Smoothing of the thermal stability of DNA duplexes by using modified nucleosides and chaotropic agents

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ABSTRACT
The effect of alkyltrimethylammonium ions on the thermostability of natural and modified DNA duplexes has been investigated. We have shown that the use of tetramethylammonium ions TMA⁺ along with the chemical modification of duplexes allow the fine adjustment of Tm and the possibility of obtaining several duplex systems with varied isostabilized temperatures, some of which show greater stability than those of natural DNA. This approach could be very useful for DNA sequencing by hybridization.

INTRODUCTION
The new method of DNA analysis and sequencing based on the hybridization of the nucleic acid targets with a complete set of oligonucleotides of a given length, immobilized as an ordered array on a solid surface, has become a promising tool in molecular biology (1–8). This strategy which implies the specific detection of hybrids without mismatches faces one basic difficulty due to the large differences in duplex stability according to their base compositions. A perfect hybrid built with an AT-rich sequence would therefore have a similar or even lower stability than that of hybrids built with GC-rich sequences involving one mismatch (9).

To solve this problem, many studies were undertaken, particularly hybridization studies using tetramethylammonium chloride (TMACl) (10–19). Melchoir and Von Hippel have shown that the presence of tetramethylammonium (TMA⁺) and tetraethylammonium (TEA⁺) cations will remove the base composition dependence of native DNA helix melting (11). These results were confirmed for long DNA duplexes (12,13) and later for shorter ones (14) (up to 16 bp) whose GC composition varied from 31 to 81%. However, these small cations were not very efficient for equalizing the stability of short DNA duplexes having <14 bp (14). Recent studies realized with DNA dumb-bells (18) showed that there was not a single concentration of TMA⁺ ion whereby all DNA duplexes exhibited the same melting temperature. Likewise, extensive studies performed in a heterogeneous phase showed that the effect of TMA⁺ cation on the thermal stability of DNA duplexes not only depends on their sequences but also on their lengths (15,16).

In order to smooth DNA duplex thermal stability, we have recently designed a new approach based on the modification of one or both base pairs with a view to obtaining a base pair whose stability is very close to that of the other. This work allowed us to select two sets of base pairs among the numerous modified base pairs studied (9,20–22). The first one involves N4-ethyl-2′-deoxycytidine which hybridizes specifically with 2′-deoxyguanosine leading to a G₄EtC base pair whose stability is very similar to that of the natural AT base pair. Melting studies showed that duplexes built with AT and/or G₄EtC base pairs exhibit thermal stability independent of their base content (9,22).

The second isostable set of base pairs (base pairs having similar stability), which has a thermal stability located between that of natural AT and GC base pairs, involves G₄MeC (guanine/N4-methyl-cytosine) and A₅P₅U [adenine/5-(1-propynyl)-uracil] base pairs (21).

These results prompted us to study the effect of chaotropic agents on hybridization properties of modified oligonucleotides. In fact, the use of TMA⁺ ions which have a differential effect on the stability of AT and GC base pairs along with the approach based on modified oligonucleotides would allow a fine adjustment of DNA thermal stability. Moreover, by carrying out hybridization studies of modified oligonucleotides in chaotropic solvents, we hoped to find conditions under which other natural or modified AT and GC base pairs would be thermally isostable. In this paper, we describe the effect of TMA⁺ and alkyltrimethylammonium (ATMA⁺) cations on the thermal stability of some natural and modified duplexes.

MATERIALS AND METHODS
Synthesis of oligonucleotides
Oligonucleotides were synthesized on a solid support according to phosphoramidite chemistry (23) using commercial phosphoramidite or modified phosphoramidite as described in preceding papers (9,22).

Synthesis of alkyl-, allyl- and propargyltrimethylammonium chloride salts
Anhydrous trimethylamine (1 mol) was added to 200 ml of acetonitrile at –20°C. The alkyl-, allyl- or propargyl chloride (1 mol)

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was slowly added at −20°C. The reaction mixture was maintained at 0°C for 10 min and then kept at room temperature until ATMA⁺, Cl⁻ precipitated. ATMA⁺, Cl⁻ salts were obtained as a white solid after filtration and washing with acetonitrile (yield 60%). These compounds were characterized by NMR.

