Ag⁺与 Hg²⁺对 G-四链体 DNA 结构的破坏及其对构筑 DNA 逻辑门的应用

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摘要: 本工作利用圆二色光谱研究了 Ag⁺与 Hg²⁺对 4 种代表性 G-四链体 DNA 结构的破坏作用。结果表明 Ag⁺可能通过与碱基 G 螯合从而破坏 G-四链体结构;Hg²⁺能通过形成 T-Hg²⁺-T 碱基对,及其他方式破坏 G-四链体结构。含巯基(-SH)的半胱氨酸与 Ag⁺与 Hg²⁺可以发生较强的配位作用,从而使被 Ag⁺与 Hg²⁺破坏后的 G-四链体 DNA 结构得以回复。基于此,一个新颖的 Ag⁺/Hg²⁺ -半胱氨酸-DNA 逻辑门得以构筑。

关键词: G-四链体;DNA 逻辑门;银离子;汞离子;半胱氨酸;圆二色光谱

Disruption of G-Quadruplex DNA by Ag⁺ and Hg²⁺ and Its Application to DNA Logic Gate

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Abstract: The G-quadruplex-disrupting abilities of Ag⁺ and Hg²⁺ ions were investigated by utilizing the metal ions-mediated circular dichroism (CD) spectrum changes of four representative G-quadruplexes. The results show that Ag⁺ ion can disrupt G-quadruplexes by chelating to G bases and thus can be used as a general G-quadruplex-disrupting agent. The results also suggest that Hg²⁺ ion may disrupt G-quadruplexes by formation of T-Hg²⁺-T base pairs as well as other species. The G-quadruplexes disrupted by Ag⁺ and Hg²⁺ can be recovered by cysteine (Cys), a thiol-containing amino acid, due to the tight binding interactions between Cys and the metal ions. Thus, the system by using Ag⁺ (or Hg²⁺) and Cys as two inputs and CD signal as output can behave as a DNA IMPLICATION logic gate.

Key words: G-quadruplex; DNA logic gate; Ag⁺; Hg²⁺; cysteine; circular dichroism

It is widely accepted that genomic DNA is mainly presented as classic Watson-Crick double helix. However, as for some DNAs with specific sequences, they may adopt some particular structures[1]. For example, some G-rich DNA sequences can form a wide variety of inter- or intramolecular four-stranded

structures by Hoogsteen-type base pairing, termed G-quadruplexes[2-5]. G-quadruplexes are unique high-ordered nucleic acid structures attracting more and more attention[6-8], since G-rich sequences with the potential to form quadruplexes have been found in several biologically important regions such as telomeric DNA and many gene promoter regions[9]. It has been reported that the formation of G-quadruplex in telomere regions can inhibit telomerase function and the reagents with the ability to promote G-quadruplex formation can be used as potential anti-cancer drugs[10]. Most studies on G-quadruplexes are focused on the promotion of G-quadruplex formation. Whereas, there have been rare report on the agents that can inhibit the formation of the G-quadruplex or disrupt G-quadruplex structures. However, the study on these agents should also be helpful for the treatment of some sort of diseases[11]. For example, the promotion of telomerase activity by disrupting the G-quadruplex formation by telomere sequence may play an important role in the treatment of liver cirrhosis, etc[12].

Some cations, such as K^+, Na^+, NH_4^+, Mg^{2+}, Pb^{2+}, Sr^{2+} and Tl^+, can promote the G-quadruplex formation and impart G-quadruplex stabilization. The effects of these ions on G-quadruplex stability and polymorphism have been widely investigated[13-15]. To the best of our knowledge, only two ions, Hg^{2+} ion and Ag^+ ion, have been reported to have the ability to disrupt G-quadruplex

structures in the presence of high concentration of K^+ [16-19]. Hg^{2+} can destroy the G-quadruplex formed by the G-rich sequence containing T bases via forming T-Hg^{2+}-T base pair[16-17], and Ag^+ ion can chelate to N7 and C6O groups in G bases and can greatly interfere with the formation of G-quadruplexes (Fig.1)[18-19]. Some G-quadruplex DNAzyme-based sensors for Hg^{2+} and Ag^+ have been developed by utilizing the G-quadruplex -disrupting abilities of these two ions[16-19]. It is necessary to investigate the abilities of the two ions to disrupt the G-quadruplex structures in detail. Such studies will provide valuable information for the investigation in the interactions between the metal ions and G-quadruplexes and the design of metal complex-based G-quadruplex disrupting reagents, and will also help to develop some important analytical applications of these interactions, for example, constructing DNA logic gates.

Recently, molecular switches and molecular logic gates have attracted considerable attention owing to the importance of the development of miniaturized devices[20-21]. As a new generation of molecular logic gates, DNA molecular logic gates have attracted increasing interest in recent years due to the well-regulated structures of DNAs and their abilities to store genetic information[22].

