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Aptamer selection for fishing of palladium ion using graphene oxide-adsorbed nanoparticles

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ABSTRACT

A new aptamer selection method using graphene oxide (GO)-adsorbed nanoparticles (GO-adsorbed NPs) was employed for specific fishing of palladium ion. High affinity ssDNA aptamers were isolated through 13 rounds of selection and the capacity of the selected DNA aptamers for palladium ion uptake was measured, clarifying that DNA01 exhibits the highest affinity to palladium ion with a dissociation constant (K_d) of 4.60 ± 1.17 μM. In addition, binding ability of DNA01 to palladium ion was verified against other metal ions, such as Li^+, Cs^+, Mg^{2+}, and Pt^{2+}. Results of the present study suggest that future modification of DNA01 may improve palladium ion-binding ability, leading to economic recovery of palladium from water solution.

Metal sequestering from water is a topic covering a wide range of scientific and practical applications for such purposes as the removal of toxic molecules and the collection of noble elements and compounds.[1] Metal-specific chelating agents are especially known to be more effective than simple ion exchange materials because they exhibit higher selectivity and better complexation constants toward target metal ions and they are practically useful for treatment of drinking or waste water as well as for extraction of the target metal ions from water where reactions are performed at very low concentrations, such as nanomolar.[2] A good example is to extract uranium ion from seawater,[3] as we previously demonstrated that a DNA-based aptamer could be utilized for uranyl ion extraction,[2d] which is considerable in that the ocean contains 1000 times as much uranium as is buried in deposits on land, with approximately 4.5 billion tons of uranium in seawater.[3a]

From this point of view, palladium is another important metal ion, because the production of palladium is relatively limited to a few production sites and the recent demand for palladium is rapidly increasing because of its wide use in electrical equipment, dental materials, and automobile catalysts.[4] Therefore, the development of separation/extraction methods for palladium or palladium ion (Pd^{2+}), that is, selective capture strategy development for palladium ion from waste or water solution, including recycling, extraction and refining technology, has been of great importance, since it can allow us to recover these precious metals from water solution and to meet the future demand.[4]

In the present study, to find selective and effective molecular recognition probes for palladium ion, we discovered a new palladium ion-specific DNA aptamers, by employing a new graphene oxide (GO)-based method as a selection strategy.[5] Aptamers are single-stranded oligonucleotides that bind to targets ranging from metal ions to cells with high affinity and selectivity.[6] Despite their potential as molecular recognition elements for extraction of small molecules including metal ions, relatively few aptamers exist that selectively bind to metal ions, presumably because target immobilization is technically challenging in consideration that the separation of target-bound sequences from those with no affinity for the target is a critical step in the conventional systematic evolution of ligands by exponential enrichment (SELEX) process.[2d,5] In this regard, methods development with no necessity of target immobilization for aptamer selection against small molecules including metal ions is interesting, because small molecules are important targets for investigation due to their diverse biological functions as well as their clinical and commercial uses.

There has been indeed a growing need for rapid, robust, and inexpensive method for aptamer selection, and in an effort to address this issue, a GO-based SELEX method was recently reported,[5] demonstrating that the unique feature of GO, of which the ability is to separate free short ssDNAs in heterogeneous solution,[7] could be used as a platform for screening of aptamers that bind to their target with high affinity and specificity. In brief, Park et al. added GO to a preincubated mixture of random ssDNA library

