Hg²⁺-Trapping Beads: Hg²⁺-Specific Recognition through Thymine–Hg(II)–Thymine Base Pairing

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Mercury pollution poses a severe threat to human health. To remove Hg²⁺ from contaminated water, we synthesized Hg²⁺-trapping beads that include oligo-thymidine functionalities that can form thymine–Hg(II)–thymine base pairs on the solid support. The beads can selectively trap Hg²⁺ even in the presence of other metal cations. More interestingly, Hg²⁺-trapping efficiency was higher in the presence of the co-existing cations. Thus, the developed Hg²⁺-trapping beads can capture Hg²⁺ without affecting the mineral balance of water so much. The Hg²⁺-trapping beads presented here show promise for removing Hg²⁺ from environmental water.

Key words mercury removal; thymine; thymine–Hg(II)–thymine base pair; DNA–metal interaction; oligo-nucleotide

Mercury is a reputable highly toxic element.^{1–3)} Notably, World Health Organization (WHO), Environmental Protection Agency (EPA), and U.S. Food and Drug Administration (FDA) reported that certain kinds of fishes accumulate high level of mercury which is harmful enough to fetuses, babies and children.^{3,4)} Such an accumulation of mercury in fishes occurred through a food chain.^{2,5)} Based on these facts, the provisional tolerable weekly intake (PTWI) for methylmercury and the reference dose (RfD) for mercuric chloride have been set at 1.6 µg/kg/week and 0.3 µg/kg/d, respectively.³⁾ In any case, mercury originates from natural sources (such as volcanoes) and human activities (such as industrial wastes). To reduce mercury contamination in the environment, it is necessary to reduce and remove mercury from environments (oceans, rivers, and lakes).

For this purpose, various techniques have been reported. These include bioremediation,^{5,6)} precipitation,⁷⁾ non-specific adsorption (using activated carbon,^{8,9)} aerogels,¹⁰⁾ and chitosan^{8,11)} as sorbents, and metal chelations (using imidazoles,¹²⁾ thiols,¹³⁾ and DNA/nucleobases^{14–19)}). However, these techniques generally trap several kinds of metals, resulting in the loss of mineral balance in the treated water. It is thus indispensable to develop methods for the selective removal of mercury.

For this purpose, thymine–Hg(II)–thymine (T–Hg(II)–T) base pairing system which was used in a DNA-based Hg²⁺-specific sensor by the Ono and Togashi²⁰⁾ gave us a hint, since thymine is a highly Hg(II)-specific ligand. The key structural element of the T–Hg(II)–T base pair was originally proposed by Katz,²¹⁾ based on pre-existing studies on Hg²⁺–DNA inter-

actions.^{22–25)} New insights were recently reported by Ono and colleagues.^{20,26,27)} Although extensive structural and theoretical studies on the structure of this base pair in a DNA oligomer have been performed,^{28–41)} the experimental structure of the T–Hg(II)–T base pair from NMR and Raman spectroscopy was recently reported^{27,42)} (see the chemical structure of the T–Hg(II)–T base pair for that shown within Fig. 1b). Thermal denaturation experiments⁴³⁾ showed that the T–Hg(II)–T base pair is as stable as the Watson–Crick A–T base pairs. More importantly, it was demonstrated that a T–T mismatch can specifically bind to Hg²⁺ without binding to other divalent metal cations.²⁶⁾ Thus, such Hg²⁺-specificity of thymine base is suitable to develop an Hg²⁺-specific trapping system. We thus prepared Hg²⁺-trapping beads based on this concept (Fig. 1), and examined their trapping efficiency.

The Hg²⁺-trapping beads consist of oligo-thymidine (T20) attached onto the polystyrene (PS) beads (solid support), as shown in Fig. 1a. Following capture of the Hg²⁺ ions by T20, the Hg²⁺-trapped PS beads can be easily separated from the solution by filtration (Fig. 1b). To give mobility to T20, polyethylene glycol 400 (PEG 400) was used as a linker to attach T20 onto the PS beads, since PEG 400 is long enough to give attached molecules their mobility on the polymer surface.⁴⁴⁾ The detailed preparation is given in Chart S1 and Supplemental Methods in Supplemental Information.

Inductively coupled plasma atomic emission spectroscopy (ICP-AES) was used to assess the performance of the prepared Hg²⁺-trapping beads. This technique is suitable for the quantification of low levels of Hg²⁺ as well as the simultaneous determination of co-existing metal cations in solution.

