NH(CH_2)_3N(CH_3)_2$	C_2H_5			OCH_3		+9	ref 29c
23e	$-NH(CH_2)_3N(C_2H_5)_2$	CH_3			OH		+10	ref 29b
24e	$-NH(CH_2)_3N(C_2H_5)_2$	CH_3			OCH_3		+16	ref 29b
25e	$-NH(CH_2)_3N(C_2H_5)_2$	CH_3	CH_3				+3	ref 29b
26e	$-NH(CH_2)_3N(C_2H_5)_2$	CH_3	CH_3		OCH_3		+12	ref 29b
27e	$-NH(CH_2)_3N(CH_3)(CH_2)_3NH_2$	CH_3			OCH_3		+16	N

[a] Substituents refer to the numbering scheme described in Figure 1. ΔT_{max} (±0.5 °C) refers to the stabilization of the triplex-to-duplex transition. Ref indicates the references where the synthesis of the compound is described. N indicates that the synthesis of the compound was not previously reported and is described in the Experimental Section.

Table 2. Structure and Results for Benzo[*g*]pyridoindoles

	R7	R11	R3	R4	ΔT_{max}^{3-2} (°C)	ref
1g	-H		OCH_3		+13	N
2g	$-NH_2$		OCH_3		+17	N
3g	$-NH(CH_2)Ph$		OCH_3		+18	N
4g	$-NH(CH_2)_2CH(CH_3)_2$		OCH_3		+22	N
5g	$-NH(CH_2)_3NH_2$		OCH_3		+28	N
6g	$-NH(CH_2)_3N(CH_3)_2$				+24	ref 29b
7g	$-NH(CH_2)_3N(CH_3)_2$			OH	+21	ref 29b
8g	$-NH(CH_2)_3N(CH_3)_2$			OCH_3	+19	ref 29b
9g	$-NH(CH_2)_3N(CH_3)_2$	CH_3			+19	ref 29b
10g	$-NH(CH_2)_3N(CH_3)_2$	CH_3		OH	+11.5	ref 29b
11g	$-NH(CH_2)_3N(CH_3)_2$	CH_3		OCH_3	+18	ref 29b
12g	$-NH(CH_2)_3N(CH_3)_2$		OCH_3		+28	N
13g	$-NH(CH_2)_3N(C_2H_5)_2$				+24	ref 29b
14g	$-NH(CH_2)_3N(C_2H_5)_2$		OCH_3		+28	ref 29b
15g	$-NH(CH_2)_3N(C_2H_5)_2$	CH_3	OH		+16	ref 29b
16g	$-NH(CH_2)_3N(C_2H_5)_2$	CH_3	OCH_3		+17	ref 29b
17g	$-NH(CH_2)_3N(C_2H_5)_2$	CH_3		OCH_3	+17	ref 29b
18g	$-NH(CH_2)_3N(CH_3)(CH_2)_3NH_2$		OCH_3		+31	N

Table 3. Structure and Results for Benzo[*f*]pyridoindoles

	R1	R9	ΔT_{max}^{3-2} (°C)	ref
1f	$-NH(CH_2)_3N(C_2H_5)_2$		+4.5	ref 29a
2f	$-NH(CH_2)_3N(CH_3)_2$	OCH_3	+4	N
3f	$-NH(CH_2)_3N(CH_3)_2$	OH	+0	N

thermodynamic study of this kind of system is not easy to perform because ligands bind to both double- and triple-helical structures, with a different number of intercalation sites and different affinities.[22] Self-association of these compounds could also compete with triplex binding.[19b] We always used a concentration of dye which was 10-fold higher than that of the target triplex. We checked that this concentration provided the maximum stabilization for the ligands investigated.[16]

The stabilization of the triple helix induced by the ligand was estimated from the difference between the melting temperature

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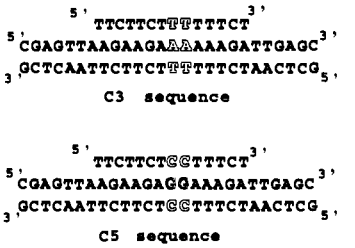

Figure 2. Sequence of the two triplexes studied in this report. The two sequences differ by only two consecutive triplets (bold).

in the presence of 15 μM of BPI and that of the triplex alone (ΔT_{max}^{3-2}) (Figure 3). This difference was determined on the curves representing the derivatives of the melting curves versus temperature. Results are summarized in Tables 1−3 for the triplex formed with oligonucleotide 14C3. They show that the geometry of the molecule plays a crucial role for triple helix affinity. Benzo[*e*]pyridoindoles (BePIs) and benzo[*g*]pyridoindoles (BgPIs) considerably stabilize triple helices, although the role of their various substituents is different. However, the benzo[*f*] derivatives have poor triple helix-stabilizing properties.

