Application of Gold Nanoparticles to Spectrophotometric Sensing of Hydrophilic Anions Based on Molecular Recognition by Urea Derivative

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We have prepared gold nanoparticles (GNPs) modified with a thiol compound that possesses a phenyleurea moiety for the spectrophotometric sensing of hydrophilic anions, such as dihydrogen phosphate, based on changes in the surface plasmon absorption of the GNP. We examined the spectral change of phenyleurea-modified GNP in dichloromethane upon the addition of various anions as tetrabutylammonium salts to the solution. The GNP showed increasing plasmon intensity with the concentration of dihydrogen phosphate. For a control experiment with an inactive hexanethiolate-modified GNP, such an ion-selective change in the plasmon band was not observed. Furthermore, in order to realize the spectrophotometric detection of hydrophilic anions in water using GNP with the urea functionality, we attempted to prepare bifunctional GNP modified with both the phenyleurea derivative and a water-soluble thiol (e.g., L-cysteine). The resulting bifunctional GNP showed anion-selective changes in the plasmon band accompanied by increasing absorbance at a longer wavelength due to GNPs aggregation.

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Introduction

Anionic species play very important roles in biological systems, and are also environmental pollutant substances. Thus, detection systems of specific anions are required for diagnostic assay and environmental analysis. In spite of recent progress in supramolecular and host-guest chemistry for molecular recognition, it is of great difficulty to attain anion recognition by artificial host compounds. Especially, highly selective anion detection in water or aqueous solutions is still a challenging issue. As has been reviewed,1-3 several types of synthetic host molecules possessing selective binding ability with specific anions have been designed. For example, fluorescent4 and colorimetric5 chemosensors based on Zn2+-dipicolylamine complexes, which bind toward phosphorylated species selectively by coordination to the central Zn2+, have been recently reported. Anion recognition by electrically neutral host molecules, which is based on the formation of hydrogen bonds between an anion and a host molecule, is also of scientific and practical interest. Organic compounds bearing amide or (thio)urea groups can provide efficient hydrogen-bonding sites, since their N-H and C=O groups act as proton-donating and proton-accepting groups for anion binding, respectively. Thus, a variety of these artificial host molecules with specific hydrogen-bonding ability have been reported for sensing hydrophilic anions.1,3-7,13

As mentioned above, anion recognition under aqueous conditions, and therefore effective sensing systems of hydrophilic anions, are still challenging to study. This difficulty in anion sensing can be ascribed to the low charge density, geometrical variety, and high hydration energy of anions.3 Thus, there have been some efforts to solve such issues while aiming at developing efficient chemical sensor systems for anionic species, especially hydrophilic ones. The anion-binding event by synthetic anion receptors at interfaces, such as air-water, liquid-liquid, and solid-liquid can enhance the detection efficiency of anions in an aqueous medium. Kunitz’s group reported on the enhanced binding-ability of guanidinium-monolayers at an air-water interface.10,11 Anion recognition by a thiourea-based chromoiononophore loaded in a cationic vesicle has also been reported.12 Isothiouuronium-derived Langmuir-Blodgett (LB) film at an air-water interface has been investigated to detect the interfacial binding of H2PO4− in aqueous subphase by UV-vis spectroscopy.14 Additionally, it was reported that LB film of a highly selective thiourea derivative for H2PO4− on a highly oriented pyrolytic graphite (HOPG) electrode was used to detect hydrophilic anions based on the ion-channel mimic electrochemical system using a redox marker ion.12 Recently, Echegoyen’s group has reported that hydrophilic anions could be detected in an aqueous solution by electrochemical impedance spectroscopy using gold electrodes modified chemically with a self-assembled monolayer (SAM) of a calixarene compound having amide groups as an anion-binding site.19 Very recently, thiourea–20 and amino–21 functionalized silane SAMs on silica surfaces for colorimetric sensing of aqueous anions have been reported. In these cases, sophisticated interfaces in contact with aqueous medium have been constructed for binding specific anions and reporting sensor signals. Thus, such an interfacial binding system functionalized with synthetic anion receptors would be one of the promising candidates for achieving effective anion sensors operating in an aqueous medium.

