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Extension of the range of DNA sequences available for triple helix formation: stabilization of mismatched triplexes by acridine-containing oligonucleotides

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ABSTRACT

Triple helix formation usually requires an oligopyrimidine•oligopurine sequence in the target DNA. A triple helix is destabilized when the oligopyrimidine•oligopurine target contains one (or two) purine•pyrimidine base pair inversion(s). Such an imperfect target sequence can be recognized by a third strand oligonucleotide containing an internally incorporated acridine intercalator facing the inverted purine•pyrimidine base pair(s). The loss of triplex stability due to the mismatch is partially overcome. The stability of triplexes formed at perfect and imperfect target sequences was investigated by UV thermal denaturation experiments. The stabilization provided by an internally incorporated acridine third strand oligonucleotide depends on the sequences flanking the inverted base pair. For triplexes containing a single mismatch the highest stabilization is observed for an acridine or a propanediol tethered to an acridine on its 3′-side facing an inverted A•T base pair and for a cytosine with an acridine incorporated to its 3′-side or a guanine with an acridine at its 5′-side facing an inverted G•C base pair. Fluorescence studies provided evidence that the acridine was intercalated into the triplex. The target sequences containing a double base pair inversion which form very unstable triplexes can still be recognized by oligonucleotides provided they contain an appropriately incorporated acridine facing the double mismatch sites. Selectivity for an A•T base pair inversion was observed with an oligonucleotide containing an acridine incorporated at the mismatched site when this site is flanked by two T•A*T base triplets. These results show that the range of DNA base sequences available for triplex formation can be extended by using oligonucleotide intercalator conjugates.

INTRODUCTION

Shortly after the structure of double-helical DNA was discovered, nucleic acids were found to be also capable of forming a triple-helical structure (1). Such structures were widely studied in the 1960s with RNA and DNA homopolymers of repeating nucleotide sequences. It was assumed that the third strand was located in the major groove and associated with the duplex via non-Watson–Crick hydrogen bonding interactions now known as Hoogsteen or reversed Hoogsteen pairing. However, it is only recently that oligonucleotides were demonstrated to form defined short triple helices by binding to the major groove of double-helical DNA at oligopyrimidine•oligopurine sequences giving rise to a local triple helix through sequence-specific hydrogen bonds (2,3).

If the triplex forming oligonucleotide (TFO) contains pyrimidines, it forms Hoogsteen hydrogen bonds in a parallel orientation with respect to the Watson–Crick paired purine strand giving rise to isomorphous T•A*T and C•G*C^+ base triplets. Since cytosine in the third strand has to be protonated to form two hydrogen bonds with guanine, the stability of this so-called (T,C)-motif triplex strongly depends on pH (4,5). If the TFO contains purines, it forms reverse Hoogsteen bonds in an anti-parallel orientation with respect to the purine strand of the Watson–Crick duplex generating T•A*A and C•G*G base triplets. The stability of such triplexes depends on the presence of bivalent metal cations especially magnesium (6,7, for a review see 8). For (G,T)- containing third strands the orientation depends on the base sequence (9)

Oligonucleotide-directed triple helix formation has created new prospects for the sequence-specific recognition of dsDNA. The high specificity of TFO-DNA recognition has led to the development of the 'anti-gene' strategy aimed at modulating gene expression *in vivo* using TFOs (10). Therefore, a considerable interest in DNA triplexes has been raised due to their potential biological and therapeutical applications (for review, see 11). TFOs have been proposed to control gene expression by competing with proteins for binding to DNA (12,13), and shown to inhibit transcription initiation (14–18). The possibility of targeting sequences downstream of the transcription initiation site has been recently demonstrated using oligonucleotide analogues containing phosphoramidate linkages (19). TFOs have access to their target DNA sequences within cell nuclei (20,21)

The requirement of an oligopyrimidine•oligopurine sequence for triplex formation has been one of the major limitations of the 'anti-gene' strategy. Interruption in the oligopyrimidine•oligopurine target sequences by one or more inverted purine•pyrimidine base

