Development of a novel device to trap heavy metal cations: Application of the specific interaction between heavy metal cation and mismatch DNA base pair

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ABSTRACT

We have already found that Hg(II) cation specifically binds to T:T mismatch base pair in heteroduplex DNA, which increases the melting temperature of heteroduplex DNA involving T:T mismatch base pair by about 4 °C. We have also found that Ag(I) cation specifically binds to C:C mismatch base pair in heteroduplex DNA, which increases the melting temperature of heteroduplex DNA involving C:C mismatch base pair by about 4 °C. Using the specific interaction, we developed a novel device to trap each of Hg(II) and Ag(I) cation. The device is composed of 5'-biotinylated T-rich or C-rich DNA oligonucleotides, BIO-T20: 5'-Bio-T\(_{20}\)-3' or BIO-C20: 5'-Bio-C\(_{20}\)-3' (Bio is a biotin), immobilized on streptavidin-coated polystyrene beads. When the beads trapping Hg(II) or Ag(I) cation were collected by centrifugation, almost all of Hg(II) or Ag(I) cation were removed from the solution. Also, when the BIO-C20-immobilized beads were added to a solution containing Ag(I) cation, and the beads trapping Ag(I) cation were collected by centrifugation, almost all of Ag(I) cation were removed from the solution. We conclude that, using the novel device developed in this study, Hg(II) and Ag(I) cation can be effectively removed from the solution.

INTRODUCTION

Contamination with heavy metal cations may have severe effects on human health and the environment. To increase our understanding of water pollution by heavy metal cations, numerous efforts have been made to develop novel strategies that can remove heavy metal cations from the environment. We have already found that Hg(II) cation specifically binds to T:T mismatch base pair in heteroduplex DNA with high affinity and specificity, which increases the melting temperature of heteroduplex DNA involving T:T mismatch base pair by about 4 °C.\(^{1,2}\) We have also found that Ag(I) cation specifically binds to C:C mismatch base pair in heteroduplex DNA with high affinity and specificity, which increases the melting temperature of heteroduplex DNA involving C:C mismatch base pair by about 4 °C.\(^{5-8}\) Here, using the specific interaction, we developed a novel device to trap each of Hg(II) and Ag(I) cation. The device is composed of 5'-biotinylated T-rich or C-rich oligonucleotides immobilized on streptavidin-coated polystyrene beads. When the beads are added to a solution containing Hg(II) or Ag(I) cation, Hg(II) or Ag(I) cation can be trapped by the immobilized T-rich or C-rich oligonucleotides through the T-Hg-T or C-Ag-C specific interaction. After the beads trapping Hg(II) or Ag(I) cation are collected by centrifugation, Hg(II) or Ag(I) cation can be removed from the solution. Using this novel device, Hg(II) or Ag(I) cation can be removed from the solution.

MATERIALS AND METHODS

We purchased 20-mer 5'-biotinylated T-rich or C-rich DNA oligonucleotides, BIO-T20: 5'-Bio-T\(_{20}\)-3' or BIO-C20: 5'-Bio-C\(_{20}\)-3' (where Bio is a biotin), from Tsukuba Oligo Service Co. (Japan). We also purchased streptavidin-coated polystyrene beads, Dynabeads M-280 streptavidin, from Veritas Corp. (Japan). BIO-T20 and BIO-C20 were immobilized on Dynabeads M-280 streptavidin.

The concentration of the remaining Hg(II) cation after removing by the BIO-T20-immobilized Dynabeads was determined by adding a dye-labeled T-rich DNA oligonucleotide, F2T6W2D: 5'-Fam-T\(_{20}\)CT\(_{20}\)CT\(_{20}\)CT\(_{20}\)G T\(_{1}\)-Dabcyl-3', where 6-carboxyfluorescein (Fam) is a fluorophore and Dabcyl is a quencher. Also, the concentration of the remaining Ag(I) cation after removing by the BIO-C20-immobilized Dynabeads was determined by adding a dye-labelled C-rich DNA oligonucleotide, F2C6W2D: 5'-Fam-C\(_{20}\)CT\(_{20}\)CT\(_{20}\)CT\(_{20}\)AC\(_{20}\)AC\(_{20}\)-Dabcyl-3'. The presence of Hg(II) or Ag(I) cation may induce the formation of the hairpin structure of F2T6W2D or F2C6W2D by the T-Hg-T or C-Ag-C specific interaction. Because the hairpin structure should bring the fluorophore, Fam, and the quencher, Dabcyl, into close enough proximity for energy transfer, fluorescence resonance energy transfer (FRET)-mediated quenching between Fam and Dabcyl may be observed. Because the presence of Hg(II) or Ag(I) cation may decrease the intensity of Fam emission at 520 nm in a concentration-dependent manner,
the concentration of each of Hg(II) and Ag(I) cation can be determined from the intensity of Fsm emission at 520 nm.

RESULTS AND DISCUSSION
When the dye-labeled T-rich DNA oligonucleotide, F2T6W2D, was added to a solution without Hg(II) cation, the intensity of Fsm emission at 520 nm was large (Fig. 1). On the other hand, when F2T6W2D was added to a solution with 200 nM Hg(II) cation, the intensity of Fsm emission at 520 nm was small due to the FRET-mediated quenching between Fsm and Dabcyl (Fig. 1). When F2T6W2D was added to a solution after the removal of Hg(II) cation by the BIO-T20-immobilized Dynabeads, the intensity of Fsm emission at 520 nm significantly increased in comparison with that observed for a solution with 200 nM Hg(II) cation (Fig. 1). The intensity of Fsm emission at 520 nm after the removal of Hg(II) cation by the BIO-T20-immobilized Dynabeads was quite similar to that observed for a solution without Hg(II) cation. Thus, the addition of the BIO-T20-immobilized Dynabeads into a solution containing Hg(II) cation removed almost all of Hg(II) cation from the solution.

When the dye-labeled C-rich DNA oligonucleotide, F2C6W2D, was added to a solution without Ag(I) cation, the intensity of Fsm emission at 520 nm was large (Fig. 2). On the other hand, when F2C6W2D was added to a solution with 240 nM Ag(I) cation, the intensity of Fsm emission at 520 nm was small due to the FRET-mediated quenching between Fsm and Dabcyl (Fig. 2). When F2C6W2D was added to a solution after the removal of Ag(I) cation by the BIO-C20-immobilized Dynabeads, the intensity of Fsm emission at 520 nm significantly increased in comparison with that observed for a solution with 240 nM Ag(I) cation (Fig. 2). The intensity of Fsm emission at 520 nm after the removal of Ag(I) cation by the BIO-C20-immobilized Dynabeads was quite similar to that observed for a solution without Ag(I) cation. Thus, the addition of BIO-C20-immobilized Dynabeads into a solution containing Ag(I) cation removed almost all of Ag(I) cation from the solution.

CONCLUSION
We conclude that, using the novel device developed in this study, Hg(II) and Ag(I) cation can be effectively removed from the solution.

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Fig. 1 Fluorescence spectra of F2T6W2D. Straight line: solution without Hg(II) cation. Broken line: solution with 200 nM Hg(II) cation. Dotted line: solution after the removal of Hg(II) cation by the BIO-T20-immobilized Dynabeads.

Fig. 2 Fluorescence spectra of F2C6W2D. Straight line: solution without Ag(I) cation. Broken line: solution with 240 nM Ag(I) cation. Dotted line: solution after the removal of Ag(I) cation by the BIO-C20-immobilized Dynabeads.