Thermodynamic and spectral properties of DNA miniduplexes with the terminal G·A mispairs and 3′ or 5′ dangling bases

S.G. Lokhov*, D.V. Pyshniy
Institute of Bioorganic Chemistry, Siberian Division, Russian Academy of Science, pr. Akademika Lavrentyeva 8, Novosibirsk 630090, Russia

Received 23 October 1997; revised version received 14 November 1997

Abstract The tetradexoxyribonucleotide pAGCG in 1 M NaCl forms duplexes with terminal non-canonical pA·G pairs with stability significantly exceeding that for the duplex (pAGCT)2 and lower than that for the duplex (pCGCG)2. The deoxyriboduplex (pAGCG)2 is considerably stabilized by 3'-Y and slightly by 5'-X dangling bases. Therefore, the stability of duplexes with 3' dangling bases decreases in the order (pAGCG)2 > (pCGCG)2 > (pAGCTA)2. The sum of the independent stabilizing effect of the of 5'-G and 3'-A dangling bases on the (pAGCG)2 core duplex is higher than that of the additional terminal pG·A pair in pG·A·G·A tandem of the duplex (pAGCGA)2.

@ 1997 Federation of European Biochemical Societies.

Key words: Deoxyriboduplex; Thermodynamics; GA mismatch; Dangling base

1. Introduction

The tandems of G·A mispairs take part in secondary structure formation of different types of natural RNAs [1,2]. In particular they have been found as main functional elements in the ribosome RNA sequences [3–5] with catalytic activity being observed also for DNA analogs [6]. It is well known that the thermodynamic parameters of duplex formation including tandems of G·A mispairs are sequence dependent. The highest thermodynamic increment of these mispairs in the nucleic acid double helix was observed in the 5'-Py-G·A-Pu/5'-Pu-G·A-Py- nearest-neighbor surrounding especially for the DNA duplexes, which are in some cases more stable than that of parent duplexes with the canonical A·T pairs in the same surrounding (5'-Py-T-A-Pu/5'-Pu-T-A-Pu) [7,8]. As it was shown, these sequences provide an unusual conformation of the G·A base pair with an amino mode of pairing ('shared' G·A) and with highly effective interstrand cross-stacking [9,10]. On the other hand, any variation of the 5'-Py-G·A-Pu/5'-Pu-G·A-Py consensus sequence results in the conformational polymorphism of the G·A base pair and a drastic decrease of duplex thermostability [7,11–14]. A few data suggest that the tandem G·A base pairing also thermodynamically stable at the end of the duplex in the contexts 5'-G·A-Pu/5'-Py-G·A or Py-G·A-3'/5'-A-Pu' [15]. It seems reasonable to expect that a G·A base pair in the terminal position of a duplex may show unusual properties as a consequence of structural flexibility of the helix terminus.

In this work we present the results of thermodynamic and spectral studies of duplexes with single and double terminal G·A mispairs in the (pAGCG)2 and (pAGCGA)2 duplexes respectively. Stabilizing effects of the 5'-X (X=A,G,T,C) dangling bases in (pXAGCG)2 and 3'-Y (Y=A,G,T) dangling bases in (pAGCGXY)2 series of duplexes versus the (pAGCG)2 core duplex, and especially for 5'-G and 3'-A, as the elements of the terminal G·A tandems in the hexameric duplex (pAGCGA)2, were shown. These properties of the above duplexes are more profound in comparison with the parent duplexes (pCGCG)2, (pAGCG)2, (pCGCGA)2 and (pAGCTA)2 containing canonical G·C or A·T terminal base pairs.

