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ARTICLE · APRIL 2003

DOI: 10.1002/jccs.200300026

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A Carbazole Derivative Synthesis for Stabilizing the Quadruplex Structure

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A new molecule of 3,6-Bis(1-methyl-4-vinylpyridium iodine)carbazole (**BMVC**) was synthesized for stabilizing the quadruplex structure of human telomeric sequence of $d(T_2AG_3)_4$ *in vitro*. Mixing **BMVC** with the DNA can raise the melting temperature of the $d(T_2AG_3)_4$ by ~ 13 °C, implying that **BMVC** could be a useful telomerase inhibitor. In addition, the fluorescence of the **BMVC** increased significantly upon interacting with the $d(T_2AG_3)_4$ which may be useful as a G-quadruplex specific marker.

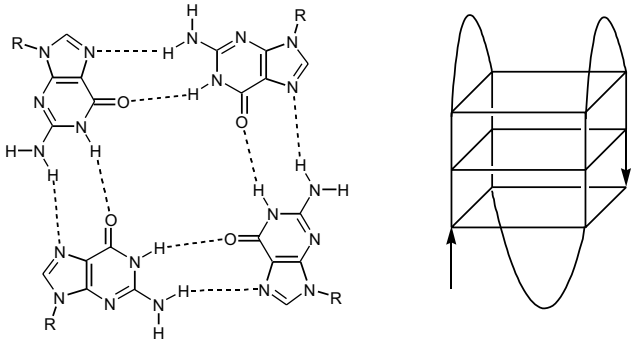
Keywords: Human telomere; Quadruplex stabilizer; Biomarker.

INTRODUCTION

Telomeres, the ends of chromosomes, are essential for the stability and replication of eukaryotic chromosomes.[1] The progressive loss of telomere repeats related to aging and the elongation of telomere length linked to tumor development are of particular interest. The telomeric sequences will be shortened during cell division since conventional DNA synthesis cannot fully replicate the extreme ends of chromosomes. A reduction in the telomere length to a critical level can lead to genomic instability, aberrant chromosome fusion and cellular senescence.[2] In contrast, telomeres of tumor cells do not shorten in replication. This is because the activity of the enzyme telomerase consisting of a reverse transcriptase and an endogenous RNA template allows the addition of the T_2AG_3 repeats to elongate telomeric DNA at the tips of chromosomes.[3] Although telomerase is not expressed in most somatic cells, it appears in more than 85% of tumor cells.[4] These observations have led to the proposal that telomerase is an attractive target for cancer diagnosis and chemotherapy.[5]

Telomeres generally consist of many tandem repeats of guanine-rich (G-rich) motifs, for example, the repeated subunits of T_2AG_3 for the human telomere.[6] Of particular interest is that the 3′-overhang G-rich single strand adopts an intramolecular G-quadruplex structure *in vitro*. The quadruplex structure is stabilized by the π-π interaction of the cyclic G-quartets stacked on top of each other, and the G-quartet is formed by Hoogsteen hydrogen bonding among four guanine bases.[7] Since the folding of telomeric DNA into G-quadruplexes has been shown to inhibit telomerase activity *in vitro*,[8] the G-quadruplexes have also been suggested as potential targets for the design of antitumor agents.[9] Molecules that stabilize G-quadruplex have the potential to interfere with telomere replication and could serve as anti-tumor agents. Recently, a number of small aromatic molecules have been investigated for their ability to stabilize quadruplex structures.[10] Scheme I shows the structure of G-quartet and the G-quadruplex structure of $d(T_2AG_3)_4$ (abbreviated as Hum).

Scheme I



G-quartet Hum

Recently, Mergny and coworkers[11] have studied a series of dibenzophenanthroline derivatives and reported that the melting temperature of quadruplex structure can be increased by 2-20 °C. In addition, their results showed a good correlation between quadruplex stabilization and telomerase inhibi-

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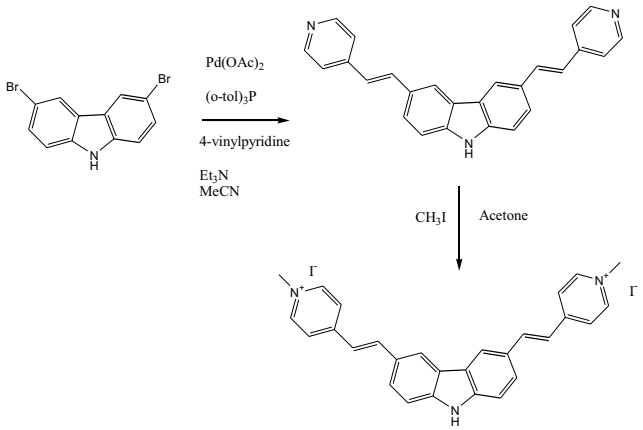
tion. Here, a new molecule of 3,6-Bis(1-methyl-4-vinylpyridium iodine)carbazole (**BMVC**) was synthesized to stabilize the G-quadruplex of human telomeres. The melting temperature of a unimolecular quadruplex of $d(T_2AG_3)_4$ can be increased by ~ 13 °C upon interaction with the **BMVC** ligand. It appears that the **BMVC** can thermally stabilize the human telomere, which could be a useful telomerase inhibitor. In addition, the fluorescence of the **BMVC** increases significantly upon interacting with the $d(T_2AG_3)_4$ which may be useful as a G-quadruplex specific marker.

