Triplex Formation by Oligonucleotides Bearing Organomercurated Base Moieties

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Abstract

Homothymine oligonucleotides bearing a single 5-mercuricytosine or 5-mercuriuracil residue at their termini have been synthesized and their capacity to form triplexes has been examined with an extensive array of double-helical targets. UV and CD melting experiments revealed formation and thermal denaturation of pyrimidine·purine*pyrimidine –type triple helices with all oligonucleotide combinations studied. Nearly all triplexes were destabilized on mercuration of the 3′-terminal residue of the triplex forming oligonucleotide (TFO), in all likelihood due to competing intramolecular Hg(II)-mediated base pairing. Two exceptions from this general pattern were, however, observed: 5-mercuricytosine was stabilizing when placed opposite to a T·A or A·T base pair. The stabilization was further amplified in the presence of 2-mercaptoethanol (but not hexanethiol, thiophenol or cysteine), suggesting a stabilizing interaction other than Hg(II)-mediated base pairing.

Introduction

Triplex forming oligonucleotides (TFOs) bind in the major groove of oligopyrimidine·oligopurine DNA sequences, either by Hoogsteen[1] or reverese Hoogsteen[2] base pairing. TFOs allow sequence-specific targeting of genomic DNA without unwinding of the double helix and have already found use as versatile tools in biotechnology.[3, 4] They also hold great, if yet to be fulfilled, promise as therapeutic agents in antigene strategy.[5]

In addition to the challenges faced by any oligonucleotide-based therapy,[6] antigene strategy is further limited by its requirement of a homopurine target sequence as well as the relatively low hybridization affinity of TFOs. The first limitation is actually not as severe as would first appear, owing to the abundance of appropriate target sequences at promoter regions of the human genome.[7] Weak hybridization is a bigger problem and considerable effort has been invested to overcome it.[4, 8] Approaches explored to improve the hybridization affinity of TFOs include introduction of positive charges on the sugar or base moieties,[9] replacement of the negatively charged sugar – phosphate backbone with an uncharged one,[10] conformational restraints[11] and attachment of triplex stabilizing conjugate groups, such as intercalators[12] or aminoglycosides.[13, 14]

The potential of metal mediated base pairing to stabilize double helices has been repeatedly demonstrated with a number of transition metal ions.[15] Corresponding studies on triple helices are scarce but encouraging results have nonetheless been obtained with homopyridine TFOs incorporating a single N7-platinated guanosine residue.[16] Cu(II) and Ag(I) have been proposed to coordinate between cytosine-N3 and guanine-N7, allowing C*G Hoogsteen pairing under conditions where cytosine is not appreciably protonated.[17] Finally, it is interesting to speculate on whether some early results on sequence-dependent stabilization of triple helices by divalent transition metal ions[18] could also be explained by metal mediated base pairing.

We have recently described base pairing properties of 5-mercuricytosine as a first example of an organometallic nucleobase surrogate.[19] Like platinated bases, organomercurated bases also resist dissociation under metal-deficient conditions but still allow rapid exchange of the ligand *trans* to the C donor, thus avoiding off-target crosslinking. Within double-helical oligonucleotides, 5-mercuricytosine favored pairing with thymine and guanine, i.e. bases with a donor atom (N3 and N1, respectively) that is readily deprotonated on coordination of Hg(II).[19] Such donor atoms are not available on the Hoogsteen face but adenine-N7 seems like a reasonable alternative given the high stability of the respective MeHg(II) complex.[20] In the present study we report on the hybridization of TFOs bearing a 3´-terminal 5-mercuricytosine or 5-mercuriuracil residue with target duplexes varied at the base pair directly opposite to the mercuracted base. Curiously, considerable triplex (and duplex) stabilization by the 5-mercuricytosine modification was observed not only with A·T but also with T·A as the target base pair.

Results and Discussion

Synthesis of the mercurated TFOs

The sequences of the oligodeoxynucleotides used are summarized in Table 1. The target duplexes (ON1x·ON2y, Fig. 1) featured a 15-mer homoadenine·homothymine sequence flanked by two extra base pairs at both ends to ensure sufficient thermal stability as well as formation of an antiparallel (rather than parallel) duplex. The homoadenine·homothymine sequence, in turn, was chosen to allow triplex formation at physiological pH. The base pair immediately upstream of the homothymine stretch was varied to test the sequence-specificity of triplex formation. The TFOs (ON3c, ON3c-Hg, ON3u and ON3u-Hg, Table 1), in turn, had a 15-mer homothymine sequence followed by a 3´-terminal 2´-deoxycytidine or 2´-deoxyuridine, either unmodified or mercurated at C5.

Mercuration of ON3c and ON3u was carried out at 2000 mM concentration of mercuric acetate and 150 mM concentration of the oligonucleotide. Under these conditions, covalent mercuration only takes place at the 3´-terminal cytosine or uracil base.[21] The reactions were run at 55 °C for 24 h, after which the mercurated products were purified by RP-HPLC. Repeated purifications were needed for complete removal of excess mercury salts, undoubtedly owing to the high affinity of Hg(II) for thymine-N3.[22] Finally, incorporation of a single Hg(II) ion was confirmed by ESI-MS analysis.

