Anal. Chem. **2009,** *81,* 2678–2684

Fluorescent Sensor for Monitoring Structural Changes of G-Quadruplexes and Detection of Potassium Ion

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G-rich sequences with the potential for quadruplex formation are common in genomic DNA. Considering that the biological functions of G-quadruplexes may well depend on their structures, the development of a sensitive structural probe for distinguishing different types of quadruplexes has received great attention. Crystal violet (CV) is a triphenylmethane dye, which can stack onto the two external G-quartets of a G-quadruplex. The ability of CV to discriminate G-quadruplexes from duplex and single-stranded DNAs has been reported by us. Herein, the ability of CV to discriminate parallel from antiparallel structures of a G-quadruplex was studied. The binding of CV to an antiparallel G-quadruplex can make its fluorescence intensity increase to a high level because of the protection of bound CV from the solvent by quadruplex end loops. The presence of side loops in parallel G-quadruplexes cannot provide bound CV such protection, causing the fluorescence intensity of CV/G-quadruplex mixture to be obviously weaker when the G-quadruplex adopts a parallel structure than that when the G-quadruplex adopts an antiparallel structure. Therefore, CV can be developed as a sensitive fluorescent biosensor for the discrimination of antiparallel G-quadruplexes from parallel G-quadruplexes and for monitoring the structural interconversion of G-quadruplexes. In addition, considering that some G-rich DNA sequences can adopt different G-quadruplex structures under Na^+ or K^+ ion conditions, a novel, cheap and simple K^+ ion detection method was developed. This method displays a high K^+ ion selectivity against Na^+ ion, the change of 200 mM in Na^+ ion concentration only causes a similar fluorescent signal change to 0.3 mM K^+ ion.

Genomic DNA usually exists as a canonical right-handed double helix, but it can also form other structures. A G-rich nucleic acid sequence in particular is predisposed to form higher order structures: e.g., a G-quadruplex.[1] G-quadruplexes are unique higher-order structures formed by G-rich nucleic acid sequences based on stacked arrays of G-quartets connected by Hoogsteen-type base pairing. Formation of G-quadruplexes requires runs of guanine on four, two, or one strand, resulting in linear (four strands) or folded (two or one strand) structures.[2] Even with the same number of associated molecules, G-quadruplexes can reveal many topological structures differing by mutual strand orientation (Scheme 1).[3]

G-rich sequences with the potential for quadruplex formation are common in genomic DNA and have been identified in several biologically important regions such as telomeric DNA and in many gene promoter regions.[4−8] Considering that the biological functions of G-quadruplexes may well depend on their structures,[9−12] the development of a sensitive structural probe for distinguishing the different types of quadruplexes has received great attention.[13−16]

Malachite green (MG) and crystal violet (CV) are both triphenylmethane dyes. It has been reported that MG exhibits a strong increase in fluorescence upon binding to intramolecular quadruplexes.[17] Recently, we have reported that CV could be used as a sensitive fluorescent biosensor for the discrimination of

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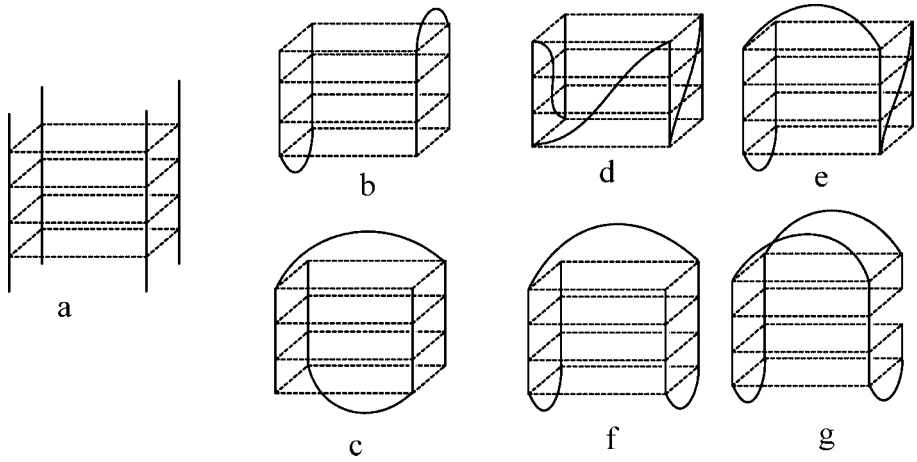
10.1021/ac802558f CCC: $40.75
Published on Web 03/09/2009

Scheme 1. Schematic Representation of Different Types of G-Quadruplex[a]



[a] (a) Intermolecular parallel-stranded quadruplex; (b,c) intermolecular dimer-hairpin-folded quadruplexes; (d,e) intramolecular quadruplexes, (d) intramolecular parallel quadruplex and (e) intramolecular mixed-type quadruplex; (f,g) intramolecular antiparallel quadruplex.