Melting experiments
Spectrophotometric studies were conducted in 1 M NaCl solution, pH 7, containing 10⁻² M sodium cacodylate and 2 × 10⁻⁸ M EDTA or in buffered ATMA⁺ solutions containing 50 mM Tris–HCl, 2 mM EDTA, pH 8. Solutions were filtered through a 0.2 μm pore size filter (Schleicher & Schuell). Changes in absorbance with temperature of 2 μM duplexes in the respective hybridization solutions were measured at λ = 260 nm on a UVikon 941 cell changer spectrophotometer equipped with a Huber PD 415 temperature programmer connected with a cryothermostat ministat circulating water bath (Huber). The samples and reference were slowly heated at a rate of 0.5°C/min from 0 to 80°C. Melting temperatures (Tm) were taken as the temperature corresponding to the half-dissociation of the complexes. The Tm values were determined using the first and second derivatives; the margin of error on Tm values was approximately ±1°C. The molar extinction coefficient of the sequences was determined as described in the literature (24).

RESULTS
Among the tetraalkylammonium cations, only the smallest TMA⁺ and TEA⁺ cations have the ability to reduce the base pair composition dependence of DNA thermal melting transition (11). At high concentration, TMA⁺ ion increases the stability of duplexes built with AT-rich sequences while it decreases those made with GC-rich sequences. On the other hand, at a high concentration of TEA⁺, a general destabilization was observed, greater for GC-rich sequences than for AT-rich ones. At the isostabilizing point (conditions in which the Tm values of duplexes are similar), Tm values of DNA duplexes are higher in 3.3 M TMA⁺ than in 2.4 M TEA⁺ (11,12,14). Larger molecular weight cations, which greatly decrease the hybrid’s stability, did not exhibit the differential effect on the stability of AT and GC base pairs. This was attributed to the fact that they are too large for hydrophobic interactions to take place in the major groove of AT-rich DNA (11).

ATMA⁺ ions, whose structure and steric requirements are very similar to those of TMA⁺, were prepared in order to study their effect on the stability of natural and modified DNA hybrids. The synthesis of ‘alkyl’-trimethylammonium chloride was easily carried out by quaternization of trimethylamine with alkyl, allyl and propargyl chloride in acetonitrile.