Table 1. Sequences of the oligodeoxynucleotides used in this study.

	Sequence[a]
ON1a	5´-G<u>A</u>T TTT TTT TTT TTT TTG C-3´
ON1c	5´-G<u>C</u>T TTT TTT TTT TTT TTG C-3´
ON1g	5´-G<u>G</u>T TTT TTT TTT TTT TTG C-3´
ON1t	5´-G<u>T</u>T TTT TTT TTT TTT TTG C-3´
ON2a	5´-GCA AAA AAA AAA AAA AA<u>A</u> C-3´
ON2c	5´-GCA AAA AAA AAA AAA AA<u>C</u> C-3´
ON2g	5´-GCA AAA AAA AAA AAA AA<u>G</u> C-3´
ON2t	5´-GCA AAA AAA AAA AAA AA<u>T</u> C-3´
ON3c	5´-TTT TTT TTT TTT TTT <u>C</u>-3´
ON3c-Hg	5´-TTT TTT TTT TTT TTT <u>C^{Hg}</u>-3´
ON3u	5´-TTT TTT TTT TTT TTT <u>U</u>-3´
ON3u-Hg	5´TTT TTT TTT TTT TTT <u>U^{Hg}</u>-3´

[a] C^{Hg} refers to 5-acetoxymercuricytosine and U^{Hg} to 5-acetoxymercuriuracil. In each sequence, the residue varied in the hybridization studies has been underlined.

Hybridization studies

Figure 1 outlines the hybridization assay used. Besides canonical Watson-Crick base pairs, homo base pairs were also tested at the variable site (X•Y), for a total of eight different target duplexes. Hybridization affinity of the TFOs with these duplexes was assessed by thermal denaturation experiments at pH 7.4 (20 mM cacodylate buffer) and 0.10 M ionic strength (adjusted with sodium perchlorate). All of the oligonucleotides were used in 1.0 mM concentration. To rule out the possibility of stabilization (or destabilization) by free Hg(II), measurements involving the unmercurated TFOs were repeated in the presence of 1.0 mM mercuric perchlorate.

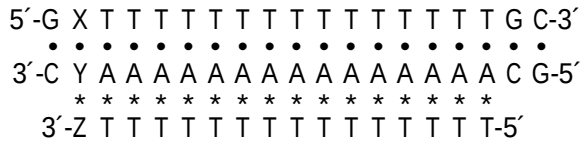

Figure 1 General outline of the hybridization assay used. X and Y are any of the canonical nucleobases and Z either cytosine, uracil, 5-acetoxymercuricytosine or 5-acetoxymercuriuracil. Watson-Crick base pairs are indicated by bullets and Hoogsteen base pairs by asterisks.

Most of the triplexes studied exhibited clearly biphasic melting profiles (Fig. 2 for ON1t•ON2a*ON3c and ON1t•ON2a*ON3c-Hg, the other profiles are presented in the Supporting Information), allowing reliable determination of the Watson-Crick and Hoogsteen melting temperatures. In a few cases (ON1a•ON2a*ON3c, ON1a•ON2t*ON3u, ON1c•ON2c*ON3u, ON1c•ON2c*ON3u-Hg, ON1t•ON2t*ON3u and ON1t•ON2t*ON3u-Hg), Hoogsteen melting could not be detected unambiguously. This phenomenon has been reported previously and attributed to the similarity in absorbance between a triplex and its constituent Watson-Crick duplex and TFO.[23, 24]

