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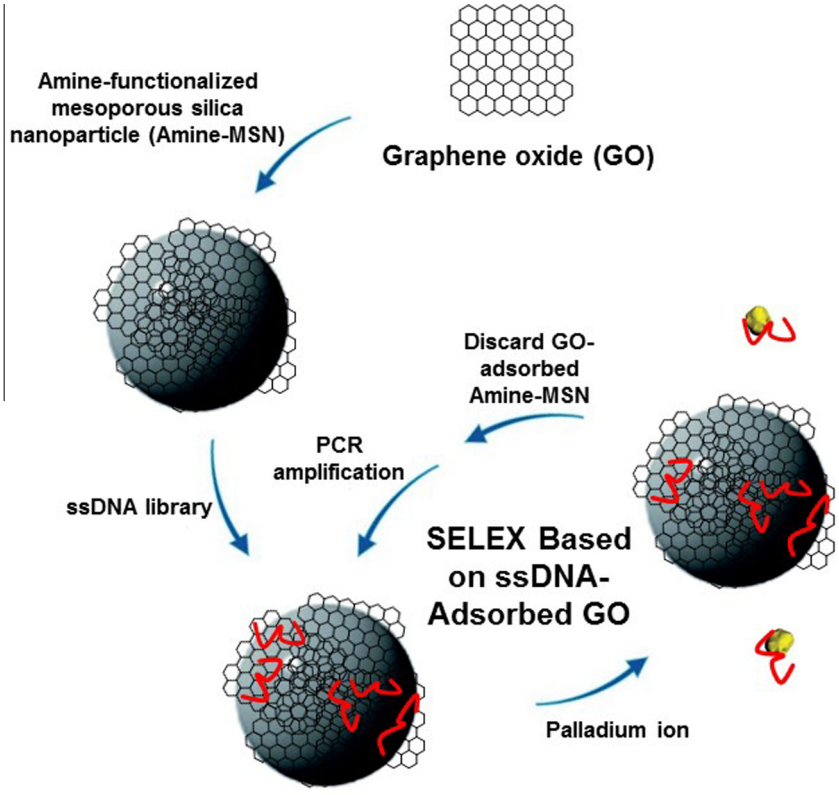
and target (nicotinamide phosphoribosyl transferase) in the first separation step of their 'GO-SELEX' procedure.[5] After discarding the unbound ssDNAs adsorbed on the surface of GO, they recovered, amplified, and purified the target-bound ssDNAs for further 'GO-counter SELEX' process, which resulted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