Iodine-Mediated Etching of Gold Nanorods for Plasmonic ELISA Based on Colorimetric Detection of Alkaline Phosphatase

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Supporting Information

ABSTRACT: Here, we propose a plasmonic enzyme-linked immunosorbent assay (ELISA) based on highly sensitive colorimetric detection of alkaline phosphatase (ALP), which is achieved by iodine-mediated etching of gold nanorods (AuNRs). Once the sandwich-type immunocomplex is formed, the ALP bound on the polystyrene microwells will hydrolyze ascorbic acid 2-phosphate into ascorbic acid. Subsequently, iodate is reduced to iodine, a moderate oxidant, which etches AuNRs from rod to sphere in shape. The shape change of AuNRs leads to a blue-shift of longitudinal localized surface plasmon resonance. As a result, the solution of AuNRs changes from blue to red. Benefiting from the highly sensitive detection of ALP, the proposed plasmonic ELISA has achieved an ultralow detection limit (100 pg/mL) for human immunoglobulin G (IgG). Importantly, the visual detection limit (3.0 ng/mL) allows the rapid differential diagnosis with the naked eye. The further detection of human IgG in fetal bovine serum indicates its applicability to the determination of low abundance protein in complex biological samples.

KEYWORDS: gold nanorods, etching, iodine, alkaline phosphatase, plasmonic ELISA

INTRODUCTION

In the last two decades, gold nanoparticles (AuNPs) have received considerable attention in colorimetric sensing because of their strong localized surface plasmon resonance (LSPR) extinction in the visible light region.¹ An obvious color change from red to blue can be induced by aggregation of AuNPs.² This property provides a simple platform for the colorimetric sensing of various targets.^{3–7} However, they often suffer from strong false positive results (autoaggregation) and time-consuming modification of AuNPs. To solve these problems, another sensing platform based on etching of gold or silver nanoparticles is proposed using the shape-dependent property of LSPR.^{8–10} In addition, this platform allows the making of economical and stable test paper.¹¹ Unfortunately, only a few targets have been detected using this platform.^{10–18} Most recently, some researchers have started to develop the nanoparticle-etching-based sensors for biomolecules by changing the shape of silver nanoprisms.^{19–21} The shortcoming of this method is that the shape of silver nanoprisms is easily influenced by light, temperature, and biological thiol.^{22,23} Therefore, it is still challenging to establish a reliable strategy to expand nanoparticle-etching-based sensors.

Enzyme-linked immunosorbent assay (ELISA) is the main format of immunoassay, which is based on the antigen–antibody recognition and biocatalytic property of an enzyme.

ELISA has been widely used in clinical diagnosis, environmental analysis, and food test, as well as laboratory research.^{24–27} In principle, ELISA can be used to detect any target if the corresponding antibody is available. Developing a nanoparticle-etching-based ELISA can expand the scope of the nanoparticle-etching-based sensors. Alkaline phosphatase (ALP) is one of the most commonly used enzyme label in ELISA due to its high catalysis activity and low cost. An efficient and effective detection method for ALP is the key to the ALP-based ELISA. For detection of ALP, even though many methods, such as fluorescence,^{28–30} chemiluminescence,³¹ surface enhanced Raman scattering,³² and electrochemistry,³³ possess high sensitivity, they often lack robustness and simplicity. In contrast, colorimetric methods are usually simple and stable, but most of them are not sensitive.^{34–38}

In this work, we propose a plasmonic ELISA based on highly sensitive colorimetric detection of ALP, which is achieved by iodine-mediated etching of gold nanorods (AuNRs). As the intermediate of enzyme-triggered reaction, iodine etches AuNRs into Au(I) and leads to a blue-shift of LSPR of AuNRs. The peak shift can be used for quantification of human

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immunoglobulin G (IgG), which is chosen as a model protein in this work. The detection limit to IgG is calculated to be 100 pg/mL, which is much lower than that of other similar methods^{39–41} and even comparable with the results obtained from many sensitive electrochemical methods.^{42–45} The successful detection of human IgG in fetal bovine serum indicates that this method is applicable to the determination of low abundance protein in complex biological samples.