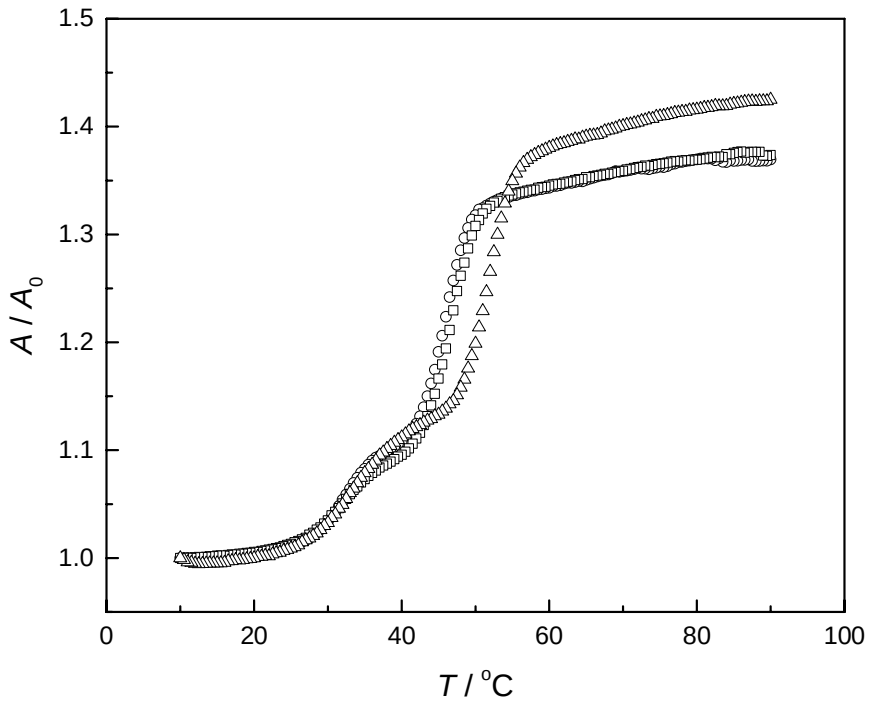

Figure 2 Melting profiles for triplexes ON1t•ON2a*ON3c-Hg (r) and ON1t•ON2a*ON3c in the absence (™) and presence (£) of 1.0 eq. of mercuric perchlorate; pH = 7.4 (20 mM cacodylate buffer); *I*($NaClO_4$) = 0.10 M; [oligonucleotides] = 1.0 mM; [$Hg(ClO_4)_2$] = 0 / 1.0 mM.

Hoogsteen and Watson-Crick melting temperatures of all of the triplexes studied are summarized in Tables 2 and 3, respectively. With triplexes formed by the unmercurated TFOs, Watson-Crick T_m values ranged from 45 to 50 °C for the matched and from 40 to 44 °C for the mismatched duplexes and were largely independent on the sequence of the TFO (ON3c or ON3u). The Hoogsteen T_m values, on the other hand, ranged from 31 to 32 °C with ON3c and from 33 to 35 °C with ON3u and did not show any clear dependence on the sequence of the Watson-Crick duplex. Addition of 1.0 eq. of mercuric perchlorate selectively stabilized the ON1t•ON2t duplex, undoubtedly by the well-documented T-Hg(II)-T base pairing.[25] Besides this expected stabilization, a somewhat anomalous increase of the Hoogsteen T_m of the ON1a•ON2t*ON3c triplex was observed, the other melting temperatures being largely unaffected.

Table 2 Hoogsteen melting temperatures of triplexes formed by ON3c, ON3c-Hg, ON3u and ON3u-Hg with various target duplexes; pH = 7.4 (20 mM cacodylate buffer); $I(NaClO_4)$ = 0.10 M; [oligonucleotides] = 1.0 mM; $[Hg(ClO_4)_2]$ = 0 / 1.0 mM.

	Hoogsteen T_m / °C					
	ON3c	ON3c + Hg[a]	ON3c-Hg[a]	ON3u	ON3u + Hg[a]	ON3u-Hg[a]
ON1t•ON2a	31.8 ± 0.5	31.8 ± 0.8 (±0.0)	33.7 ± 0.7 (+1.9)	34.7 ± 0.5	33.8 ± 0.4 (-0.9)	26.2 ± 0.9 (-8.5)
ON1a•ON2t	30.8 ± 0.6	34.1 ± 0.2 (+3.3)	34.6 ± 0.2 (+3.8)	n/a[b]	n/a[b]	30.0 ± 0.9
ON1g•ON2c	30.9 ± 0.7	30.5 ± 0.6 (-0.4)	27.5 ± 0.2 (-3.4)	33.3 ± 0.5	32.7 ± 0.6 (-0.6)	27.1 ± 0.4 (-6.2)
ON1c•ON2g	31.0 ± 0.8	32.1 ± 0.6 (+1.1)	26.1 ± 0.7 (-4.9)	33.8 ± 0.3	33.2 ± 0.2 (-0.6)	27.8 ± 0.6 (-6.0)
ON1a•ON2a	n/a[b]	n/a[b]	26.8 ± 0.9	35.0 ± 0.9	34.4 ± 0.5 (-0.6)	28.4 ± 0.8 (-6.6)
ON1c•ON2c	31.9 ± 0.2	31.3 ± 0.2 (-0.6)	25.8 ± 0.7 (-6.1)	n/a[b]	n/a[b]	n/a[b]
ON1g•ON2g	32.4 ± 0.2	31.9 ± 0.5 (-0.5)	29.0 ± 0.6 (-3.4)	34.5 ± 0.4	33.5 ± 0.3 (-1.0)	27.4 ± 0.4 (-7.1)
ON1t•ON2t	32.2 ± 0.4	32.8 ± 0.5 (+0.6)	27.9 ± 1.2 (-4.3)	n/a[b]	n/a[b]	n/a[b]

[a] Values in parentheses refer to change in T_m relative to the value obtained with the unmercurated TFO (ON3c or ON3u) in the absence of Hg(II).

[b] Hoogsteen T_m could not be determined reliably from the UV melting profile.

Table 3 Watson-Crick melting temperatures of triplexes formed by ON3c, ON3c-Hg, ON3u and ON3u-Hg with various target duplexes; pH = 7.4 (20 mM cacodylate buffer); $I(NaClO_4)$ = 0.10 M; [oligonucleotides] = 1.0 mM; $[Hg(ClO_4)_2]$ = 0 / 1.0 mM.

