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A Fluorescent Carbazole Derivative: High Sensitivity for Quadruplex DNA

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We have synthesized a novel molecule, 3,6-bis(1-methyl-4-vinylpyridium)carbazole diiodide (BMVC), for recognizing specific quadruplex structures, particularly the quadruplex of human telomeric sequence d(T_2AG_3)$_4$. The fluorescence intensity of the BMVC molecule increases from 1 to almost 2 orders of magnitude upon interacting with various DNAs. At a concentration of BMVC of 10 μM, fluorescence bands with different colors of BMVC in electrophoresis gels of various DNAs can be observed. The fluorescence of BMVC can be used to discriminate between duplex and quadruplex DNAs. At the low concentration of 0.1 μM BMVC in prestained gels, the fluorescence is observed in the presence of quadruplexes with anti−anti−anti−anti and anti−anti−syn−syn arrangements. However, no fluorescence band is detected upon interacting with duplexes and quadruplexes with anti−syn−anti−syn arrangement. Moreover, the sensitivity assays show that as little as 0.2 pmol of quadruplex of d(T_2AG_3)$_4$ can be revealed by BMVC.

The use of fluorescent dyes is a key factor for the highly sensitive detection of nucleic acids in electrophoresis gels and fluorescence microscopy. The design and synthesis of new fluorescent molecules has become a widespread means of detecting nucleic acids. A number of dyes, such as ethidium bromide, YOYO, and SYBR green I, have been developed mostly for double-stranded DNA. A common feature of these dyes is the significant enhancement of their fluorescence upon binding to DNA. However, fluorescent dyes have not been extensively studied for guanine-rich (G-rich) quadruplex structures. These DNA quadruplexes have recently received great attention because G-rich sequences are often found in telomeres and because of their potential application in the development of antitumor agents.

Telomeres, the ends of chromosomes, are essential for the stability and replication of eukaryotic chromosomes.[1,2] The telomeric DNA sequences generally consist of many tandem repeats of G-rich motifs. For example, the T_2G_4, T_4G_4, and T_2AG_3 are the repeated subunits of the telomeres in *Tetrahymena*, *Oxytricha*, and vertebrates, respectively. There are G-rich sequences that can self-associate to form quadruplex structures, especially in the presence of specific salts. The quadruplex structure is stabilized by the $\pi-\pi$ interaction of cyclic G-quartets stacking on top of each other. Each G-quartet is formed by Hoogsteen hydrogen bonding among four guanine (dG) bases.[3] Three types of quadruplex structures, dependent on the orientation of the guanine in the strands, have been characterized in vitro.[4] Four G-rich strands form a linear parallel tetramer by the arrangement of four anti dG residues in each quartet (Chart 1a). The anti−syn−anti−syn alternation forms the antiparallel quadruplex and has lateral loops (Chart 1b−d). The anti−anti−syn−syn alternation makes another type of antiparallel quadruplex with diagonal loops (Chart 1e and f). Knowing the structure is essential to the design of sequence-specific DNA-binding ligands. Recently, we reported that the interaction of a ligand with the loops or tails of the quadruplexes could be important in stabilizing the external binding.[5] A sensitive DNA-binding ligand is developed for distinguishing different types of quadruplexes and recognizing a specific human telomeric sequence. Chart 1 shows some of the possible quadruplex structures.

Studies of binding interactions of small molecules with DNA quadruplexes are useful not only for better understanding of molecular recognition but also for the development of anticancer therapeutic agents.[6−8] Recently, we have synthesized a novel molecule, 3,6-bis(1-methyl-4-vinylpyridium)carbazole diiodide (BMVC), to stabilize the quadruplex structure of d(T_2AG_3)$_4$ (Hum24) and to inhibit telomerase activity.[9,10] In addition, the fluorescence of BMVC strongly increases upon interacting with Hum24. Here we report that visible fluorescence bands of BMVC upon binding various DNAs are observed from green to red in electrophoresis gels. Moreover, using 0.1 μM BMVC, one can reveal specific DNA quadruplexes in the prestained gels. It implies that BMVC could be used as a sensitive biosensor, particularly