Sequence effects were investigated by comparing ΔT_{max} for the C3 and C5 sequences for five leading compounds, for which only the side chain is changed (Table 4). Interactions with double helices were also investigated, and the corresponding ΔT_{max}^{2-1} (the increase of melting temperature for opening of the double helix) are reported in Table 4.

Discussion

Compounds **9e** and **14g** have been shown to intercalate into double and triple helices by fluorescence energy transfer and viscosimetry measurements.[16,19a] Other compounds will be assumed to bind in the same way. The structural modifications introduced in the BePI and BgPI derivatives investigated in the present study involve only a modification of the alkylamine side chain or the addition of methyl or hydroxyl groups. The

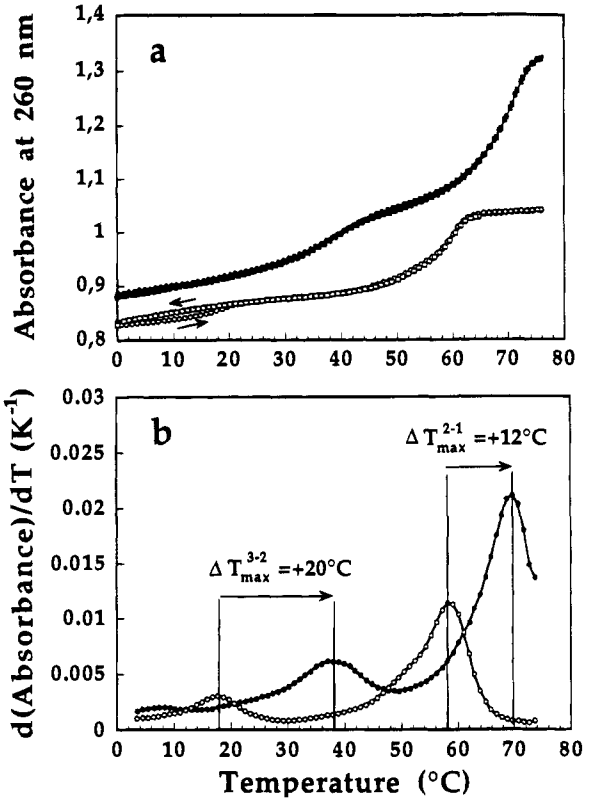

Figure 3. (a) Denaturation profiles of the mixtures of the 14C3 oligonucleotide (1.8 μM) and the 26-bp Watson–Crick C3 duplex (1.5 μM) in the absence (open symbols) and in the presence (closed symbols) of BPI derivative 9e (15 μM). The transition at lower temperature was not reversible in the absence of ligand. Arrows indicate the direction of the temperature variations. Temperature was changed at a rate of 0.1 °C/mn. Squarres represent the curves obtained by cooling the sample whereas circles represent the curves obtained by heating. (b) First derivative of the denaturation profile shown in part (a). Two maxima are observed for both curves. The one at low temperature corresponds to the dissociation of the third strand from the double helix, whereas the one at higher temperature is due to melting of the duplex into single strands. Difference between the maximum of the first derivative curves in the presence and the absence of ligand allowed us to determine a ΔT_{max} for each transition, which is indicative of the stabilization induced by the ligand for the triplex-to-duplex or for the duplex-to-single strands transitions (see text).

intercalative binding mode does not exclude that BPI derivatives could bind to duplex–triplex junctions, as does an ellipticine[23] or an acridine derivative,[24] but the same stabilization was observed with a blunt-end triple helix, suggesting that stabilization arises from interactions with base triplets and not from binding at triplex–duplex junctions. Moreover, changing the sequence affects the stabilization as will be discussed below. At pH 6.2, aliphatic amines are protonated. Spectroscopic measurements have shown that aromatic nitrogens (N10 for BePIs, N8 for BgPIs) are also protonated at pH below 8.[19] Therefore, all these molecules are polyaromatic cations bearing one positive charge on the aromatic system and one or two more charges on the aliphatic amines of the side chain.

