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Highly sensitive gold nanoparticle-based colorimetric probe for phytate detection with high selectivity over various phosphate derivatives

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ABSTRACT

A competitive-assay-based colorimetric sensing system for phytate was developed using a combination of $[Zn_3$(1,3,5-tris[bis(pyridine-2-ylmethyl)aminomethyl]-2,4,6-triethylbenzene)$]^{6+}$ as the receptor unit for the phytate and 11-mercaptoundecylphosphoric acid functionalized gold nanoparticles as the reporter unit. The detection limit of this assay system is ~100 times more sensitive than that reported for colorimetric probes and is compatible with the high sensitivity of previous fluorescent probes. The proposed sensing system can easily detect phytate at less than ~300 nM with the naked eye. Unlike previous sensing systems, this design provides a high selectivity for phytate over various phosphate derivatives, including ATP, pyrophosphate, and inositol trisphosphate, and works well over a broad pH range.

Phytate, *myo*-inositol hexakisphosphate (IP_6), is a fully phosphorylated form of inositol. It is widely distributed, and it serves as the principal storage source of phosphorus in many plants.[1] Numerous daily dietary foods contain high amounts of IP_6, such as cereals, soybeans, almonds, legumes, oil seeds, pollens, and nuts, and the daily intake of IP_6 in a Western-style diet has been estimated to vary from ~0.3 to 2.6 g.[2] IP_6 chelates essential trace minerals, such as Fe, Zn, and Ca, which inhibits the absorption of the minerals, leading to calcium, iron, and zinc deficiencies under certain dietary condition.[3] Therefore, for decades, IP_6 has been regarded as an antinutrient. However, a variety of recent epidemiological studies have discovered that IP_6 has several beneficial properties, such as blood-glucose-lowering and lipid-lowering effects, antioxidative properties, anticancer activities, and pathological calcification prevention.[4] In addition, IP_6 reduces the toxicity of harmful mineral elements, such as Pb and Cd, and facilitates their excretion.[5]

The potentially detrimental and beneficial effects of IP_6 have led to the development of various methods for the determination of IP_6 in food and biological samples. Various detection methods have been developed based on high-performance liquid chromatography (HPLC), mass spectrometry, and nuclear magnetic resonance (NMR) spectroscopy.[6] Although these methods are very useful in the analysis of IP_6, each system has particular limitations. For example, HPLC is a very useful tool for the analysis of IP_6 in various matrixes, but exhibits low sensitivity and requires a laborious set-up process, including the selection of the column and eluent conditions and the flow rate of the eluent. To address these problems, a fluorogenic assay method for IP_6 has been developed using Fe^{3+}–methylcalcein blue complex, Cu^{2+}–gelatin complex, and Fe^{3+}–1,10-phenanthroline complex.[7] These methods were based on a ligand exchange in which metal ions are removed from metal complexes by IP_6, which has high binding affinities to metal ions, which results in changes in the fluorescence of these metal complexes depending on the IP_6 concentration. Although these methods have some advantages over HPLC, such as high sensitivity, low cost, and ease of application, they are very sensitive to the conditions of the assay medium, such as pH. They exhibit low selectivity over other phosphate derivatives because the binding affinity of IP_6 to metal ions is sensitive to the conditions of the assay medium and because other phosphate derivatives, such as pyrophosphate and adenosine triphosphate, also have high binding affinities to the metal ions.[8] Recently, a fluorogenic chemosensors for IP_6 was developed, but these chemosensors were still not able to overcome these problems.[9]

Compared with the fluorometric assay method, colorimetric chemosensors are attractive because they allow naked eye detection in an uncomplicated and inexpensive manner. However, the few colorimetric chemosensors for IP_6 that have been developed have exhibited low sensitivity and selectivity.[10] In addition, these chemosensors are unable to detect IP_6 in physiological samples,

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such as urine and tissues, because in contrast to food samples, the concentration of IP_6 in physiological samples is sub-micromolar. Therefore, it is desirable to develop a colorimetric probe that is able to detect IP_6 at sub-micromolar concentrations with high selectivity.