Effect of TMA⁺ and ATMA⁺ ions on the thermal stability of natural duplexes
Thermal stability studies were carried out with duplexes involving nine AT or GC base pairs representing the maximum of base composition variations. These duplexes were formed by a triplet of bases repeated three times. Two dangling TT arms were added at the 3′- and 5′-extremities of one oligonucleotide to prevent the possible concatenation of duplexes and for mimicking hybrids formed between an oligonucleotide probe and a longer nucleic acid sequence. Duplexes involving nine AT base pairs were made with (homopurine and (homopyrimidine oligodeoxyribonucleotides (duplexes 4 and 5) or by purine→pyrimidine transitions (duplexes 1–3). For duplexes containing nine GC base pairs, only sequences involving purine→pyrimidine transitions (duplexes 6–8) gave a cooperative thermal dissociation of duplexes into single strands. When one strand is made with a nonadeoxyguanylate sequence, it does not form a double helix with the complementary sequence nonadeoxycytidin but rather self-associated complexes, particularly G tetrads (22), as has been described for other G-rich sequences (25–27). Among quaternary ammonium cations, we studied TMA⁺ ions, which are usually used in hybridization studies, and ethyltrimethylammonium ions (ETMA⁺), n-propyltrimethylammonium ions (PTMA⁺), allyltrimethylammonium ions (ATMA⁺) and propargyltrimethylammonium ions (PgTMA⁺), which are structurally very close to TMA⁺ ions. In fact, these compounds were obtained by replacement of one methyl group of TMA⁺ with an ethyl (E), n-propyl (P), allyl (Al) or propargyl (Pg) group. The results are summarized in Table 1. In the presence of TMA⁺ ions, melting temperatures of duplexes 1–5 involving AT base pairs increased with the cation concentration as described in the literature (10–17). We observed a very important sequence effect on duplex stability as in the presence of NaCl. In fact, duplexes 1–3 involving A↔T transitions were less stable than duplexes 4 and 5. These results, which corroborate those already observed in classical buffers, were attributed to the particular structure of duplexes d(A)n→d(T)n (28,29). Note that duplex 4 involving a dodecathomymidylate and a nonadeoxyadenylate could adopt concatamer structures. This could explain the higher thermal stability of duplex 4 compared with the one obtained with duplex 5. Moreover, Tm values of duplexes 4 and 5 involving a (homodeoxyadenylate–(homothyrimidylate structure increased stronger with the TMA⁺ concentration than those of duplexes 1–3 having A↔T transitions (Fig. 1). This phenomenon could be due to the high cooperativity of interactions between TMA⁺ ions and AT (duplex 5) or TA base pairs (duplex 4) when the latter are regularly distributed along the double helix, contrary to the case where they are interrupted by A↔T transitions (duplexes 1–3). As expected, the Tm values of duplexes 6–8 made with nine GC or CG base pairs decreased when the concentration of TMA⁺ was increased. Curves representing the Tm variation of (homodeoxyadenylate–(homothyrimidylate duplexes 4 and 5 diverge clearly from those of duplexes 1–3 involving A↔T transitions. We can note that there is not a single concentration of TMA⁺ where the natural duplexes studied 1–8 are isostable. However, a concentration of ~4.5 M TMA⁺ allows a significant reduction in the stability difference of duplexes according to their base content. If we set apart (homodeoxyadenylate–(homothyrimidylate duplexes 4 and 5, which are highly stable due to their special structure, we observed that a TMA⁺ concentration of ~5–5.5 M is necessary to smooth the Tm of duplexes involving nine AT or GC base pairs and the purine→pyrimidine transitions (duplexes 1–3 and 6–8) (Fig. 1). The isostabilizing concentration of 5.5 M TMA⁺ determined in this work with short natural duplexes involving 9 bp is clearly higher than the 3.3 M described in the literature for DNA duplexes whose lengths are superior or equal to 16 bp (11–14). This isostabilizing concentration difference could be due both to the sequence effect and the length of duplexes studied.

Concerning the effect of ATMA⁺ ions with the same ionic strength on the thermal stability of natural duplexes, replacement of one methyl group of TMA ion by an ethyl, propyl, allyl or propargyl group, led to a decrease in duplex stability (Table 1). The
Tm were determined at an oligomer strand concentration of 2 µM in 10−2 M sodium cacodylate pH 7 buffer containing 1 M NaCl and 2 × 10−4 M EDTA or in 50 mM Tris–HCl, pH 8, buffer containing TMACl or TMACl and 2 × 10−3 M EDTA.

*a All sequences are in ‘deoxy’ series.

Table 1. Melting temperatures of natural duplexes

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<th>Sequence*</th>
<th>Tm (°C)</th>
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<td>T₃[T₁{A₄T₄/T₄A₄}]</td>
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<tr>
<td>7</td>
<td>T₃[T₁{G₄C₄/C₄G₄}]</td>
<td>63.5</td>
</tr>
<tr>
<td>8</td>
<td>T₃[T₁{G₄C₄/C₄G₄}]</td>
<td>59</td>
</tr>
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</table>

Tm were determined at an oligomer strand concentration of 2 µM in 10−2 M sodium cacodylate pH 7 buffer containing 1 M NaCl and 2 × 10−4 M EDTA or in 50 mM Tris–HCl, pH 8, buffer containing TMACl or TMACl and 2 × 10−3 M EDTA.