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Recently, gold nanoparticles (GNPs) have attracted a great deal of interest for developing novel materials based on its unique optical properties.\textsuperscript{22-24} GNP shows an absorption band in the visible region around 520 nm owing to its surface plasmon (SP) resonance. From the viewpoint of the analytical applications, GNP is potentially applicable to a colorimetric sensing probe, since it has a very large molar absorption coefficient and a high sensitivity of its SP band toward the changes in its states in the dispersion (e.g., refractive index of its local environments or aggregates formation), resulting in absorption-spectral changes. Specifically, the aggregation of dispersed GNP s causes a distinct color change from red to blue owing to plasmon coupling, as has been reported in pioneering studies.\textsuperscript{25,27} Thus, it has already been reported that highly sensitive and selective colorimetric sensing can be attained by chemically modified GNP s with binding sites tailored for specific analytes based on aggregation-induced changes in the plasmon band. For example, there have been several reports on colorimetric sensors using GNP s functionalized with a phenanthroline derivative for Li\textsuperscript{+},\textsuperscript{28} crown ethers for K\textsuperscript{+}, Na\textsuperscript{+},\textsuperscript{29,30} and Pb\textsuperscript{2+},\textsuperscript{31} carboxylic acids for heavy-metal ions,\textsuperscript{32} such as Hg\textsuperscript{2+},\textsuperscript{33} and DNAzymes for Pb\textsuperscript{2+}.\textsuperscript{34,35} Moreover, Watanabe \textit{et al.} have reported on the GNP modified with amide ligands for enhanced optical anion-sensing in dichloromethane\textsuperscript{36} and thioglucose-capped GNP for F\textsuperscript{-} detection in aqueous solution.\textsuperscript{37} Isothiouronium-modified GNP s responsive to oxoanion\textsuperscript{18} and hydrophobic anions\textsuperscript{19} were also reported.

In this paper, we report that the spectrophotometric sensing of hydrophilic anions, such as dihydrogen phosphate, in dichloromethane can be attained using chemically modified GNP with a thiol compound possessing a phenylurea group as an anion-binding site. Furthermore, we attempted to develop water-soluble GNP with phenylurea moieties by introducing l-cysteine as a water-soluble component, and examined its spectrophotometric sensing-ability of hydrophilic anions in water.

\section*{Experimental}

\textbf{Reagents and chemicals}

2-Aminoethanethiol, 6-mercaptohexane (Tokyo Kasei, Tokyo, Japan), phenyl isocyanate (Nacalai Tesque Inc., Kyoto, Japan), and hydrogen tetrachloroaurate(III) tetrahydrate (Kanto Kagaku, Tokyo, Japan) were used as received. 2-(N'-Pheny lureido)ethanethiol (PhUET) was synthesized by the reaction of 2-aminoethanethiol and phenyl isocyanate, as described elsewhere.\textsuperscript{40} Other chemicals were of reagent grade and used as received.

\textbf{Preparation of phenylurea-modified GNP}

Phenyurea-modified GNP (PhU-GNP) was prepared according to a literature method for preparing thiol-capped GNP s.\textsuperscript{41} Briefly, PhUET dissolved in 30 mL of ethanol was added to 10 mL of an ethanol solution containing 15 mM of HAuCl\textsubscript{4} and then mixed. Then, 40 mL of a freshly prepared aqueous solution containing 40 mM NaBH\textsubscript{4} was added to the ethanol solution with vigorous stirring. After further stirring for 3 h, the solution was kept at \textdegree C for 30°C in a refrigerator. The GNP was recovered by centrifugation of the suspension and purified by repetitive cycles of its re-dispersion in ethanol and centrifugation to yield the desired GNP s.

\textbf{Preparation of the bifunctional GNP s with phenylurea and l-cysteine}

GNP (approximately 20 nm in diameter) was prepared by the chemical reduction of AuCl\textsubscript{4} with sodium citrate. Two and half milliliters of 2 mM L-cysteine aqueous solution and 2.5 mL of 3 mM PhUET ethanol solution were added to the citrate-stabilized GNP aqueous solution (45 mL) with stirring. After additional stirring for 1 h, the resulting GNP aqueous solution was filtered with a membrane filter and purified by dialysis to remove unbounded adsorbates.

\textbf{Apparatus}

The absorption spectra of the GNP s were recorded at room temperature by a UV-visible spectrophotometer (V-550, JASCO Corp., Tokyo, Japan). The diameter of the GNP was measured from its micrographs obtained by a transmission electron microscope (TEM, H-8000, Hitachi High-Technologies Corporation, Tokyo, Japan). TEM specimens were obtained by depositing a dispersion of the GNP s and evaporating the solvent. Thermogravimetric analyses (TGA) of the chemically-modified GNP s were carried out at a heating rate of 10°C min\textsuperscript{-1} using a thermogravimetric analyzer (EXSTAR 6000 TG/DTA, Seiko Instruments Inc., Tokyo, Japan).

