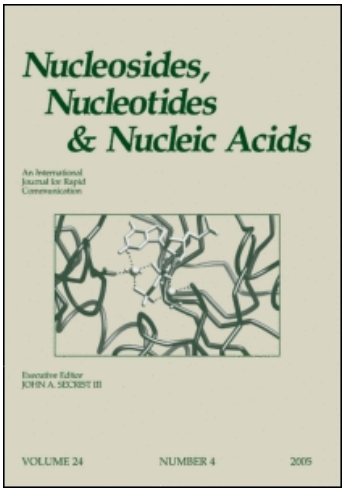

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Relative Stability of Quadruplexes Containing Different Number of G-Tetrads

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RELATIVE STABILITY OF QUADRUPLEXES CONTAINING DIFFERENT NUMBER OF G-TETRADS

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▫ *The aim of this work is to compare the physicochemical properties of three oligonucleotidic sequences, d(TGGGT), d(TGGGGT) and d(TGGGGGT), which assemble to form quadruplex structures with the same molecularity, but containing three, four, and five G-quartets, respectively. The addition of one or two G-tetrads greatly increases both the enthalpy and T_m values of the quadruplex dissociation.*

Keywords DNA Quadruplexes, Circular Dichroism, Gel Electrophoresis

INTRODUCTION

Telomeres are DNA protein structures that exist at the end of chromosomes and are essential for chromosomal stability. Although most of the telomeric DNA is double-stranded, the extreme 3′-end of the telomere consists of a single-stranded G-rich DNA overhang.[1,2] Telomeric G-rich DNAs can adopt unusual DNA structures, called G-quadruplex DNA. The fundamental building block of these unusual structures is the G-tetrad (also called the G-quartet). The G-tetrad consists

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of a planar arrangement of four guanine bases associated through a cyclic array of hydrogen bonds in which each guanine base both accepts and donates two hydrogen bonds. The resulting square-planar array is unique due to the "hole" that is created in the center. The G-quadruplex structures have generated considerable interest due to their potential role in the maintenance of eukaryotic chromosome.[3]

In this work, we compare the physicochemical properties of three oligonucleotidic sequences, d(TGGGT), d(TGGGGT) and d(TGGGGGT), where the deoxyoligonucleotide d(TGGGGT) derives from the 3′ overhang of *Oxytricha* telomere. The three oligonucleotides assemble to form quadruplex structures with the same molecularity, but containing three, four and five G-quartets, respectively. The characterization was made by poliacrylamide gel electrophoresis (PAGE) and circular dichroism (CD).

The quadruplexes were formed by dissolving the oligonucleotides in the appropriate buffer and heating the solution at 90°C for 5 min. The solution was slowly cooled to room temperature, and then equilibrated for 1 day at 4°C. The buffer used was 10 mM NaH_2PO_4, 200 mM NaCl, and 0.1 mM EDTA at pH = 7.0.

Nondenaturing 20% polyacrylamide gel electrophoresis (PAGE) was then carried out at 25°C for 5 h at 100 V (10 V/cm) using an electrophoresis buffer containing 45 mM Tris-borate and 1 mM EDTA (pH 8.0). The gel stained with ethidium bromide was photographed with a Panasonic DMC-LC40 Camera. The gel electrophoresis, shown in Figure 1, demonstrates that each sequence forms a single quadruplex.

FIGURE 1 Nondenaturing 20% polyacrylamide gel electrophoresis of the $[d(TGGGGT)]_4$ (A); $[d(TGGGGT)]_4$ (B); and $[d(TGGGT)]_4$ (C).