RESULTS AND DISCUSSION

Principle for Iodine-Mediated Etching of AuNRs. The absorption spectra in Figure 1 show the responses of AuNRs to

Figure 1. SEM (scanning electron microscopy) images (A–C) and absorption spectra (color inset) of AuNRs after incubation with 0 (A, a), 10 (B, b) and 30 (C, c) μM I_2 in glycine/HCl buffer solution (pH 2.2, 50 mM) containing 10 mM CTAB, 1.0 mM I^- .

0 (curve a), 10 (curve b), and 30 (curve c) μM I_2 in the presence of I^- and cetyltrimethylammonium ions (CTA^+). With the increase in I_2 concentration, the longitudinal LSPR shifts to shorter wavelength gradually, which is attributed to the decrease in aspect ratio of AuNRs (SEM images A–C in Figure 1).⁴⁶ Although the iodine-induced morphological change of AuNRs has been widely reported, the mechanism for this phenomenon has not reached a consensus.^{47–49} On the basis of our results, we propose a new mechanism of CTA^+ -assisted oxidation of gold. The reaction is proposed as follows:

To verify the hypothesis, we added a high concentration of I_2 (1.0 mM) to the concentrated gold nanorods solution (2.8 nM). After incubation at 50 °C for 10 min, the solution became colorless, indicating that the product is Au(I) .¹¹ Then, the obtained solution was measured to record the mass spectrum. A full mass spectrum can be seen in Figure 2. Peaks corresponding to five components of the etching of AuNRs are present: CTA^+ at m/z 284.52, $\text{Cl}^-(\text{CTA}^+)_2$ at m/z 603.46, $\text{Br}^-(\text{CTA}^+)_2$ at m/z 649.2, $\text{I}^-(\text{CTA}^+)_2$ at m/z 695.29, and $\text{AuI}_2^-(\text{CTA}^+)_2$ at m/z 1018.88. This result indicates that the product in this reaction is $\text{AuI}_2^-(\text{CTA}^+)_2$, which directly validates the reaction equation mentioned above.

Figure 2. Mass spectrum of AuNRs solution (2.8 nM) after incubation with 1.0 mM I_2 in glycine/HCl buffer solution containing 10 mM CTAB, 1.0 mM I^- .

To further confirm the accelerative role of CTA^+ in the etching process, several control experiments were conducted. The addition of 1.0 mM CTAB or CTAC greatly increased the etching of tween 20-stabilized gold, whereas the addition of 1.0 mM NaBr or NaCl had little effect on the etching process (Figure S1A). These results are consistent with our previous reports.^{11,17} Experimental results also indicate that CTA^+ and I^- (halide ions) have synergy effects on the etching of gold nanoparticles (Figure S1B).

Theoretically, the etching product is dependent on both the reduction potential of Au(I) halide/Au and the concentration of halide ions. On the one hand, the reduction potential of AuI_2^-/Au (0.578 V vs NHE) is much lower than that of $\text{AuBr}_2^-/\text{Au}$ (0.959 V vs NHE) and $\text{AuCl}_2^-/\text{Au}$ (1.154 V vs NHE). These values well explain that the main product in this reaction is $\text{AuI}_2^-(\text{CTA}^+)_2$, but not $\text{AuCl}_2^-(\text{CTA}^+)_2$ (m/z 836) or $\text{AuBr}_2^-(\text{CTA}^+)_2$ (m/z 924.52). On the other hand, the concentration of halide ions has a great effect on the product. For example, when the Br^- concentration is much higher than I^- (Figure S4, Supporting Information), the $\text{AuBr}_2^-(\text{CTA}^+)_2$ will become the domain product.