G-quadruplexes from duplex and single-stranded DNAs.[18] In this work, the ability of CV to discriminate G-quadruplexes with different structures was investigated. The results show that CV can also be used as a sensitive biosensor for the discrimination of antiparallel G-quadruplexes from parallel G-quadruplexes and for monitoring the cation dependence of quadruplex structure interconversion. In addition, considering that some G-rich DNA sequences can adopt different G-quadruplex structures under Na^+ or K^+ ion conditions, the suitability of CV/G-quadruplex complexes for K^+ ion detection was determined.

EXPERIMENTAL SECTION

Materials. Crystal violet was obtained from Sigma and used without further purification. All oligonucleotides were purchased from Etiwu Biotech. Co. Ltd. (Tianjin China). The concentrations of these oligonucleotides were represented as single-stranded concentrations unless otherwise noted. Single-stranded concentrations were determined by measuring the absorbance at 260 nm. Molar extinction coefficients were determined using a nearest neighbor approximation (http://www.idtdna.com/analyzer/Applications/OligoAnalyzer). In order to ensure the formation of G-quadruplex structures, the oligonucleotide samples were dissolved in a buffer solution consisting of 25 mM Tris-HCl (pH = 7.0) and the indicated levels of NaCl and KCl. The solutions were heated to 90 °C for 5 min, cooled slowly to room temperature, and then incubated at room temperature overnight.

Fluorescence Spectroscopy. All fluorescence measurements were made using Hitachi model RF-4500 spectrofluorimeter. Reaction mixtures with different concentrations of oligonucleotides were mixed with 15 μM CV in 25 mM Tris-HCl buffer (pH = 7.0) containing the indicated levels of NaCl and KCl. To ensure the interaction between G-quadruplexes and the dyes, the mixtures were kept at 30 °C for 1 h before spectrometric detection. The spectra were excited at 580 nm and monitored from 610 to 700 nm. The fluorescence titration measurements were performed by keeping the concentration of CV at 15 μM while varying the DNA concentrations. The emission at 635 nm was monitored as a function of DNA concentration.

Competition Dialysis Assay. The buffer (25 mM Tris-HCl, pH = 7.0, 100 mM KCl or 100 mM NaCl) that was used in the fluorescence assays was also used in the competition dialysis assay. Dialysate solution (200 mL) containing 1 μM CV was placed into a beaker. A volume of 100 μL of each of the DNA samples was pipetted into a silide-A-lyzer miniature dialysis unit (molecular-weight cutoff of 3500; Thermo Scientific). All of the dialysis units were inserted into a float and then placed in the beaker containing the dialysate solution. In order to minimize the effect of evaporation of the dialysate solution, the beaker was covered with preservative film and wrapped in aluminum foil. The dialysate solution was continuously stirred for 24 h at room temperature. DNA samples or dialysate solution (90 μL of each) were then carefully removed to a PCR tube, and 10% (w/v) Triton X-100 (10 μL) was added to dissociate CV from the DNA structures. The total concentration of CV within each dialysis unit (C_t) and the free concentration of CV within dialysate solution (C_f) were determined spectrophotometrically using an absorbance wavelength of 595 nm and a constructed standard curve. Appropriate correction was made for the small dilution resulting from the addition of the Triton X-100 stock solution. The bound CV concentration (C_b) for each dialysis unit was then determined by difference as $C_b = C_t - C_f$.

Circular Dichronism (CD) Study. CD spectra were measured on a Jasco J-715 spectropolarimeter at room temperature. Oligonucleotide solutions (7.5 μM) were prepared in 25 mM Tris-HCl buffer (pH 7.0) containing the indicated levels of NaCl and KCl. To ensure the formation of quadruplex structures, the solutions were heated to 90 °C for 5 min, cooled slowly to room temperature, and then incubated at room temperature overnight. Spectra were recorded between 220 and 320 nm in 1 mm path length cuvettes. Spectra were averaged from 3 scans,

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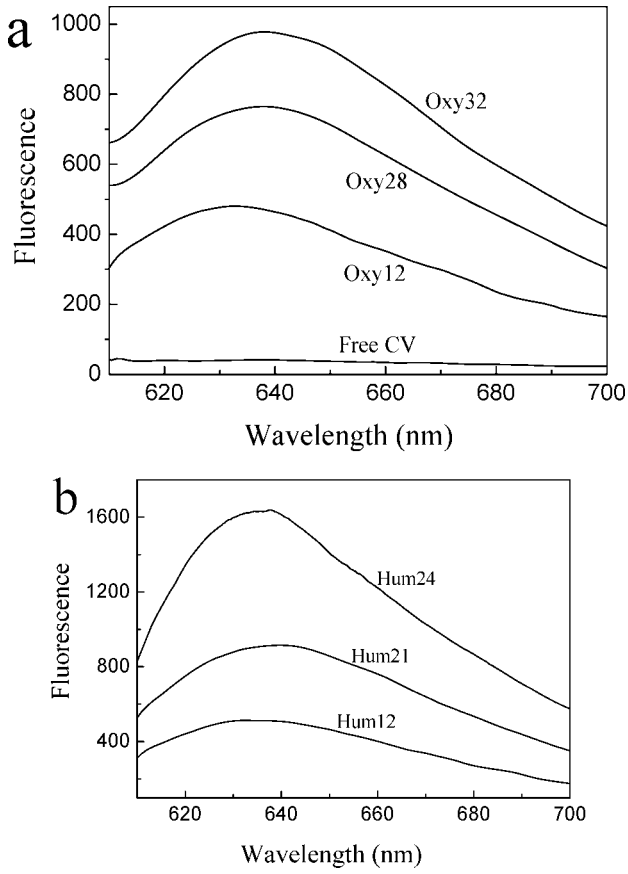