Prior to conducting the Hg²⁺-trapping experiments, we confirmed the linearity between the Hg²⁺-concentration and the ICP-AES peak intensities, measured at the 253 nm

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Fig. 4. Residual Ratios of the Co-existing Metal Cations after Hg²⁺-Trapping

The emission wavelengths of the corresponding co-existing metal cations are listed in Table S3. All the experiments were performed in 50 μ M HgCl₂, 5.0 mM MgCl₂, and 0.5 mM of the respective co-existing metal cation except for sample Mg²⁺* whereby the experiment was conducted in the absence of the co-existing cations.

Mg²⁺, see Fig. S2.

The non-specific binding of the co-existing metal cations by the Hg²⁺-trapping beads was then examined (Fig. 4, Figs. S3, S4, Tables S3, S4). The residual ratios (%) of the co-existing cations after Hg²⁺-trapping are shown in Fig. 4 and Table S3. As observed, the remaining percentages of all the co-existing metal cations in the corresponding supernatant solutions, after Hg²⁺-trapping, were very high.

The DNA-based Hg²⁺-trapping beads effectively removed mercury in both the presence of Mg²⁺ only, and several co-existing metal cations that included Mg²⁺ (Fig. 3). In contrast to this effective Hg²⁺-trapping, non-specific trapping of co-existing cations did not occur so much (Fig. 4). The results are consistent with our previous study that showed the specific binding of Hg²⁺ by thymine bases.²⁶⁾ Thus the herein developed Hg²⁺-trapping beads are suitable for use in natural conditions that involve a variety of mineral ingredients.

The enhanced performance of the Hg²⁺-trapping beads in the presence of co-existing metal cations is explained as follows. The formation of T–Hg(II)–T base pairs implies the formation of a DNA duplex. For the latter to form, electrostatic repulsion between the negatively-charged phosphates in the respective DNA strands needs to be suppressed. The Mg²⁺ and other co-existing cations thus suppress the electrostatic repulsion between the phosphate groups by neutralizing the negative charges (Fig. S5). Hence, in the absence of a charge compensation effect of the co-existing cations, formation of T–Hg(II)–T base pair and the resulting DNA duplex formation cannot occur efficiently. In addition, the competition between Mg²⁺ and Hg²⁺ during their phosphate-binding steps may assist preferential Hg²⁺-binding to thymine bases. This phenomenon was evident in the presence of Mg²⁺, as indicated by the considerable increase in the Hg²⁺-trapping efficiency (Figs. S2, S5c, d). A slight increase in the trapping efficiency was observed in the presence of the co-existing cations under Mg²⁺-depleted conditions (Figs. S2, S5e). These findings and some foregoing studies^{45,46)} support our hypothesis that co-existing cations stabilize the formation of a DNA duplex following formation of T–Hg(II)–T base pair. The measured residual ratio of the co-existing metal cations after Hg²⁺-trapping (Fig. S3) further supported our hypothesis that co-existing cations served as counter ions for DNA, as explained: in the ab-

sence of Mg²⁺, the other co-existing cations were captured by the Hg²⁺-trapping beads, possibly as counter ions for the phosphate groups (Fig. S5e), hence other co-existing cations rarely remained in solution in the absence of Mg²⁺ (Fig. S3). The present experimental data indicate that the Hg²⁺-trapping beads show potential for their usage under natural environmental conditions, particularly in seawater with a high salt concentration.

The present findings were compared to those obtained by other reported methods (bioremediation,^{5,6)} precipitation,⁷⁾ non-specific adsorbents,^{8–10)} and some metal chelators^{12,13)}. In these studies, the Hg²⁺-specificity was not indicated nor was it expected on the basis of metal-specificities of the ligands.^{5–10,12,13)} Only Hg²⁺-trapping beads, containing thymine moieties,^{14–19)} showed good Hg²⁺-selectivity and these data are consistent with our previous results.²⁶⁾ As an exception, chitosan-based Hg²⁺-trapping beads showed Hg²⁺ selectivity,¹¹⁾ however, the origin of the Hg²⁺-specificity is unclear on the basis of an intrinsic metal-specificity of chitosan.

In conclusion, the prepared Hg²⁺-trapping beads can exclusively capture Hg²⁺ even in the presence of co-existing cations. More interestingly, the Hg²⁺-trapping efficiency of the beads improved in the presence of co-existing cations. The Hg²⁺-trapping beads are suitable for the elimination of Hg²⁺ from environmental water, especially seawater. As a practical usage of the Hg²⁺-trapping beads, the Hg²⁺-decontamination from industrial wastes would be possible.

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