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Chart 1

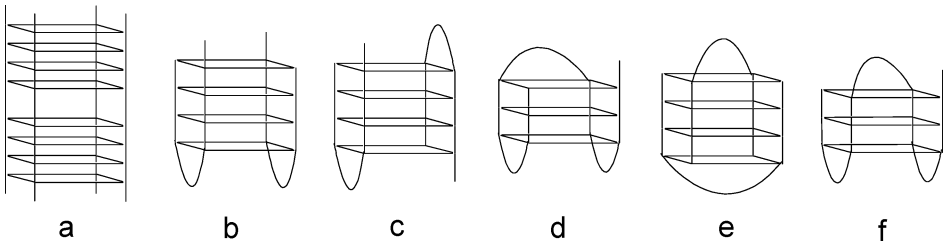
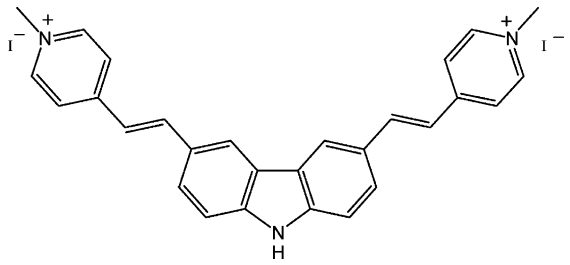


Table 1. Oligonucleotides Used in This Work

	sequence	abbreviation	structures[a]	ref
1	calf thymus DNA	ct-DNA		
2	5′-GCGCA_2T_2GCGC	LD		
3.	5′-$(GC)_6$	GC		
4.	5′-$(AT)_6$	AT		
5	5′-$(T_2G_4)_2$	Tet12	a−c	19, 20, 24
6	5′-$G_4T_4G_4$	Oxy12	e	22, 23
7	5′-$(T_2AG_3)_2$	Hum12	c	21
8	5′-$G_2T_2G_2TGTG_2T_2G_2$	Apt	d	15, 16
9	5′-$G_4(T_4G_4)_3$	Oxy28	f	14
10.	5′-$(T_2AG_3)_4$	Hum24	f	17, 18
11	5′-TG_4T	LQ1	a	5, 25
12	5′-T_4G_4	LQ4	a	25

[a] Refers to Chart 1.

Chart 2



for the quadruplex of Hum24. Furthermore, our results clearly indicate that at least two binding modes characterized by different fluorescence properties are involved. Table 1 lists the DNA sequences studied in this work.

EXPERIMENTAL SECTION

Chemical and Sample Preparation. Synthesis of the BMVC molecule from 3,6-dibromocarbazole has been described elsewhere.[9,10] The chemical structure is shown in Chart 2. All oligonucleotides were purchased from Applied Biosystems. Solutions of 10 mM Tris-HCl (pH 7.5) and 150 mM NaCl mixed with each DNA were heated to 90 °C for 2 min, cooled slowly to room temperature, and then stored for more than 2 days at 4 °C before use.

Absorption, Fluorescence, and Circular Dichroism Spectra. Absorption spectra were taken on a Hitachi U3200 UV−visible spectrophotometer, and fluorescence spectra were recorded on a Hitachi F4010 spectrofluorometer with a 2-nm band-pass in a 1-cm cell length at room temperature. The CD spectra were averages of 10 scans on a Jasco J-715 spectropolarimeter with a 2-nm bandwidth. The scan speed was 50 nm/min with a step resolution of 0.2 nm.

Fluorescence Titration. The fluorescence titration of BMVC by adding Hum24 was conducted at room temperature. Two different concentration ranges of DNA, from 0.015 to 6 μM and from 0.1 to 40 nM, were titrated into 15 μM and 10 nM solutions of BMVC, respectively. The total increase in sample volume during a titration was ∼2.5%. Each fluorescence spectrum was taken after incubation for 10 min.

PAGE. The PAGE was conducted in 10 mM Tris-HCl and 150 mM NaCl (pH 7.5) with 20% native gels. Electrophoresis gels were carried out at 100 V/cm for 15 h at 4 °C. The DNA concentration was determined by the absorbance at 260 nm and was adjusted to ∼10 μM per unit structure. The gels were photographed under UV light at 254 nm by a Bio-Rad imaging detector. After photographing with UV shadowing, gels were poststained in a solution containing 10 μM BMVC, 10 mM Tris-HCl, and 150 mM NaCl (pH 7.5) for 10 s at room temperature, rinsed with distilled water, and then photographed under UV light at 254 nm by a digital camera. The prestained gels were studied by incubation with either 10 or 0.1 μM BMVC with 10 μM concentrations of various DNA for 10 min before running the gels to present the selectivity of BMVC. To study the sensitivity of DNA detection, 0.1 μM BMVC incubated with various concentrations of DNA from 2.5 to 0.005 μM in the prestained gels at 100 V/cm for 6 h at 4 °C were examined.