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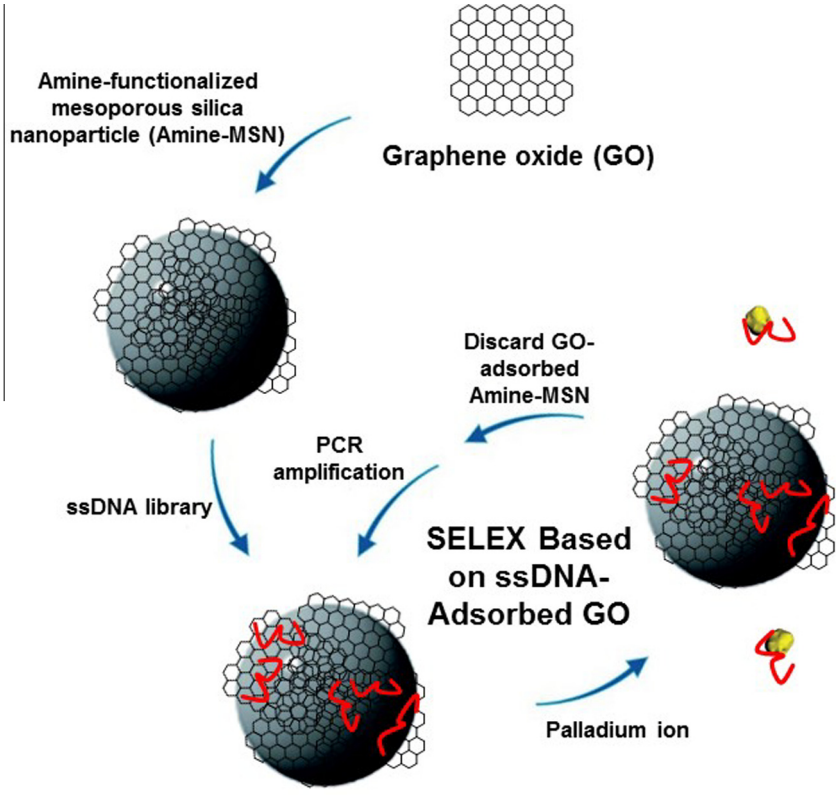
and target (nicotinamide phosphoribosyl transferase) in the first separation step of their 'GO-SELEX' procedure.[5] After discarding the unbound ssDNAs adsorbed on the surface of GO, they recovered, amplified, and purified the target-bound ssDNAs for further 'GO-counter SELEX' process, which resulted in the final 15 hit materials for specific recognition of the target protein.[5]

Inspired by the work, we modified their GO-based selection method in order to find $PdCl_4^{2-}$-specific DNA aptamers (Scheme 1), which could solve the problem of selective and effective molecular recognition of aptamers for palladium ion, as an alternative aptamer selection method to our previously published one by converting in vitro-selected UO_2^{2+}-specific catalytic DNAs to UO_2^{2+}-specific DNA-based aptamers.[2d] Whereas the method reported by Park et al. made use of free GO for separation of unbound nucleic acids,[5] our present method took advantage of the properties of GO adsorbed onto the surface of amine-functionalized mesoporous silica nanoparticles (Amine-MSN) prior to the conventional SELEX process.[7c] Our method using GO-adsorbed nanoparticles (GO-adsorbed NPs) allowed better separation of free target-bound ssDNAs from target-unbound and GO-adsorbed ssDNAs when metal ions are the targets for aptamer selection.

After preparation of GO-adsorbed NPs according to the literature,[7c] a ssDNA library for palladium ion capturing that contained 40-mer random DNA sequences flanked by two PCR primer sequences was utilized,[8] and $PdCl_4^{2-}$ was used as target in a solution containing $NaHCO_3$ (21 mM) and HEPES (0.1 M) at pH 8.02, because palladium is known to mainly forms chlorocomplex in chloride medium.[4] During the 13th round SELEX processes, the specifically bound ssDNAs were enriched by excluding and subsequently discarding nonspecific binders. After 10 cycles, negative selection using NaCl, just in case of aptamer-based extraction of palladium ion from seawater, along with positive selection was applied. In combination with a negative selection step, nonspecific variants could be eliminated from the ssDNA pools and palladium ion-specific aptamer species were generated. The aptamer selection progress was monitored by comparing the concentration of all elutes. Comparison of the amount of DNA elutes from the entire 13 round selections showed that the enrichment of the selected ssDNA pools was increasing proportionally to the round number and that the concentration of the enriched ssDNA pool at the 13th round of selection was high enough for the following cloning procedures (data not shown). Thus, the 13th round ssDNAs were chosen for the cloning experiments and sequenced to identify individual aptamer candidates, and the four 40-mer aptamers (DNA01–DNA04) selected from the 10 blue colonies resulting from the cloning experiments were examined in equilibrium dialysis-based binding assays (Table 1).

