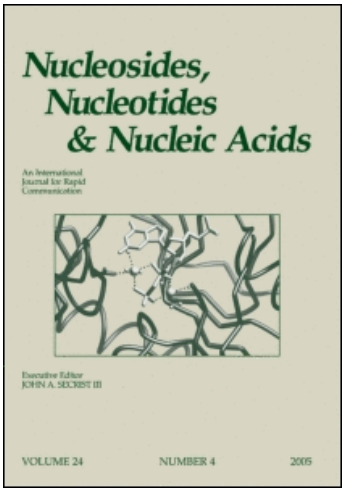

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EFFECT OF THE INCORPORATION OF 2′-DEOXY-8-(HYDROXYL)ADENOSINE ON THE STABILITY OF QUADRUPLEXES FORMED BY MODIFIED HUMAN TELOMERIC DNA

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□ *Differential scanning calorimetry (DSC) and circular dichroism (CD) techniques were used to investigate the physico-chemical properties of the quadruplexes formed by the two different truncations of human telomeric sequence d(TAGGGT) and d(AGGGT), where the adenines were substituted by 2′-deoxy-8-(hydroxyl)adenosine ($A \rightarrow A^{OH}$). CD spectra show that the modified sequences are able to form parallel-stranded quadruplex structure. Analysis of the thermodynamic parameters reveals that the introduction of the modified adenine affects in different way the thermal stability of the $[d(TAGGGT)]_4$ and $[d(AGGGT)]_4$ quadruplexes.*

Keywords G-quadruplex; modified nucleotide; differential scanning calorimetry (DSC); circular dichroism (CD)

The guanine-rich oligonucleotides can adopt G-quadruplex structure stabilized by quartet layers of four Hoogsteen-paired guanines (the G-tetrads).[1] These guanine-rich sequences are found in biologically relevant regions of the genome such as telomeres, immunoglobulin switch regions, and sequences associated with human disease.[2,3] Many of these sequences have been shown to form quadruplex structures in *vitro*. The level of interest in these peculiar structures has increased, due to the identification of their potential biomedical applications.[4] G-quadruplex formation at telomeric ends of chromosomal DNA and their stabilization by specific ligands has been shown to inhibit telomerase activity in cancer cells. In addition to

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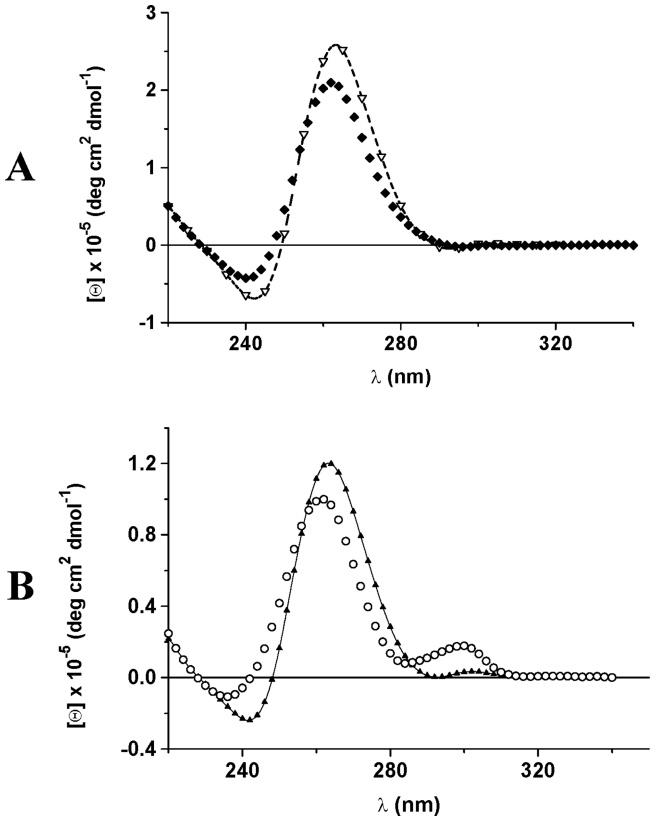

FIGURE 1 CD spectra of quadruplexes at 10°C: A (∇) $[d(TAGGGT)]_4$, (♦) $[d(TA^{OH}GGGT)]_4$, B) (▲) $[d(AGGGT)]_4$, (○) $[d(A^{OH}GGGT)]_4$.