RESULTS

Absorption and Fluorescence Spectra. Figure 1 shows the absorption and fluorescence spectra of free BMVC and its complex with DNA of AT, LD, ct-DNA, Tet12, Oxy12, Hum12, Apt, Oxy28, Hum24, LQ1, and LQ4 at room temperature. The molar ratio between BMVC and each DNA structure is 1:1 with the BMVC concentration at 5 μM. The absorption maximum of BMVC at ∼435 nm is red-shifted to ∼450 nm upon interacting with LD and ct-DNA and further red-shifted to ∼460 nm in the presence of DNA quadruplexes. In addition, the molar absorption coefficient is decreased by ∼15% for duplexes and ∼35% for quadruplexes. The spectral changes of the BMVC/DNA complexes indicate the interaction of the BMVC with these DNAs.

The fluorescence spectra recorded at $\lambda_{ex} \approx 430$ nm show that the fluorescence of free BMVC is quite weak. However, the fluorescence increases 15-fold in the presence of LQ1, 60-fold in the presence of Hum24, and 75-fold in the presence of LD and ct-DNA. Moreover, the increase is even higher at $\lambda_{ex} \approx 460$ nm. For example, it can be up to 90-fold in the presence of Hum24. Another important finding is that the strong fluorescence of the BMVC resulting from the interaction with AT, LD, and ct-DNA duplexes differs from that with DNA quadruplexes by a band shift from ∼550 to ∼575 nm.

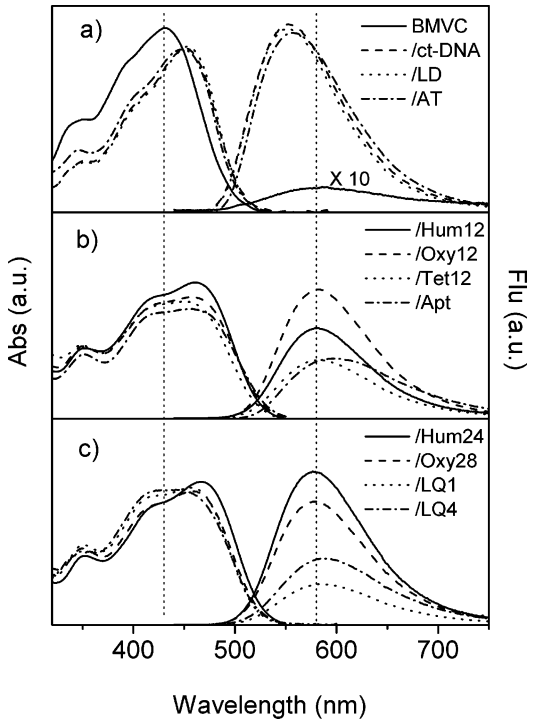


Figure 1. Absorption and fluorescence spectra of 5 μM BMVC and its complexes with AT, LD, ct-DNA, Oxy12, Hum12, Tet12, Apt, Hum24, Oxy28, LQ1, and LQ4. The concentration of DNA is adjusted to be 5 μM per unit structure in a solution of 10 mM Tris-HCl (pH 7.5) and 150 mM NaCl.

Electrophoresis Gels. A common feature of sensitive biomarkers is the significant enhancement of fluorescence upon binding to DNA. The fluorescence enhancement of BMVC prompted us to examine whether BMVC could act as a DNA marker in electrophoresis gels. Figure 2a shows the UV shadowing of a typical gel of LD, AT, GC, LQ1, LQ4, Tet12, Oxy12, Oxy28, Hum12, Hum24, and Apt. It is found that most of the DNA migrates as a single band, but Tet12 and LQ4 have two bands. After staining the gels in a solution containing 10 μM BMVC for 10 s, Figure 2b shows the different colors for the fluorescence bands in the gels under UV light at 254 nm. Note that no fluorescence band is detected for LQ1 and for the fast mobile component of LQ4, and an extremely weak band is detected for GC.

Furthermore, we have studied whether the fluorescence of BMVC can be used to present the selectivity of BMVC to various DNAs. Figure 2c shows the fluorescence bands of 10 μM BMVC in the prestained gels of 10 μM LD, Hum24, Oxy12, and LQ4, and mixtures of LD and Hum24, LD and Oxy12, LD, and LQ4, and Hum24 and Oxy12. Although the mobility of LD/BMVC and Hum24/BMVC is similar in the gels, the color of the fluorescence band suggests that BMVC has preference to Hum24 than to LD. Further study of LD with 16 mers and Hum24 incubated with BMVC clearly shows that BMVC has high selectivity to Hum24 rather than to LD. In addition, the fluorescence band of BMVC is observed in both LD and Oxy12 but is much dominated in Hum24 than in Oxy12. In the mixture of LQ4 and LD, a strong fluorescence band is only observed in LQ4. It appears that the fluorescence of BMVC can be used to discriminate between the various DNAs.