In the present study, the effects of Hg^{2+} and Ag^+ ions on four representative G-quadruplexe structures
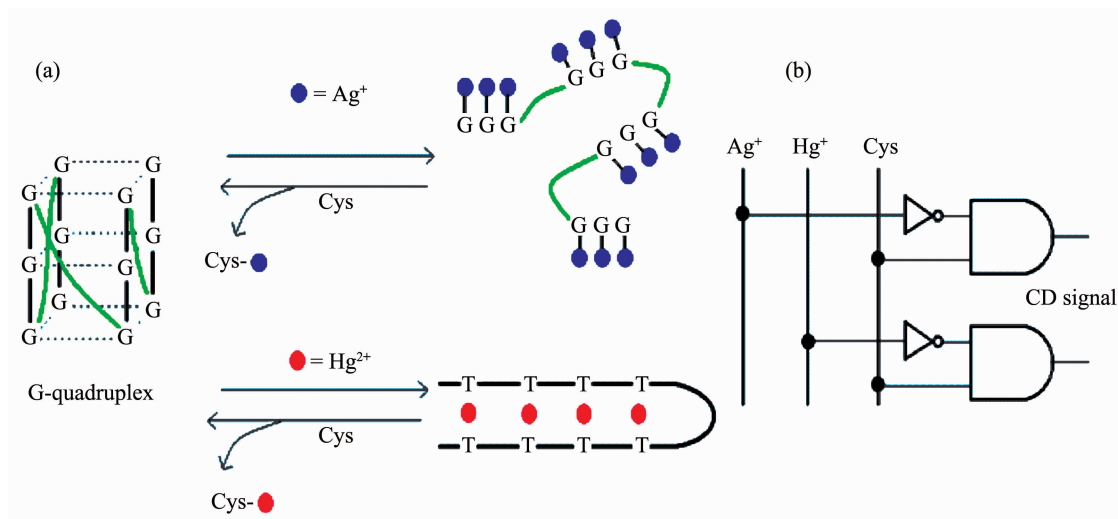

Fig.1　(a) Proposed mechanism of the disruption of G-quadruplexes by Ag^+ and Hg^{2+} ions, and the reformation of G-quadruplexes in the presence of Cysteine (Cys). (b) The design of a DNA IMPLICATION logic gate

were studied and compared by circular dichroism (CD) spectra and their G-quadruplex-disrupting mechanisms were also discussed. It is interesting that both of the two G-quadruplex-disrupting metal ions can tightly bind to the thiol group of cysteine (Cys). Thus, Cys may capture Ag^+ or Hg^{2+} from DNA-Ag^+ or DNA-Hg^{2+} complexes, promoting the reformation of G-quadruplexes. As a result, a DNA IMPLICATION logic gate was constructed by sequential addition of Ag^+ (or Hg^{2+}) and Cys into G-quadruplex solutions. In this IMPLICATION logic gate, Ag^+ (or Hg^{2+}) and Cys can be as the two inputs, and CD signal can be as the output.

1　Experimental

1.1　Materials and reagents

　　The oligonucleotides listed in Table 1 were purchased from Sangon Biotech. Co., Ltd. (Shanghai, China). The concentrations of the oligonucleotides were represented as single-stranded concentration. Single-stranded concentration was determined by measuring the absorbance at 260 nm. Molar extinction coefficient was determined using a nearest neighbour approximation (http://www.idtdna.com/analyzer/Applications/OligoAnalyzer). $AgNO_3$, Hg $(Ac)_2$ and L-cysteine (Cys) were obtained from Sigma. All chemical reagents were of analytical grade and used without further purification. Deionized and sterilized water (resistance >18 $M\Omega \cdot cm^{-1}$) was used throughout the experiments.

Table 1　Sequences of the oligonucleotides used in this work

DNAs	Sequence (from 5′ to 3′)
Hum24	TTAGGGTTAGGGTTAGGGTTAGGG
KRAS	AGGGCGGTGTGGGAAGAGGGAAGAGGGGGAGG
M3Q	GAGGGAGGGAGGGAGAGGGA
Oxy28	GGGGTTTTGGGGTTTTGGGGTTTTGGGG

1.2　Circular dichroism (CD) titration

　　3 mL reaction mixture was prepared in 10 mmol·L^{-1} Tris-HAc buffer (pH=7.4) containing 1.5 μmol·L^{-1} individual DNA oligonuculeotide and 50 mmol·L^{-1} KAc. In order to ensure the formation of G-quadruplex structures, the mixture was heated at 95 ℃ for 5 min, cooled slowly to 25 ℃ and then incubated at 25 ℃ overnight. To this mixture concentrated Ag^+ or Hg^{2+} solution was added continuously, and the mixture was thoroughly mixed by repeated aspiration and injection. CD spectra of the mixtures were recorded between 200 and 320 nm in 1 mm path length cuvettes on a Jasco J-715 spectropolarimeter. Spectra were averaged from 3 scans recorded at 100 nm·min^{-1} with a response time of 1 s and a bandwith of 1.0 nm. The CD signals and the concentrations of DNA oligonucleotides and metal ions were corrected for the amount of volume change during titration.