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pair(s) leads to a dramatic decrease in triplex stability. There have been several studies attempting to extend the range of recognition sequences (22–27). The use of non-natural nucleotide analogues has been described which allows recognition of oligopurine strands interrupted by one pyrimidine (27,28). We have recently shown that an acridine derivative incorporated within an oligopyrimidine TFO strongly stabilizes triple helices containing mismatches (29). In the present work, we have extended our studies to further examine relative affinities of the natural or modified nucleotide bases for all Watson–Crick base pairs at a single position within a (T,C)-motif triple helix. We show that the destabilization caused by single or double base pair inversions in the target sequence can be counterbalanced by using a TFO containing an appropriately incorporated intercalating agent, an acridine derivative, facing the inverted base pair(s). Nearest neighbour effects observed in the present and in the previous studies are discussed.

MATERIALS AND METHODS

Nomenclature

The following convention for TFOs and base triplets are used. The duplexes are written as 26YX•26RY, with the third strand as 14YZ. The letters X and Y stand for the bases involved at the base pair inversion site in the oligopyrimidine and oligopurine target sequences respectively. Z is the corresponding base in TFO. The acridine derivative is designated as W, thus 14WZ and 14ZW mean that the acridine was incorporated either on the 5′- or 3′-side of Z in TFOs, respectively. A base triplet is designated X•Y*Z, where X•Y is the Watson–Crick base pair and Z the base in the third strand that recognizes the purine tract through Hoogsteen hydrogen bonds (Y*Z). The base analogs propynyl-dC and propynyl-dU are designated as C and U respectively, and the propanediol linker as L. M is a 3-nitropyrrole derivative (30).

Oligonucleotides

All the oligonucleotides, including acridine-containing oligonucleotides were synthesized by Eurogentec (Belgium). An acridine phosphoramidite (Glen Research) was internally incorporated into oligonucleotides as shown in Figure 1 where the natural 3-carbon internucleotide phosphate distance was maintained. Oligonucleotides were ethanol precipitated in the presence of 0.3 M sodium acetate, washed with ethanol and used without further purification. The quality of acridine-containing oligonucleotides was checked by the fluorescence of incorporated acridine. The concentration of all oligonucleotides was determined spectrophotometrically using the extinction coefficients calculated by a nearest-neighbour method (31). An extinction coefficient of 8845 $M^{-1}cm^{-1}$ at 424 nm was used for measuring the concentrations of acridine-containing oligonucleotides (32).

UV absorption spectrometry

Triple helix stability was measured by UV spectrophotometry. All the DNA thermal denaturation experiments were performed on a UVIKON 940 Spectrophotometer using quartz cuvettes of 1 cm optical pathlength. The spectrophotometer was interfaced to an IBM-AT computer for data collection and analysis. The cell holder was thermostated with a circulating liquid (80% water 20% ethyleneglycol) in a Haake P2 water bath. The temperature

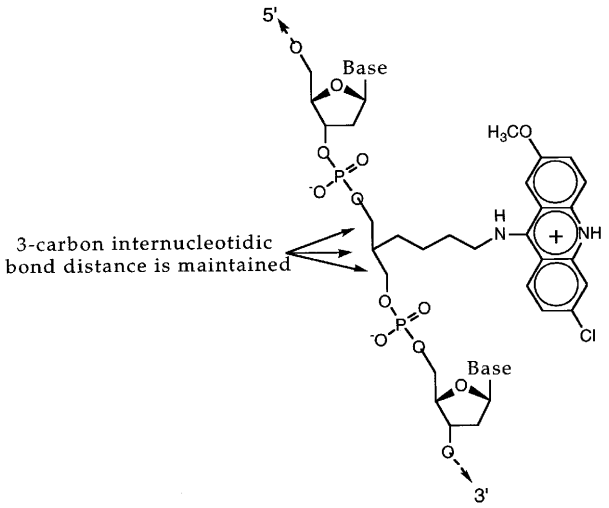

Figure 1. Chemical structure of an internally incorporated acridine derivative within an oligonucleotide in which the 3-carbon internucleotide phosphate distance is maintained at the intercalator site.

of the water bath was decreased from 80 to 0°C and then increased up to 80°C at a rate of 0.15°C/min with a Haake PG 20 thermoprogrammer. The absorbance at 260 or 425 nm was recorded every 8 min. The sample temperature was measured by a teflon-coated temperature probe immersed directly in a control cuvette. All the oligonucleotide samples were prepared in 20 mM sodium cacodylate buffer at pH 6.0 containing 100 mM sodium chloride and 10 mM $MgCl_2$. The triplexes were formed by first mixing the two strands (26YX and 26RY) of the Watson–Crick duplex, each at 1 µM concentration, and then adding 1.5 µM of the third strand. The triplex melting temperatures (T_m) were evaluated as the maximum of the first derivative of the melting profiles. The accuracy of the reported T_m values is ±1°C.