Different roles are played by the substituents of BePIs and BgPIs. In the absence of the side chain at position 11 for BePI and 7 for BgPI, BePI is more efficient for triple helix stabilization (compare **3e** and **2g**, **1e** and **1g**). For BePIs, addition of the alkylamine side chain decreases triple helix stabilization (compare **3e** and **9e**), and the destabilization is more important when the number of positive charges (compare **7e**, **9e**, and **27e**) or the size of the N-terminal substituents increases (compare **9e**, **11e**, **16e**, and **24e**). BgPI derivatives exhibit an opposite effect of the alkylamine side chain. For this class of molecules, the presence of an alkylamine side chain provides a further stabilization, particularly if it is carrying a positive charge (compare **2g**, **3g**, **4g**, and **5g**). The size of the N-terminal substituents does not influence triplex stability, since **5g**, **12g**, and **14g** provide equal stabilization. A second positive charge provides a further stabilization (compare **5g** and **18g**). Thus, it seems that hydrophobic, steric, and electrostatic effects are responsible for a negative role of the side chain of BePIs and for a positive contribution of the side chain of BgPI to triplex stabilization. Other substituents are found to affect triplex stabilization. The methoxy group at position 3 of BePIs is generally better than a hydroxyl group (see compounds **2e–3e**, **4e–5e**, **8e–9e**, **10e–11e**, **12e–13e**, **15e–16e**, **23e–24e**) or a hydrogen atom (see **11e** and **14e**), and position 3 is preferred (see **16e**, **18e**, and **19e**). For the BgPIs, the importance of the methoxy group at position 3 is less drastic. Alkyl groups at position 7 and 8 of BePI and 11 of BgPI also affect triplex stabilization.

The sequence specificity for triple helix stabilization was also investigated. As previously reported,[16] compound **9e** was found to stabilize the C3 triplex by 20 °C, whereas its effect was much less important on the C5 triplex (+3 °C) where the stretch of six T·AxT base triplets is interrupted by two C·GxC$^+$ base triplets. This result led to the conclusion that compound **9e** interacts more strongly with T·AxT triplets. This specificity was checked for other representative compounds. All BPI derivatives exhibit this preference for T·AxT triplets since the ΔT_{max} value obtained for the C5 sequence is lower than that obtained for the C3 sequence. However, the ability of ligands to stabilize the C5 sequence strongly depends on the structure and the charge of the molecule. Among the BePI derivatives, only compounds bearing no side chain (**3e**) or no charge on the side chain at position 11 (**7e**) stabilized the C5 triple helix with some efficiency (Table 4). This could be due to their ability to bind to a shorter T·AxT stretch as allowed by less important electrostatic repulsions between the molecule and protonated cytosines in the third strand. Surprisingly, the BgPI derivatives have an opposite behavior as compared to BePIs regarding their ability to stabilize the C5 sequence. Positively charged BgPI derivatives are able to stabilize this sequence much more efficiently than BePI analogues. Thus, they are able to bind triple helices containing short T·AxT stretches despite the presence of positive charges in the vicinity of T·AxT triplets.

On the basis of molecular modeling studies, the side chain of BePIs has been suggested to lie in the Watson–Hoogsteen groove[25] of triple helices[19a] (see Figure 4a). This model could account for the electrostatic repulsions described above. The positive charge of protonated cytosine is located close to the Watson–Hoogsteen groove, and the electrostatic potential in the Watson–Hoogsteen groove is much less negative near C·GxC+ triplets than near T·A*T triplets. Thus, binding of ligands bearing positive charges in the Watson–Hoogsteen groove should not be favorable near C·GxC+ triplets. A hydrophobic uncharged side chain (**7e**) allows stabilization of a sequence containing only three consecutive T·AxT triplets