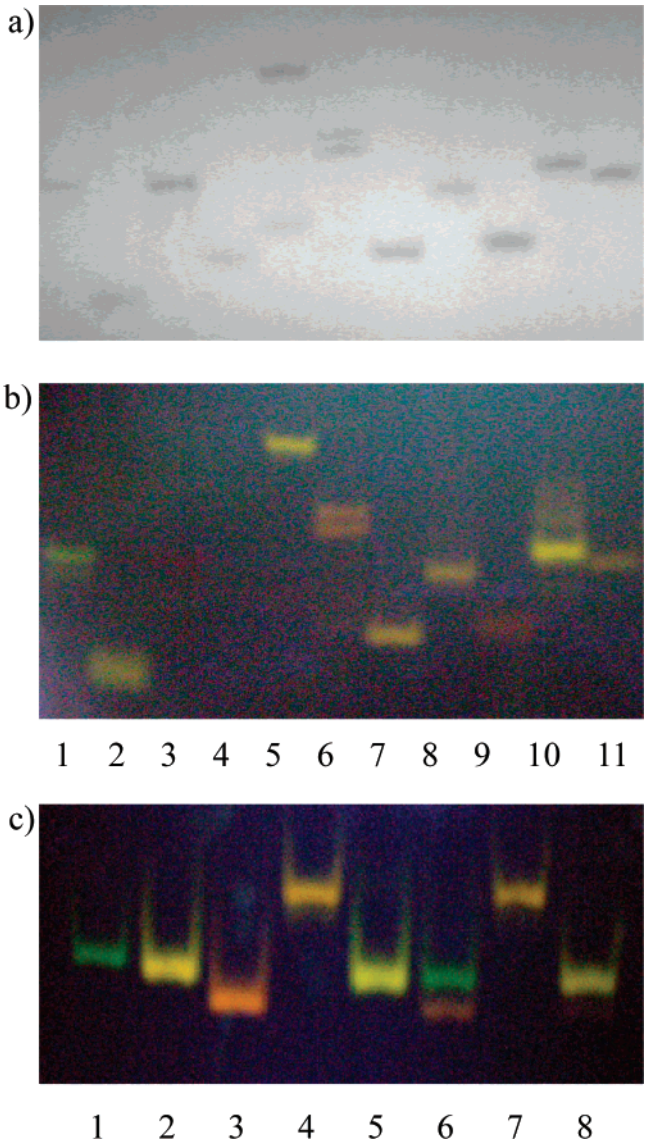


Figure 2. Selectivity assays. (a) UV shadowing in electrophoresis gels of (1) LD, (2) AT, (3) GC, (4) LQ1, (5) LQ4, (6) Tet12, (7) Oxy12, (8) Oxy28, (9) Hum12, (10) Hum24, and (11) Apt. (b) After staining the gels in a solution containing 10 μM BMVC for 10 s, different colors of BMVC result from the interaction with various DNA. (c) The prestained gels of 10 μM DNA incubated with 10 μM BMVC for 10 min, where (1) LD, (2) Hum24, (3) Oxy12, (4) LQ4, and the mixtures of (5) LD + Hum24, (6) LD + Oxy12, (7) LD + LQ4, and (8) Hum24 + Oxy12.

To measure the sensitivity of DNA detection in gels, we have recorded the fluorescence band of BMVC by using a Bio-Rad imaging detector. Figure 3a shows that the quadruplexes of LQ4, Oxy12, Oxy28, Hum24, and Tet12 can be clearly revealed in the prestained gels by the presence of the fluorescence band of BMVC at 0.1 μM under UV light at 254 nm. However, no band is detected in the presence of LD, AT, GC, LQ1, Hum12, and Apt. Of particular interest is that the third band of Tet12 revealed by the fluorescence of BMVC in the prestained gels differs from the two fast mobile bands of Tet12 observed in the UV shadowing and poststained gels. It appears that 0.1 μM BMVC is sufficient to reveal a small amount of the linear tetramer of Tet12. Another interesting feature is that the mobility of Hum24 is slower than that of Oxy28 in the poststained gels, but the mobility of BMVC/Hum24 complexes