EXPERIMENTAL SECTION

Synthesis of the 3,6-Bis(1-methyl-4-vinylpyridium iodine)carbazole is described in Scheme II. The 3,6-Dibromocarbazole (1.63 g, 5 mmole, Aldrich) was added into a high pressure bottle containing the mixture of Palladium(II) acetate (15 mg, Strem) and tri-*o*-tolyl phosphine (150 mg, Alrich), then to which was added the solvent pair (triethylamine 5 mL/tetrahydrofuran 15 mL) and 4-vinylpyridine (2 g, 20 mmole, Merck). The bottle was sealed after bubbling 10 min with nitrogen. After keeping the system under ~ 105 °C for three days, the precipitant was collected and then extracted with H_2O/CH_2Cl_2 twice. The insoluble solid was filtered and dissolved in THF, then dried by $MgSO_4$. The yellow powder 3,6-di(4-vinylpyridine)carbazole (Yield: 62%, mp > 300 °C) was collected by recrystallization from THF filtrate. Data for this compound: ^{1}H NMR (CD_3OD): δ 8.42 (d, J = 5.7 Hz, 4H), 8.25 (s, 2H), 7.65 (d, J = 8.4 Hz, 2H), 7.56 (d, J = 16.2 Hz, 2H), 7.45 (d, J = 5.7 Hz, 4H), 7.42 (d, J = 8.4 Hz, 2H), 7.07 (d, J = 16.2 Hz, 2H). EA (373 + 1.5H_2O): calc (obs %) C: 83.64 (78.20), H: 5.09 (5.14), N: 11.26 (10.38). After refluxing the compound of 3,6-di(4-vinylpyridine)carbazole with excess CH_3I in acetone, the final compound of **BMVC** (Yield: 92%, mp > 300 °C) was collected as an orange-red powder that was recrystallized from methanol twice. Data for **BMVC**: ^{1}H NMR (DMSO-d_6): δ 8.77 (d, J = 6.9 Hz, 4H), 8.59 (s, 2H), 8.19 (d, J = 6.9 Hz, 4H), 8.20 (d, J = 15.9 Hz, 2H), 7.90 (d, J = 8.7 Hz, 2H), 7.64 (d, J = 8.7 Hz, 2H), 7.53 (d, J = 15.9 Hz, 2H). EA (657 + 1.0H_2O): calc (obs %) C: 51.14 (49.87), H: 3.81 (4.03), N: 6.39 (6.32).

Scheme II



RESULTS AND DISCUSSION

During the last decade, the circular dichroism (CD) has been used to characterize the particular structures of G-rich quadruplexes.[12] It has been documented that linear parallel quadruplexes give a positive band around 260 nm and a negative band around 240 nm,[12a] while anti-parallel hairpin quadruplexes have two positive bands around 245 and 290 nm and a negative band around 265 nm.[12b] Fig. 1a shows CD spectra of Hum and its complex with the **BMVC** ligand at room tem-

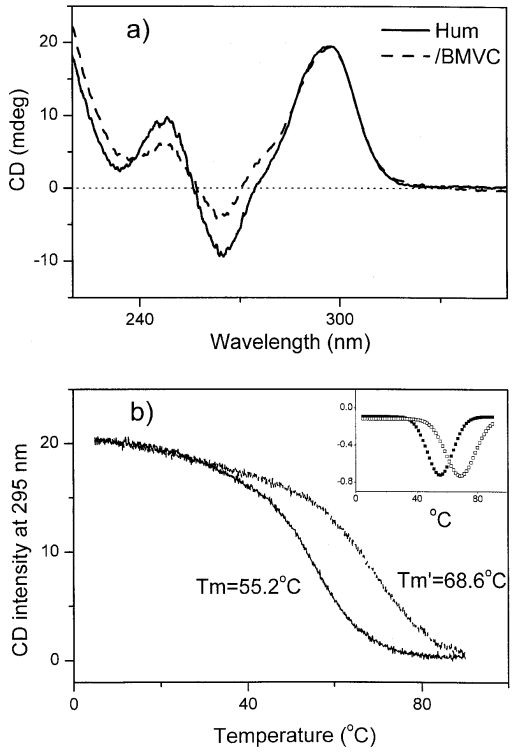


Fig. 1. (a) CD spectra of Hum and its complex with **BMVC** at room temperature. (b) Temperature-dependent CD signal at 295 nm for the measurement of *Tm* of Hum and its complex of **BMVC**. The inset shows the *Tm* measured from Gaussian fitting of the differential curve.