To obtain a palladium ion binding profile of the selected aptamers as a function of aptamer concentration, the aptamer solution contained in the dialysis caging (cutoff M.W. 5000) was equilibrated against a solution containing the metal ion (Na_2PdCl_4 (32 μM), $NaHCO_3$ (21 mM), and HEPES (0.1 M) at pH 8.02), similarly with our previous work.[2d] Briefly, commercially available dialysis casings were used to minimize concentration changes due to osmotic pressure, and by ICP-MS measurement of the concentration of the metal ion outside and inside the dialysis caging after 18-h incubation, the amount of the metal ion bound to the aptamer was obtained. As shown in Figure 1, straight lines resulting from the binding study were observed until the concentration of each aptamer was equivalent to $[PdCl_4^{2-}]_0$, indicating that DNA01 exhibits the highest affinity for palladium ion among the 4 aptamer candidates, although there is no



Scheme 1. Schematic representation of an aptamer selection method based on graphene oxide-adsorbed nanoparticles (GO-adsorbed NPs) for specific fishing of palladium ion. Prior to the conventional SELEX processes, GO was adsorbed onto the surface of amine-functionalized mesoporous silica nanoparticles (Amine-MSN). The resulting GO-adsorbed NPs were added to a preincubated mixture of random ssDNA library and target (palladium ion in this study). The palladium ion-bound ssDNAs were collected, amplified, and identified for further specificity experiments.

Table 1
Final 4 hit DNAs identified after the 13th round of GO-adsorbed NP-based aptamer selection processes were sequenced and the DNA sequences of the four groups of aptamers are shown

	Sequence
DNA01	5′-GGGCG GACGC TAGGT GGTGA TGCTG TGCTA CACGT GTTGT-3′
DNA02	5′-ACAAG GGGTG CAGGG GTCCG CGATT GTGAC TGTGT GTGCG-3′
DNA03	5′-GGGCG GACGC TAGGT GGTM GGCTG TGCTA CACGT GTTGT-3′
DNA04	5′-CCCCG CTGCC CGTGT TCCGT CCTCC CTTGC TGTGT GTGCG-3′

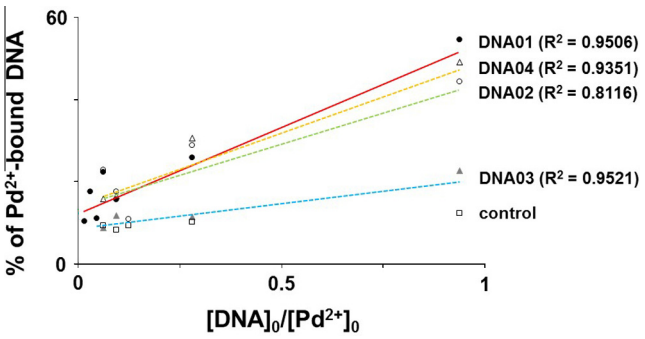


Figure 1. Binding affinity of the selected aptamers (DNA01–DNA04) to palladium ion was examined in equilibrium dialysis-based binding assays. The aptamer solution contained in the dialysis caging (cutoff M.W. 5000) was equilibrated for 18 h against a solution containing the metal ion (Na_2PdCl_4 (32 μM), $NaHCO_3$ (21 mM), and HEPES (0.1 M) at pH 8.02). By ICP-MS measurement of the concentration of the metal ion outside and inside the dialysis caging after equilibrium was reached, the amount of the metal ion bound to the aptamer was obtained. R^2 represents the coefficient of determination.