Figure 1. Fluorescence spectra of CV in the absence or presence of different G-quadruplexes. (a) Oxy12, Oxy28, or Oxy32; (b) Hum12, Hum21, or Hum24. [CV] = 15 μM, [Oxy28] = [Oxy32] = [Hum21] = [Hum24] = 7.5 μM, [Oxy12] = [Hum12] = 15 μM.

which were recorded at 50 nm/min with a response time of 1 s and a bandwidth of 0.2 nm.

RESULTS

Fluorescence and CD Spectra of Oxy12, Oxy28, and Oxy32. To investigate the ability of CV to distinguish G-quadruplexes with different structures, the fluorescence spectra of CV in the presence of Oxy12 (5′-$G_4T_4G_4$), Oxy28 (5′-$G_4(T_4G_4)_3$), and Oxy32 (5′-$G_2(T_4G_4)_3T_4G_2$) were measured. All of these three DNAs have the repeated subunit of the telomere in *Oxytricha*. Oxy12 can form an intermolecular dimer-hairpin-folded G-quadruplex structure, Oxy28 and Oxy32 can form intramolecular G-quadruplex structures.[19] As can be seen in Figure 1a, the fluorescence of free CV was very weak, and a significant increase in the emission intensity was observed at a fixed CV concentration in the presence of G-quadruplex DNAs. To investigate the fluorescence of CV as a function of DNA concentration, a fluorescence titration experiment was also performed (see Supporting Information); the results show that the binding stoichiometric ratios of CV to the three G-quadruplexes were all 2:1, and the association constants (K_a) of the G-quadruplex-CV complexes are 0.0066 ± 0.0003, 0.019 ± 0.003, and 0.012 ± 0.001 μM^{-2} and for Oxy12, Oxy28, and Oxy32, respectively. The result of energy transfer fluorescence titration experiment also demonstrated that the binding stoichiometric ratios of CV to the three G-quadruplexes were 2:1 (see Supporting Information). This 2:1 stoichiometry is consistent with a binding model based on the stacking of the dye onto the two external G-quartets of the quadruplexes.[20]

Figure 1a also shows that the fluorescence intensities of CV in the presence of the intramolecular G-quadruplexes (Oxy28 and Oxy32) are always higher than those in the presence of the intermolecular G-quadruplex (Oxy12). This result suggests that CV may be used as a biosensor to distinguish intramolecular G-quadruplexes from intermolecular G-quadruplexes when they have similar base sequences.

Both of Oxy28 and Oxy32 can form intramolecular G-quadruplexes, but the fluorescence of CV in the presence of Oxy32 is obviously higher than that in the presence of Oxy28. The reason may be attributed to the structural difference of the two G-quadruplexes. The CD spectra of Oxy28 and Oxy32 were investigated (see Supporting Information). It can be seen that both Oxy28 and Oxy32 display a major positive peak at around 295 nm with a minimum at around 260 nm, which is typical of an antiparallel arrangement of the strands.[9,21] No appreciable change in their CD patterns is observed in the presence of CV, which implies that their quadruplex structures are not significantly affected by CV. The main structural difference between Oxy28 and Oxy32 is the number and the type of loops. There are two kinds of loops in G-quadruplexes, one is an "end loop" located at the two ends of the G-quadruplex and used to link two G bases in the same G-quartet (loops in Scheme 1f,g). The other is a "side loop" located at the side of the G-quadruplex and used to link two G bases in the two terminal G-quartets (loops in Scheme 1d). End loops can also be divided into two kinds: one is a "diagonal loop" that connects two G bases located at diagonal sites in a terminal quartet. The other is a "lateral loop" that connects two adjacent G bases in a terminal quartet. According to CD spectra and previously reported results,[22,23] there are two lateral loops and one diagonal loop in the G-quadruplex formed by Oxy28 (Scheme 1f) and there are four lateral loops in the G-quadruplex formed by Oxy32 (Scheme 1g).

CV is a triphenylmethane dye where the propeller-like twisting of the phenyl rings with respect to the central triarylmethyl carbon increases the vibrational flexibility, and the total polarity of the whole molecule is poor.[24] Thus, the fluorescence yield of the dye is very low. The stacking of the dye on the face of the G-quartet increased the rigidity of the dye structure, which may increase the fluorescence of CV to some extent. As mentioned above, the fluorescence of the CV/Oxy32 mixture is obviously higher than that of the CV/Oxy28 mixture. No doubt there are also some other factors that influence the fluorescence of CV. CV binds to Oxy28 and Oxy32 with the same stoichiometric ratio and similar association constants. Thus, binding stoichiometric ratio and binding stability may not be the decisive factors. It is very possible that the protection of bound CV by end loops is an important factor

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affecting the fluorescence of CV. When the dye of CV stacks onto the two external G-quartets of the quadruplexes, it can be well-protected by end loops from the solvent and present a large increase in the emission intensity.[14] It is very possible that the cooperative protection ability of two lateral loops is stronger than the sole protection ability of a diagonal loop, causing the fluorescence of CV/Oxy32 to be higher than that of CV/Oxy28.