1.3　IMPLICATION logic operation

　　3 mL reaction mixture was prepared in 10 mmol·L^{-1} Tris-HAc buffer (pH=7.4) containing 1.5 μmol·L^{-1} individual DNA oligonuculeotide and 50 mmol·L^{-1} KAc. In order to ensure the formation of G-quadruplex structures, the mixture was heated at 95 ℃ for 5 min, cooled slowly to 25 ℃ and then incubated at 25 ℃ overnight. To this mixture concentrated Ag^+ (or Hg^{2+}) solution was added to reach a specified concentration, and CD spectrum of the mixture was recorded. Then, concentrated Cys solution was added to reach the same concentration as the metal ion, and CD spectrum of the mixture was recorded again. CD spectra were all recorded between 200 and 320 nm in 1 mm path length cuvettes on a Jasco J-715 spectropolarimeter. The spectra average was performed using the same procedure as that in section 1.2.

2　Results and discussion

2.1　Disrupting abilities of Ag⁺ and Hg²⁺ ions to the G-quadruplex formed by Hum24

　　The CD spectra of G-quadruplex-forming G-rich oligonucleotides in the presence of different concentrations of each ion were used to investigate the disruption of G-quadruplexes by the metal ions. It has been reported that CD spectra of a typical parallel G-quadruplex structure have a positive peak near 260 nm and a negative peak around 240 nm, whereas CD spectra of a typical antiparallel G-quadruplex structure have a positive peak at 295 nm and a negative peak close to 265 nm[23-24].

Hum24, KRAS, M3Q and Oxy28 (Table 1) were used as the four G-quadruplex-forming oligonucleotides. Hum24 is a G-rich oligonucleotide with the repeated subunit of the telomere from vertebrates. In the presence of 50 mmol·L^{-1} K$^+$, the CD spectrum of Hum24 displayed a positive peak at around 290 nm and a negative peak at 240 nm (Fig.2a), indicating that neither a typical antiparallel G-quadruplex nor a typical parallel G-quadruplex can be formed. It is very possible that Hum24 adopts a hybrid structure containing both *syn*- and *anti*-bonds[25-28]. As shown in Fig.2a, the addition of Ag$^+$ ion leads to a gradual decrease in the positive CD signal intensity at 290 nm, reaching a plateau when the concentration of Ag$^+$ exceeded 6 μmol·L^{-1}. According to the plot of CD signal density versus Ag$^+$ concentration, the IC$_{50}$ value, which represents the metal ion concentration required for 50% decrease of the signal intensity, can be calculated. The obtained IC$_{50}$ value is 2.2 μmol·L^{-1} (Table 2).

Table 2　Calculated IC$_{50}$ values in different G-quadruplex-metal ion systems

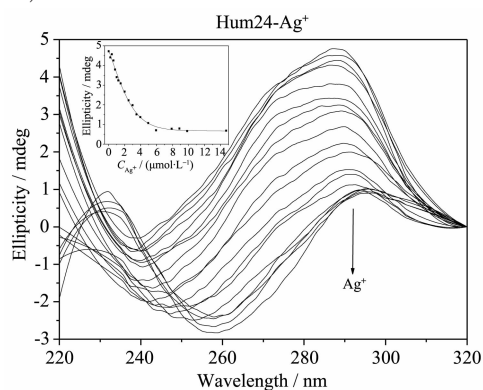| DNAs	IC$_{50}$ / (μmol·L^{-1})	
	Ag$^+$	Hg^{2+}
Hum24	2.2	7.5
KRAS	17.9	>116
M3Q	15.4	>116
Oxy28	20.5	77

Fig.2a　Ag$^+$ ion-mediated disruption of the G-quadruplex formed by Hum24. Ag$^+$ concentration-dependent change in CD spectra. The corrected concentrations of Ag$^+$ are (arrow direction): 0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0, 6.0, 7.9, 8.6, 9.8 and 14.7 μmol·L^{-1}. The inset represents the Ag$^+$ concentration-dependent change in CD signal at 290 nm. The solid line represents least square fit to the data

Under the same conditions, the addition of Hg^{2+} could also lead to a gradual decrease in the positive CD signal intensity at 290 nm (Fig.2b), and the obtained IC$_{50}$ value is 7.5 μmol·L^{-1}. (Table 2), which is higher than that of Ag$^+$, indicating that the G-
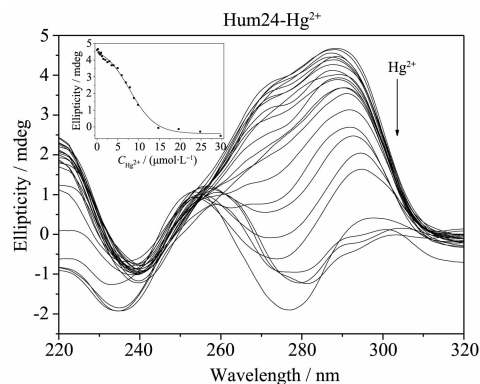
Fig.2b　Hg^{2+} ion-mediated disruption of the G-quadruplex formed by Hum24. Hg^{2+} concentration-dependent change in CD spectra. The corrected concentrations of Hg^{2+} are (arrow direction): 0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0, 6.0, 7.0, 7.9, 8.9, 9.8, 14.7, 19.6, 24.8 and 29.7 μmol·L^{-1}. The inset represents the Hg^{2+} concentration-dependent change in CD signal at 290 nm. The solid line represents least square fit to the data