Static fluorescence measurements

Fluorescence emission spectra were recorded with a Spex Fluorolog F1T11T spectrofluorimeter using quartz suprasil microcells of 1 cm pathlength. They were corrected for the wavelength dependence of the transmission and the detection systems. The areas under the fluorescence spectra were integrated to estimate the relative fluorescence intensities. The excitation wavelength was set at the maximum of absorbance of free acridine (424 nm). Temperature of cell holder was controlled with a Huber Ministat circulating water bath. Fluorescence melting curves were obtained by measuring the ratio of fluorescence intensities of acridine-containing oligonucleotide in the presence and in the absence of the target double helix at various temperatures, as previously described (33,34).

RESULTS

The studies were performed with a 26 bp long synthetic DNA fragment (26YX•26RY) containing a 14 bp oligopyrimidine•oligopurine sequence which served as a target for triplex formation by a 14mer pyrimidine TFO (14YZ). Both the oligopyrimidine and oligopurine sequences of the target duplex were interrupted at a

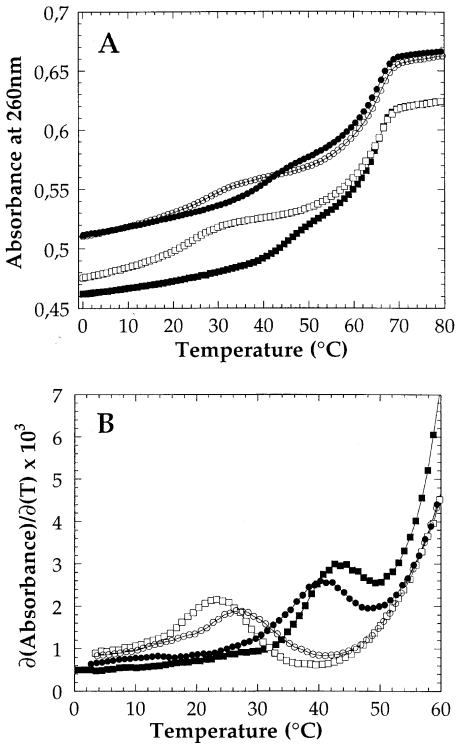

Figure 2. (**A**) Thermal denaturation profiles showing absorbance at 260 nm versus temperature for triplexes described at the top of Table 1. The following triplexes are shown: no base-pair inversion X•Y*Z = C•G*C (filled square), one base pair inversion X•Y*Z = G•C*C (open square), with TFO containing an internally incorporated acridine derivative on the 3′- (filled circles) or on the 5′-side (open circles) of Z position when Z = C. Melting curves were reversible under our experimental conditions (see Materials and Methods). (**B**) Derivatives of the thermal denaturation profiles shown in (A). The maxima of the curve peaks corresponds to the T_m of the triplex-to-duplex transition.

central position by introduction of one (or two) purine and pyrimidine bases respectively, thus causing a single (or double) base pair inversion (Table 1 or 3, upper part). The 14mer TFOs were used with or without incorporation of an intercalating agent, 2-methoxy,6-chloro,9-aminoacridine (W) (Table 2, upper part) either on the 5′- or 3′-side of the Z nucleotide [A,G,C,T,C,U, M (3-nitropyrrole) or L (propanediol linker)] facing the inverted base pair.