\section*{Results and Discussion}

\textbf{Characterization and anion-sensing ability of phenylurea-modified GNP}

We synthesized phenylurea-modified GNP, as illustrated schematically in Fig. 1, by using Brust’s method, reported as a procedure for preparing thiol-capped GNP s based on a single-phase system. We attempted to confirm the size and shape of the resulting PhU-GNP by TEM observations. The size distribution of the PhU-GNP measured from its TEM image is given in Fig. 2, indicating that nearly mono-dispersed GNP was successfully obtained. The average diameter of the PhU-GNP was determined to be 5.2 ± 1.1 nm. The amount of the thiolate ligand on the PhU-GNP was evaluated by its TGA. From the loss of mass in thermal decomposition of the PhU-GNP and its average diameter, the phenylurea ligands on one PhU-GNP were roughly estimated to be 1.6 × 10\textsuperscript{4} molecules. The corresponding surface density of the ligand, calculated from this average number per particle, is comparable to a well-known molecular density for (√\text{3} × √\text{3})R30° structure of SAMs of alkanethiols on Au(111). The slightly higher value obtained experimentally for the ligand molecules immobilized on the PhU-GNP may be ascribed to the curvature of the spherical GNP.

The PhU-GNP dispersed in CH\textsubscript{2}Cl\textsubscript{2} showed the absorption peak near 510 nm, owing to its SP absorption, as shown in Fig. 3. We examined the anion-sensing ability of the PhU-GNP

![Fig. 1 Schematic drawing of PhU-GNP prepared in this study.](image-url)
in CH₂Cl₂ by measuring the absorption spectra upon the addition of several anions as their tetrabutylammonium salt. Figure 4a provides absorption spectra around the SP band of the PhU-GNP upon adding H₂PO₄⁻. The SP band increased with increasing concentration of H₂PO₄⁻. Such spectral changes imply that the anion-binding event by the urea moieties on the PhU-GNP would cause some perturbation of the SP band. Figure 4b shows plots for the changes in the SP absorbance of the PhU-GNP at 510 nm as a function of the concentration of added anions. As can be seen from Fig. 4b, H₂PO₄⁻ among the anions tested here caused a significant increase in the SP absorption, while the others raised almost no marked perturbation of the SP. No significant change in the SP absorption was observed in control tests carried out by hexanethiolate-modified GNP s without any hydrogen-bonding groups (Fig. 4c). Thus, the anion selectivity in the increase in the SP absorption of the PhU-GNP would be attributed to selective anion binding of the urea moieties immobilized on the nanoparticle surface. In previous studies on amide-functionalized GNP s, the anion-induced changes in the SP band were ascribed to anion binding by amide groups on the GNP s. As is well known, the binding of the guest anions by (thio)urea compounds is based on the formation of hydrogen-bonding between the urea moiety and the anion. The proton of N-H group in the urea host molecules acts as a proton donor, while the anion behaves as a proton acceptor. Thus, the anions that have higher basicity can undergo more stable anion-urea bonding. The anion-binding selectivity should follow the ability of the guest anions to accept hydrogen bonding, as has been reported. Thus, the anion-selective response of our PhU-GNP probe in Fig. 4b would be governed mainly by the anion-binding affinity of the immobilized urea moieties. In addition, the selectivity may be enhanced by a possible cooperative effect of the multiple bond formation by the urea moieties assembled on the PhU-GNP, as pointed out in a previous study.

As described above, it is well known that optical responses of GNP s result from changes in the refractive index of their local environments and aggregates formation in the dispersion. The latter causes the appearance of an additional absorption peak by plasmon coupling and a decrease in the original SP absorption by screening of the GNP s in the aggregates. Taking into account the hydrogen-bonding ability of the urea groups immobilized on the PhU-GNP, it is likely that the GNP s in CH₂Cl₂ associate to each other by interparticle hydrogen bonding between the urea groups. Thus, the increase in the SP absorption of PhU-GNP by H₂PO₄⁻ can be explained by some disaggregation of the PhU-GNP s in aggregated states without any anion. Namely, the anion-binding event by the urea moieties would diminish the interparticle hydrogen bonding, resulting in the more dispersed PhU-GNP s. The binding of H₂PO₄⁻ at the PhU-GNP s surface may also have an additional effect on the SP band due to an increase in the local refractive index at the interface between the GNP and the solution, which can lead to an increase in the SP absorption.
Applicability of phenylurea-cysteine-modified GNP for anion sensing in water