quadruplex-disrupting ability of Ag$^+$ is higher than that of Hg^{2+}, at least for the G-quadruplex formed by Hum24. This can be easily interpreted by the reported G-quadruplex-disrupting mechanisms (Fig.1)[16-19]. That is, Hg^{2+} and Ag$^+$ ions can disrupt G-quadruplexes by forming T-Hg^{2+}-T base pair and by chelating to G bases, repectively. As for Ag$^+$ ion, its binding sites in the G bases are just involved in the formation of G-quartets that are the basic structural motifs of G-quadruplexes. Therefore, when a Ag$^+$ ion binds with a G base in Hum24, the G base cannot participate in the formation of the corresponding G-quartet any more, and the number of G-quartet in the G-quadruplex decreases from 3 to 2. As a result, the stability of the G-quadruplex decreases greatly or stable quadruplex cannot be formed any more.

However, Hg^{2+} ion interactes with T bases, which are located in the loops of the G-quadruplex. Thus, it is very possible that the interaction between Hg^{2+} ion and T bases has less effect on G-quadruplex stability.

2.2 Disrupting abilities of Ag^+ and Hg^{2+} ions to the G-quadruplex formed by KRAS

To further investigate the disrupting abilities of Hg^{2+} and Ag^+ ions to G-quadruplexes, another oligonucleotide KRAS was used. KRAS is a 32-nucleotide G-rich sequence, which is located in the promoter of the human KRAS gene. The mammalian KRAS gene encodes for a guanine nucleotide binding protein of 21 kDa that is involved in an important cell-growth pathway[29-30].

In the presence of 50 mmol·L^{-1} K^+, the CD spectrum of KRAS shows a positive peak at around 262 nm (Fig.3a), indicating that KRAS could form a parallel G-quadruplex[31-32]. Increasing the concentration of the added Ag^+ ion causes a monotonic decrease of the positive CD signal, and the signal intensity nearly reaches zero when 116 μmol·L^{-1} Ag^+ is added. The calculated IC_{50} value is about 17.9 μmol·L^{-1} (Table 2), which is much higher than that in Hum24-Ag^+ system. There are 12 G bases in Hum24, and all

of them can participate in the formation of G-quartets. When any one of them interacts with Ag^+ ion, the conformation and stability of the G-quadruplex will be affected greatly. However, there are 21 G bases in KRAS sequence, and according to the previous reports, only 12 G bases in KRAS participate in the formation of G-quartets[31-32]. That is to say, other 9 G bases are located in the loops of the G-quadruplex. When the G bases in the loops interact with Ag^+ ion, the G-quartets may not be destroyed, and the conformation and stability of the G-quadruplex may not be obviously affected. As a result, the KRAS-Ag^+ system has a higher IC_{50} value than Hum24-Ag^+ system.

Under the same conditions, the addition of Hg^{2+} could also lead to a gradual decrease of the positive CD signal intensity, but the decreasing rate was much lower than that in KRAS-Ag^+ system (Fig.3b). Even when 116 μmol·L^{-1} Hg^{2+} ion is added, the positive peak still remained a high signal intensity, indicating that Hg^{2+} ion can not entirely destroy the G-quadruplex structure. The calculated IC_{50} value was > 116 μmol·L^{-1} (Table 2), suggesting the weak ability of Hg^{2+} ion to disrupt the G-quadruplex structure formed by KRAS. This can be easily interpreted. The weak
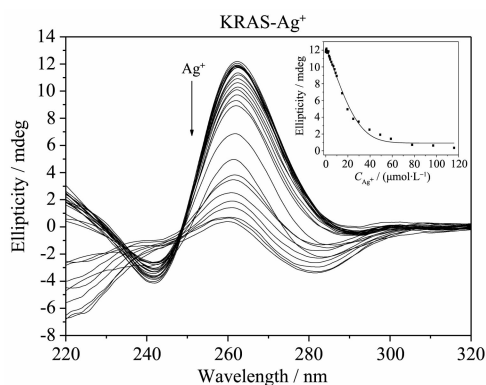