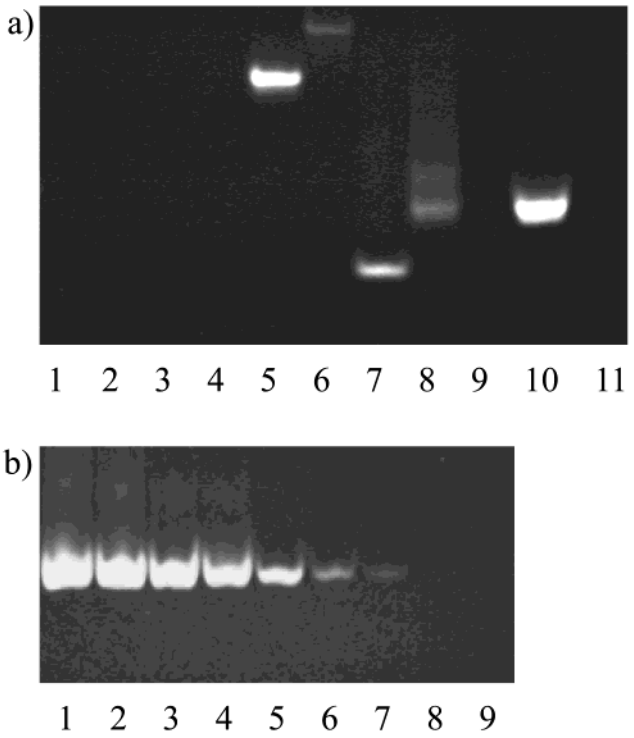


Figure 3. Sensitivity assays. (a) The prestained gels of 10 μM DNA incubated with 0.1 μM BMVC for 10 min, where (1) LD, (2) AT, (3) GC, (4) LQ1, (5) LQ4, (6) Tet12, (7) Oxy12, (8) Oxy28, (9) Hum12, (10) Hum24, and (11) Apt. (b) The prestained gels of 0.1 μM BMVC incubated with various concentrations of Hum24 at (1) 2.5, (2) 1, (3) 0.5, (4) 0.25, (5) 0.1, (6) 0.05, (7) 0.025, (8) 0.01, and (9) 0.005 μM.

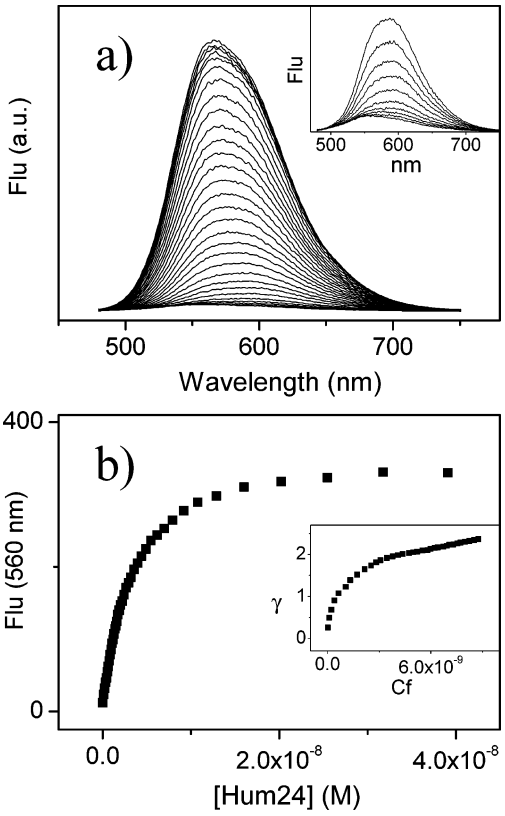


Figure 4. (a) Fluorescence spectra of BMVC at 15 μM by adding Hum24 from 0.015 to 6 μM and (b) fluorescence intensity at 560 nM BMVC with 10 nM by adding Hum24 from 0.1 to 40 nM. The inset in (a) shows several initial titration spectra, and the inset in (b) shows the binding plots of the titration.

is similar to that of BMVC/Oxy28 complexes in the prestained gels. It implies that the binding of BMVC makes Hum24 more rigid.

We have further determined the sensitivity of BMVC to Hum24. Figure 3b shows the sensitivity assays of 0.1 μM BMVC incubated with various concentrations of Hum24 from 2.5 to 0.005 μM in the prestained gels. The sensitivity assays show that 0.1 μM BMVC can clearly reveal 0.025 μM Hum24. As a matter of fact, we can visualize the BMVC fluorescence band even at 0.01 μM Hum24. It appears that the BMVC is a highly sensitive biosensor for revealing the presence of tiny amounts of Hum24. Furthermore, 0.1 μM BMVC can reveal the linear tetramer of LQ4 at 0.05 μM, the antiparallel quadruplex of Oxy28 at 0.1 μM, and particularly small amounts of linear tetramer in 0.25 μM Tet12 in the prestained gels.