Unfortunately, the aforementioned excellent sensing-ability of the PhU-GNP can not be utilized in an aqueous medium because of its poor solubility in water. We thus attempted to prepare a water-soluble GNP possessing the phenylurea functionality in order to attain urea-based spectrophotometric GNP sensors of hydrophilic anions in water. As has been shown in previous studies,14,46,47 marked versatility of a combination of GNP with SAMs makes it easy to design and fabricate a sophisticated interface on GNP, resulting in various types of functionalized GNPs. Namely, SAMs can be used to tailor the desired functionalities on GNPs. For example, Stellacci’s group has presented a “rippled” GNP modified with mixed SAMs of hydrophilic and hydrophobic alkanethiols.42 It has also been reported that the bifunctional GNP modified with binary SAMs composed of two functional thiols can be used for metal ions sensing.30,31 Thus, we have designed a bifunctional GNP covered with both PhU/ET and l-cysteine, a typical hydrophilic thiol, which has been used to prepare water-soluble GNP44 gold nanorods,45 and quantum dots.46,47 We measured the absorption spectra of the resulting GNP (PhU-Cys-GNP) in water upon the addition of several anions.

Figure 5a provides the absorption-spectral changes of the PhU-Cys-GNP in water upon the addition of H$_2$PO$_4^-$ . Before adding the anion, the PhU-Cys-GNP showed the SP band at 525 nm. The addition of H$_2$PO$_4^-$ caused a decrease in the SP band, accompanied by a marked increase in the absorbance around 600 nm, resulting in a color change from red to blue. It is well known that such spectral changes in the SP band result from plasmon coupling caused by the aggregation of GNPs. Thus, we can conclude that the spectral change in Fig. 5a would be caused by the formation of GNPs aggregates induced by the anion. Since the original absorption peak at 525 nm and the new peak at a longer wavelength are due to the dispersed and aggregated GNPs, respectively, the anion-induced aggregation of the PhU-Cys-GNP results in a decrease in the intensity of the former and an increase in that of the latter, simultaneously. Thus, this absorption-spectral change enables an effective ratiometric anion sensing based on the ratio of the absorbance at 650 and 525 nm (A$_{650}$/A$_{525}$). We used this ratiometric method to investigate the anion selectivity of the PhU-Cys-GNP in water. The spectral changes of the PhU-Cys-GNP by several anions are summarized in Fig. 5b. As can be seen, H$_2$PO$_4^-$, hydrophilic and tetrahedral anions, caused the largest increase in A$_{650}$/A$_{525}$ with increasing concentration of the anion, while ClO$_4^-$, a less hydrophilic anion, afforded no significant increase in the ratio of A$_{650}$/A$_{525}$. The addition of excess H$_2$PO$_4^-$ induced a gradual decrease in the intensities of the UV-vis spectra at any wavelength, indicating that large aggregates were formed and precipitated. Such a high selectivity of our PhU-Cys-GNP with respect to more hydrophilic anions in water can be explained in terms of the anion affinity of the urea moiety on the GNP, which is similar to that observed for the aforementioned PhU-GNP in CH$_3$Cl. Additionally, the remarkable increase in the A$_{650}$/A$_{525}$ of the PhU-Cys-GNP colorimetric probe by H$_2$PO$_4^-$ would be attributed to a favorable formation of GNPs aggregates by the tetrahedral anion, which can form interparticle multiple hydrogen bonds with the urea moieties, and act as interparticle bridges, resulting in an effective coupling of the plasmon absorption. Similar interparticle binding (formation of sandwich complexes) of metal ions, such as K$^+$ and Na$^+$, by crown-ether-modified GNPs29,30 has already been reported. The results in Fig. 5 clearly indicate the possibility of the water-soluble GNP with urea moieties as a spectrophotometric sensing probe of hydrophilic anions at millimolar levels. We believe that the anion-sensing efficiency of the PhU-Cys-GNP presented here can be improved by sophisticated control of the surface modification of the GNP.

Conclusions

We have prepared GNPs modified with phenylurea moieties while aiming to develop a novel spectrophotometric probe for hydrophilic anions. The phenylurea-modified GNP in dichloromethane showed significant anion-selectivity with respect to H$_2$PO$_4^-$ at micromolar levels. Furthermore, the watersoluble GNP with the phenylurea moiety and l-cysteine indicated a promising sensing-ability for the colorimetric detection of hydrophilic anions in water. It has been demonstrated that GNP can be used as a platform for preparing a colorimetric probe based on an anion-binding interface with electrically neutral host compounds complexing with specific anions. Further investigations for achieving more efficient anion sensing in water by GNP probes are currently underway.

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References