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Table 4. Change in Melting Temperature (ΔT_{max}) of the Transition from the Triple Helix to the Double Helix (3 → 2) and for the Double Helix to Single Strands (2 → 1) for the C3 and C5 Sequences, as a Function of the Nature of the Alkylamine Side Chain[a]

R11 for BePI, R7 for BgPI		benzo e					benzo g			
		C3		C5			C3		C5	
ΔT_{max} (±0.5 °C)	ref[b]	3 → 2	2 → 1	3 → 2	2 → 1	ref[b]	3 → 2	2 → 1	3 → 2	2 → 1
NH_2	(3e)	28	4	13	4	(2g)	19	6	6	6
$NH(CH_2)_3NH_2$	(9e)	20	12	3	10	(5g)	28	16	12	13
$NH(CH_2)_3N(CH_3)(CH_2)_3NH_2$	(27e)	16	16	1	14	(18g)	31	20	14	16
$NH(CH_2)_2CH(CH_3)_2$	(7e)	22	nd[c]	8	nd	(4g)	22	nd	10	nd
$NH(CH_2)$Phi	(5e)	21	nd	8	nd	(3g)	18	nd	8	nd

[a] T_m for the 3 → 2, transition in the absence of any ligand are 18 and 25 °C for the C3 and C5 sequences, respectively, and 56 and 60 °C for the 2 → 1 transition. Experiments were carried out in a pH 6.2 cacodylate buffer (10 mM) containing 0.1 M NaCl. Other substituents are R_8 = CH_3 and R_3 = OCH_3 for BePIs, R_{11} = CH_3, and R_3 = OCH_3 for BgPIs. [b] Ref refers to the number of Tables 1 and 2. [c] nd: not determined.

but has a destabilizing effect compared to compound **3e**, probably because the hydrophobic chain disrupts the spine of hydration of the Watson–Hoogsteen groove[26] or prevents optimal stacking interactions with the base triplets. On the basis of the results presented above, we propose that the side chain at position 7 of BgPIs lies in the Watson–Crick groove of triple helices (Figure 4b). The same model was proposed by Wilson and co-workers for their unfused aromatic cations.[18] This could explain why this side chain has opposite effects on triplex stabilization obtained with BePIs and BgPIs. For BgPIs, positive charges would enhance binding of the molecule to triplex by establishing electrostatic and van der Waals interactions within the minor groove, where the effect of the positive charge of C·GxC+ triplets is strongly attenuated. The positively charged side chain could play the same role as polyamines in triplex stabilization. It is interesting to note that the distribution of the three positive charges in compound **18g** resemble that of spermidine which can stabilize triple helices.[27] Thus, this class of molecules seems to add electrostatic contributions of the polyamine chain to stacking interactions of the polyaromatic ring. The effect of other chemical modifications were more difficult to rationalize. Molecular mechanics calculations show that stacking interactions of BePI bearing a hydroxyl group at position 3 is lower than that of BePI bearing a methoxy group, probably because of a different dipolar moment. The influence of methyl groups is difficult to study without taking into account water molecules and hydrophobic effects, which are not included in the JUMNA program.

The value of ΔT_{max}^{3-2} measured in our experiments does not reflect the affinity for the triple helix but rather the difference in affinity between triplex and duplex. If we neglect binding of the molecules to the single strands, ΔT_{max}^{2-1} reflects the affinity for double helix. For BePI derivatives, the side chains which stabilize less the triplex-to-duplex transitions are those which stabilize most the duplex-to-single strands transitions. BgPI derivatives have a different behavior; modifications that increase the stabilization of triple helices also increase stabilization of double helices. Thus, ligand **18g**, which gives the highest ΔT_{max}^{3-2} and the highest ΔT_{max}^{2-1}, is the most potent triplex ligand of the BPI family. However, if a high discrimination between triplex and duplex, associated with a poor binding to duplex is required, then compound **3e** should be preferred.