2. Materials and methods

Oligonucleotides were synthesized by the phosphotriester method in solution [16]. The buffer for all experiments was: 1 M NaCl, 0.01 M Na2HPO4, 0.1 mM EDTA, pH 7.3. The concentrations of the oligonucleotides were prepared using the extinction coefficients (ε mh) calculated as described in [17]. The optical melting curves were recorded with a heating rate of 0.7–0.9°C/min by means of a specially designed device, based on the spectrophotometric UV detector of the microcolumn chromatograph 'MilliChrom' (Russia) interfaced to a personal computer. This system allows a high informative multilength mode recording of melting curves to be used. Four wavelengths were used simultaneously in UV melting experiments. All melting curves were recorded with a Shimadzu, Japan spectrophotometer (10 mm pathlength thermostated cuvette). The temperature of the cuvette was measured with a 10-pin thermocouple battery with absolute accuracy ±0.05°C. The data files consisted of 700–800 points, which were corrected for temperature volume expansion.

Circular dichroism (CD) spectra were recorded on a J-600 spectropolarimeter (Jasco, Japan) with a 0.1 mm pathlength thermostated cuvette. UV absorbance spectra were recorded on a UV-2100 spectrophotometer (Shimadzu, Japan) with a 10 mm pathlength thermostated cuvette. The temperature of the cuvette was measured with a 10-pin thermocouple battery with absolute accuracy ±0.05°C. The temperature series of the CD and UV spectra were recorded from 1°C to 70°C with 5–10°C steps. The temperature of the cuvette was stabilized with ±0.05°C precision by means of a liquid circulative bath UH-8 (Germany). To improve spectrum analyses the data points were exported to a PC.

Thermodynamic parameters of duplex formation were obtained by two methods, the first one being a melting curve fitting procedure and the second one concentration dependence of reciprocal melting temperature.

(1) The optical melting curves were fitted to a ‘two-state’ model with sloping baselines by using the original non-linear least-squares ‘Simplex’ program (Windows ‘95 version) providing interactive operation and visualization fitting procedures in a real time mode (A.V. Ivanov, Russia). Six temperature-independent fitting parameters were used: AH°, DS°, aT, bT, aS, bS [18]. The equations used for the fitting procedure were:

*Corresponding author. Fax: (7) (3832) 351665. E-mail: lokhov@modul.bioch.nsk.su

Abbreviations: Prefix ‘d’ to designate oligodeoxyribonucleotides was omitted; G·C base pair of the opposite strands of duplex; G·C-, nearest-neighbor bases of the same strand; Py and Pu, pyrimidine and purine nucleic acid bases

FEBS 19650 24-12-97


0014-5793(97)017100-0
molecularity associates were presented up to an oligonucleotide concentration of 1.3 mM [19].

The enthalpy of the formation of the basic tetroneucleotide duplex (pAGCG)$_2$ (1) with a central G-C-G-C-core flanked by pA-G pairs is equal to $-25.1$ kcal/mol, that is 3 kcal/mol lower than for the parent duplex (pCGCG)$_2$ (10) with the canonical terminal pC-G pairs. The (pAGCG)$_2$ duplex shows intermediate thermostability between (pCGCG)$_2$ and (pAGCT)$_2$. As expected, the latter has a low $T_m$ so we cannot derive its accurate thermodynamic characteristics. It is known [20] that the thermodynamic parameters of central -G-C-G-C-core formation in deoxyriboduplexes is equal to $-11.1$ kcal/mol ($\Delta H^\circ$) and $-28.4$ cal/mol/K ($\Delta S^\circ$). Thus, the nearest-neighbor thermodynamic parameters of pA-G-C-G in the (pAGCG)$_2$ duplex may be estimated at least as $-1.6$ kcal/mol ($\Delta G^\circ_{25}$), $-7.6$ kcal/mol ($\Delta H^\circ$) and $-18.2$ cal/mol/K ($\Delta S^\circ$) taking into account the initiation parameters of a duplex formation ($0.0$ kcal/mol ($\Delta H^\circ$) and $-5.9$ cal/mol/K ($\Delta S^\circ$)) [20].