Fig.3a　Ag^+ ion-mediated disruption of the G-quadruplex formed by KRAS. Ag^+ concentration-dependent change in CD spectra. The corrected concentrations of Ag^+ are (arrow direction): 0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0, 6.0, 7.0, 7.9, 8.9, 9.8, 14.7, 19.6, 24.8, 29.7, 39.5, 49.2, 58.8, 78.0, 97.1 and 116.0 μmol·L^{-1}. The inset represents the Ag^+ concentration-dependent change in CD signal at 262 nm. The solid line represents least square fit to the data
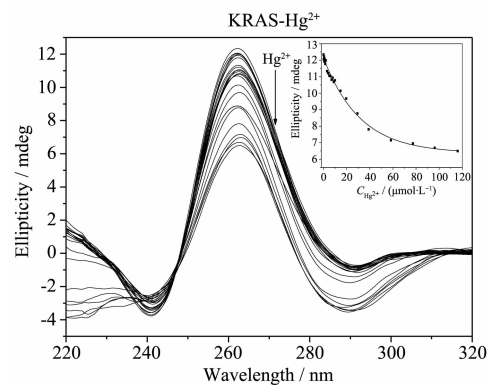
Fig.3b　Hg^{2+} ion-mediated disruption of the G-quadruplex formed by KRAS. Hg^{2+} concentration-dependent change in CD spectra. The corrected concentrations of Hg^{2+} are (arrow direction): 0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.5, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 7.9, 8.9, 9.8, 14.7, 19.6, 24.8, 29.7, 39.5, 49.2, 58.8, 78.0, 97.1 and 116.0 μmol·L^{-1}. The inset represents the Hg^{2+} concentration-dependent change in CD signal at 262 nm. The solid line represents least square fit to the data

disruption of Hg^{2+} ion should be attributed to the only 2 T bases in KRAS sequence.

2.3 Disrupting abilities of Ag^+ and Hg^{2+} ions to the G-quadruplex formed by M3Q

M3Q is a 20-nucleotide G-rich sequence located on the upstream of the initiation codon of MT3-MMP mRNA. Matrix metalloproteinases (MMPs) are zinc-dependent endopeptidases and coded by MT3-MMP mRNA. The upregulation of MMP protein expression level is associated with the invasiveness of many cancers[33-34].

In the presence of 50 mmol·L^{-1} K$^+$, M3Q also folded into a parallel G-quadruplex with a positive peak at around 262 nm in the CD spectrum (Fig.4a). With an increased concentration of Ag$^+$ ion, the CD signal intensity was decreased significantly, reaching zero at about 58 μmol·L^{-1} Ag$^+$. The calculated IC$_{50}$ value is 15.4 μmol·L^{-1} (Table 2), which is much higher than that of the Hum24-Ag$^+$ system and a little lower than that of the KRAS-Ag$^+$ system. There are 14 G bases in M3Q, and 12 of them participate in the formation of G-quartets. The other two are located in the loops of the G-quadruplex. The number of the G bases in loops is just between those in the G-quadruplexes formed by KRAS and Hum24. This result further demonstrates the proposed G-quadruplex -disrupting mechanism of Ag$^+$ ion.
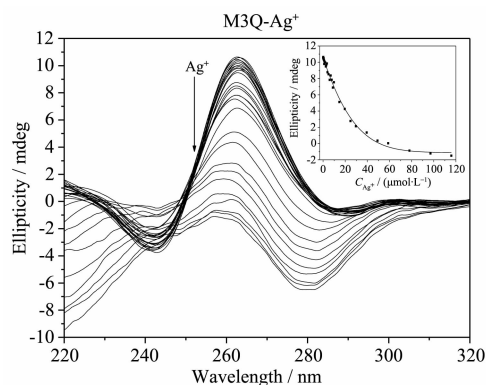

Fig.4a Ag$^+$ ion-mediated disruption of the G-quadruplex formed by M3Q. Ag$^+$ concentration-dependent change in CD spectra. The corrected concentrations of Ag$^+$ are (arrow direction): 0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0, 6.0, 7.0, 7.9, 8.9, 9.8, 14.7, 19.6, 24.8, 29.7, 39.5, 49.2, 58.8, 78.0, 97.1 and 116.0 μmol·L^{-1}. The inset represents the Ag$^+$ concentration-dependent change in CD signal at 262 nm. The solid line represents least square fit to the data

According to the reported G-quadruplex-disrupting mechanism of Hg^{2+} ion[16-17], Hg^{2+} ion should not destroy the G-quadruplex formed by M3Q, because there is no T base in M3Q at all. To our surprise, however, the CD signal intensity increases with increasing the concentration of Hg^{2+} ion in the low Hg^{2+} concentration range (Fig.4b), suggesting that low concentrations of Hg^{2+} can promote the folding of
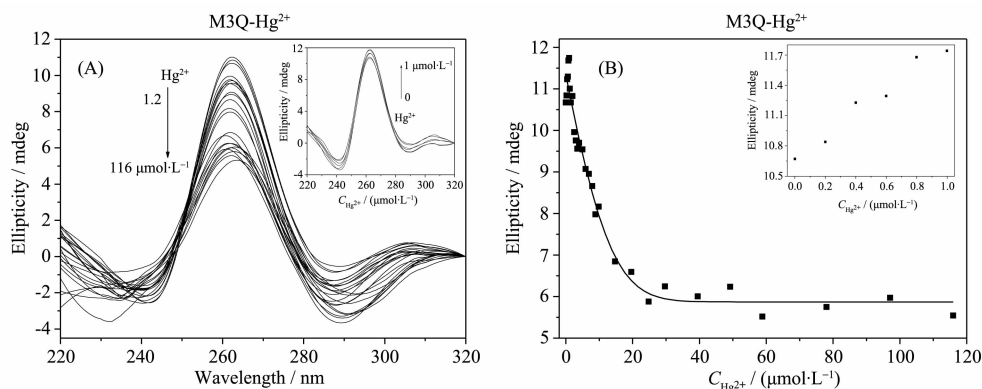