UV thermal denaturation studies

Effect of single and double mismatches on triplex stability. The effects of the various nucleotides (Z) in TFOs on the stability of 14mer triplexes were investigated by DNA thermal denaturation measurements. All the possible 36 combinations (4 X•Y bp facing 9 Z nt) were tried. The melting profiles showed a biphasic pattern (Fig. 2A), typical of triplexes for all fully complementary and non-fully complementary sequences in a buffer (20 mM sodium cacodylate pH 6.0) containing 100 mM NaCl and 10 mM $MgCl_2$. The choice of a longer DNA duplex allowed us to distinguish easily the triplex-to-duplex and the duplex-to-single strands transitions (Fig. 2). The transition at higher temperature corresponding to the dissociation of the 26 bp duplex had similar T_m values (X•Y = T•A, 63°C; A•T, 62°C; G•C, 64°C; C•G, 64°C). The T_m values of the triplex-to-duplex transition for all the combinations of third strands are summarized in Table 1.

Table 1. Melting temperature values (±1°C) of the triplexes whose sequences are described at the top of the table.

```
3' TGTCAA TTCTTCTT X TTTCT AACTCG 5'   26YX
5' ACAGTT AAGAAGAA Y AAAGA TTGAGC 3'   26RY
     5' TTCTTCTT Z TTTCT 3'            14YZ
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Tm (±1°C)

X•Y / Z	T•A	A•T	G•C	C•G
A	18	18	15	30
C	10	14	24	**45**
G	08	**28**	19	22
T	**37**	10	**26**	24
L	11	20	20	10
U	**38**	12	**26**	27
C	05	11	14	32
M	14	14	15	07
None	22	< 5	13	09

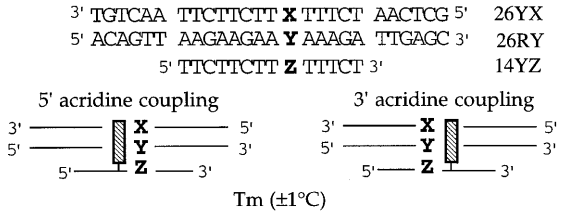
The values are obtained for all X•Y*Z triplet combinations. 'Z = None' indicates the absence of any nucleotide or base analog moiety at the Z position on the third strand. Experimental conditions are described in Materials and Methods.

For a fully complementary target, the canonical triple helix with a 14mer TFO exhibited a T_m of 37°C for X•Y*Z = T•A*T and 45°C for a C•G*C triplet. Substituting T with U (C5-propynyl U) did not markedly change the stability of the triplexes whereas C (C5-propynyl C) led to a reduction of T_m. The destabilizing effect of C has been previously described (35, see below).

In the case of a single base pair inversion (X•Y = A•T or G•C) at the center of the target duplex, a dramatic decrease of T_m values was observed as compared to the related perfectly matched triplexes. The least destabilization was observed for A•T*G and G•C*T combinations giving T_m values of 28 and 26°C, respectively. This result is in agreement with the observations reported earlier (22,24,36). It is noteworthy that the base analog U used as a Z nucleotide in TFO facing the G•C base pair inversion formed a triplex that had a similar stability to the triplex containing the G•C*T triplet.

A propanediol linker (L) was also introduced at Z position of the third strand. With inverted base pairs A•T and G•C the triplexes were more stable (T_m ~20°C) than with the T•A or C•G base pairs (T_m = 10–11°C). A propanediol linker without any base facing the inverted base pair is not as destabilizing as compared to the best of the natural bases at Z position (ΔT_m = 8°C for A•T and 6°C for G•C base pairs). When a base was missing in the third strand (Z = 'none' in Table 1) the triplexes were strongly destabilized. The moderate T_m value observed with X•Y = T•A (T_m = 22°C) is probably due to the presence of a stretch of five thymines in the 13mer allowing formation of 11

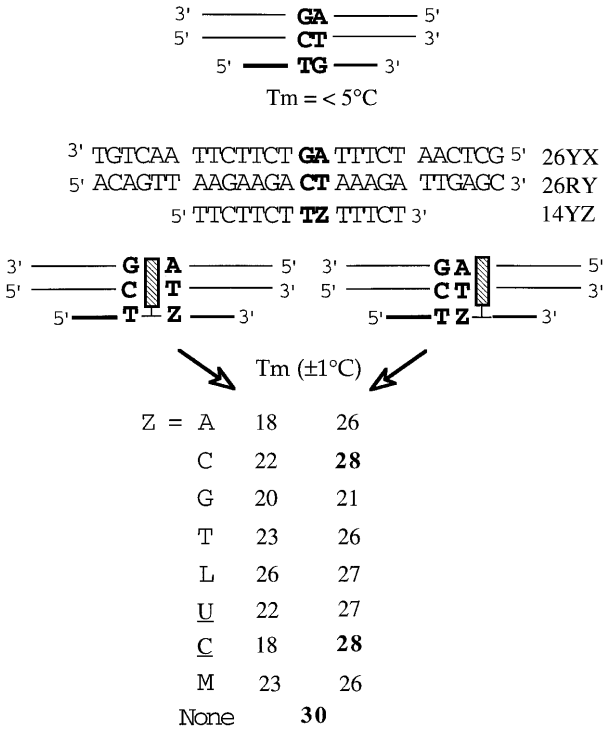
Table 2. Melting temperatures (±1°C) of the triplexes formed upon binding of acridine-containing third strand oligonucleotides to a 26 bp oligopyrimidine•oligopurine DNA fragment in which a single base pair inversion was introduced