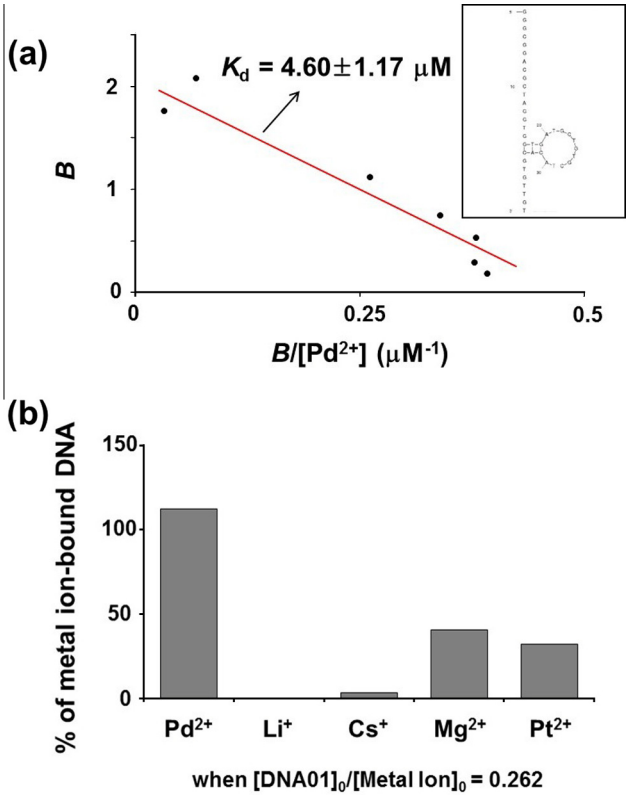


Figure 2. (a) Scatchard plot resulting from palladium ion binding experiment of DNA01, indicating that B can be approximated to be 2 at complete saturation binding when [Pd^{2+}] is in large excess and all receptor binding sites of DNA01 are occupied and that the binding follows the ideal independent double-site, equal affinity binding model. The data from the binding experiment of DNA01 demonstrate that the dissociation constant (K_d) for palladium ion complexes can be estimated to be 4.60 μM. Inset: Secondary structure of DNA01, predicted using the MFold program. It should be noted that DNA03, of which the sequence is very similar with that of DNA01, are not allowed to form this type of secondary structures owing to the base difference in positions 19 and 21. (b) Binding experiments were further performed against Li^+, Cs^+, Mg^{2+}, and Pt^{2+}, respectively, for the determination of the binding ability between DNA01 and each of the four metal ions. The data resulting from 18-h incubation imply that DNA01 has the great binding ability for palladium ion over the four metal ions used in this study, while single-charged metal ions, such as Li^+ and Cs^+, can barely bind to DNA01. Our results reveal that approximately 30% and 40% of DNA01 can be bound to Pt^{2+} and Mg^{2+}, respectively, in comparison to Pd^{2+}, under the same dialysis-based binding assay conditions.

significant difference in binding affinity between DNA01, DNA02 and DNA04. As a non-specific palladium ion-binding control, a 40-mer oligonucleotide (control in Fig. 1) was used to measure the amounts of palladium ion that could be bound to the control, clearly demonstrating that palladium ion could be specifically bound to the DNA aptamer selected in this study. Thus, the binding affinity of DNA01 was investigated by ICP-MS and the dissociation constant (K_d) for the $PdCl_4^{2-}$ complexes of DNA01 was calculated from the data of Figure 2(a), according to the Scatchard equation,[9] revealing that DNA01 forms 1:2-type complexes with palladium ion and that it can bind to palladium ion with K_d of 4.60 ± 1.17 μM. We carried out HPLC experiments using anion-exchange column before and after 18-h palladium ion binding, and found no self-cleavage of DNA01 caused by the palladium ion binding.

To further demonstrate the binding specificity of DNA01 over other metal ions, selectivity experiments were performed against Li^+, Cs^+, Mg^{2+}, and Pt^{2+}. As indicated in Figure 2(b), DNA01 has the great selectivity for palladium ion over the four metal ions used in this study, while non-sensitive targets, such as Li^+ and Cs^+, can barely bind to DNA01. Very importantly, DNA01 was able to better recognize palladium ion than platinum ion. Based on the developed method and the information provided by the present study, determination of the structure of the aptamer-target complex and further validation of the palladium ion specificity of DNA01 in the presence of other metal ions are in progress in this laboratory.