Fig.4b Hg^{2+} ion-mediated disruption of the G-quadruplex formed by M3Q. (A) Hg^{2+} concentration-dependent change in CD spectra. The corrected concentrations of Hg^{2+} are (arrow direction): 0, 1.0, 1.2, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0, 6.0, 7.0, 7.9, 8.9, 9.8, 14.7, 19.6, 24.8, 29.7, 39.5, 49.2, 58.8, 78.0, 97.1 and 116.0 μmol·L^{-1}. The inset represents the CD spectrum change in the Hg^{2+} concentration range of 0~1.0 μmol·L^{-1}. The corrected concentrations of Hg^{2+} are (arrow direction): 0, 0.2, 0.4, 0.6, 0.8 and 1.0 μmol·L^{-1}. (B) Hg^{2+} concentration-dependent change in CD signal at 262 nm. The solid line represents least square fit to the data. The inset represents the CD signal change in the Hg^{2+} concentration range of 0~1.0 μmol·L^{-1}

M3Q into G-quadruplex structure or increase the stability of the G-quadruplex. Further addition of Hg^{2+} could also decrease the CD signal intensity though the signal intensity still remains at a very high level even when 116 $\mu mol \cdot L^{-1}$ Hg^{2+} is added. That is to say, besides the formation of T-Hg^{2+}-T base pairs, Hg^{2+} ion can also disrupt G-quadruplex structures through other way(s).

2.4 Disrupting abilities of Ag⁺ and Hg²⁺ ions to the G-quadruplex formed by Oxy28

As for the three oligonucleotides mentioned above, KRAS and M3Q adopt parallel G-quadruplex structure, Hum24 adopts a hybrid G-quadruplex structure. To investigate the disrupting abilities of Hg^{2+} and Ag^{+} ions to G-quaduplexes with antiparallel structure, Oxy28 was used. Oxy28 is a 28-nucleotide G-rich sequence with the repeated subunit of the telomere from Oxytricha. In the presence of 50 mmol$\cdot$ L^{-1} K^{+}, the CD spectrum of Oxy28 displays a positive peak at around 293 nm and negative peak at around 260 nm, which is typical of antiparallel G-quadruplexes. As shown in Fig.5a, the CD signal intensity of Oxy28 also monotonically decreases with the increment of Ag^{+}. The calculated IC_{50} value is

20.5 $\mu mol \cdot L^{-1}$ (Table 2), which is the highest one in the four G-quadruplexes studied in this work. This is not surprised. Because the G-quadruplex formed by Oxy28 contained four G-quartets, the stability of the G-quadruplex formed by Oxy28 is certainly higher than the G-quadruplexes containing three G-quartets. The higher stability might increase the difficulty of Ag^{+}-mediated disruption of G-quadruplex structures. When one G-quartet is destroyed by Ag^{+} and the number of G-quartet decreases from four to three, the stable G-quadruplex still exists.

Oxy28 is a T-rich oligonucleotide. The addition of Hg^{2+} should has significant effect on the G-quadruplex formed by Oxy28 through T-Hg^{2+}-T base pair formation. As shown in Fig.5b, the addition of Hg^{2+} indeed leads to the decrease of the CD signal intensity, but the decreasing rate is not so great as we expect. The calculated IC_{50} value is 77 $\mu mol \cdot L^{-1}$, which is lower than those of KRAS and M3Q, but much higher than that of Hum24. The lower IC_{50} value in comparison to KRAS and M3Q can be interpreted by the higher T-base number in Oxy28. The higher IC_{50} value in comparison to Hum24 may be attributed to the higher stability of the G-quadruplex formed by
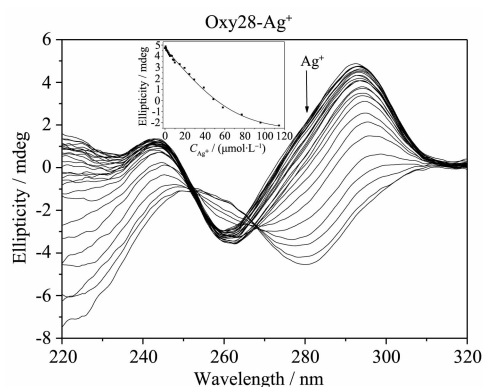