Both BePI and BgPI series of molecules exhibit some specificity for A·Tbase pairs in duplexes, as the ΔT_{max}^{2-1} obtained with the C5 sequence is smaller than that obtained with the C3 sequence. It has been documented that the electrostatic potential of the minor groove is more electronegative than that of the major groove in double helices and that the potential in the minor groove is more electronegative for A·T rich sequences.[28] A positively charged side chain increases duplex stabilization for both BePIs and BgPIs. This could be explained by a model in which the side chain lies in the minor groove of the double helix for both BePIs and BgPIs. Such a model was already suggested for BePI[19] and would explain the selectivity for A·T base pairs.

The effects of coralyne which was recently described by Lee et al.[17] to stabilize triple helices were tested on both of our systems. Coralyne increases the T_m of the triplex-to-duplex transition with ΔT_{max}^{3-2} of 8 °C for triplex C3 and 7 °C for triplex C5. Thus, at the same concentration as the one used with BPI, coralyne appears to be a much less efficient triplex stabilizer than are some BPI derivatives, but this molecule is also one of the less selective between the two types of sequences. It binds also less efficiently to double helices as the ΔT_{max}^{2-1} measured for the C3 and C5 sequences are + 3 and + 2 °C, respectively.

BePIs were originally studied among other polyaromatic cations as potential triple helix ligands. Their structure involves four rings with a crescent shape that could optimize stacking interactions with base triplets (see Figure 1) and made them good candidates as triple helix-specific intercalators. BgPIs possess a more linear geometry, and the lower stacking interactions can be compensated by very important electrostatic interactions of the side chain. BfPIs do not seem to be able to stabilize triple helices. Their shape is similar to that of ellipticine derivatives which also do not provide any stabilization. With the side chain placed in one of the grooves of the triple helix, models show that BfPI derivatives cannot provide as much stacking interactions with base triplets as BePI and BgPI. The results presented in this study point to the role of stacking as well as hydrophobic and electrostatic interactions of both the aromatic ring and the substituents in the binding of triplex-specific intercalators. These studies should provide a rational basis for the design of sequence specific triple helix ligands.

Conclusion

This study has shown that benzo[*e*]- and benzo[*g*]pyrido[4,3-b]indole derivatives can bind to DNA triple helices, thus providing a strong stabilization of the triple-helical structure. Both molecules display high affinity and selectivity for triple helices. However, the role played by the positively charged alkylamine side chain is different for BePIs and BgPIs. For BePIs, this chain is unfavorable to triplex formation, whereas it contributes positively to triplex stability for BgPIs. This suggest that BePIs and BgPIs form two very different intercalation complexes with the side chain located in different grooves of the triple helix.