In order to know the TMA+ effect on the stability of duplexes involving natural or modified base pairs

destabilizing effect is stronger for the n-propyl, allyl and propargyl groups than for the ethyl one (at a high concentration of AITMA+ and PgTMA+ ions, Tm of some duplexes were very low and could not be determined). The Tm variation of natural duplexes studied as a function of the ammonium cation concentration was similar for TMA+ (Fig. 1) and ETMA+ (Fig. 2). In the presence of ETMA+ ions, the high stability of (homo)deoxyadenylate–(homo)thymidylylate duplexes 4 and 5 was again observed as in the presence of TMA+ ions. If we set aside the latter, we observe that the ETMA+ ion seems to have an interesting differential effect on the stability of AT and GC base pairs. In fact, a concentration of ~4 M ETMA+ lower than its saturation concentration (~6 M), allows efficient reduction of the thermal stability of different duplexes 1, 3 and 6–8.

TMA+ ion effect on the stability of duplexes involving natural or modified base pairs

In order to know the TMA+ effect on the stability of duplexes made with either nine AT base pairs (duplexes 1, 3 and 5), GC base pairs (duplexes 6–8), G₄MeC base pairs (duplexes 11 and 12), G₄MeC base pairs (duplex 15), A₅PU base pairs (duplex 16) or AU base pairs (duplex 17), which represent the maximum of base composition variations, and with natural duplexes 9 and 10 and modified duplexes 13 and 14 having three AT and six GC or G₄MeC base pairs, respectively. Results shown in Table 2 and Figures 4 and 5 allow the following observations to be made.
Figure 2. Melting temperature variation of natural duplexes according to the
ETMACl concentration. Dark purple dotted line with diamond, duplex 1
(ATA/TA T); magenta dotted line with square, duplex 3 (TTA/AA T); red
dotted line with square, duplex 5 (AAA/TTT); yellow dotted line with
triangle, duplex 6 (CGCGGC); blue dotted line with circle, duplex 7
(CCGG GCC); black dotted line with rectangle, duplex 8 (CGG GCC).
Duplexes 5'-d[T2(abc)3T2]3'-d(def)3'5' are symbolized by abc/def.

(i) In TMA+ solution, for duplexes containing G 4MeC or G 4Et C
base pairs, the presence of a methyl (duplex 15) or an ethyl group
(duplexes 11 and 12) at position 4 of cytosine decreased the stability
of modified duplexes compared with that of natural ones
duplexes 6–8), as has been observed in classical buffer
containing NaCl (9,22), whatever the TMA+ concentration used.
The stability of natural and modified duplexes involving nine GC
duplexes 6 and 7), G 4MeC (duplex 15) or G 4Et C (duplex 11) base
pairs decreased when the TMA+ concentration was increased from 1 to 4 M.
For sequences built with three AT and six GC base
pairs (duplexes 9 and 10), their stability decreased from 4 M
TMA+, higher than the 3 M TMA+ obtained with duplexes 13 and
14 containing three AT and six G 4Et C base pairs (Fig. 4).

(ii) Concerning duplexes involving AT, A 5P U and AU base pairs,
substitution of the methyl group at position 5 of thymine with a
1-propynyl group led to an important increase in the stability of
modified duplexes compared with that of thymine one in
TMACl or NaCl buffer (21,30). The 1-propynyl group is
co-planar to uracil, thus allowing its stacking to the adjacent 5
base which would increase the stacking interaction between base
pairs. Thermal stability of modified duplex 16 containing
A 5Prop U base pairs increased with TMA+ concentration as for the
natural duplexes 1, 3 and 5 involving AT base pairs. However, this
stabilizing increase in function of the TMA+ concentration is
higher for the modified duplex 16 than for the natural duplexes
1, 3 and 5. The replacement of thymines (duplex 5) by uracil
devoid of methyl groups at position 5 (duplex 17) led to much
destabilization due to the abolition of the hydrophobic interaction
of the methyl group in the major groove. However, the stability
of duplex 17 composed of AU base pairs related to the TMA+
concentration was maintained (Fig. 5).