Iodine-Mediated Etching of AuNRs for Sensing of Reducing Targets. Normally, reducing agents are used to reduce gold ions into gold nanoparticles. However, in this work, the presence of reducing agents can conversely induce the oxidation of the gold nanoparticles into gold ions. Scheme S1 (Supporting Information) schematically shows the strategy for sensing of reducing targets based iodine-mediated etching of AuNRs. KIO_3 is first transformed into I_2 by reducing targets, and then the produced I_2 further oxidizes Au into Au(I) . As a result, the longitudinal LSPR of AuNRs shifts to short wavelength. Several normal reducing targets including S^{2-} , I^- , $\text{S}_2\text{O}_3^{2-}$, *o*-dihydroxybenzene, hydrazine hydrate, and ascorbic acid (AA) were tested using this sensing strategy. As shown in Figure S2 (Supporting Information), the LSPR peak does not change in the presence of KIO_3 and CTAB (curve a). This is because IO_3^- can hardly react with Br^- under the conditions. Otherwise, the AuNRs would be etched by the produced Br_2 , accompanied by a blue-shift of LSPR. In contrast, the presence of 10 μM concentration of these reducing targets induces a large peak shift of longitudinal LSPR (about 50 nm) owing to the etching of AuNRs by the produced I_2 (curves b–f).

To demonstrate the production of I_2 , the UV/vis spectra of KIO_3 solution in the presence of different substances were

measured (Figure S3, Supporting Information). KIO_3 produced negligible absorption (curve a), and the solution appeared colorless. The addition of AA resulted in a big absorption in the range from 400 to 550 nm (curve b) and the solution changed into yellow, suggesting the production of large amounts of I_2 . The yellow solution turned to dark blue after addition of starch (photo d), which further indicates that large amounts of I_2 were produced.

On the basis of the above results and our previous work,^{11,50} the reaction for the AA-induced etching of AuNRs can be described as follows:

Although this sensing strategy does not have specificity for certain targets, it is still possible to detect some targets under given conditions, such as H_2S in the air.⁵¹ More importantly, this strategy provides an indirect sensing method for some targets via a reducing intermediate.

Colorimetric Sensing of ALP. Inspired by the above results, we developed a colorimetric method for sensing of ALP. Figure 3A shows the sensing mechanism for the colorimetric

Figure 3. (A) Schematic illustration for colorimetric detection of ALP based on etching of AuNRs. (B) Absorption spectra and optical images of AuNRs before (curve a, blue image) and after (curve b, red image) incubation with 10 U/L of ALP. TEM (transmission electron microscope) images of the AuNRs (C) before and (D) after treatment with ALP separately.

detection of ALP. ALP hydrolyzes ascorbic acid 2-phosphate (AA-p) into AA due to the dephosphorylation. Subsequently, AA induces the etching of AuNRs in the presence of KIO_3 (images C and D in Figure 3). As a result, the etching leads to the blue-shift of longitudinal LSPR with a significant color change from blue to red (Figure 3B). This color change provides a visual sensing method for ALP. It is worth noting that the high stability and unique optical property of AuNRs plays an important role for the visual detection. Zeta potential and dynamic light scattering (DLS) of AuNRs in the buffer solution indicate that the AuNRs can keep stable in the sensing

system (Figure S5, Supporting Information). In addition, the maximum absorption of the used AuNRs also benefits the visual detection. The aspect ratio (length/width $\approx$ 2:1) of the used AuNRs results in a maximum absorption at 660 nm, which is close to the center of the visible range (390–780 nm). Therefore, the color of the used AuNRs is very sensitive to human eyes.