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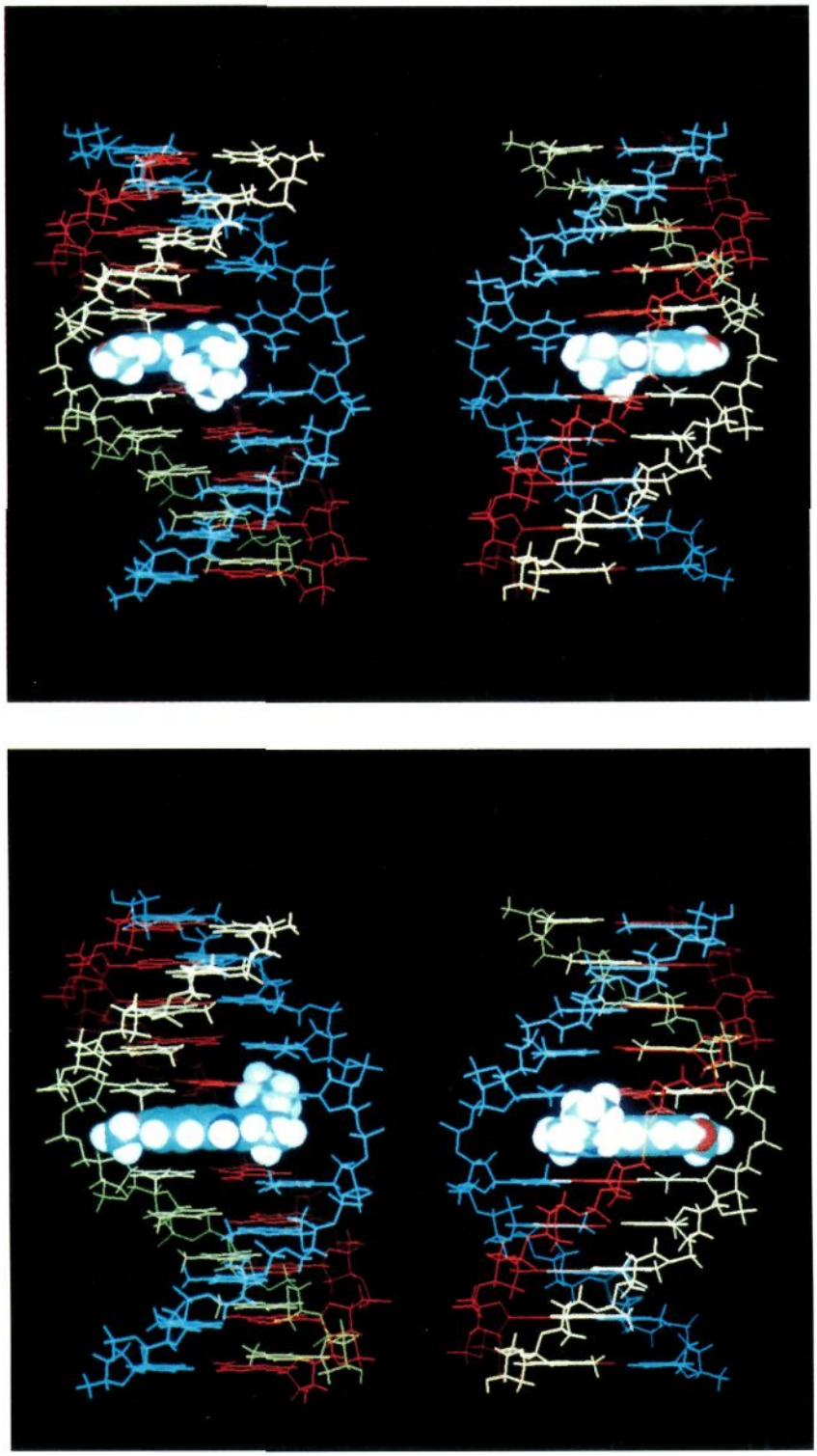

Figure 4. Proposed model for BePI (a, top) and BgPI (b, bottom) intercalated in a triple helix. The structure is derived from energy minimization using the JUMNA programm. The left view shows the major groove of the triple helix, whereas the right view show the same triple helix rotated by 180°, allowing one to see the minor groove. Watson−Crick target duplex is colored in blue (pyrimidine strand) and red (purine strand), and the third strand is colored in yellow.

Triple helices can also be formed when an oligonucleotide containing G and T or G and A binds to an oligopurine-oligopyrimidine sequence in an antiparallel orientation with respect to the purine sequence. Some applications of triple helices might involve RNA or 2′-*O*-methyl-containing strands. Preliminary results suggest that benzopyridoindoles interact with these different types of structures. The effect of benzopyridoindoles on triple helices containing a third strand with modified phosphodiester backbones such as methylphosphonates or phosphorothioates is also worth investigating.

Covalent linkage of a benzopyridoindole derivative to an oligonucleotide forming a local triple helix upon binding to double-helical DNA should provide a strong stabilization of the triple helix. The use of such a conjugate would probably eliminate the cytotoxicity of the free molecule. It was shown earlier[12] that an acridine derivative linked to an oligonucleotide