perature. The spectra were obtained by averaging 10 scans on a Jasco J-715 spectropolarimeter using a 1-cm cell for CD spectra of DNA with a 2 nm bandwidth. Buffer solutions of 10 mM Tris-HCl (pH 7.5) and 150 mM NaCl mixed with oligonucleotides were heated to 90 °C for 2 min, cooled slowly to room temperature, and then stored at 4 °C for more than 2 days before use. The molar ratio between **BMVC** ligand and *ss*-DNA is 1:1 with the ligand concentration of 5 μM. The positive band around 295 nm and the negative band around 265 nm shown in Fig. 1a suggested that the structure of Hum is predominated by the anti-parallel quadruplex. Our results show no appreciable change in the CD patterns upon mixing with the ligand, implying that the $d(T_2AG_3)_4$ quadruplex is not appreciably distorted by the **BMVC** ligand.

The change of melting temperature (*Tm*) in the folded and unfolded quadruplex structures upon interacting with ligand provides evidence of thermal stabilization of DNA structure. Here the *Tm* was measured by monitoring the 295 nm CD signal that is a characteristic of an anti-parallel quadruplex. The temperature was ramped from 5 to 90 °C at a rate of 0.8 °C/min. Fig. 1b shows temperature-dependent CD intensity at 295 nm of Hum and its complex with **BMVC**. The magnitude of *Tm* of Hum increases from ~ 55 °C to ~ 68 °C upon interaction with **BMVC**, indicating that the binding of **BMVC** can thermally stabilize the quadruplex structure of Hum. In general, the ligand stabilization of the quadruplex is accompanied with telomerase inhibition. The increase of *Tm* of the quadruplex structures by ~ 13 °C upon interacting with **BMVC** suggests that this ligand could be a useful telomerase inhibitor.

Fluorescent dyes are useful to probe specific DNA from staining gels to visualizing chromosomes. Fig. 2 shows the absorption and fluorescence spectra of the free **BMVC** and its complex with $[d(GC)_6]_2$ (GC) and Hum at room temperature. The absorption maximum of **BMVC** at ~ 435 nm is red-shifted to ~ 465 nm in the presence of each DNA. In addition, a ~ 30% decrease in molar absorption coefficient was observed in both cases. These spectral changes of the absorption band indicate the interaction of **BMVC** with DNA. Furthermore, the corresponding fluorescence spectra recorded at λ_{ex} ~ 430 nm showed more interesting features. The fluorescence of **BMVC** increases in the presence of GC by an order of magnitude and upon interacting with Hum increases by two orders of magnitude. We consider that the **BMVC** ligand has the potential to act as a biomarker for recognizing some specific structures of DNA.

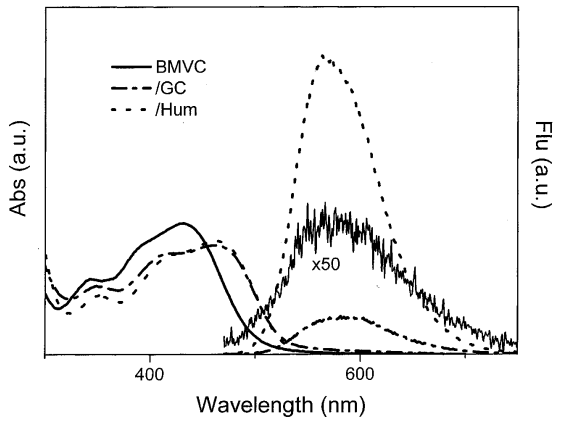


Fig. 2. Absorption and fluorescence spectra of **BMVC** and its complex with GC and Hum.

In summary, we have synthesized a new molecule of **BMVC**. This new molecule is of special interest in stabilizing the quadruplex of $d(T_2AG_3)_4$ structure and in increasing its fluorescence significantly upon interacting with $d(T_2AG_3)_4$. A conclusive application for anti-tumor agent and molecular biosensor definitively deserves more study on the selectivity, activity and specificity of the **BMVC** ligand to various DNA. Experiments are underway to further investigate these findings on **BMVC**, as well as to design more active analogues.

ACKNOWLEDGMENTS

This work was supported by Academia Sinica and the National Science Council of the Republic of China (Grants NSC 91-2113-M001-028).

Received March 17, 2003.

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