X•Y =	T•A		A•T		G•C		C•G	
Position of acridine with respect to Z	5'	3'	5'	3'	5'	3'	5'	3'
Z = A	22	40	26	36	23	33	24	38
C	26	34	29	36	27	**41**	41	46
G	23	34	28	35	30	32	24	46
T	**45**	46	32	35	28	37	27	37
L	30	34	34	38	31	35	30	34
U	44	**50**	32	35	26	36	36	38
C	22	36	26	32	24	40	38	**48**
M	23	32	30	36	28	34	24	34
None	33		**41**		34		34	

The intercalation sites of acridine derivative (hatched rectangle) and its position with respect to Z are indicated. The T_m values of best combinations in each case are highlighted in bold. 'Z = None' denotes that no nucleotide or linker except acridine (W) was added facing the base pair inversion site. Experimental conditions are described in Materials and Methods.

Table 3. Melting temperatures of the triplexes formed when acridine containing oligonucleotides (centre and right) bind to the duplex target (shown at the top) containing a double base pair inversion.



Z =		
A	18	26
C	22	**28**
G	20	21
T	23	26
L	26	27
U	22	27
C	18	**28**
M	23	26
None	**30**	

The T_m values of other triplex combinations formed in the absence of acridine were found to be less than 5°C. A representative example is shown where A•T*Z = A•T*G. Experimental conditions are described in Materials and Methods.

contiguous base triplets upon shifting the third strand by one nucleotide as compared to the 14mer.

When Z was a 3-nitropyrrole (M), it was shown to form triplexes with similar low stability for X•Y = T•A, A•T and G•C base pairs (T_m ~14–15°C). With X•Y = C•G one of the lowest stabilities was observed.

Introduction of two purine and two pyrimidine bases at the central position of the oligopyrimidine•oligopurine duplex causing a double base pair inversion in the target duplex further destabilized the triplexes. The triplexes formed had a very poor thermal stability and the corresponding T_m values in all cases were found to be below 5°C. A representative example showing triplex sequence and T_m value is given in Table 3.

Effect of single and double mismatches on the stability of triplexes formed by acridine-containing TFOs. The binding affinity of TFOs to their target duplex sequences has been shown to be improved upon covalent attachment of a DNA intercalating agent at the end of TFOs (33,37,38). In the present study we have used TFOs with an internally incorporated acridine derivative (W) (Table 2, upper part) on the 5′- or 3′-side of the Z nucleotide. The incorporation of acridine was made in such a way that the 3-carbon internucleotide phosphate distance was maintained (Fig. 1). Previous thermal denaturation experiments have described the effect of such acridine-containing oligonucleotides on the stability of triplexes in which the flanking base triplets were T•A*T and C•G*C on the 5′- and 3′-side of base pair inversion sites respectively (29). In the present study, both flanking triplets are T•AxT, and the T_m values for all triplex combinations are shown in Table 2.

A general survey of the T_m values shows that incorporation of an acridine derivative at an internal site of the third strand stabilizes these triplexes. The extent of stabilization is always greater when acridine is located on the 3′-side of the Z base. The triplex containing canonical base triplets (X•Y*Z = T•A*T, T_m = 37°C) was stabilized by 9°C with the third strand containing acridine (Z = 5′-TpW-3′), raising the T_m value to 46°C. Such an effect was not found significant for the triplex containing a C•G*C triplet at the central position where the T_m value remained nearly unchanged (46°C verses 45°C) even in the presence of acridine on the 3′-side of Z (Z = 5′-CpW-3′, in Table 2). This result could be expected due to electrostatic repulsion