Solvent extraction has become an effective technique in the recovery and separation of palladium,[4,10] and thus, the design of host molecules for palladium ion is inevitably linked to the economic importance of selective extraction of palladium from water.[10] The conventional methods reported so far for palladium recovery, however, are not so reliable for routine extraction because of the drawbacks such as operating conditions (emulsion formation leading to many problems for the separation, slow equilibrium, and so forth).[4,10] Moreover, these techniques have turned out to unsatisfactorily lack the required specificity for palladium ion due to interference with other metal ions.[4,10] Taking these into account, our results in the present study strongly imply that DNA01 or its derivatives may overcome the present problems on extraction of palladium ion from water.

To summarize, a new aptamer selection method based on GO-adsorbed NPs was applied to find palladium ion-specific aptamers. DNA01 with high affinity to palladium ion was identified through 13 rounds of selection, which was also found to be able to more strongly bind to palladium ion than the other metal ions studied, suggesting that our DNA01 may be a starting point for developing a DNA aptamer that is specific for palladium ion. Both the GO-adsorbed NP-based aptamer selection method for palladium ion and the aptamer-based recovery strategy of palladium ion can be modified or scaled up and may have diverse applications in palladium-industry.

Acknowledgements

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8. Reagents were obtained from commercial suppliers and were used without further purification, and double-distilled deionized water was used for all experiments. Graphene oxide solution (1 g/L) was obtained from Graphene Square, Inc., Korea), and disodium tetrachloropalladate (Na_2PdCl_4) from Sigma was used as a palladium ion source. DNA and palladium ion concentrations were measured by absorbance at 260 nm using Agilent 8453 UV–Visible spectrophotometer and by inductively coupled plasma mass spectroscopy (ICP-MS) using a PerkinElmer ELAN6100 model, respectively. Amine-functionalized mesoporous silica nanoparticles was prepared according to the literature (see Ref. 7c).
The aptamers were generated using in vitro selection and amplification procedures in the literature (see Ref. 5) from a random DNA library designed and prepared by Bioneer, Korea (5′-CGT ACG GAA TTC GCT AGC-N40-GGA TCC GAG CTC CAC GTG-3′, chemical synthesis, purified by PAGE). Asymmetric PCR was used to preferentially amplify one strand of the original DNA more than the other. The asymmetric PCR products were then separated and purified using streptavidin for single-stranded DNA (ssDNA) preparation. The concentration of ssDNA was determined using Nanodrop spectrophotometer (Thermo Scientific, Wilmington, DE). The aptamer pool from the selection round 13 including negative selection using NaCl along with positive selection at the 8th round was subsequently amplified by PCR using the unmodified primers. Cloning, sequence analysis and alignments were performed using RBC T&A cloning kit (Real Biotech Corp., Taiwan). The selected 4 aptamer candidates (40-mer, DNA01-DNA04) and a 40-mer control (5′-CCCCC CCCCC CCCCC CCCCC CCCCC CCCCC CCCCC CCCCC-3′, used as a control to show whether any of the palladium ion could be bound to non-specific DNA) were purchased from Integrated DNA Technologies and purified by gel electrophoresis followed by ethanol precipitation.
The aptamer solution contained in the dialysis caging (cutoff M.W. 5000) was equilibrated for 18 h against a solution containing the metal ion (Na_2PdCl_4 (32 μM), LiCl (32 μM), CsCl (32 μM), $MgCl_2$ (32 μM), or K_2PtCl_4 (32 μM)), $NaHCO_3$ (21 mM), and HEPES (0.1 M) at pH 8.02. Commercially available dialysis casings (Slide-A-Lyzer G2 Dialysis Cassettes, Thermo) were used to minimize concentration changes due to osmotic pressure. By ICP-MS measurement of the concentration of the metal ion outside and inside the dialysis caging after 18-h incubation, the amount of the metal ion bound to the aptamer was obtained. The resulting data were used to calculate the values of B and K_d according to the Scatchard equation.
All experiments were performed in duplicate.
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