Fluorescence and CD Spectra of Hum12, Hum21, and Hum24. Under the same conditions, the fluorescence spectra of CV in the presence of Hum12 (5′-$(T_2AG_3)_2$), Hum21 (5′-$G_3(T_2AG_3)_3$), and Hum24 (5′-$G(T_2AG_3)_3T_2AG_2$) were also measured. These three oligonucleotides have the repeated subunit of the telomere from vertebrates. Hum12 can form the intermolecular dimer-hairpin-folded G-quadruplex structure. Hum21 and Hum24 can form intramolecular G-quadruplex structures. As shown in Figure 1b, the fluorescence intensities of CV in the presence of the intramolecular G-quadruplexes (Hum21 and Hum24) are also higher than that in the presence of the intermolecular G-quadruplex (Hum12). This result further suggests that CV may be used as a biosensor to distinguish intramolecular G-quadruplexes from intermolecular G-quadruplexes. In the case of the intramolecular G-quadruplexes, Hum24 can increase the fluorescence intensity of CV to a much greater extent than Hum21. The results of fluorescence titration experiment show that the binding stoichiometric ratios of CV to these three G-quadruplexes are all 2:1, and the association constants (K_a) of the G-quadruplex-CV complexes are 0.0033 ± 0.0008, 0.036 ± 0.008, and $0.059 \pm 0.018\ \mu M^{-2}$ and for Hum12, Hum21, and Hum24, respectively (see Supporting Information). Although the binding stability of CV/Hum24 is a little higher than that of CV/Hum21, the fluorescence difference between CV/Hum24 and CV/Hum21 can also be interpreted to be due to the protection of bound CV by end loops. The CD spectrum of Hum24 indicates that Hum24 adopts a typical antiparallel structure with four lateral loops (Scheme 1g). The CD spectrum of Hum21 displays a positive peak at around 295 nm and a negative peak at near 240 nm (see Supporting Information), indicating that neither a typical antiparallel G-quadruplex nor a typical parallel G-quadruplex can be formed. Some reports suggest that Hum21 may adopt a hybrid structure as in Scheme 1e.[25−28] In this structure, there are two lateral loops and one side loop. It is very probable that a side loop has no protective ability or only provides a much weaker protection to the bound CV than end loops. Thus, it is not surprising that the fluorescence of the CV/Hum24 mixture is much greater than that of the CV/Hum21 mixture.

Fluorescence and CD Spectra of d(5′-$G_3T_iG_3T_jG_3T_kG_3$-3′) Sequences. To further reinforce the results mentioned above, the suitability of CV to discriminate different structures of the G-quadruplexes formed by d(5′-$G_3T_iG_3T_jG_3T_kG_3$-3′) sequences was assessed (Table 1). Such sequences have been used to study the influence of short loop lengths on the stability and topology of intramolecular DNA G-quadruplexes, and the results show that loop lengths can strongly influence the topology and stability of intramolecular G-quadruplexes.[4,9] To simplify the names, sequences were named $T_iT_jT_k$ according to the number of T bases in loops connecting the four tracts of guanines (for example, TT_2T_3 corresponds to d(5′-$G_3TG_3T_2G_3T_3G_3$-3′)).

To investigate the suitability of CV as a structural sensor, the CD spectra of such sequences under different ionic conditions (100 mM K^+ or 100 mM Na^+) were measured (see Supporting Information). According to the results of CD measurement, these 27 DNA sequences can be divided into five groups. There are 10 DNA sequences in group I whose total lengths of the loops are not more than five nucleotides. The G-quadruplex structures formed by them are not affected by ionic conditions. No matter which ion (K^+ or Na^+) was present in the solution, their CD spectra displayed a positive peak at around 260 nm and a negative peak at near 240 nm, suggesting that all of them form quadruplexes with the same topology, which is likely to be a parallel structure. For each DNA sequence, considering that a similar G-quadruplex structure was formed in the presence of 100 mM K^+ ion or 100 mM Na^+ ion, the corresponding CV/DNA mixture should have a similar fluorescence levels under the two ionic conditions, which is demonstrated by the results of fluorescence determination (Table 1). In addition, as mentioned above, there is no end loops in the parallel G-quadruplex structures and side loops can not provide effective protection to bound CV, thus the fluorescence of CV can only be enhanced to a relatively low level in the presence of the DNA sequences in group I. The fluorescence intensities listed in Table 1 supports this speculation.