According to the mechanism, the sensing performance of ALP is directly dependent on the sensitivity for detection of AA. Thus, the AA sensing conditions, including pH, concentration of Br^- , incubation temperature, and time, were first optimized (Figures S6–S9). It should be noted here that the addition of Br^- to the sensing system improved the sensitivity greatly (Figure S7). MS experiment showed that the component of $\text{AuI}_2^-(\text{CTA}^+)_2$ almost disappeared, accompanied by the appearance of a new component, $\text{AuI}_2^-(\text{CTA}^+)_2$ (Figure S4). The formation of $\text{AuI}_2^-(\text{CTA}^+)_2$ is probably attributed to the replacement reaction, as shown in reaction 3. The formation of $\text{AuI}_2^-(\text{CTA}^+)_2$ decreases the reduction potential of $\text{Au(I)}/\text{Au}$ further and, therefore, makes the oxidation of AuNRs more thorough. Under optimal experimental conditions, the responses of the AuNRs to different concentrations of AA were tested (Figure S10). A linear relationship between the absorbance at 670 nm ($A_{670\text{ nm}}$) and AA concentrations from 0.1 to 0.8 μM was obtained. The detection limit was calculated as 0.1 μM (1.0 μM for visual detection limit) according to the 3 σ rule.

The high sensitivity for AA provides a good prerequisite for ALP detection. After optimizing the substrate (AA-p) concentration and bioreaction time (Figures S11 and S12), we investigated the sensing performance for the colorimetric sensing of ALP. As shown in Figure 4A, the solution changes gradually from blue to red with the increase in ALP concentration. The obvious color changes comes from the change in the absorption spectra of AuNRs. As shown in Figure 4B, the $A_{670\text{ nm}}$ decreases with increasing ALP concentration (Figure S13) and there is a linear relationship between $A_{670\text{ nm}}$ and ALP concentrations in the range from 0.01 to 0.4 U/L (Figure 4C). A detection limit of 0.01 U/L is calculated based on the 3 σ rule. To the best of our knowledge, the limit of detection is among the lowest reported for the nanoparticle-based colorimetric detection methods (Table 1).^{34–38}

As mentioned above, the sensing strategy does not have specificity for certain reducing agents. Interferences, such as dopamine, adrenaline, and AA, will hinder the direct sensing of ALP in biological samples. In consideration of the successful application of ALP in ELISA, the high sensitivity for sensing of ALP probably is of great benefit to improve the sensitivity of ALP-labeled sandwich ELISA. (The sensitivity of sandwich immunoassay can be increased by using a tracer with higher detectability. The factors that determine the ultimate sensitivity of competitive immunoassays are the antibody affinity constant and the experimental errors, but not the detectability of tracer.⁵²)

Plasmonic ELISA with the Naked Eye. Figure 5A schematically illustrates the mechanism of the visual plasmonic sandwich ELISA. ALP was used as the readout enzyme, and AA-p was chosen as the substrate. After formation of a sandwich-type immunocomplex, the ALP bound on the polystyrene microwells triggers iodine-mediated etching of AuNRs accompanied by a color change from blue to red.

Figure 4. (A) Color changes and (B) absorption spectra of AuNRs after incubation with different concentrations of ALP. (C) Linear relationship between logitudinal LSPR of AuNRs and ALP concentrations.

Table 1. Comparison of the Performance of Different Plasmonic Sensors for ALP

method	material	mechanism	detection limit	ref
colorimetry	AuNPs	nanoparticle aggregation	1.7 μ M	34
colorimetry	AuNPs	nanoparticle aggregation	8000 U/L	35
colorimetry	AuNPs	nanoparticle aggregation	10 U/L	36
colorimetry	AuNPs	nanoparticle aggregation	0.2 U/L	37
colorimetry	AuNRs	growth of AuNRs@Ag	3.3 U/L	38
colorimetry	AuNRs	etching of AuNRs	0.01 U/L	this study