	Watson-Crick T_m / °C					
	ON3c	ON3c + Hg[a]	ON3c-Hg[a]	ON3u	ON3u + Hg[a]	ON3u-Hg[a]
ON1t•ON2a	45.7 ± 0.3	46.2 ± 0.7 (+0.5)	51.8 ± 0.2 (+6.1)	47.3 ± 0.3	46.7 ± 0.4 (-0.6)	46.3 ± 0.4 (-1.0)
ON1a•ON2t	45.3 ± 0.3	46.5 ± 0.7 (+1.2)	51.5 ± 0.4 (+6.2)	46.8 ± 0.7	46.8 ± 0.3 (±0.0)	45.4 ± 0.3 (-1.4)
ON1g•ON2c	47.1 ± 0.5	47.7 ± 0.7 (+0.6)	46.9 ± 0.6 (-0.2)	48.3 ± 0.2	47.7 ± 0.2 (-0.6)	47.5 ± 0.3 (-0.8)
ON1c•ON2g	48.1 ± 0.6	48.9 ± 0.4 (+0.8)	47.9 ± 0.5 (-0.2)	49.5 ± 0.2	49.2 ± 0.2 (-0.3)	48.4 ± 0.3 (-1.1)
ON1a•ON2a	40.9 ± 0.3	41.3 ± 0.8 (+0.4)	40.9 ± 0.4 (±0.0)	42.0 ± 0.4	41.3 ± 0.4 (-0.7)	41.3 ± 0.2 (-0.7)
ON1c•ON2c	41.8 ± 0.2	42.3 ± 0.5 (+0.5)	41.7 ± 0.6 (-0.1)	42.3 ± 0.3	41.5 ± 0.3 (-0.8)	41.6 ± 0.4 (-0.7)
ON1g•ON2g	43.2 ± 0.4	43.3 ± 0.6 (+1.0)	43.0 ± 0.6 (+0.2)	44.4 ± 0.3	43.4 ± 0.3 (-1.0)	43.1 ± 0.4 (-1.3)
ON1t•ON2t	41.8 ± 0.3	45.2 ± 0.2 (+3.4)	44.0 ± 0.3 (+2.2)	42.4 ± 0.4	44.3 ± 0.3 (+1.9)	43.0 ± 0.7 (+0.6)

[a] Values in parentheses refer to change in T_m relative to the value obtained with the unmercurated TFO (ON3c or ON3u) in the absence of Hg(II).

A very different pattern was observed with triplexes formed by the mercurated TFOs. While the Watson-Crick T_m values were in most cases unaffected by mercuration of the 3´-terminal base, the Hoogsteen T_m values were decreased by 3 – 9 °C. This destabilization was more pronounced with ON3u-Hg than with ON3c-Hg and probably stems from competition between Hoogsteen base pairing and intrastrand Hg(II)-mediated base pairing of the mercurated residue with one of the fifteen thymine bases. Interestingly, two mercurated triplexes stood out from the general pattern: with ON1t•ON2a*ON3c-Hg and ON1a•ON2t*ON3c-Hg, both Hoogsteen and, especially, Watson-Crick melting temperatures were higher than with the respective unmercurated triplexes. Mutual dependence of the triplex and duplex stabilities on each other has been reported previously for a number of related systems[14, 26] and a common origin for the increased Hoogsteen and Watson-Crick T_m values appears likely also in the present case.

The sequence-specificity of the observed triplex stabilization on covalent mercuration of the 3´-terminal cytosine residue could be explained by Hg(II)-mediated Hoogsteen-type base pairing, although the absence of similar stabilization on mercuration of a uracil residue appears enigmatic. This possibility was further explored by repeating the melting temperature experiments described above in the presence of 100 mM 2-mercaptoethanol, a very strong ligand for Hg(II).[27] Under these conditions, Hg(II)-mediated base pairing is effectively precluded while canonical Watson-Crick base pairing is unaffected. Melting profiles of ON1t•ON2a*ON3c and ON1t•ON2a*ON3c-Hg are presented in Fig. 3 and the other profiles in the Supporting Information.