Recently, we developed a competitive assay-based colorimetric pyrophosphate probe using phosphate-functionalized gold nanoparticles (Phos-AuNPs) and a pyrophosphate receptor.[11] Compared with previous organic-dye-based colorimetric pyrophosphate probes, this probe enhanced sensitivity by utilizing the unique optical properties of AuNPs of extremely high extinction coefficients and distance-dependent surface plasmon resonance, and pyrophosphate could be detected with a high sensitivity similar to that of fluorescent probes.[11,12] In this Letter, the strategy of the pyrophosphate probe is expanded to develop a colorimetric IP_6 probe with high sensitive and selectivity. The probe consists of 11-mercaptoundecylphosphoric-acid-functionalized 13 nm gold nanoparticles (Phos-AuNPs) as a signal unit and $[Zn_3$(1,3,5-tris[bis(pyridine-2-ylmethyl)aminomethyl]-2,4,6-triethylbenzene)$]^{6+}$ (**1**) as a receptor unit because Phos-AuNPs have a high extinction coefficient of $\sim 10^9$ and the phosphate ions of the Phos-AuNPs provide binding sites for **1**, and **1** has a high binding affinity to inositol phosphate derivatives.[10,11] Furthermore, **1** binds with Phos-AuNPs and causes them to aggregate. However, when **1** is pre-incubated with IP_6 and then exposed to the Phos-AuNPs, the color of the Phos-AuNPs is maintained because **1** is transformed into a $[\mathbf{1}(IP_6)]^{6-}$ complex having a weak binding affinity for the phosphate groups on the Phos-AuNPs, as shown in Scheme 1.

First, titration of **1** was conducted using a 3 nM solution of Phos-AuNP in an aqueous buffer at pH 7.0 to determine the optimal concentration of **1** in this assay system. UV/vis spectra of Phos-AuNP in the presence of varied concentrations of **1** are shown in Figure 1. The addition of **1** induced absorbance changes, as shown in Figure 1 inset, when saturated with approximately 1.5 μM of **1**. With this information, the assay system for IP_6 was prepared with 3 nM of Phos-AuNPs and 1.5 μM of **1**. UV/vis spectra of the Phos-AuNP solution were recorded after the addition of **1** pretreated with various concentrations of IP_6 to determine the sensitivity of this method. In a typical experiment, 0.5 mL of **1** (3.0 μM) was pretreated for 5 min with various concentrations of IP_6 in buffer solution (10 mM HEPES, 10 mM NaCl) and then added to 0.5 mL of Phos-AuNPs solution (AuNPs 6 nM) in the same buffer solution. The changes in the absorbances of the assay mixtures were recorded at 579 nm after the addition of **1** pretreated with various concentrations of IP_6. Figure 1b shows the change in the absorbance of the assay solution in the presence of **1** pretreated with various concentrations of IP_6 ions. The observed absorbance intensity at 579 nm decreased approximately proportionally with the IP_6 concentration. From the titration results, the IP_6 detection limit of this assay was estimated to be 209 nM (see Supplementary data). This detection limit is ~100 times more sensitive than that reported for colorimetric probes and is compatible with the sensitivities of previous fluorescent probes. To the best of our knowledge, this method is currently the most sensitive colorimetric sensing system for IP_6.[7,9,10]