(iii) When we compared the stability of duplexes containing nine
AT or A 3P U base pairs with those composed of nine GC, G 4MeC
or G 4Et C base pairs having the same purine→pyrimidine
transitions (Fig. 5 and Table 3), we observed several systems of
duplexes thermally isostable. The TMA+ concentration necessary
to obtain the different isostabilizing sets of base pairs varied from
1 M for the [AT/G 4Et C] or [A 5P U/G 4Me C] sets (duplexes 1 and
11 and duplexes 16 and 15, respectively) to 5.5 M for the [AT/GC]
set (duplexes 1 and 7). This isostabilizing concentration difference
of TMA+ reflects the difference in thermal stability between the
base pairs within a set of base pairs in classical buffers containing

<table>
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<th>n°</th>
<th>Sequence*</th>
<th>Tm (°C)</th>
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<tr>
<td>1</td>
<td>[A T] [A T]</td>
<td>18.5</td>
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<tr>
<td>2</td>
<td>[A T] [A T]</td>
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<td>3</td>
<td>[A T] [A T]</td>
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<tr>
<td>4</td>
<td>[G C] [G C]</td>
<td>62.5</td>
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<tr>
<td>5</td>
<td>[G C] [G C]</td>
<td>63.5</td>
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<tr>
<td>6</td>
<td>[G C] [G C]</td>
<td>59</td>
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<tr>
<td>7</td>
<td>[G C] [G C]</td>
<td>46</td>
</tr>
<tr>
<td>8</td>
<td>[G C] [G C]</td>
<td>49</td>
</tr>
<tr>
<td>9</td>
<td>[G C] [G C]</td>
<td>27</td>
</tr>
<tr>
<td>10</td>
<td>[G C] [G C]</td>
<td>23</td>
</tr>
<tr>
<td>11</td>
<td>[G C] [G C]</td>
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</tr>
<tr>
<td>12</td>
<td>[G C] [G C]</td>
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<td>13</td>
<td>[G C] [G C]</td>
<td>33.5</td>
</tr>
<tr>
<td>14</td>
<td>[G C] [G C]</td>
<td>31</td>
</tr>
<tr>
<td>15</td>
<td>[G C] [G C]</td>
<td>11.5</td>
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</table>

Table 2. Melting temperatures of natural and modified duplexes involving
dMeC, dEtC or dPU.

Tm were determined at an oligomer strand concentration of 2 µM in 10–2 M
sodium cacodylate pH 7 buffer containing 1 M NaCl and 2 × 10–4 M EDTA
or in 50 mM Tris–HCl, pH 8, buffer containing TMACl and 2 × 10–3 M EDTA.

*All sequences are in ‘deoxy’ series.
NaCl (Table 3). Among the different sets of base pairs, the [A\(^{3P}\)U/GC] set is especially interesting. In fact, in the presence of 3.5 M TMACl duplexes 16 and 7 composed of nine A\(^{3P}\)U and GC base pairs, respectively, are more stable than the natural duplexes 1 and 7 in 5.5 M TMACl solution. Moreover these new base pairs require a TMACl concentration of 3.5 M, clearly lower than the one necessary for natural oligomers (5.5 M). It is important to note that 5.5 M TMA\(^+\) is nearly its saturation concentration which makes the solution very viscous and hence difficult to manipulate.

Stability of duplexes built with AT and/or G\(^{4EC}\) base pairs in the presence of TMA\(^+\) and Na\(^+\) ions

To know the sequence effect on the stability of duplexes built with AT and/or G\(^{4EC}\) base pairs in 1 M TMA\(^+\) solution, which is close to the isostabilizing concentration, studies were carried out with duplexes having different sequence and base compositions. Table 4 gives \(T_m\) data for 15 duplexes (duplexes 1, 2 and 5 containing nine AT base pairs; duplexes 18–23 composed of six AT and three G\(^{4EC}\) base pairs; duplexes 13, 14, 24 and 25 built with three AT and six G\(^{4EC}\) base pairs and duplexes 11 and 12 involving nine G\(^{4EC}\) base pairs) in the presence of 1 M TMACl or 1 M NaCl. With the exception of duplex 23, all duplexes built with AT and AT/G\(^{4EC}\) base pairs have \(T_m\) slightly higher in the presence of 1 M TMACl than in the presence of 1 M NaCl, while duplexes 11 and 12 containing nine G\(^{4EC}\) base pairs are slightly less stable in 1 M TMACl solution than in NaCl solution. The \(T_m\) difference observed between the more stable duplex (duplex 20, \(T_{m20} = 32^\circ\)C) and the less stable duplex (duplex 13, \(T_{m13} = 18^\circ\)C) in the presence of TMACl is 14\(^\circ\)C, which is close to the 12\(^\circ\)C melting range determined between duplex 25, the most thermally stable (\(T_{m25} = 29.5^\circ\)C), and duplexes 13 and 18, the least thermally stable (\(T_{m13} = T_{m18} = 17.5^\circ\)C), in 1 M NaCl solution. These results suggest that the hybridization properties of oligonucleotides built with AT and/or G\(^{4EC}\) base pairs are similar in either 1 M TMACl or NaCl solution. At the isostabilizing concentration of 1 M, TMA\(^+\) (representing a ratio of 68 000 cations/phosphate) has a slight effect on the stabilization of AT sequences or on the destabilization of G\(^{4EC}\) sequences.