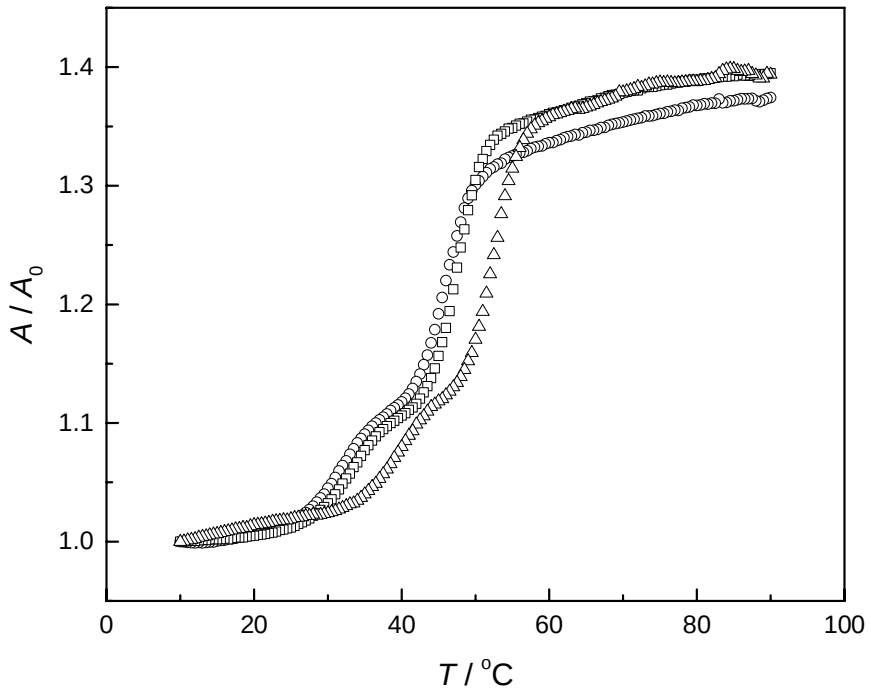

Figure 3 Melting profiles for triplexes ON1t•ON2a*ON3c-Hg (r) and ON1t•ON2a*ON3c in the absence (™) and presence (£) of 1.0 eq. of mercuric perchlorate; pH = 7.4 (20 mM cacodylate buffer); *I*($NaClO_4$) = 0.10 M; [oligonucleotides] = 1.0 mM; [$Hg(ClO_4)_2$] = 0 / 1.0 mM; [2-mercaptoethanol] = 100 mM.

Hoogsteen and Watson-Crick melting temperatures obtained in the presence of 100 mM 2-mercaptoethanol are summarized in Tables 4 and 5, respectively. As expected, melting temperatures of the unmercurated triplexes were insensitive to the presence of 2-mercaptoethanol. Stabilization of the ON1t•ON2t duplex by mercuric perchlorate was no longer observed, consistent with disruption of the T-Hg(II)-T base pair. Increase of the Hoogsteen T_m of ON1a•ON2t*ON3c was also less marked than in the absence of 2-mercaptoethanol but still detectable.

Table 4 Hoogsteen melting temperatures of triplexes formed by ON3c, ON3c-Hg, ON3u and ON3u-Hg with various target duplexes; pH = 7.4 (20 mM cacodylate buffer); $I(NaClO_4)$ = 0.10 M; [oligonucleotides] = 1.0 mM; $[Hg(ClO_4)_2]$ = 0 / 1.0 mM; [2-mercaptoethanol] = 100 mM.

	Hoogsteen T_m / °C					
	ON3c	ON3c + Hg[a]	ON3c-Hg[a]	ON3u	ON3u + Hg[a]	ON3u-Hg[a]
ON1t•ON2a	31.7 ± 0.4	32.8 ± 0.8 (+1.1)	39.0 ± 0.6 (+7.3)	34.0 ± 0.6	34.9 ± 0.8 (+0.9)	n/a[b]
ON1a•ON2t	30.5 ± 0.3	32.5 ± 0.7 (+2.0)	38.1 ± 0.5 (+7.6)	n/a[b]	n/a[b]	32.5 ± 0.8
ON1g•ON2c	31.3 ± 0.1	31.1 ± 0.6 (+0.2)	30.4 ± 0.2 (-0.9)	n/a[b]	33.5 ± 0.4	31.3 ± 0.5
ON1c•ON2g	33.0 ± 0.2	32.8 ± 0.6 (-0.2)	32.3 ± 0.5 (-0.7)	32.6 ± 0.3	34.3 ± 0.6 (+1.7)	33.1 ± 0.4 (+0.5)
ON1a•ON2a	n/a[b]	n/a[b]	34.1 ± 0.4	n/a[b]	35.3 ± 0.7	n/a[b]
ON1c•ON2c	31.4 ± 0.4	32.5 ± 0.4 (+0.9)	30.6 ± 0.4 (-0.8)	n/a[b]	n/a[b]	n/a[b]
ON1g•ON2g	32.5 ± 0.5	32.8 ± 0.3 (+0.3)	32.0 ± 0.5 (-0.5)	32.8 ± 0.2	34.2 ± 0.5 (+1.4)	32.7 ± 0.7 (+0.1)
ON1t•ON2t	33.2 ± 0.4	33.0 ± 0.4 (-0.2)	31.8 ± 0.2 (-1.4)	n/a[b]	n/a[b]	n/a[b]

[a] Values in parentheses refer to change in T_m relative to the value obtained with the unmercurated TFO (ON3c or ON3u) in the absence of Hg(II).

[b] Hoogsteen T_m could not be determined reliably from the UV melting profile.

Table 5 Watson-Crick melting temperatures of triplexes formed by ON3c, ON3c-Hg, ON3u and ON3u-Hg with various target duplexes; pH = 7.4 (20 mM cacodylate buffer); $I(NaClO_4)$ = 0.10 M; [oligonucleotides] = 1.0 mM; $[Hg(ClO_4)_2]$ = 0 / 1.0 mM; [2-mercaptoethanol] = 100 mM.