Table 5

ref[a]	chemical prep. method	heating temp (°C)	time of reaction[b]	yield %	mp °C[c]	formula	mass spectra[f]
1e	A	20	48 h	75	>260	$C_{17}H_{14}N_2O$ (C,H,N)	
2e	C[h]	rfx	5 h	60	>260	$C_{16}H_{13}N_3O$; 0.5 H_2O (C,H,N)	263 (100%)
3e	D	65	24 h	24	>260	$C_{17}H_{15}N_3O$ (C,H,N)	
4e	C	rfx	4 h	29	180−182	$C_{23}H_{19}N_3O$; $C_4H_4O_4$; 2H_2O (C,H,N)	55 (100%); 353 (27.3%)
5e	B	160	24 h	91	224−226	$C_{24}H_{21}N_3O$; $C_4H_4O_4$ (C,H,N)	
7e	B	rfx	28 d	82	214−216	$C_{22}H_{25}N_3O$; $C_4H_4O_4$; 0.5 H_2O (C,H,N)	304 (100%); 347 (49.2%)
10e	C	rfx	4 h	41	120−125	$C_{20}H_{22}N_4O$; 2$C_4H_4O_4$; H_2O (C,H,N)	54 (100%); 334 (6.2%)
11e	B	rfx	24 h	72	173−175	$C_{21}H_{24}N_4O$; 2$C_4H_4O_4$ (C,H,N)	
19e	E[g]	rfx	3 d	45[j]	196−198	$C_{22}H_{26}N_4O$; 2$C_4H_4O_4$; H_2O (C,H,N)	58 (100%); 362 (7.8%)
27e	B	140	18 h	70	124−126	$C_{24}H_{31}N_5O$; 3$C_4H_4O_4$; H_2O (C,H,N)	277 (100%); 405 (4.8%)
2f	B[g]	rfx	48 h	78	138−140	$C_{23}H_{28}N_4O$; 2$C_4H_4O_4$; 0.25 H_2O (C,H,N)	58 (100%); 376 (13.4%)
3f	C[g]	rfx	2.5 h	59	122−4	$C_{22}H_{26}N_4O$; 2$C_4H_4O_4$; H_2O (C,H,N)	291 (100%); 362 (11.6%)
1g	A	65	24 h	81	>260	$C_{17}H_{14}N_2O$ (C,H,N)	
2g	D	65	24 h	25	>260	$C_{17}H_{15}N_3O$; 0.33 H_2O (C,H,N)	277 (100%)
3g	B	160	72 h	60	>260	$C_{24}H_{21}N_3O$ (C,H,N)	
4g	B[g]	190[i]	7 d	71	226−8	$C_{22}H_{25}N_3O$; 2$C_4H_4O_4$; H_2O (C,H,N)	304 (100%); 347 (54.9%)
5g	B	rfx	48 h	64	193−195	$C_{20}H_{22}N_4O$ (C,H,N)	
12g	B[g]	170[i]	24 h	55	198−200	$C_{22}H_{26}N_4O$; 2$C_4H_4O_4$ (C,H,N)	
18g	B	190[i]	6 d	68	95−7	$C_{24}H_{31}N_5O$; 3$C_4H_4O_4$; 2.5H_2O (C,H,N)	277 (100%); 405 (20%)

[a] Ref refers to the numbering of Tables 1 and 2. [b] h, hours; d, days. [c] Melting pionts (mp) were determined with a Köfler apparatus. [d] Elemental analysis was performed by Service Central de Microanalyses du CNRS, 91190 Gif sur Yvette, France. The results are within ±0.4% of the theoretical values corresponding to the mentioned empirical formulas for the mentioned elements. [e] ^{1}H-NMR spectra were recorded on a Bruker AC 200 spectrometer in Me_2SO-d_6. For all the newly obtained compounds described in this table, they are fully consistent with the assigned structures. [f] Mass spectra (EI) were only performed for the new derivatives whose elemental analysis correspond to hydrated or partially hydrated compounds, using a Kratos MF80 mass spectrometer (70 eV). [g] Further purifications of the free bases by using flash chromatography were performed on a alumina column with a 98−2 methylene chloride−ethanol mixture as eluent. [h] As mentioned in footnote *g* using a 80−18−2 methylene chloride−ethanol−triethylamine mixture as eluent. [i] In a steel vessel. [j] Yield of the last step.

lost its intercalating properties in DNA due to electrostatic repulsions between the negatively charged oligonucleotide "tail" and DNA. Only at sequences allowing for sequence-specific binding of the oligonucleotide did the acridine intercalate.[12] The proposed model for the side chain position within the triple helix grooves is an important parameter to consider when synthesizing conjugates of benzopyridoindole derivatives with oligonucleotides.

Experimental Sections

The synthesis of some benzopyridoindole derivatives was previously reported[29] (see Table 1−3 for references). New compounds were synthesized using one of the five following methods.