Fig.5a　Ag⁺ ion-mediated disruption of the G-quadruplex formed by Oxy28. Ag⁺ concentration-dependent change in CD spectra. The corrected concentrations of Ag⁺ are (arrow direction): 0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0, 6.0, 7.0, 7.9, 8.9, 9.8, 14.7, 19.6, 24.5, 29.3, 39.0, 48.5, 58.1, 77.0, 95.8 and 114.5 $\mu mol \cdot L^{-1}$. The inset represents the Ag⁺ concentration-dependent change in CD signal at 293 nm. The solid line represents least square fit to the data

Fig.5b　Hg²⁺ ion-mediated disruption of the G-quadruplex formed by Oxy28. Hg²⁺ concentration-dependent change in CD spectra. The corrected concentrations of Hg²⁺ are (arrow direction): 0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0, 6.0, 7.0, 7.9, 8.9, 9.8, 14.7, 19.6, 24.5, 29.3, 39.0, 48.5, 58.1, 77.0, 95.8 and 114.5 $\mu mol \cdot L^{-1}$. The inset represents the Hg²⁺ concentration-dependent change in CD signal at 293 nm. The solid line represents least square fit to the data
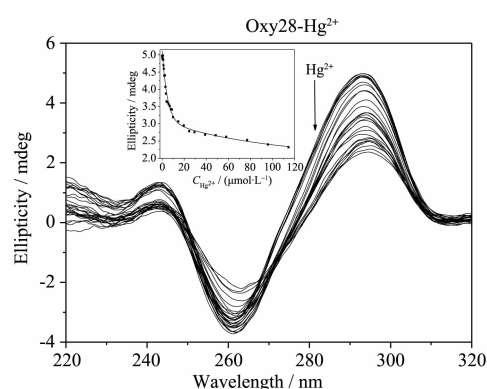
Oxy28.

Comparing with K^{+}, Na^{+}, NH$_4$[+], Mg^{2+}, Pb^{2+}, Sr^{2+} and Tl[+], Hg^{2+} and Ag^{+} show effective disruption abilities to G-quadruplex structures as described by above experiments. The ionic radius is a parameter that aptly describes how well guanine tetrads are stabilized by various cations, and K^{+} and Sr^{2+} with similar ionic radii of approximately 0.13 nm imparting G-quadruplex prominent stabilization are believed to fit exceptionally well in the cavities between guanine tetrads[13]. The ionic radii of Hg^{2+} and Ag^{+} are similar with that of Na^{+} (0.1 nm). According to the above fit model, they should show the similar effect on the stabilization of G-quadruplex structures with Na^{+}. So we believe that the disruption abilities of Hg^{2+} and Ag^{+} to G-quadruplex structures may be due to the formation of the coordinate bonds between Hg^{2+} or Ag^{+} with the nitrogen and/or oxygen atoms in the bases of the strands, instead of the interaction with guanine tetrads.

2.5 Construction of DNA IMPLICATION logic gates

The experiments mentioned above show that both Ag^{+} and Hg^{2+} can disrupt G-quadruplex structures, though the disrupting abilities of them are highly dependent on the stability and base component of G-quadruplexes. It is reported that Cys, a thiol-containing amino acid, can tightly bind to Ag^{+} or Hg^{2+} [35-36]. Thus the interactions between the two metal ions and DNA bases could be influenced by Cys. Based on this, many highly sensitive and specific Cys detection methods have been reported[37-40]. Herein, using Ag^{+} and Cys as the two inputs and the CD signal as output, a DNA IMPLICATION logic gate is designed. Fig.6 shows the operation of the IMPLICATION logic gate utilizing the influence of Ag^{+} or/ and Cys on the CD spectrum of Hum24. Similar spectrum variation can be observed in the operation to KRAS, M3Q and