	Watson-Crick T_m / °C					
	ON3c	ON3c + Hg[a]	ON3c-Hg[a]	ON3u	ON3u + Hg[a]	ON3u-Hg[a]
ON1t•ON2a	45.5 ± 0.3	47.0 ± 0.5 (+1.5)	52.0 ± 0.4 (+6.5)	46.3 ± 0.5	47.6 ± 0.7 (+1.4)	47.1 ± 0.4 (+0.8)
ON1a•ON2t	45.3 ± 0.3	47.2 ± 0.6 (+1.9)	51.6 ± 0.4 (+6.3)	45.7 ± 0.2	47.3 ± 0.7 (+1.6)	46.5 ± 0.6 (+0.8)
ON1g•ON2c	48.2 ± 0.2	48.3 ± 0.3 (+0.1)	47.8 ± 0.3 (-0.4)	47.7 ± 0.2	48.5 ± 0.4 (+0.8)	48.3 ± 0.6 (+0.6)
ON1c•ON2g	49.2 ± 0.2	49.7 ± 0.4 (+0.5)	48.8 ± 0.2 (-0.4)	48.8 ± 0.3	49.9 ± 0.7 (+1.1)	49.5 ± 0.6 (+0.7)
ON1a•ON2a	42.3 ± 0.2	42.4 ± 0.4 (+0.1)	42.0 ± 0.4 (-0.3)	41.8 ± 0.3	41.7 ± 0.4 (-0.1)	41.3 ± 0.3 (-0.5)
ON1c•ON2c	41.8 ± 0.2	42.6 ± 0.4 (+0.8)	41.6 ± 0.6 (-0.2)	41.8 ± 0.4	41.9 ± 0.4 (+0.1)	42.2 ± 0.4 (+0.4)
ON1g•ON2g	45.5 ± 0.6	44.2 ± 0.2 (-1.3)	43.0 ± 0.5 (-2.5)	42.6 ± 0.4	44.1 ± 0.5 (+1.5)	43.2 ± 0.2 (+0.6)
ON1t•ON2t	42.7 ± 0.2	43.1 ± 0.4 (+0.4)	41.3 ± 0.2 (-1.4)	41.6 ± 0.4	41.9 ± 0.6 (+0.3)	41.7 ± 0.4 (+0.1)

[a] Values in parentheses refer to change in T_m relative to the value obtained with the unmercurated TFO (ON3c or ON3u) in the absence of Hg(II).

In the presence of 100 mM 2-mercaptoethanol, triplexes formed by the mercurated and unmercurated TFOs exhibited very similar melting temperatures with almost all target duplexes. In other words, decrease of the Hoogsteen T_m observed with most triplexes on covalent mercuration of the 3´-terminal residue of the TFO was negated on addition of 2-mercaptoethanol, again consistent with disruption of the competing Hg(II)-mediated base pairing. In striking contrast, the selective stabilization of ON1t•ON2a*ON3c-Hg and ON1a•ON2t*ON3c-Hg was even more pronounced (+7.3 and +7.6 °C for the Hoogsteen and +6.5 and +6.3 °C for the Watson-Crick T_m, respectively) in the presence of 2-mercaptoethanol than in the absence thereof. Evidently stabilization of these triplexes cannot be attributed to Hg(II)-mediated base pairing.

The unexpected stabilization of the mercurated triplexes ON1t•ON2a*ON3c-Hg and ON1a•ON2t*ON3c-Hg in the presence of 2-mercaptoethanol prompted us to further investigate the role of the exchangeable ligand of Hg(II). To this end, UV melting profile of ON1t•ON2a*ON3c-Hg was recorded in the presence of 100 mM hexanethiol, thiophenol and cysteine. Hexanethiol has no functional groups besides the sulfhydryl donor and would be expected to stabilize the triplex only through hydrophobic effect. Coordination of thiophenol, in turn, would considerably expand the stacking surface of 5-mercuricytosine and could thus stabilize the triplex through intercalation. Finally, the amino and carboxylate functions of a cysteine ligand should allow more extensive hydrogen bonding and/or electrostatic interactions than the hydroxy function of 2-mercaptoethanol.

Hoogsteen and Watson-Crick melting temperatures of triplex ON1t•ON2a*ON3c-Hg in the presence of 2-mercaptoethanol, hexanethiol, thiophenol and cysteine, as well as in the absence of any thiols, are presented in Fig. 4. Respective values for the unmercurated counterpart ON1t•ON2a*ON3c are included for reference. The extra stabilization observed in the presence of 2-mercaptoethanol could not be reproduced with any of the other thiols studied. In fact, both Hoogsteen and Watson-Crick T_m values decreased back to the levels observed with the respective unmercurated triplex ON1t•ON2a*ON3c.