The thermodynamic contribution of the 3'-$Y$ dangling base to the stability of (pAGCGG)$_2$ (2-4) is more significant than that of 5'-$Y$ dangling bases to (pXAGCGG)$_2$ (5-8). The ratio $\Delta G^\circ_{25}(3'-$Y$/\Delta G^\circ_{25}(5'-$X$)) and $\Delta H^\circ(3'-$Y$/\Delta H^\circ(5'-$X$)) is equal to 11.2, reaching more than 2 in the cases of 3'-$G$ and 5'-$G$ ($\Delta G^\circ_{25} = -1.47$ and $-0.71$ kcal/mol, $\Delta H^\circ = -6.0$ and $-2.6$ kcal/mol, respectively). Obviously, the origin of the stabilization derives from a favorable enthalpic change both for the 5'-$X$ and 3'-$Y$ dangling bases. Thus, more or less close enthalpic effects were found for the 5'-$X$ and 3'-$Y$-positions of $T$ and $C$. For example, the $\Delta H^\circ$ values for the 3'- or 5'-position of $T$ dangling base are equal to $-4.6$ or $-4.1$ kcal/mol, respectively.

Interestingly, the observed tendency of thermostabilization of duplexes with terminal pA-G pairs by the 3'-$Y$ or 5'-$X$ dangling bases is inversely related to the well-known trend for the oligodeoxyriboduplexes with Watson-Crick terminal pairs, in which 3'-Y has a lower stabilization effect, if at all, than 5'-X [21]. As was shown in this work, $\Delta G^\circ_{25}(3'$-$\Delta$) for (pCGCGA)$_3$ (11) versus (pCGCG)$_2$ (10) is equal to $-0.54$ kcal/mol, which is only of the same order of magnitude as a 5'-phosphate effect [22]. On the other hand, the stabilization