Method A. Compounds **1e** and **1g** were prepared by dechlorination of 11-chloro-3-methoxy-8-methyl-7*H*-benzo[*e*]pyrido[4,3-*b*]indole and 7-chloro-3-methoxy-10-methyl-11*H*-benzo[*g*]pyrido[4,3-*b*]indole, respectively.[29b]

A solution of the required chloro-derivative (2 mmol) in absolute ethanol (100 mL) was hydrogenated in the presence of 10% palladium on charcoal (500 mg) at the temperature and for the time indicated in Table 5. The catalyst was filtrated and washed with hot ethanol, and the filtrate was evaporated to dryness. Water (50 mL) was added to the residue, and the pH of the solution was adjusted to 14 with 2 N NaOH (3 mL). The precipitate was collected by filtration and washed with water. Pure product was then obtained by recrystallization from aqueous ethanol.

Method B. The new BePI derivatives **5e**, **7e**, **11e**, and **27e**, Bg PI **3g** and **11g**, and BfPI **2f** were synthesized from 11-chloro-3-methoxy-8-methyl-7*H*-benzo[*e*]pyrido[4,3-*b*]indole,[29b] 7-chloro-3-methoxy-10-methyl-11*H*-benzo[*g*]pyrido[4,3-*b*]indole,[29b] and 1-chloro-9-methoxy-5,6-dimethyl-5*H*-benzo[*f*]pyrido[4,3-*b*]indole,[29a] respectively.

The mixture of the required chloro derivative (10 mmol) and the primary amine (30 mL, large excess) was heated under N_2 at the temperature and for the time indicated in Table 5. Excess amine was evaporated under reduced pressure; the residue was taken up in diluted ammonia and extracted with methylene chloride. After evaporation of the solvent, the residue was washed with *n*-hexane (50 mL) providing the pure free base. Maleate salts were prepared by treatment of the free bases with an excess of maleic acid (3 equiv) in acetone as solvent.

Method C. The mixture of the methoxylated starting compound (1 g) and concentrated hydrobromic acid (d = 1.47; 25 mL) was stirred under N_2 and heated under reflux for the period indicated in Table 5. Evaporation to dryness under reduced pressure provided a solid residue, which was taken up in water and basified with an excess of ammonia. The aqueous layer was saturated with solid NaCl, extracted with ethyl acetate, dried (magnesium sulfate), and evaporated. Maleate salts were prepared under the conditions described in method B.

Method D. To the solution of the required benzylamino derivative (100 mg) in acetic acid (16 mL) was added palladium on charcoal (10%; 100 mg). The mixture was stirred at 65 °C under hydrogen atmosphere at normal pressure for 24 h. The catalyst was then filtered off, washed with hot acetic acid and the solvent was removed under reduced pressure. To the residue was added water (30 mL), and the pH of the solution was adjusted to 14 with aqueous ammonia. The precipitate was collected and washed with water. Pure product was then obtained after flash chromatography on an alumina column with a 95−5 methylene chloride−ethanol mixture as eluent.

Method E. Compound 19e was prepared by substitution of 11-chloro-4-methoxy-8-methyl-7*H*-benzo[*e*]pyrido[4,3-*b*]indole with 3-dimethylaminopropylamine according to the method B described above. This intermediate chloro-derivative was obtained with an overall yield of 23% in a four-step procedure starting from 4-hydrazino-5-methyl-1*H*-pyridin-2-one and 5-methoxy-2-tetralone, according to the previously described techniques.[29b]

The compounds which were synthesized as maleate salts were dissolved in bidistilled water at the required concentration, usually 2 mM. The free bases were dissolved in DMSO at a concentration of 2 mM. The final concentration of DMSO obtained in the melting experiments (0.75% in the solution used for spectroscopic measurements) had no effect on the melting profiles.

Other Materials. Oligonucleotides were purchased from Eurogentec (Seraing, Belgium). They were ethanol precipitated, and their concentration was calculated using a nearest-neighbor model.[30] Coralyne chloride was purchased from Pharmacia.

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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 maintained 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 with 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 an hypochromism at this wavelength. The maxima of the derivatives of the melting curves (T_{max}) gave a good approximation of the half-dissociation temperatures (T_m) and allowed us to analyze the results in an easy and reproducible way. Thus, these values were used to characterize the stability of the complexes.

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