DISCUSSION

Sequencing by hybridization requires DNA duplexes with similar stability. Among the several approaches developed to achieve this objective, the one based on the use of chaotropic solvents (11–19,31,32) and the recently reported one based on the modification of oligonucleotides (9,20–22) possess real potential applications. However, the first approach requiring high...
concentrations of TMA\(^+\) ions leads to very viscous solutions and manipulation difficulties. Moreover, its effect on the stability of DNA duplexes is complex and not very efficient for short sequences (14), which are used in the strategy of sequencing by hybridization in order to reduce the number of probes to be immobilized. The use of [AT/G\(^{4Et}\)C] or [A\(^5\)P U/G\(^{4Me}\)C] base pair sets allowed DNA duplexes to be obtained whose thermal stability is independent of their base content in a classical buffer, containing NaCl. However, this approach based on the chemical modulation of the relative stability of AT and GC base pairs not only involves difficult synthetic work but is also inflexible.

The use of moderate concentrations of TMACl along with the chemical modification of duplexes allow a fine adjustment of \(T_m\) and the possibility of obtaining other duplex systems with varied isostabilized temperatures. This approach considerably increases the number of choices concerning the chemical nature of duplexes and hybridization conditions. Among the quaternary ammonium cations used, TMA\(^+\) ion has the greatest stabilizing effect on AT-rich sequences, while its destabilizing effect on GC-rich sequences is weaker compared with that of its ETMA\(^+\) analog. The differential stabilizing effect of small ammonium cations on AT and GC base pairs was attributed to the decreasing polar character of the solvent leading to a general destabilization of the base pair stacking interactions compensated by preferential hydrophobic interactions of TMA\(^+\) with AT sequences (10).

### Table 3. TMACl concentration leading to different systems of duplexes, involving nine natural or modified AT or GC base pairs, thermally isostable

<table>
<thead>
<tr>
<th>n(^a)</th>
<th>Duplex(^a)</th>
<th>NaCl 1 M</th>
<th>TMACl</th>
<th>(T_m) (°C)</th>
<th>(T_m) (°C)</th>
<th>Molarity (M)</th>
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<td>1</td>
<td>(T_1 \ [A-T \ A \ T_j] \ T_1^j)</td>
<td>18.5</td>
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<td>11</td>
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<td>27</td>
<td>23.5</td>
<td>(\approx 2)</td>
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<tr>
<td>3</td>
<td>(T_1 \ [T \ T \ A \ T \ A \ T_j] \ T_1^j)</td>
<td>19</td>
<td>29</td>
<td>(\approx 2)</td>
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<tr>
<td>15</td>
<td>(T_1 \ [G \ A \ T \ G \ A \ T_j] \ T_1^j)</td>
<td>33.5</td>
<td>29.5</td>
<td>(\approx 1.1)</td>
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<td>16</td>
<td>(T_1 \ [G \ T \ A \ T \ A \ T_j] \ T_1^j)</td>
<td>31</td>
<td>(\approx 35)</td>
<td>(\approx 1.1)</td>
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<td>(T_1 \ [G \ A \ T \ G \ A \ T_j] \ T_1^j)</td>
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<td>(\approx 3.5)</td>
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\(^a\)All sequences are in ‘deoxy’ series.