### Table 1

Thermodynamic parameters of duplex formation in 1 M NaCl$^+$ solution

<table>
<thead>
<tr>
<th>No.</th>
<th>Oligonucleotide</th>
<th>$\Delta H^\circ$ (kcal/mol)</th>
<th>$\Delta S^\circ$ (cal/mol/K)</th>
<th>$\Delta G^\circ_{25}$ (kcal/mol)</th>
<th>$T_m$ (°C)</th>
<th>$\Delta H^\circ/2$ (kcal/mol)</th>
<th>$\Delta S^\circ/2$ (cal/mol/K)</th>
<th>$\Delta G^\circ_{25}/2$ (kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>pAGCG</td>
<td>25.1 ± 1.0</td>
<td>70.7 ± 3.6</td>
<td>4.8 ± 0.2</td>
<td>9.5</td>
<td>36.8 ± 0.7</td>
<td>-5.8 ± 0.5</td>
<td>-20.7 ± 6.4</td>
</tr>
<tr>
<td>2</td>
<td>pAGCGA</td>
<td>10.4 ± 1.0</td>
<td>10.5 ± 3.5</td>
<td>7.5 ± 0.2</td>
<td>12.5</td>
<td>37.5 ± 0.7</td>
<td>14.6 ± 0.7</td>
<td>7.6 ± 0.7</td>
</tr>
<tr>
<td>3</td>
<td>pAGCGG</td>
<td>34.2 ± 0.9</td>
<td>82.5 ± 4.5</td>
<td>49.5 ± 0.4</td>
<td>7.4</td>
<td>37.4 ± 0.7</td>
<td>14.5 ± 0.7</td>
<td>7.5 ± 0.7</td>
</tr>
<tr>
<td>4</td>
<td>pAGCGGG</td>
<td>30.5 ± 1.4</td>
<td>83.2 ± 3.2</td>
<td>30.2 ± 0.1</td>
<td>7.8</td>
<td>39.1 ± 0.7</td>
<td>14.1 ± 0.7</td>
<td>7.9 ± 0.7</td>
</tr>
<tr>
<td>5</td>
<td>pAGCGG</td>
<td>30.3 ± 1.0</td>
<td>83.2 ± 3.1</td>
<td>5.0 ± 0.2</td>
<td>7.4</td>
<td>39.7 ± 0.8</td>
<td>14.7 ± 0.7</td>
<td>8.0 ± 0.7</td>
</tr>
<tr>
<td>6</td>
<td>pAGCGG</td>
<td>30.3 ± 1.0</td>
<td>83.2 ± 3.1</td>
<td>5.0 ± 0.2</td>
<td>7.4</td>
<td>39.7 ± 0.8</td>
<td>14.7 ± 0.7</td>
<td>8.0 ± 0.7</td>
</tr>
<tr>
<td>7</td>
<td>pAGCGG</td>
<td>30.3 ± 1.0</td>
<td>83.2 ± 3.1</td>
<td>5.0 ± 0.2</td>
<td>7.4</td>
<td>39.7 ± 0.8</td>
<td>14.7 ± 0.7</td>
<td>8.0 ± 0.7</td>
</tr>
<tr>
<td>8</td>
<td>pAGCGG</td>
<td>30.3 ± 1.0</td>
<td>83.2 ± 3.1</td>
<td>5.0 ± 0.2</td>
<td>7.4</td>
<td>39.7 ± 0.8</td>
<td>14.7 ± 0.7</td>
<td>8.0 ± 0.7</td>
</tr>
<tr>
<td>9</td>
<td>pAGCGG</td>
<td>30.3 ± 1.0</td>
<td>83.2 ± 3.1</td>
<td>5.0 ± 0.2</td>
<td>7.4</td>
<td>39.7 ± 0.8</td>
<td>14.7 ± 0.7</td>
<td>8.0 ± 0.7</td>
</tr>
<tr>
<td>10</td>
<td>pAGCGG</td>
<td>30.3 ± 1.0</td>
<td>83.2 ± 3.1</td>
<td>5.0 ± 0.2</td>
<td>7.4</td>
<td>39.7 ± 0.8</td>
<td>14.7 ± 0.7</td>
<td>8.0 ± 0.7</td>
</tr>
<tr>
<td>11</td>
<td>pAGCGG</td>
<td>30.3 ± 1.0</td>
<td>83.2 ± 3.1</td>
<td>5.0 ± 0.2</td>
<td>7.4</td>
<td>39.7 ± 0.8</td>
<td>14.7 ± 0.7</td>
<td>8.0 ± 0.7</td>
</tr>
<tr>
<td>12</td>
<td>pAGCGG</td>
<td>30.3 ± 1.0</td>
<td>83.2 ± 3.1</td>
<td>5.0 ± 0.2</td>
<td>7.4</td>
<td>39.7 ± 0.8</td>
<td>14.7 ± 0.7</td>
<td>8.0 ± 0.7</td>
</tr>
<tr>
<td>13</td>
<td>pAGCGG</td>
<td>30.3 ± 1.0</td>
<td>83.2 ± 3.1</td>
<td>5.0 ± 0.2</td>
<td>7.4</td>
<td>39.7 ± 0.8</td>
<td>14.7 ± 0.7</td>
<td>8.0 ± 0.7</td>
</tr>
</tbody>
</table>

$^a$The data were calculated from $1/T_m$ vs. ln$C$ dependencies and are shown with $\pm 2$ standard deviations intervals.

$^b$was calculated for an oligonucleotide concentration of $10^{-4}$ M.

$^c$The $\Delta H^\circ$, $\Delta S^\circ$, $\Delta G^\circ_{25}$, $T_m$ were calculated relatively to: (c) (pCGCGA)$_3$, (d) (pAGCGA)$_3$, (e) (pAAGCG)$_2$.
Effect ($\Delta G^\circ_{298}$) of the 5'-A dangling base in (pACGCG)$_2$ (12) is equal to $-1.01$ kcal/mol, that is 2.4-fold higher than for the 3' dangling base.