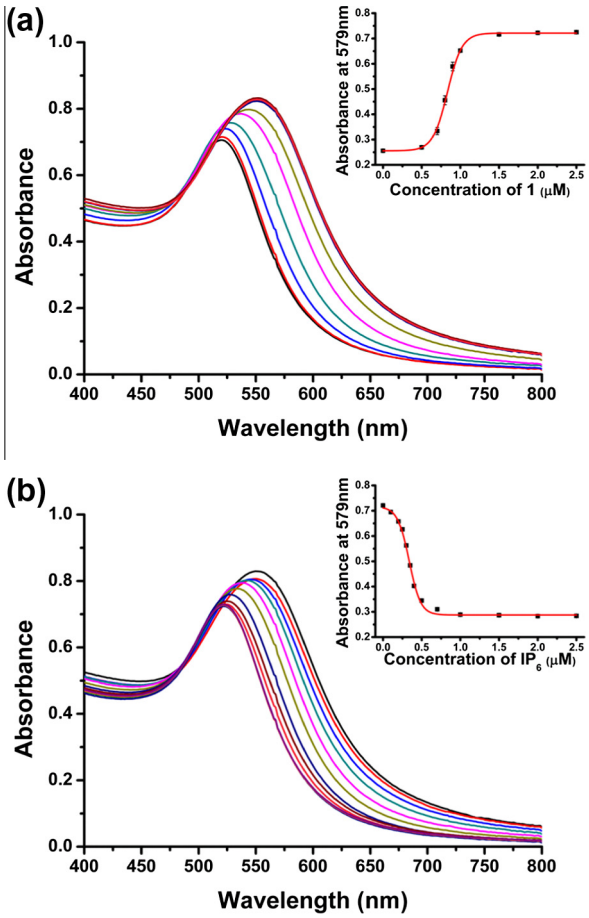
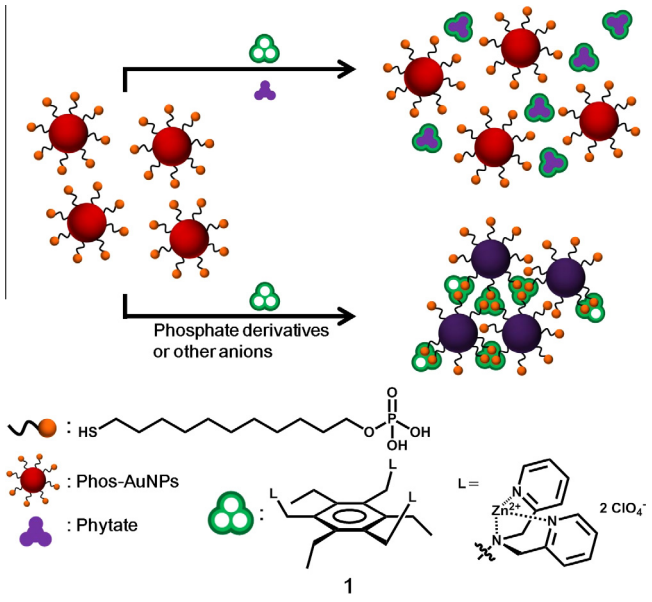


Figure 1. (a) Absorbance spectra of Phos-AuNPs (3 nM) were recorded 1 min after the addition of various concentrations of **1**. Inset: Plot of absorbance intensities of Phos-AuNPs (3 nM) versus **1** concentration; (b) absorbance changes of **1** pretreated with IP_6 in pH 7.0 buffer solution (10 mM HEPES, 10 mM NaCl) containing Phos-AuNPs (3 nM). Inset: Plot of assay solution absorbance intensities at 579 nm versus IP_6 concentrations.



Scheme 1. Schematic representation of the colorimetric probe for IP_6 using a competition assay.

Another important property of this method is its high selectivity for IP_6 over other anions, including various phosphate derivatives, such as pyrophosphate, ATP, and inositol trisphosphate. To evaluate IP_6 selectivity, absorbance changes in the Phos-AuNPs caused by other anions were measured. UV/vis spectra of Phos-AuNPs solutions were recorded 1 min after the addition of **1** (1.5 μM) pretreated with 0.5 μM of other anions (see Supplementary data). No other anions, including various phosphate derivatives, caused any significant changes. This method showed a high selectivity for IP_6 over the other anions. This is significant because all previous assay methods for IP_6 have exhibited low selectivity