### Table 4. Melting temperatures of duplexes involving AT and/or G\(^{4Et}\)C

<table>
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</tr>
<tr>
<td>(T_1 \ [G \ A \ T \ G \ A \ T_j] \ T_1^j)</td>
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<tr>
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<td>27.5</td>
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<tr>
<td>(T_1 \ [G \ A \ T \ G \ A \ T_j] \ T_1^j)</td>
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<tr>
<td>(T_1 \ [G \ A \ T \ G \ A \ T_j] \ T_1^j)</td>
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<td>6</td>
</tr>
<tr>
<td>(T_1 \ [G \ A \ T \ G \ A \ T_j] \ T_1^j)</td>
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<td>6</td>
</tr>
<tr>
<td>(T_1 \ [G \ A \ T \ G \ A \ T_j] \ T_1^j)</td>
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<tr>
<td>(T_1 \ [G \ A \ T \ G \ A \ T_j] \ T_1^j)</td>
<td>23</td>
<td>6</td>
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</table>

\(T_m\) were determined at an oligomer strand concentration of 2 \(\mu\)M in 10\(^{-2}\) M sodium cacodylate pH 7 buffer containing 1 M NaCl and 2 \(\times\) 10\(^{-5}\) M EDTA or in 50 mM Tris–HCl, pH 8, buffer containing 1 M TMACl and 2 \(\times\) 10\(^{-3}\) M EDTA.

\(^a\)All sequences are in ‘deoxy’ series.
However, $T_m$ values of duplexes built with GC base pairs are equivalent in 4 M TMACl and 2 M ETMACl. This observation suggests that the destabilization of GC base pairs by ETMACl, which is more important than expected, is not only due to the decrease in polarity of the solvent when one methyl of TMA is replaced with one ethyl group. Moreover, differential disruptions of the hydration degree of AT and GC sequences by TMA$^+$ ion may also be considered in the presence of chaotropic solvents.

NMR studies (33) of the helix-to-coil transition of poly(dA-dT) may also be considered in the presence of chaotropic solvents. The differential stabilizing effect of TMA$^+$ on natural base pairs is observed with modified A$^H$U, AU, G$^H$Mc and G$^H$Ec base pairs as well. The examination of the structure of natural and modified base pairs used in this work according to their stability in the presence of TMA$^+$ ions shows that the presence of a methyl group (as in thymine) or a 1-propynyl group [as in 5-(1-propynyl)-uracil] at position 5 of uracil is not necessary for the stabilization by the TMA$^+$ ion. This observation suggests that the hydrophobic interactions of TMA$^+$ with the lipophilic group at the 5 position of uracil in the major groove is not a decisive factor. On the other hand, substitution of one hydrogen of the exocyclic amino group at position 4 of cytosine, which could potentially take part in hydration, by a methyl or ethyl hydrophobic group has little or no effect on the destabilization of sequences involving G$^H$Mc and G$^H$Ec base pairs by TMA$^+$ ions. Others studies carried out on modified base pairs by substituting either one hydrogen atom at position 2 of guanine with one alkyl group or by replacing one nitrogen atom at position 3 or 7 of purine with a CH group will be necessary to determine the site(s) implied in the mechanism of action of TMA$^+$ ions.

In conclusion, the use of TMA$^+$ along with the chemical modification of duplexes allow a fine adjustment of $T_m$ and the possibility of obtaining other duplex systems with varied and high isostabilized temperatures. This enables the use of short oligonucleotides in a reverse hybridization process in order to reduce the sequence number to be immobilized and to obtain the best discrimination between perfect hybrids and those with mismatches. Moreover, this approach makes it possible to design numerous new base pair sets thermally isostable. In addition it could be possible to use modified nucleosides such as 7-deaza-2'-deoxyguanosine (20,34) to reduce or avoid the formation of self-associated complexes observed with G-rich sequences which disturb or prevent DNA hybridization.

**ACKNOWLEDGEMENT**

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**REFERENCES**