The properties of the terminal tandem G-A mispairs were studied with the use of the hexanucleotide duplex (pAGCCG)$_2$ (9). The thermodynamic contribution of the additional terminal pG-A pair relative to (pAGCG)$_2$ is equal to $-1.62$ kcal/mol ($\Delta G^\circ_{298}$) and $-6.4$ kcal/mol ($\Delta H^\circ$) (Table 1). These values are close to the Watson-Crick nearest-neighbor parameters of the interaction of -G-A/-T-C- in deoxyriboduplexes ($-1.65$ kcal/mol and $-7.7$ kcal/mol, respectively) but 1.5-fold less than the interaction parameters of -G-C/-G-C- [20]. These relationships are in agreement with thermodynamic stability of deoxyriboduplexes containing the internal tandem of the G-A, T-A or G-C base pairs in the same 5'-3'


To determine the possible structure of the terminal G-A mispairs in the series of mismatched duplexes (1–9) in 1 M NaCl solution (1–9) circular dichroism (CD) studies were carried out (Fig. 1). To calculate CD profiles of double-stranded forms ($F=1$) the following data were used: (a) the respective temperature series of CD spectra, (b) the thermodynamic parameters of the duplexes formation ($\Delta H^\circ$, $\Delta S^\circ$) (Table 1), and (c) straight baseline approximation which is the same as used for UV melting curve fitting (Eqs. 3 and 4). The spectra of the G-A family duplexes have identical spectral characteristics, that is well-resolved bands with a high positive amplitude at 270, 280 and 290 nm. In addition, a shoulder at 260 nm for duplexes (6, 9) was observed [23]. These data are in good agreement with the earlier published CD spectra of the mismatched duplexes containing -Py-G-A-Pu/-Py-G-A-Pu- sequences whose conformations were defined by 2D-NMR spectroscopy [15,24]. Based on these results we propose that the conformation of G-A pairs in all the duplexes studied is G(anti-)A(anti-) with amino-type base pairing. Detailed analysis of the first derivative CD spectra has revealed a very low amplitude shoulder at 305 nm, which was found for all recorded spectra of G-A family duplexes. It should be especially emphasized that the UV optical melting curves (305 nm) of these duplexes are distinguished by unexpected hypochromic effects (~40 to ~80%), as shown, for example, in Fig. 2 for pAGCGA. Spectral characteristics listed before exclusively referred to G-A family duplexes, instead of parent duplexes with G-C or A-T base pairs (Fig. 1c). These properties can be used in both analytical and structural investigations. It allows one to estimate thermodynamics of the highly concentrated oligonucleotide solution, for example of NMR samples, by UV melting experiments.

4. Discussion

The contribution of the 3'-Y dangling bases to the thermodynamics of the (pAGCG)$_2$ deoxyriboduplex formation is the same as for the 3' dangling bases A or G over the terminal G-C base pair in riboduplexes [25]. This means that the 3'-Y stabilizing effect (G > A > T) is the result of the optimal conformation of the dangling base for the stacking interaction with the terminal pA-G mispair. This influence is higher even than the thermodynamic contribution of the 5'-A dan-
gling base to the stability of parent duplex (pAGCGG)2 (Table 1). There are a number of reasons explaining the efficiency of the 3'-Y dangling base. First, the A(anti-)/G(anti-) conformation with amino-type pairing results in a Y/A optimal interstrand cross-stacking interaction. This well-known type of base pairing occurs in the Py-G-A-Pu/-Pu-G-A-Pu- internal sequences [7] and in the hairpin structure formed by d(GCGAAGC) [26]. Obviously, the same type of interaction of the A-G terminal base pair with 3'-Y dangling bases could be completely realized in the (pAGCGY)2 duplexes (as shown in Scheme 1).

\[
\begin{align*}
5' & - Py-G-A-Pu- & 3' \\
3' & - Pu-A-G-Py- & 5' \\
& - G-C-G-A-Y & - \vphantom{Py-G-A-Pu-}
\end{align*}
\]

Scheme 1.