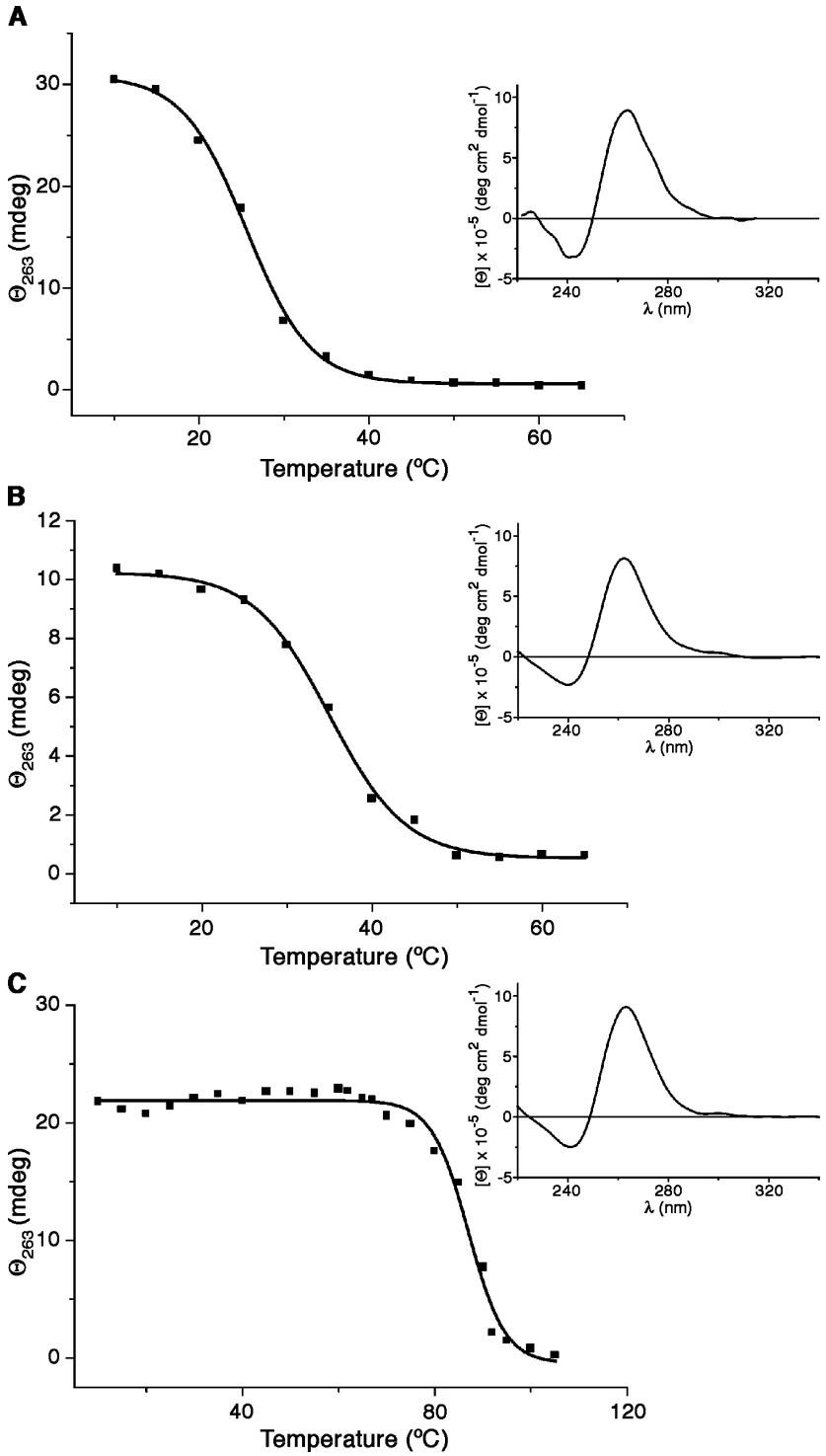

FIGURE 2 CD melting curves at 263 nm of the $[d(TGGGT)]_4$ (A), $[d(TGGGGT)]_4$ (B), and $[d(TGGGGGT)]_4$ (C). The inserts show the CD spectra of quadruplexes at 20°C.

TABLE 1 Temperature and Enthalpy Values for the Dissociation Process of Three Quadruplexes

	T_m (°C)	$\Delta H°$ (kJ mol^{-1})
$[d(TGGGT)]_4$	25.6 ± 0.2	231 ± 8
$[d(TGGGGT)]_4$	35.0 ± 0.2	320 ± 8
$[d(TGGGGGT)]_4$	87.2 ± 0.2	–

Circular dichroism (CD) studies were performed in the 10 mM NaH_2PO_4, 200 mM NaCl and 0.1 mM EDTA buffer at pH = 7.0 at the final quadruplex concentration of 5×10^{-5} M. CD spectra of the studied sequences were characteristic of parallel-stranded, tetramolecular quadruplexes. Taking into account that the rates of quadruplex formation/dissociation are extremely slow, to avoid a kinetic influence on the collected data, we allowed thermodynamic equilibrium to be reached at each temperature as follows. The equilibrium melting curves for all the quadruplexes were obtained by collecting data in the range 5–105°C using a temperature step of 5°C and leaving the samples to equilibrate for a suitable time after each temperature step before recording the CD. The CD signal at 263 nm was reported as function of temperature for all the studied quadruplexes (Figure 2). The thermodynamic parameters of quadruplex dissociation, listed in Table 1, were obtained by fitting the experimental data. It was not possible to estimate the enthalpy change for the $[d(TGGGGGT)]_4$ because the end of transition occurred at temperatures that were not experimentally accessible. The addition of one G-tetrad greatly increases both the enthalpy and T_m values of the quadruplex dissociation. The enthalpy values per tetrad were 76 and 80 kJ mol^{-1} for the $[d(TGGGT)]_4$ and the $[d(TGGGGT)]_4$, respectively. The subtraction of one G-tetrad to the telomeric sequence d(TGGGGT) decreases the thermal stability by about 12°C, whereas the addition of one G-tetrad strongly increases the thermal stability by about 49°C.

In conclusion, a thermodynamic study is the first step to understand the factors that govern the quadruplex formation processes, being careful about the time dependence of the melting experiments. Thermodynamic study can be useful when considering the design of synthetic oligonucleotides for therapeutic applications.

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