There is only one DNA sequence (TT_3T_2) in group II, and the total length of the loops is six nucleotides. Under the two ionic conditions, the CD spectra of TT_3T_2 display a positive peak around 280 nm rather than 295 or 260 nm, indicating that no G-quadruplex structures are formed. It is very possible that TT_3T_2 exists in a randomly coiled configuration. It has been demonstrated that CV can be developed as a biosensor to distinguish G-quadruplexes from duplex and single-stranded DNAs,[19] and the fluorescence increase of CV upon addition of duplex or single-stranded DNAs is much lower than that upon addition of G-quadruplexes. Table 1 shows that the fluorescence intensities of CV in the presence of TT_3T_2 under both ionic conditions are only half of that in the presence of the DNA sequences in group I. These results are perfectly consistent with the conclusions that have been reported.

There are five DNA sequences in group III whose total lengths of the loops are six or seven nucleotides. According to CD spectra, these sequences adopt different G-quadruplex structures under the two ionic conditions. In the presence of 100 mM K^+ ion, they can form typical parallel G-quadruplexes. In the presence of 100 mM Na^+ ion, their CD profiles display two positive peaks at around 265 nm and around 295 nm or display a positive peak at around 265 nm (or 295 nm) and a shoulder peak at around 295 nm (or 265 nm). This suggests that they are probably composed of mixtures of parallel and antiparallel G-quadruplexes or exist as a hybrid of parallel/antiparallel G-quadruplex structures. We assign the two situations the same name of "mixed-type". There is no end loop in parallel G-quadruplex structures, but end loops undoubtedly exist in both the parallel/antiparallel G-quadruplex mixtures and parallel/antiparallel

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Table 1. Fluorescence of CV/DNA Mixtures and G-Quadruplex Structures of DNAs

		fluorescence intensity			G-quadruplex structure	
group	DNAs	100 mM K^+	100 mM Na^+	ratio[a]	100 mM K^+	100 mM Na^+
I	TTT	90.51	88.87	0.9819	parallel	parallel
	T_2TT	101.0	100.7	0.9970	parallel	parallel
	TT_2T	72.29	81.97	1.134	parallel	parallel
	TTT_2	96.82	115.0	1.188	parallel	parallel
	T_3TT	138.8	150.4	1.084	parallel	parallel
	TT_3T	133.6	137.5	1.029	parallel	parallel
	TTT_3	120.2	133.6	1.111	parallel	parallel
	T_2T_2T	117.3	120.3	1.026	parallel	parallel
	TT_2T_2	107.8	115.0	1.067	parallel	parallel
	T_2TT_2	112.9	147.6	1.255	parallel	parallel
II	TT_3T_2	59.39	57.35	0.9657	none[b]	none[b]
III	TT_2T_3	287.3	671.6	2.338	parallel	mixed-type
	$T_2T_2T_2$	151.1	268.1	1.774	parallel	mixed-type
	T_3TT_2	204.6	1089	5.323	parallel	mixed-type
	T_2TT_3	144.3	455.6	3.157	parallel	mixed-type
	$T_3T_2T_2$	229.3	460.6	2.009	parallel	mixed-type
IV	T_2T_3T	185.5	442.4	2.385	parallel	antiparallel
	T_3T_2T	176.5	358.1	2.029	parallel	antiparallel
	TT_3T_3	270.9	596.0	2.201	parallel	antiparallel
	T_3TT_3	146.4	854.9	5.839	parallel	antiparallel
	T_3T_3T	443.3	1123	2.533	parallel	antiparallel
	$T_3T_3T_2$	145.7	324.5	2.227	parallel	antiparallel
V	$T_2T_2T_3$	401.1	407.0	1.015	mixed-type	antiparallel
	$T_2T_3T_2$	493.1	560.1	1.136	mixed-type	antiparallel
	$T_2T_3T_3$	374.6	456.2	1.218	mixed-type	antiparallel
	$T_3T_2T_3$	520.6	722.1	1.387	mixed-type	antiparallel
	$T_3T_3T_3$	614.2	1088	1.771	mixed-type	antiparallel

[a] The value is the ratio of the fluorescence intensity in the presence of 100 mM Na^+ ion to that in the presence of 100 mM K^+ ion. [b] None represents no G-quadruplex is formed.

hybrid G-quadruplexes. The presence of end loops should be helpful for the protection of bound CV from the solvent, producing enhanced fluorescence signal. As shown in Table 1, the fluorescence intensities of the CV/DNA mixtures nearly doubled when the ionic condition was changed from 100 mM K^+ ion to 100 mM Na^+ ion.

There are six DNA sequences in group IV whose total lengths of loops are six or more nucleotides. They also adopt different G-quadruplex structures under the two ionic conditions. In the presence of 100 mM K^+ ion, they can form typical parallel G-quadruplexes. In the presence of 100 mM Na^+ ion, they exist in a typical antiparallel G-quadruplex form. There are zero and three end loops in typical parallel and antiparallel G-quadruplex structures, respectively. The protection of bound CV by antiparallel G-quadruplexes is certainly stronger than that by parallel G-quadruplexes. As shown in Table 1, in the presence of 100 mM K^+ ion, the fluorescence intensities of the CV/DNA mixtures were at low levels but much higher fluorescence intensities were obtained in the presence of 100 mM Na^+ ion. The fluorescence intensity of the CV/T_3TT_3 mixture increased nearly 5 times when the ionic condition is changed from 100 mM K^+ ion to 100 mM Na^+ ion.