Previous studies have identified dehydration of the bridging Hg(II) ion and the resulting alleviation of the entropic penalty as the main driving force for Hg(II)-mediated base pairing.[19, 28] Such dehydration could take place when the mercurated residue gets embedded in the major groove, even if the Hg(II) ion is not directly coordinated to the nucleobases of the target duplex. Unfortunately, the Hoogsteen and Watson-Crick melting temperatures are too close to each other for reliable determination of the respective thermodynamic parameters. It is also not obvious how Hg(II) dehydration could result in the sequence-specific stabilization observed and detailed structural studies, beyond the scope of this paper, would be needed to elucidate this matter. Based on the data

at hand, the exact nature of the stabilizing interaction(s) in ON1t•ON2a*ON3c-Hg and ON1a•ON2t*ON3c-Hg remains elusive.

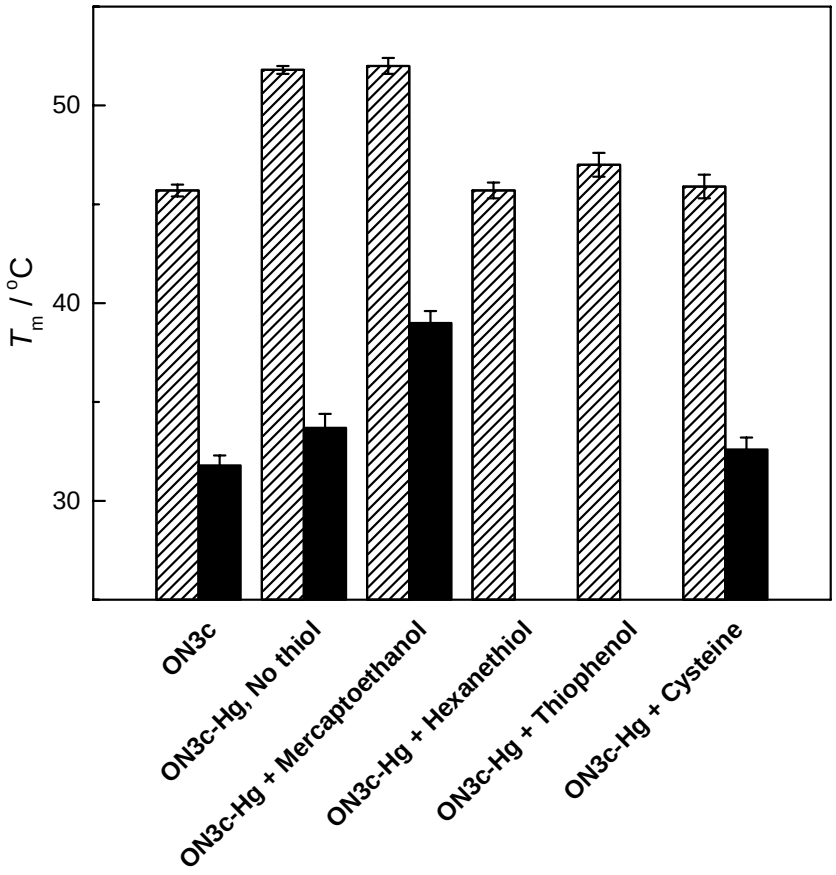

Figure 4 Hoogsteen (solid black bars) and Watson-Crick (hashed bars) melting temperatures of triplexes ON1t•ON2a*ON3c and ON1t•ON2a*ON3c-Hg in the presence of 2-mercaptoethanol, hexanethiol, thiophenol and cysteine and in the absence of any thiols; pH = 7.4 (20 mM cacodylate buffer); *I*($NaClO_4$) = 0.10 M; [oligonucleotides] = 1.0 mM; [thiols] = 0 / 100 mM.

The unmercurated and mercurated triplexes were also characterized CD spectropolarimetrically to verify folding into the expected pyrimidine•purine*pyrimidine secondary structure. The measurements were carried out over a temperature range of 10 – 90 °C at 10 °C intervals under otherwise the same conditions as used for the T_m measurements. All spectra obtained at 10 °C were characteristic of pyrimidine•purine*pyrimidine triple helices,[23, 29] with minima at 248 nm and maxima at 260 and 284 nm (Fig. 5 for ON1t•ON2a*ON3c-Hg, the other profiles are presented in the Supporting

Information). On increasing temperature, the minima at 248 nm and the maxima at 260 nm diminished and the maxima at 284 nm shifted toward shorter wavelengths, consistent with sequential Hoogsteen and Watson-Crick melting processes. Biphasicity of the CD melting profiles was evident even with triplexes for which a Hoogsteen T_m could not be detected but, unfortunately, the relatively low number of data points did not allow reliable supplementation of the UV melting temperatures with CD melting temperatures.