between the C•G*C^+ triplet and the acridine derivative which are both positively charged (29). The incorporation of an acridine on the 3′-side of guanine significantly stabilized the triplex formed with the C•G*G triplet (T_m = 46°C), while the T_m of the same triplex but without acridine was 22°C (Table 1). The triplexes containing a T•A*U or a C•G*C base triplet with acridine on the 3′-side were stabilized by 12 and 16°C, respectively, as compared to triplexes without acridine. It came out that Z = 5′-UpW-3′ and Z = 5′-CpW-3′ provided the most stable triplex facing T•A and C•G base pairs, respectively. It is noteworthy that the destabilization afforded by the C5-propyne

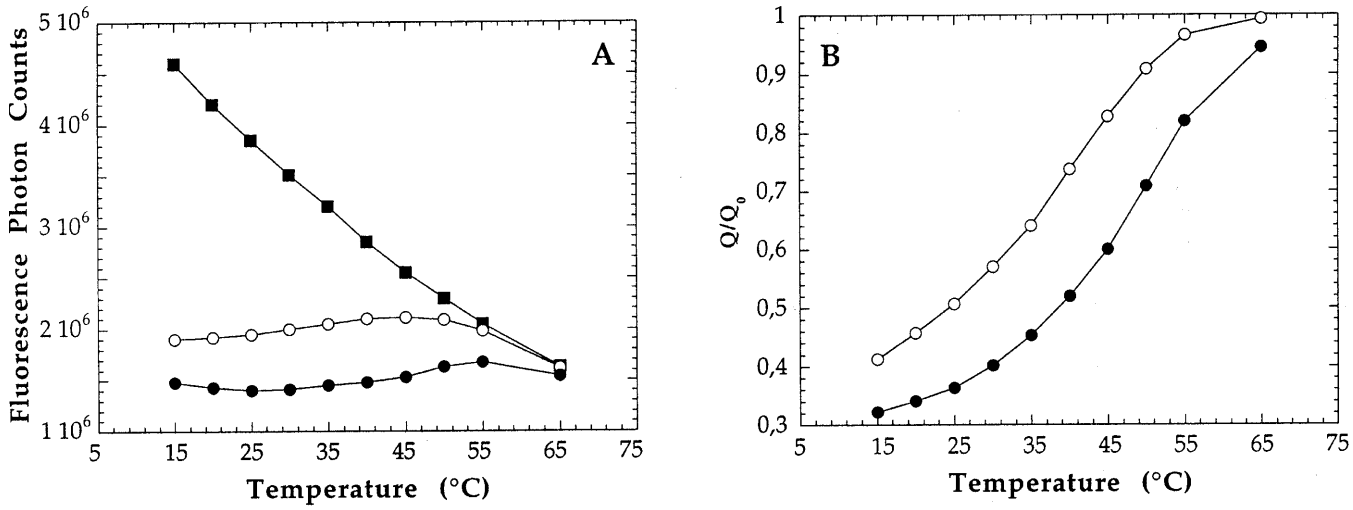


Figure 3. (**A**) Fluorescence intensity of a free acridine-containing oligonucleotide 14CW (filled square), and that of the same oligonucleotide in the presence of the target double helix 26YC•26RG (filled circle) or 26 YG•26RC (open circle) at various temperatures. Experimental conditions are described in Materials and Methods. (**B**) The ratio of fluorescence intensity of the acridine-containing oligonucleotide in the presence and in the absence of the target double helix at various temperatures.

substituent in the third strand of the C•G*C^+ triplet was more than compensated when an acridine was incorporated on the 3′-side of this modified base.

At the A•T base pair inversion site, the highest stabilization was obtained when an acridine moiety alone was incorporated facing the A•T base pair (Z = none, T_m = 41°C). At the site of G•C base pair inversion, the highest stabilization was obtained when an acridine was incorporated on the 3′-side of a cytosine facing the A•T base pair (Z = 5′-CpW-3′, T_m = 41°C), whereas the best choice was Z = T or U (T_m = 26°C) in the absence of acridine.

The triplexes formed with a double base pair inversion in the target sequences showed very poor thermal stability. A representative example is shown in Table 3 where, even when the two least destabilizing A•T*G and G•C*T triplets were used, the T_m value was <5°C. Such triplexes were stabilized when acridine was incorporated in TFOs at the position between or on the 3′-side of the two bases facing the doubly inverted base pairs (Table 3). In general, it was observed that incorporation of an acridine derivative between the two bases facing inverted base pairs, was less effective in stabilizing triplexes than acridine incorporated on the 3′-side of Z position. The highest stabilization was achieved when Z = None (T_m = 30°C). This observation was similar to that found in the case of single base pair inversion, where an acridine moiety alone at Z nucleotide position on the third strand facing A•T base pair provided the highest stabilization.

Fluorescence quenching

The fluorescence properties of acridine were used to monitor triple helix formation. It was previously reported that the fluorescence of 2-methoxy,6-chloro,9-aminoacridine is quenched when it intercalates into double and triple helices and stacks with guanines (33,34). We have exploited this property to investigate the binding of acridine containing oligonucleotides to double-helical DNA. As shown on Figure 3A an apparent quenching of the acridine fluorescence at room temperature is observed in the samples containing the acridine oligonucleotide conjugate 14CW and the target DNA double-helical sequence in which a C•G or G•C base pair (26YC•26RG or 26YG•26RC) is located at the central position facing the cytosine on the 5′-side of the acridine in 14CW. This fluorescence quenching is totally relieved at high temperature. It should be noted that the fluorescence intensity