There are five DNA sequences in group V whose total lengths of loops are seven or more nucleotides. They also adopt different G-quadruplex structures under the two ionic conditions. In the presence of 100 mM K^+ ion, they may exist as a parallel/antiparallel G-quadruplex mixture or a parallel/antiparallel hybrid G-quadruplex. In the presence of 100 mM Na^+ ion, they form typical antiparallel G-quadruplexes. The number of end loops in antiparallel G-quadruplexes is higher than that in the parallel/antiparallel G-quadruplex mixtures or parallel/antiparallel hybrid G-quadruplexes. However, except for the fluorescence intensity of the CV/$T_3T_3T_3$ mixture nearly doubled when the ionic condition was changed from 100 mM K^+ ion to 100 mM Na^+ ion, the fluorescence intensities of the mixtures of CV and other four DNA sequences showed no obvious change.

The results of this study suggest that CV might be developed as a biosensor to distinguish G-quadruplexes from randomly coiled DNAs, parallel G-quadruplexes from antiparallel G-quadruplexes, parallel G-quadruplexes from mixed-type G-quadruplexes but is not suitable for distinguishing mixed-type G-quadruplexes from antiparallel G-quadruplexes.

Monitoring Structural Changes of G-Quadruplexes. Since CV can be developed as a biosensor for discrimination of parallel from antiparallel G-quadruplexes, it may also be used to monitor the structural changes of G-quadruplexes induced by the change of ionic conditions. Using these 27 DNA sequences (Table 1) as model analytes, the ability of CV to monitor the structural changes of G-quadruplexes was further investigated. In this experiment, the total concentration of K^+ and Na^+ ions was kept constant at 100 mM, the fluorescence intensities of the CV/DNA mixtures as a function of the concentration ratio of Na^+/K^+ were followed. Figure 2 shows representative experimental results. For the DNA sequences in groups I and II, they adopt similar secondary structures under K^+ ion or Na^+ ion conditions. The fluorescence intensities of the CV/DNA mixtures show no significant change as a function of the ratio of $[Na^+]/[K^+]$. However, for the DNA sequences in groups III and IV that adopt parallel G-quadruplex structures in

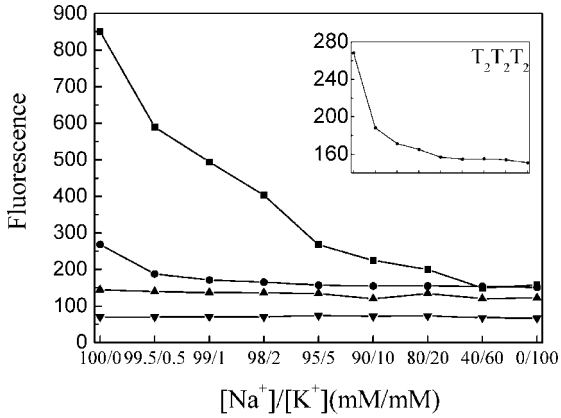

Figure 2. Plots of the fluorescence intensity of CV against the concentration ratio of Na^+ ion to K^+ ion in the presence of TT_2T_2 (▲), TT_3T_2 (▼), $T_2T_2T_2$ (●), or T_3TT_3 (■). Insert: the fluorescence intensity change plot of the CV/$T_2T_2T_2$ mixture. The vertical axis is amplified to highlight the differences.

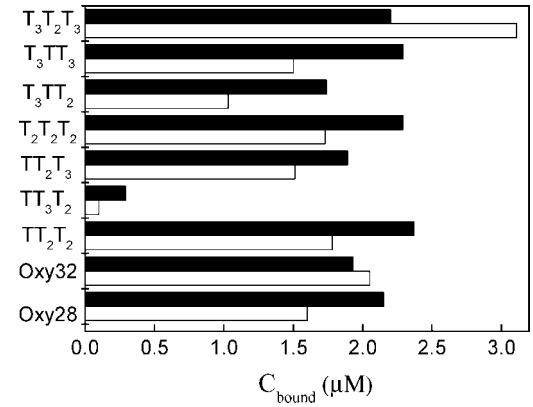

Figure 3. Results obtained by the competition dialysis method for CV in the presence of 100 mM K^+ ion (black bars) or 100 mM Na^+ ion (white bars).

the presence of 100 mM K^+ ion and form mixed-type G-quadruplexes or antiparallel G-quadruplexes in the presence of 100 mM Na^+ ion, the fluorescence intensities of the CV/DNA mixtures decreased gradually and reach a plateau with the increase of K^+ ion concentration in the solution. The decrease in fluorescence intensity may be attributed to the structure interconversion of G-quadruplexes from mixed-type or antiparallel structures to parallel structures, which was confirmed by CD spectra determination (see Supporting Information). These results show that CV can be used as a fluorescent probe to monitor the structural changes of G-quadruplexes.