Fluorescence Titration. We have performed fluorescence titration of BMVC by Hum24 for the study of binding affinity. Figure 4a shows the fluorescence titration of BMVC at 15 μM by adding Hum24 from 0.015 to 6 μM. The fluorescence peak is first shifted from 550 to ~580 nm and then shifted back to ~565 nm, as shown in the inset, indicating that the binding is not a single process. The strong enhancement of the fluorescence upon interaction with DNA allows us to study the binding by using fluorescence titration at a very low concentration. Figure 4b shows the fluorescence intensity at 560 nm of BMVC with 10 nM by adding Hum24 from 0.1 to 40 nM. The titration data are then applied to construct the binding plots of γ versus C_f, as shown in the inset. The binding ratio γ is defined as C_b/C_{DNA}, where C_f, C_b, and C_{DNA} are the molar concentrations of free ligand, bound ligand, and DNA, respectively. The difference between C_t and C_b gives the magnitude of C_f, where C_t is the total concentration of ligand. No clear convergence for the curve of the binding plots suggests that the binding of BMVC to Hum24 is a complex process. It probably involves both nonspecific binding and specific binding sites.[11] Although we are not able to determine the binding constant, the small amounts of nanomole concentrations used for the fluorescence titration indicates a high binding affinity for Hum24.

Circular Dichroism (CD). During the past decade, CD has been used to characterize different structures of G-rich quadruplexes.[12−14] It has been documented that parallel four-stranded quadruplexes give a positive band around 260 nm and a negative band around 240 nm,[12] while antiparallel folded quadruplexes have two positive bands around 245 and 290 nm and a negative band around 265 nm.[13] Figure 5 shows the CD spectra of Apt, Tet12, Hum12, Hum24, Oxy12, Oxy28, LQ1, and LQ4 in the absence and presence of BMVC. The CD results confirm that antiparallel quadruplexes dominate in Hum12, Hum24, Oxy12, Oxy28, and Apt and that linear tetramers are present in LQ1 and LQ4.

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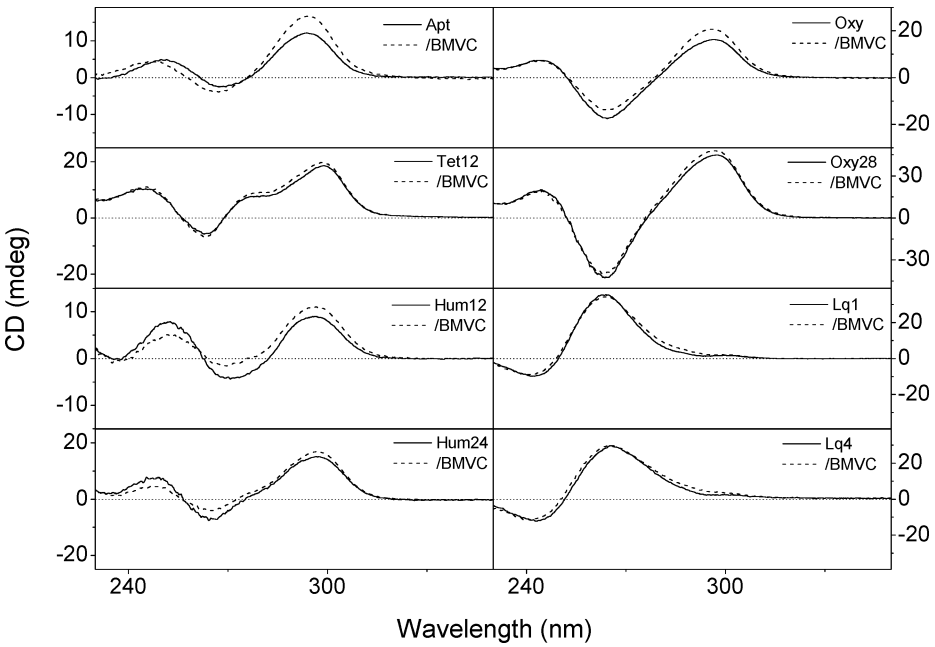

Figure 5. CD spectra of Apt, Tet12, Hum12, Hum24, Oxy12, Oxy28, LQ1, LQ4, and their complexes with BMVC at room temperature.