Here, human IgG was chosen as a model analyte in the proposed plasmonic ELISA. The photos in Figure 5B show the responses of AuNRs to different concentrations of human IgG. The solution turned from blue to red gradually with the increase in human IgG concentration. The naked-eye detectable limit for human IgG is as low as 3 ng/mL, allowing the rapid diagnosis with the naked eye. In contrast, the multiple color changes in this method are much easier to distinguish than the single color change which was used in the ALP-based commercial ELISA kit. Figure S14 (Supporting Information) shows the absorption spectra of AuNRs in the presence of different concentrations of human IgG. Two parameters can be used for quantification of human IgG: the absorbance at 670 nm, and the peak shift of logitudinal LSPR. A linear relationship was obtained between the absorbance at 670 nm and logarithm of human IgG concentrations in the range from 0.1 to 10 ng/mL (Figure S15, Supporting Information). This linear relationship can be only used for the low concentration range from 0 to 10 ng/mL. By comparison, using peak shift, two different linear relationships could be obtained, respectively: one between the peak shift and human IgG concentrations (0.1–10 ng/mL) and the other between the peak shift and logarithm of human IgG concentrations (10–10 000 ng/mL) (Figure 5B). According to the 3σ rule, the detection limit for human IgG is calculated to be 100 pg/mL, which is at least 1 order of magnitude lower than that of other plasmonic methods^{39–41} and even comparable with the results obtained from many sensitive electrochemical methods.^{42–45} Additionally, compared to other nanoparticle-based ELISA methods, this method also exhibits many advantages: (1) it is easier to adapt to the conventional ELISA platforms directly, since ALP-labeled antibodies are commercially available;^{21,45,53,54} (2) there is no false positive

signal coming from nanoparticles autoaggregation,^{37,54} as the signal does not come from nanoparticle aggregation; (3) it is label-free for AuNRs, making it much easier to operate;^{37,54} and (4) AuNRs are much more stable to light, temperature, and biological thiol than silver nanoprimers.^{21,22}

Determination of Human IgG in Fetal Bovine Serum.

It is important to detect low concentrations of proteins (e.g., biomarkers) in biological samples with the presence of an abundance of other proteins. Since the normal concentration of human IgG in serum reaches mg/mL levels, the direct detection of human IgG in human serum cannot show the applicability of low abundant protein detection in biological samples. In consideration of the similar component and concentration in mammals (including humans), the applicability of the proposed plasmonic ELISA was evaluated by the analysis of low concentrations of human IgG spiked in fetal bovine serum. As the fetal bovine serum contains many potential interferences such as proteins, salt, and other small molecules (including glucose, ascorbic acid, etc.), the resistance to interferences can be also tested by this assay at the same time. The recoveries of the spiked human IgG ranged from 83.3% to 103.8% (Table 2). The results indicate that such a method is applicable to the quantification of low abundant protein in complex biological samples, avoiding the interference from protein, salt, and other small molecules.

CONCLUSION

In conclusion, we present a plasmonic ELISA based on colorimetric detection of ALP using iodine-mediated etching of AuNRs. Benefiting from the excellent sensing performance for ALP with a detection limit of 10 mU/L, the visual plasmonic ELISA achieved a naked-eye detectable limit of 3 ng/mL of

Figure 5. (A) Schematic illustration for visual plasmonic ELISA based on ALP-triggered etching of AuNRs. (B) Color of AuNRs after incubation with different concentrations of human IgG and linear relationship between the peak shift of AuNRs and human IgG concentrations.

Table 2. Determination of Human IgG in Simulated Biological Samples

sample	spiked (ng/mL)	detected (ng/mL)	recovery (%)	RSD (%)
1	1.0	0.833	83.3	13.8
2	7.0	6.4	91.4	8.2
3	100.0	103.8	103.8	5.5

human IgG. The detection limit is much lower than that of other similar methods. In addition, this method is able to avoid some disadvantages in other plasmonic ELISA methods, such as additional modification of antibodies, autoaggregation, labeling of nanoparticles, and low stability. The successful detection of human IgG in fetal bovine serum indicates that it is feasible for the proposed method to detect low abundance protein in complex biological samples. From the perspective of principle, we certify a CTA⁺-assisted etching mechanism of AuNRs, giving a better understanding of the intrinsic principle for the oxidation of AuNRs.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsami.5b07344.

Experimental detail information for synthesis of gold nanorods and detecting procedures, figures for etching

proof and optimization of experimental conditions, and scheme for the reducing target sensing (PDF)

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Notes

The authors declare no competing financial interest.

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