Competition Dialysis Assay. Competition dialysis has proved to be a powerful and versatile tool for the study of the binding selectivity of ligands to different DNA structures.[29−32] In order to compare the binding affinity of CV to the G-quadruplexes with different structures, some representative DNA sequences, such as Oxy28, Oxy32, TT_2T_2, TT_3T_2, TT_2T_3, $T_2T_2T_2$, T_3TT_2, T_3TT_3, and $T_3T_2T_3$ were dialyzed against CV. The results are shown as a bar graph (Figure 3), in which the amount of CV bound to each nucleic acid sample is plotted. Regardless of Na^+ or K^+ ion conditions, the level of CV binding to TT_2T_2, TT_2T_3, $T_2T_2T_2$, T_3TT_2, T_3TT_3, or $T_3T_2T_3$ is much higher than that to TT_3T_2. This result is consistent with the fact that TT_2T_2, TT_2T_3, $T_2T_2T_2$, T_3TT_2, T_3TT_3, and $T_3T_2T_3$ can form G-quadruplexes whereas TT_3T_2 cannot, indicating the binding preference of CV to G-quadruplex DNAs. This result further demonstrates the ability of CV to discriminate G-quadruplex structures from randomly coiled structures. The change of ionic condition did not have much effect on the binding ability of CV to the six oligonucleotides (TT_2T_2, TT_2T_3, $T_2T_2T_2$, T_3TT_2, T_3TT_3, or $T_3T_2T_3$) that can form G-quadruplex structures. Although the fluorescence intensities of CV/TT_2T_3, CV/$T_2T_2T_2$, CV/T_3TT_2, and CV/T_3TT_3 mixtures in the presence of 100 mM Na^+ ion were much higher than those in the presence of 100 mM K^+ ion, the results of the competition dialysis experiment show that the binding preference of CV to TT_2T_3, $T_2T_2T_2$, T_3TT_2, or T_3TT_3 under Na^+ ion conditions is not much higher than that under K^+ ion conditions. In fact, the binding preference under Na^+ ion conditions is a little lower than that under K^+ ion condition. This result, together with the result of competition dialysis of Oxy28 and Oxy32, further demonstrates that the fluorescence difference of CV in the presence of G-quadruplexes with different structures is not due to the binding ability of CV to different G-quadruplex structures but may be attributed to the protection ability of different G-quadruplex loop structures to bound CV.

Fluorescence Detection of K^+ Ion. Considering that a great difference in fluorescence intensity was observed for some CV/DNA mixtures when the ionic condition was changed from 100 mM K^+ ion to 100 mM Na^+ ion, (for example, a nearly 5-fold fluorescence change was obtained for the mixture of CV and T_3TT_3), they may be developed as a potassium sensor. It is well-known that the K^+ ion plays an important role in biological systems, and it is important to develop a sensing system for the reliable detection of this ion.[33] To date, numerous studies concerning K^+ ion assays have been reported, but for most of these methods, poor selectivity against the Na^+ ion make it difficult in practical use. It is generally accepted that the stabilizing ability of the K^+ ion to nucleic acid G-quadruplexes is much higher than that of Na^+ ion.[34,35] Thus, for a G-rich sequence that adopts different G-quadruplex structures under K^+ or Na^+ ion conditions, the addition of a small quantity of K^+ ion into a large quantity of Na^+ ion might also cause great changes in G-quadruplex structures. On the basis of this, a new method for K^+ ion detection with good selectivity against Na^+ ion might be developed.

To minimize the effect of Na^+ ion on K^+ ion detection, the effect of Na^+ ion on the fluorescence of CV/G-quadruplex mixtures was investigated. The results show that as the concentration of Na^+ ion increased, the fluorescence of the CV/T_3TT_3 mixture dramatically increased, reaching a plateau when the concentration of Na^+ ion exceeded 600 mM (see Supporting Information). That is to say, when the concentration of Na^+ ion is higher than 600 mM, the effect of Na^+ ion concentration on the fluorescence of the CV/T_3TT_3 mixture reaches saturation; further addition of Na^+ ion has no significant effect on the fluorescence of the mixture. Keeping

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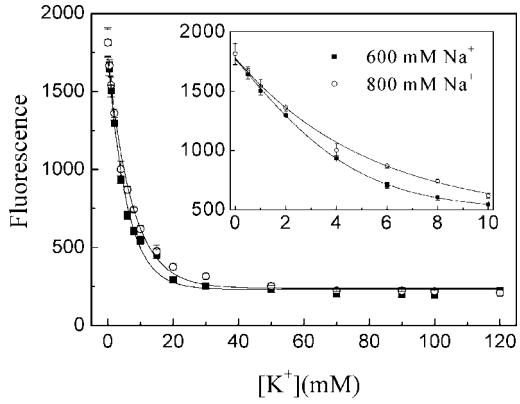