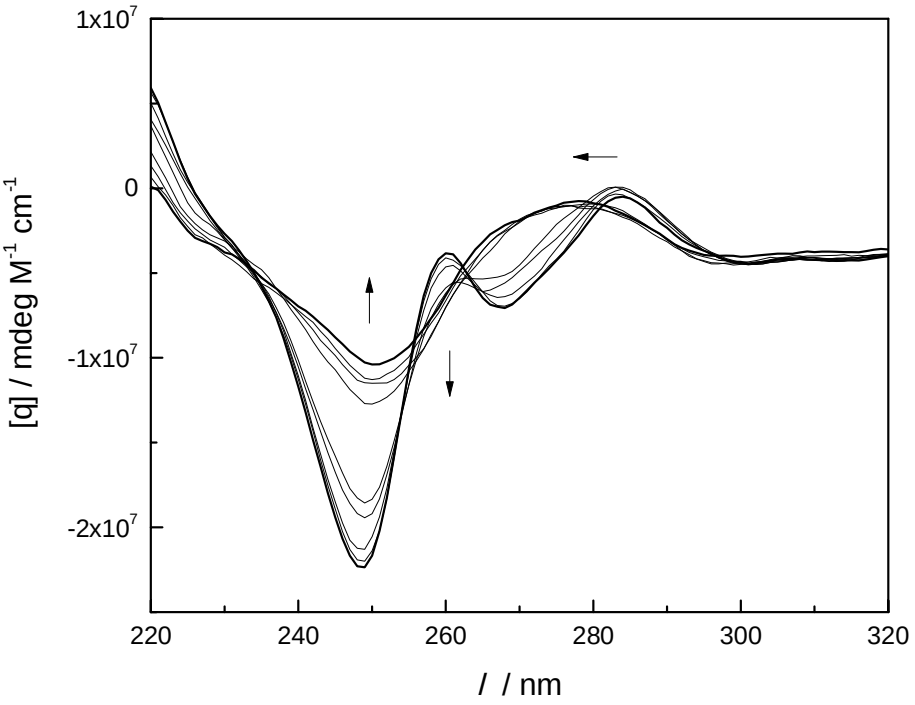

Figure 5 CD spectra of ON1t•ON2a*ON3c-Hg, recorded at 10 °C intervals between 10 and 90 °C; pH = 7.4 (20 mM cacodylate buffer); I($NaClO_4$) = 0.10 M; [oligonucleotides] = 1.0 mM. Spectra acquired at the extreme temperatures are indicated by thicker lines and the thermal shifts of the minima and maxima by arrows.

Conclusions

The hybridization affinity of TFOs bearing covalently mercurated pyrimidine bases tends to be lower than the hybridization affinity of their unmercurated counterparts, owing to competition by intrachain Hg(II)-mediated base pairing. Significant increase in both Hoogsteen and Watson-Crick melting temperatures was, however, observed when 5-mercuricytosine was placed opposite to an A•T or T•A base pair. This stabilization was negated in the presence of hexanethiol, thiophenol or cysteine but,

curiously, amplified in the presence of 2-mercaptoethanol. While the origin of the stabilization remains elusive the results nonetheless suggest that metalated nucleobases could prove a valuable addition to the repertoire of modifications for increasing the hybridization affinity of TFOs.

Experimental Section

General Methods

Mass spectra were recorded on a Bruker Daltonics micrOTOF-Q ESI mass spectrometer, UV spectra on a Perkin-Elmer Lambda 35 UV/Vis spectrophotometer and CD spectra on an Applied Photophysics Chirascan spectropolarimeter. Freshly distilled triethylamine was used for preparation of the HPLC elution buffers. The other reagents, including the unmodified oligonucleotides, were commercial products that were used as received.

Synthesis of the mercurated TFOs ON3c-Hg and ON3u-Hg

Aqueous solutions (200 mL) of ON3c or ON3u (150 mM) and $Hg(OAc)_2$ (2000 mM) were incubated at 55 °C for 24 h. The crude mercurated oligonucleotides thus obtained were purified by RP-HPLC on a Hypersil ODS C18 column (250 × 4.6 mm, 5 µM) eluting with a linear gradient of acetonitrile (10 – 40% over 35 min, flow rate 1.0 ml min^{-1}) in 50 mmol L^{-1} aqueous triethylammonium acetate. Repeated purifications were needed for complete removal of free Hg(II). The purified oligonucleotides were characterized by ESI-TOF-MS (mass spectra presented in the Supporting Information) and quantified UV spectrophotometrically using molar absorptivities calculated by an implementation of the nearest-neighbors method.

Melting temperature measurements

All samples were prepared by mixing the appropriate oligonucleotides (1.0 µM) in 20 mM cacodylate buffer (pH 7.4), the ionic strength of which was adjusted to 0.10 mmol L^{-1} with $NaClO_4$. When applicable, $Hg(ClO_4)_2$ and thiols (2-mercaptoethanol, hexanethiol, thiophenol and cysteine) were used in 1.0 and 100 mM concentration, respectively, and added after mixing the oligonucleotides. Before each experiment, the samples were annealed by heating to 90 °C and then allowing to gradually cool down to room temperature. UV melting curves were acquired by monitoring the absorbance at 260 nm over a temperature range of 10 – 90 °C, sampling at 0.5 °C intervals. The Hoogsteen and Watson-Crick T_m values were determined as inflection points on the UV melting curves.

CD measurements

Samples used in the CD spectropolarimetric measurements were identical to those used in the melting temperature measurements. CD spectra were recorded between 220 and 400 nm over a temperature range of 10 – 90 °C, sampling at 10 °C intervals. At each temperature, samples were allowed to equilibrate for 120 s before recording the spectrum.

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