Although two bands are revealed for LQ4 in electrophoresis gels, the CD patterns suggest that the linear tetramer is the major component of LQ4. At present, the positive shoulder around 275 nm in Tet12 is not clear. However, the prominent 295-nm CD band suggests that the antiparallel quadruplex predominates in Tet12. In addition, the fact that there is no appreciable change in their CD patterns upon mixing with BMVC implies that their quadruplex structures are not appreciably distorted by BMVC.

DISCUSSION

Specific Quadruplexes Recognized by BMVC in Prestained Gels. The detailed structural information available for these DNAs provides a basis for the interpretation of our results. The Apt adopts a very stable unimolecular quadruplex with two G-quartets connected by one lateral TGT loop at one end and two parallel TT loops at the other end.[15,16] Hum24 in solution adopts a unimolecular quadruplex with one diagonal T_2A loop at one end and two parallel T_2A loops at the other end of G-quartets.[17,18] In the prestained gels, the fluorescence band of 0.1 μM BMVC can easily reveal the presence of Hum24 at 0.025 μM, but no band is detected for Apt even at 10 μM. In addition, dimeric hairpin quadruplexes with lateral loops has been reported in both Tet12[19,20] and Hum12,[21] but dimeric hairpin quadruplex with a diagonal loop at each end of the quartets has been documented for Oxy12.[22,23] Again, the prestained gels show a fluorescence band in Oxy12 but not in Tet12 and Hum12. Note that the quadruplex structures of Hum24, Oxy12, and Oxy28 have at least one diagonal loop at the end of G-quartets but no diagonal loop in the antiparallel quadruplex structures of Apt, Tet12, and Hum12. It is suggested that BMVC could recognize the antiparallel quadruplexes with diagonal loops in prestained gels. BMVC can be applied to distinguish the two major conformations of anti−anti−syn−syn and anti−syn−anti−syn of the antiparallel quadruplexes.

Figure 3a clearly shows a small amount of linear tetramer of Tet12 revealed by BMVC in the prestained gels, although it is not observed in the UV shadowing and poststained gels. With reference to the early studies on Tet12 by Sen and Gilbert,[19,24] the one band observed in the prestained gels is ascribed to a linear tetramer and the two bands detected in the poststained gels are due to two different antiparallel quadruplexes. For the LQ4 linear tetramers, there are two components characterized by two fluorescent bands in the UV shadowing. The upper band is more dominated than the lower one. The CD patterns indicate that the linear tetramer is the predominant component of LQ4.[25] This upper band is therefore likely due to the linear tetramer of LQ4. In addition, the fact that the mobility of the upper band of LQ4 is slightly faster than the linear tetramer of Tet12 and is a lot slower than the linear tetramer of LQ1 supports this assignment. The prestained gels show a bright fluorescence band in the presence of LQ4, but not in the presence of LQ1, implying that the length of the four tails could play an important role in the binding of BMVC to the linear tetramers. Furthermore, the prestained gels also show a bright fluorescence band of BMVC upon interacting with $[d(T_2G_4T_2)]_4$ but no band in the presence of $[d(G_{10})]_4$ (data not shown). It appears that BMVC can also recognize linear tetramers with a certain length of tails. Nevertheless, the linear tetramers and antiparallel quadruplexes can be distinguished by the CD spectra.

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Binding Sites. Electrophoresis gel is not only invaluable in distinguishing various quadruplex structures but also useful in providing binding information. Since both LQ1 and LQ4 are dominated by linear tetramers, a fluorescence band of BMVC detected in the presence of LQ4, but not in LQ1, in prestained gels implies that this band is unlikely due to the binding modes of intercalation or groove binding. It is more likely that the ring moiety of BMVC stacks to the end of the G-quartet and the pendant group of BMVC interacts with the T_4 tails. Note that external stacking to the end of a G-quartet is a popular model for planar aromatic molecules binding to the antiparallel quadruplexes.[5,26−29]

The minor component of the linear tetramer of Tet12 revealed in prestained gels and the two major components of antiparallel quadruplexes of Tet12 observed in poststained gels indicate that at least two binding modes are involved in the BMVC/Tet12 complexes. One binding mode is more stable since it can overcome the running of electrophoresis gels. Another binding mode revealed in the poststained gels is kinetically favored. It is rational that similar binding modes of BMVC to LQ4 are also involved with the linear tetramer of Tet12. The kinetically favored binding mode may be mainly due to ionic interaction between them.