Figure 4. Fluorescence of CV/T_3TT_3 mixture as a function of K^+ ion concentration in the presence of 600 mM Na^+ ion (■) or 800 mM Na^+ ion (○). The insert shows the fluorescence change of the CV/T_3TT_3 mixture in the K^+ ion concentration range from 0 to 10 mM. The solid lines represent nonlinear least-squares fits to the data.

the concentration of Na^+ ion at 600 mM, the effect of K^+ ion concentration on the fluorescence of the CV/T_3TT_3 mixture was studied. As shown in Figure 4, the addition of K^+ ion obviously decreased the fluorescence of the mixture. For example, the addition of 1 and 2 mM K^+ ion produced ~17% and ~29% decreases in the fluorescence intensities, respectively. When the concentration of Na^+ ion was kept at 800 mM, the fluorescence of the CV/T_3TT_3 mixture also decreased with the increase of K^+ ion concentration. The extent of fluorescence decrease is a little lower than that in the presence of 600 mM Na^+ ion, which may be attributed to the competition between K^+ and Na^+ ions in adjusting G-quadruplex structures. That is to say, the change of Na^+ ion concentration has an effect on K^+ ion detection, but this effect is very small. As shown in Figure 4, the extent of fluorescence decrease in the presence of 2.0 mM K^+/800 mM Na^+ is comparable to that in the presence of 1.7 mM K^+/600 mM Na^+. That is to say, the effect of 200 mM Na^+ ion on the fluorescence of the CV/T_3TT_3 mixture is comparable to that of 0.3 mM K^+ ion, indicating that this K^+ ion detection method has an attractively high selectivity against Na^+ ion.

DISCUSSION

Crystal violet (CV) is a triphenylmethane dye that can stack onto the two external G-quartets of the G-quadruplexes with a binding stoichiometry of two CV per G-quadruplex. When CV binds to a G-quadruplex with an antiparallel structure, the bound CV is well protected from the solvent because of the presence of end loops in the antiparallel G-quadruplex, causing a large increase in the emission intensity. However, in the case of parallel G-quadruplexes, the presence of side loops cannot effectively protect the bound CV from the solvent. Thus, the fluorescence of the CV/G-quadruplex mixture is obviously higher when the G-quadruplex adopts an antiparallel structure than that when the G-quadruplex adopts a parallel structure. Therefore, CV can be developed as a sensitive fluorescent biosensor for the discrimination of antiparallel G-quadruplexes from parallel G-quadruplexes and for monitoring the structural interconversion of G-quadruplexes.

According to the results of quadruplex structural interconversion as a function of cation condition and corresponding fluorescence change of the CV/G-quadruplex mixture, a simple, cheap and highly selective K^+ ion detection method has been developed. Recently, several K^+ ion detection methods based on the formation of a quadruplex structure from a random coil in the presence of K^+ ion have been developed.[36−43] However, to our knowledge, a K^+ ion detection method based on G-quadruplex structure interconversion between parallel and antiparallel structures has never been reported. In the reported K^+ ion detection methods, dual fluorescent-labeled G-rich DNA oligonucleotides were required, which will potentially increase the cost of detection. In our method, no labeled DNA oligonucleotides are needed. To increase the K^+ ion selectivity against Na^+ ion, we let the effect of Na^+ ion on fluorescent signal reach a saturation point. Thus, the K^+ ion detection can be performed in the presence of a large amount of Na^+ ions. In other words, this K^+ ion detection method can tolerate the presence of a large amount of Na^+ ion. In addition, in most of K^+ ion detection methods, the estimation of K^+ ion selectivity against Na^+ ion is performed in the presence of only K^+ ion or Na^+ ion. The selectivity was judged by the concentration ratio of the two ions when the signal reaches the same level. However, if the competition between the two ions is ignored, it is very possible that the actual selectivity cannot be reflected correctly. In this paper, the fluorescence signal change as a function of K^+ ion concentration was studied in the presence of 600 and 800 mM Na^+ ions, respectively. The results show that the change of 200 mM in Na^+ ion concentration causes a similar fluorescent signal change to 0.3 mM K^+ ion, indicating that this method has a good K^+ ion selectivity against Na^+ ion.

ACKNOWLEDGMENT

This work was supported by the National Natural Science Foundation of China (Grant No. 20675041), the National Basic Research Program of China (Grant No. 2006CB705700), the National Natural Science Foundation of Tianjin (Grant No. 08JCZDJC21200).

SUPPORTING INFORMATION AVAILABLE

Details of fluorescence titrations and energy transfer fluorescence titrations by Oxy12, Oxy28, Oxy32, Hum12, Hum21, and Hum24; CD spectra of Oxy12, Oxy28, Oxy32, Hum12, Hum21, Hum24, and d(5′-$G_3T_iG_3T_jG_3T_kG_3$-3′) sequences; CD spectra of TT_2T_2, TT_3T_2, $T_2T_2T_2$, and T_3TT_3 in the presence of different concentration ratios of Na^+ ion to K^+ ion; and fluorescence of the CV/T_3TT_3 mixture as a function of Na^+ ion concentration. This material is available free of charge via the Internet at http://pubs.acs.org.

Received for review December 10, 2008. Accepted February 16, 2009.

AC802558F

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