of the free oligonucleotide decreases when the temperature is raised. No significant quenching is observed when the duplex contains a T•A or an A•T base pair at the central position (26T•A or 26YA•RT) instead of a C•G or a G•C base pair (data not shown). Figure 3B shows the temperature dependence of the ratio of fluorescence intensities (Q) of the complex formed between the duplex 26YC•26RG or 26YG•26RC and the TFO (14CW), relative to that of the free TFO (Q_0). The melting profiles obtained by fluorescence measurement (Fig. 3B) provide T_m values similar to those obtained in the UV thermal denaturation study (see above). The decrease in fluorescence intensity at low temperature reaches ~70%. It should be pointed out that the excitation wavelength (424 nm) was not chosen at the isosbestic point observed when acridine intercalates into double and triple helices. However, the hypochromism at 424 nm is <30% for a fully intercalated molecule, as previously reported (33) and cannot be the only phenomenon responsible for the observed fluorescence decrease. Therefore, the fluorescence quenching observed when the triplex is formed is likely due to stacking of the acridine ring with a neighbouring guanine. The absence of quenching when X•Y = T•A or A•T despite binding of the third strand oligonucleotide supports this interpretation. These results provide strong evidence that the stabilization of the triple-helical complexes observed with acridine-containing oligonucleotides is due to intercalation of acridine between two base triplets.

DISCUSSION

In the present work, it is shown that the decrease of triplex stability caused by an interruption of the oligopurine target sequence by one or two pyrimidine bases, can be compensated by

using a TFO containing an internally incorporated intercalator on the 3′-side of the natural or modified base (Z) facing the imperfect target sequence. The incorporation of an acridine on the 3′-side of the Z position on TFOs, gives the best stabilization of perfect and imperfect sequences. This is probably due to the creation of a 5′-YpR-3′ step in the oligopurine sequence which is a favored intercalation site for many intercalators including acridine derivatives (39–41). The fluorescence quenching study shows that acridine is likely intercalated in the triplex as its fluorescence quantum yield drops upon binding due to stacking with the guanine residue located in the neighboring base pair.

TFOs containing base analogues U and C which have been shown to be effective antisense inhibitors of gene expression as a result of enhanced binding to the target RNA sequence (42,43), are also shown to bind to perfect or imperfect duplex DNA targets by triple helix formation (35). The TFO containing a single U residue (14YU) behaves similarly to its analog containing a natural T residue (14YT), whereas a single C residue in TFO (14YC) destabilizes triplex in all cases as compared to natural C residue (14YC) (Table 1). The destabilizing effect of C in the triplex involving a C•G*C^+ triplet (in which C^+ is protonated) was previously interpreted as a decrease of its pKa due to electron withdrawing at the N3 atom subsequent to the C5-propyne substitution (35). However, this could not explain the destabilization observed in other mismatched triplets (T•A*C, A•T*C and G•C*C) in which the protonation of C is not expected. It should be noted that: (i) TFOs in which an acridine is incorporated on the 3′-side of C (5′-CpW-3′) exhibit a stability similar to that of its natural analog C (5′-CpW-3′); (ii) TFOs containg 5′-CpW-3′, 5′-GpW-3′ and 5′-CpW-3′ triplets form triplexes with similar stability (Table 2).