the use of G-quadruplex structures as targets for biomedical applications, these molecules have been tested as therapeutic agents themselves. Indeed, aptamers based on quadruplex motives can specifically bind and inhibit proteins. To enhance the affinity of aptamers toward selected targets and to increase their stability in biological fluids, many modified nucleotides have been employed.[4] Biophysical methods, such as CD spectroscopy and calorimetry, can be easily applied, allowing the determination of the influence of nucleotide modification on the conformation and thermal stability of different G-quadruplex structures. In this work, the adenines of the human telomeric repeat sequences d(TAGGGT) and d(AGGGT) were substituted by 2′-deoxy-8-(hydroxyl)adenosine ($A \rightarrow A^{OH}$).

The oligonucleotides were synthesized on a Millipore Cyclon Plus DNA synthesizer, using solid phase β-cyanoethyl phosphoramidite chemistry and by using commercially available 5′-DMT-aminoprotected-8-(hydroxyl)adenosine-3′-phosphoramidite. The quadruplexes were formed by dissolving the purified oligonucleotides in the appropriate buffer and heating the solution at 90°C for 5 min. The solution was slowly cooled to

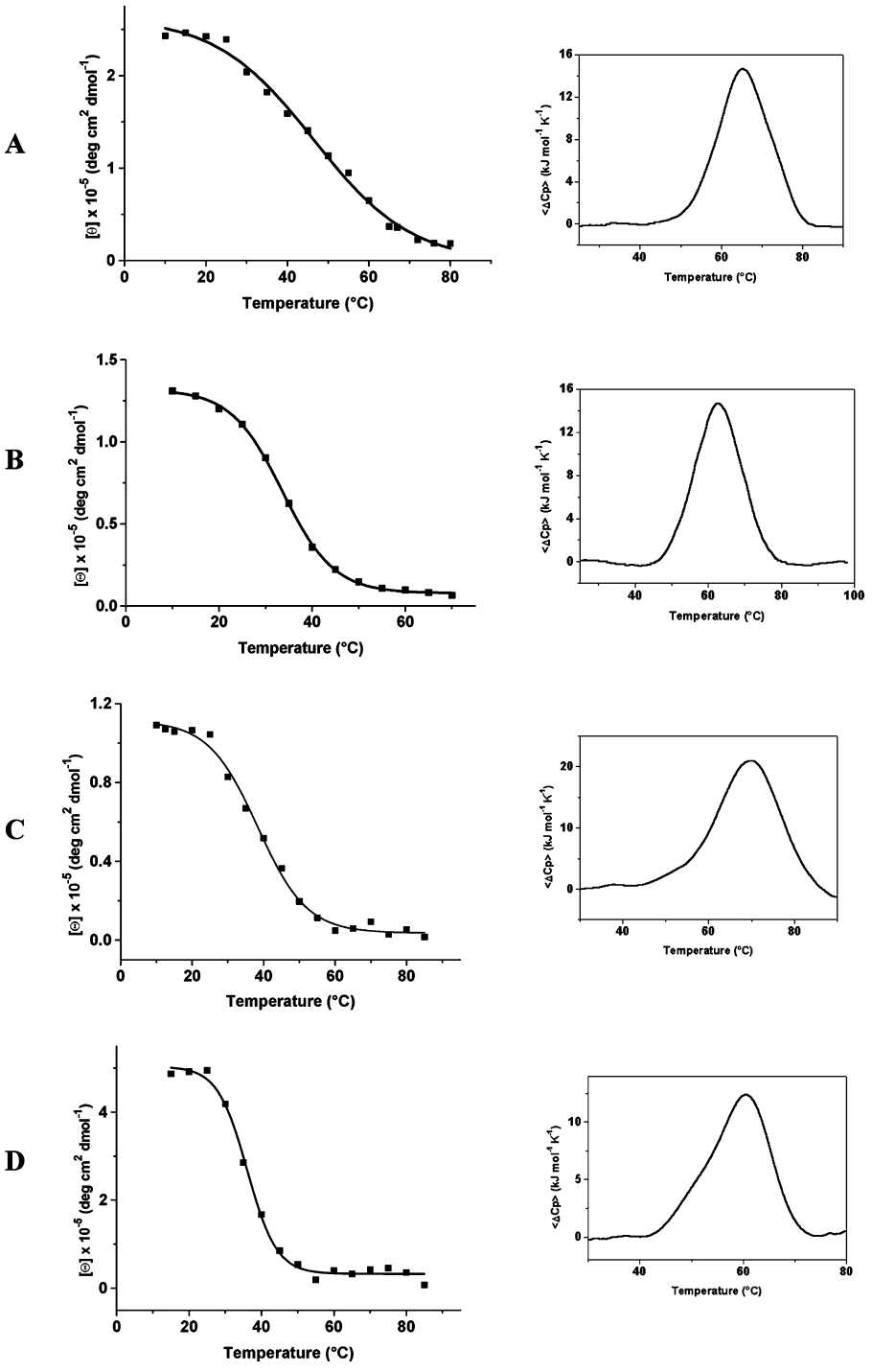

FIGURE 2 CD melting curves (on the left) and DSC profiles (on the right) for $[d(TAGGGT)]_4$ (A), $[d(TA^{OH}GGGT)]_4$ (B), $[d(AGGGT)]_4$ (C), and $[d(A^{OH}GGGT)]_4$ (D).

TABLE 1 Thermodynamic parameters for the dissociation process of the quadruplexes

Quadruplex	Tm (°C) (± 1)	ΔH° kJ mol^{-1}	ΔS° kJ mol^{-1}K^{-1}	ΔG° (298 K) kJ mol^{-1} (± 1)
[d(TAGGGT)]$_4$	51	210 ± 10	0.43 ± 0.03	81
[d(TA^{OH}GGGT)]$_4$	38	180 ± 9	0.33 ± 0.03	82
[d(AGGGT)]$_4$	37	280 ± 12	0.68 ± 0.04	77
[d(A^{OH}GGGT)]$_4$	37	280 ± 12	0.68 ± 0.04	77

room temperature and then equilibrated for one day at 4°C. The buffer 10 mM KH_2PO_4, 1.0 M KCl and 0.1 mM EDTA at pH 7.0 The CD spectra for all sequences were recorded at T = 20°C and at single strand concentration of 2×10^{-4} M (Figure 1). These spectra are diagnostic of parallel-stranded quadruplex structures.[5]