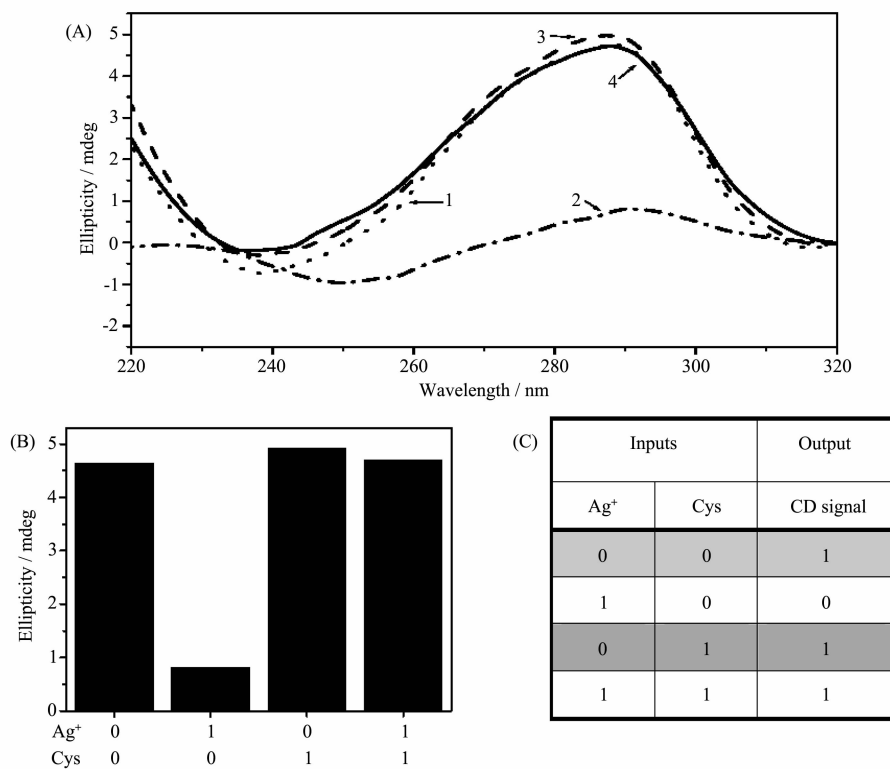|	Inputs		Output
	Ag^{+}	Cys	CD signal
	0	0	1
	1	0	0
	0	1	1
	1	1	1

Fig.6 Operation of the IMPLICATION logic gate utilizing the influence of Ag^{+} and/or Cys on the CD spectrum of Hum24. (A) CD spectra of Hum24 in the presence of different inputs. 1: C_{Ag^+}=0 μmol·L^{-1}, C_{Cys}=0 μmol·L^{-1}; 2: C_{Ag^+}=8 μmol·L^{-1}, C_{Cys}=0 μmol·L^{-1}; 3: C_{Ag^+}=0 μmol·L^{-1}, C_{Cys}=8 μmol·L^{-1}; 4: C_{Ag^+}=8 μmol·L^{-1}, C_{Cys}=8 μmol·L^{-1}. (B) The CD signal intensities at 290 nm in the presence of different inputs. The dashed line represents the defined threshold value. (C) Truth table for the DNA IMPLICATION logic gate

Oxy28. As shown in Fig.6, with no input or with Cys input alone, CD signal maintains at high levels with an output of 1. With Ag^+ input alone, the CD signal decreases sharply, giving an output of 0. With both Ag^+ and Cys inputs, the CD signal is recovered to almost its initial level with an output of **1**, suggesting the disruption of the Ag^+-DNA complex and the reformation of G-quadruplex structure. A bar graph of the output signals of the system is in Fig.6(B), and the truth table is in Fig.6 (C). Using Hg^{2+} and Cys as the two inputs, similar IMPLICATION logic gate can also be designed.

3　Conclusions

In conclusion, the G-quadruplex-disrupting abilities of Hg^{2+} and Ag^+ ions were studied using four representative G-quadruplex-forming sequences. According to the experimental results, some primary conclusions can be drawn: (1) Hg^{2+} and Ag^+ ions both exhibit the disrupting abilities to the representative G-quadruplex-forming sequences. Compared to Hg^{2+} ion, Ag^+ ion can display higher G-quadruplex-disrupting ability. (2) Ag^+ ion can be used as a general G-quadruplex-disrupting reagent. It can disrupt G-quadruplexes by interacting with G bases. Because G bases are necessary for G-quadruplex formation, the G-quadruplex-disrupting ability of Ag^+ ion can be applicable to any G-quadruplexes. (3) The G bases located in the loops of G-quadruplexes can also interact with Ag^+ ion. As a result, the presence of G bases in G-quadruplex loops can decrease the G-quadruplex-disrupting ability of Ag^+ ion. (4) The G-quadruplex-disrupting ability of Hg^{2+} to T-rich G-quadruplexes is higher than that to T-poor G-quadruplexes, demonstrating that the formation of T-Hg^{2+}-T base pairs is one important way for Hg^{2+} ion to disrupt T-rich G-quadruplexes. But because Hg^{2+} ion can also disrupt G-quadruplexes without T bases (M3Q), Hg^{2+} ion may also disrupt G-quadruplexes by other way(s). (5) The stability of the G-quadruplex has a great effect on the G-quadruplex-disrupting abilities of Ag^+ and Hg^{2+} ions. These results may provide some important information for the development of highly efficient G-quadruplex-disrupting reagents, for example metal complexes-based G-quadruplex-disrupting reagents.

The G-quadruplex-disrupting abilities of Ag^+ and Hg^{2+}, together with their strong interactions with Cys, provide the possibility to carry out an IMPLICATION logic opertation. Thus, using Ag^+ (or Hg^{2+}) and Cys as the two inputs, CD signal as the output, a DNA IMPLICATION logic gate has been constructed. To the best of our knowledge, this logic gate is the third example of a molecular logic gate using CD signal as output[41-42], and the first example of a DNA IMPLICATION logic gate using CD signal as output.

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