In addition, Arthanari and Bolton[30] and Hurley et al.[31] have shown that porphyrins can catalyze the conversion of antiparallel quadruplex structures into parallel strand structures. Here the difference between the prestained and poststained gels of Tet12 could be due to conversion from antiparallel quadruplexes into parallel quadruplexes by BMVC. If it is so, the binding sites could be very different before and after the conversion. However, no appreciable change around 260 nm in the CD pattern of Tet12 upon mixing with BMVC suggests that the conversion from antiparallel to parallel quadruplexes can be neglected in this work. Nevertheless, the catalytic activity of BMVC deserves further detailed study.

In our previous study of absorption titration of Hum24 to BMVC,[10] the clear absence of an isosbestic point indicates that the binding is a complex process, with at least two binding modes characterized by different spectral properties. Here the fluorescence titration confirms that at least two binding modes are involved. The fluorescence bands of BMVC in the prestained gels suggest that the quadruplexes with either diagonal loops or the linear tetramers with at least two bases in each tail are essential for binding stability. On the other hand, the fluorescence bands of BMVC in the poststained gels of Tet12, Hum12, and Apt imply that the quadruplexes with lateral loops can also interact with BMVC. We anticipate that the two binding modes are probably due to two different loop configurations at the ends of the Hum24 quadruplex.

Neidle et al.[29] have applied molecular modeling to determine the binding mode of 3,6-disubstituted acridine derivatives to the Hum24 quadruplex. They found that the ligand externally stacks between the diagonal loop and the end of the G-quartet. It is conceivable that this binding mode is also possible for the G-quadruplex interaction with BMVC. The carbazole moiety of BMVC externally stacks to the end of the G-quartet through $\pi-\pi$ interaction. In addition, ionic interaction of the positive charge in the pendant group of pyridium with a negative DNA phosphate group plays an important role in the binding stability. The details of the binding modes deserve further investigation.

Different colors of the fluorescence bands of BMVC suggest that BMVC might be a useful DNA biomarker. In poststained gels, the fluorescence bands of BMVC are greenish in the presence of LD and AT but are extremely weak and red in the sequence of GC. It appears that BMVC has preference to bind to AT than to GC in DNA duplexes. On the other hand, the fluorescence bands of BMVC upon interacting with most quadruplex structures are characterized by the colors from yellow to orange. The interaction of BMVC with the antiparallel quadruplex of Tet12 is characterized by an orange band and differs from that of Hum24, which is characterized by a yellow band in poststained gels, implying that their binding modes are different. More experiments are required for better understanding of the relationship between the fluorescence properties and the binding modes of BMVC to various DNA.

Sensitive Biosensor. Recently, Koeppel and co-workers[32] measured the sensitivity of ethidium derivatives to quadruplex DNA. The highest sensitivity found in their work is that a 0.1 μM concentration of an ethidium derivative could reveal 0.12 μM quadruplex DNA, indicating that tiny amounts of quadruplex DNA at 2.4 pmol can be revealed by this ligand. Here we observe that 0.1 μM BMVC could reveal 0.01 μM Hum24, indicating that a tinier amount of a quadruplex, Hum24 at 0.2 pmol, can be revealed by BMVC. To the best of our knowledge, BMVC is one of the most sensitive fluorescent dyes for recognizing the quadruplex of human telomeric sequence of $d(T_2AG_3)_4$ to date. Increasing the detection sensitivity of small molecules that bind to specific DNA might find utility as probes to elucidate these DNA structures and their functional significance. One of the potential applications is to determine the structure of human telomere in living cells.

In summary, we have illustrated the fluorescence properties of BMVC upon mixing with various nucleic acids. The fluorescence of BMVC increases significantly upon interacting with DNA, up to 90-fold with Hum24 at $\lambda_{ex} \approx 460$ nm. Moreover, visible fluorescence bands from green to red in electrophoresis gels are detected upon interacting with various DNA. This finding may be useful in the application of fluorescence imaging for high-throughput screening. The fluorescence of BMVC can be used to discriminate between duplex and quadruplex DNAs. In addition, 0.1 μM BMVC in prestained gels could be used to identify some

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specific quadruplex structures. By combining with CD spectra, one can distinguish three types of quadruplexes. Of particular interest is that a very tiny amount of Hum24 at 0.2 pmol can be revealed by BMVC. The high sensitivity of BMVC to a specific DNA indicates that BMVC might be an excellent biosensor. We consider that BMVC is not only useful as a quadruplex stabilizer and telomerase inhibitor but also is invaluable as a sensitive biosensor.

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