Differential scanning calorimetry (DSC) measurements were carried out on a second generation Setaram Micro-DSC. The experiments were performed at constant single strand concentration of 8×10^{-4} M. The melting temperatures for each system were influenced by scan rate. This arises when the complexes are not at thermodynamic equilibrium during the temperature changes and it is due to slow rates of dissociation and/or association process. In this non-equilibrium conditions only the enthalpy change relative to the quadruplex dissociation process may be directly obtained by differential scanning microcalorimetry measurements. The equilibrium melting temperatures were obtained by circular dichroism (CD) monitored denaturation experiments following a previously reported procedure.[6] The CD and DSC melting profiles for the modified and unmodified quadruplexes are shown in Figure 2. The thermodynamic parameters for the quadruplex dissociation are collected in Table 1. The enthalpy value for the [d(AGGGT)]$_4$ is 70 kJ mol^{-1} higher than the enthalpy change reported for the [d(TAGGGT)]$_4$. This result is in perfect agreement with the NMR data that indicate the formation of an A-tetrad in the quadruplex [d(AGGGT)]$_4$ but not in the [d(TAGGGT)]$_4$ quadruplex.[7] For the modified sequences, inspection of Table 1 reveals that the introduction of the hydroxy group in position 8 of adenosine in the [d(AGGGT)]$_4$ quadruplex does not alter the thermodynamic stability of the resulting quadruplex. On the other hand, the same modification in the [d(TAGGGT)]$_4$ quadruplex causes a decrease of both the entalpic and entropic terms. Further, the melting temperature of the [d(TA^{OH} GGGT)]$_4$ quadruplex is 13°C lower than the melting temperature obtained for the unmodified quadruplex. These data seem to indicate that the presence of the A^{OH} residue in the [d(TA^{OH}GGGT)]$_4$ significantly affects the quadruplex conformation whereas the structure of the [d(A^{OH}GGGT)]$_4$ quadruplex is probably very similar to the unmodified quadruplex. To give further insight into the conformational features of the modified quadruplexes, molecular modelling studies are currently in progress in our laboratories.

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