Second, the terminal 5'-phosphate can stabilize a duplex structure in the high ionic strength solution and destabilize it at a low salt concentration due to electrostatic repulsion [22]. In the series of duplexes (pAGCGY)2 the 5'-phosphate is located close both to the terminal A-G pair and to a 3'-Y dangling base. In contrast, the 5'-phosphate group in the (pXAGCGY)2 series is out of duplex terminus elements. Unusual properties of the 5'-phosphate emerged from thermodynamic stability studies of the (pAGCGY)2 duplexes under extremely decreased ionic strength of the solution. Among the duplexes listed in Table 1 only (pAGCGG)2, (pAGCGG)2, (pAGCGA)2, and (pAGCGA)2 were sufficiently stable in deionized water. The \( T_m \) values of the duplexes were found to be 14, 15, 16, and 17°C, respectively (\( C = 10^{-4} \text{ M} \)). The data obtained for the (pAGCGY)2 duplex series suggest the possibility of some additional interaction between the 5'-phosphate and 3'-Y dangling base. It can be partly explained by hydrogen bonding between the phosphate and 2-amino group of G in the opposite strand [27,28], which is impossible in the case of the canonical base pairs. As an original result, the mismatched duplexes (pAGCGY)2 are more stable than the parent duplexes with Watson-Crick terminal base pairs: (pCGCGA)2, (11) and (pAGCTA)2, (13) Table 1).

It is well known that the internal tandem 5'-G-A-3'/5'-G-A-3' is a thermodynamically favorable structural element of the double helix [7]. Our results suggest that this is also true for terminal tandem G-A mismatches. A free energy increment of the additional terminal pG-A base pair, \( \Delta \Delta G^{\circ} \) (pG-A), in (pAGCGA)2 (9) relatively to the core duplex (pAGCGG)2, (1) is equal to -1.62 kcal/mol and the enthalpy increment, \( \Delta H^\circ \) (pG-A), is equal to -6.4 kcal/mol (Table 1). However, these values are unexpectedly low, approximately 3/4 times, than the sum of the independent 5'-pG and 3'-A dangling bases increments in the duplexes (pAGCGA)2 and (pAGCGA)2, respectively \( \Delta \Delta G^{\circ} \) (pG-A) = 1.62 < 0.71 + 1.38 = 2.09 kcal/mol; \( \Delta H^\circ \) (pG-A) = 6.4 < 2.65+8.8 = 8.4 kcal/mol (Table 1). It is important to note that the sum of the internal stabilizing effects of the 5' and 3' dangling bases over the canonical base pair does not exceed the effect of the additionally formed corresponding base pair [25]. Thus, the formation of the second G-A mismatch in the terminal 5'-G-A-3'/5'-G-A-3' duplex region may be characterized by an anticooperative interaction between 5'-pG and 3'-A at the end of the duplex (pAGCGA)2. Obviously, the single dangling base can adopt the optimal position for interstrand stacking with the terminal G-A pair, that is spatially hampered when both bases exist at the duplex terminus. The data obtained allow us to propose the crucial role of A/A (rather than G/G) cross-stacking interaction in a thermodynamic increment of tandem 5'-G-A-3'/5'-G-A-3' formation. Thus, it should be taken into account that the single G-A mispairing is able to cause unexpected duplex stability in the case of the C-G/A-G dinucleotide terminal helix domain. This can be the most conspicuous in the presence of the dangling bases and the 5'-terminal phosphate group. The weak salt dependence of the nucleic base interaction can lead to the preference for G-A mismatched duplexes over Watson-Crick duplexes at a low ionic strength of the solution. Moreover, the unusual properties of the single terminal G-A base mispair probably can play a role in some biological systems where the terminus of the double helix is involved in processes such as strand elongation in the course of replication.

Acknowledgements: We would like to thank professor D.G. Knorre for helpful discussion and comments on the manuscript. We are grateful for the excellent technical help from T.Yu. Bushueva.

References