The present results can be compared with data reported in an earlier study (29): the base pair inversion sites were flanked by a T•A*T triplet on the 5′-side in both works, but a T•A*T triplet (present study) and a C•G*C triplet (earlier study) were present on the 3′-side. Therefore, sequence effects can be primarily ascribed to the nature of flanking triplets. In the absence of base pair inversion, the target T•A base pair at X•Y position in both studies gave most stable triplexes when Z position in TFO contained T as expected for a T•A*T base triplet. In addition, in the present study the U base analog gives higher stability than the natural base T. It should also be pointed out that, in the present study, the C•G target site is recognized better by the C base analog than by natural bases C and G in the presence of acridine (Table 2). At an A•T base pair inversion site, an acridine derivative alone facing the A•T base pair (present work), or an acridine derivative incorporated internally on the 3′-side of a propanediol linker (L) in the third strand (previous work) provides the highest stabilization of the triplex. At the G•C inversion site, 5′-CpW-3′ and 5′-WpG-3′ are the best choices, in the present and the previous works, respectively. One simple explanation of flanking sequence effect could be the charge repulsion between the positively charged C•G*C^+ triplet and acridine derivative. Therefore, when the acridine is attached to the 3′-side of the Z nucleotide one could anticipate that a C•G*C^+ and a T•A*T triplet, respectively, on the 5′- and 3′-sides, would behave similarly to T•A*T triplets on both sides. The presence of C•G*C^+ triplets on both sides is expected to give only a marginal increase of stability with internally incorporated acridine.

It should be noted that the use of an intercalator reduces sequence selectivity to some extent depending on the sequence. The absence of selectivity was anticipated due to the absence of any recognition element in the intercalating agent. However, the combination of intercalator (W) with Z nucleotide still provides some selectivity. In the present work, it appears that the 5′-UpW-3′, 5′-W-3′ and 5′-GpW can afford reasonably good selectivity for X•Y = T•A (>12°C difference with other base pairs), A•T (>7°C) and C•G (>11°C), respectively. The G•C base pair cannot be recognized specifically since the most stable triplets (5′-CpW-3′ and 5′-CpW-3′) recognize also the C•G base pair. For inverted base pairs we observed good selectivity when acridine alone (Z = none) was incorporated at the site facing the inverted A•T base pair.

CONCLUSION

The incorporation of acridine within TFOs allows stable triple helix formation and can tolerate the presence of single and/or double base pair inversion in the oligopyrimidine•oligopurine target sequences. The insertion of an acridine on the 3′-side of the nucleotide facing the inverted base pair generally provides higher stability. Based on the present and the previous studies (29), the observed effect of flanking base triplets could be primarily ascribed to charge repulsion. These works open a possibility of extending the range of DNA sequences which can be recognized by intercalator-containing oligonucleotides. A number of triplex-specific intercalators have been described (44–46). Work is under way to incorporate triplex-specific intercalators instead of acridine into TFOs in order to enhance the stabilizing effect. Some encouraging results have recently been reported using a benzopyridoindole or a benzopyridoquinoxaline derivative linked through a reduced Schiff base bond at an abasic site (47,48).

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