analytical chemistry

ARTICLE

pubs.acs.org/ac

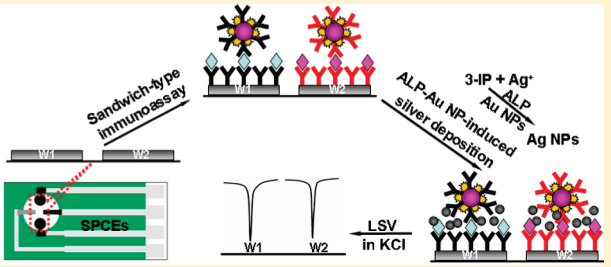
Ultrasensitive Multiplexed Immunoassay with Electrochemical Stripping Analysis of Silver Nanoparticles Catalytically Deposited by Gold Nanoparticles and Enzymatic Reaction

Guosong Lai,[†] Feng Yan,[‡] Jie Wu,[†] Chuan Leng,[†] and Huangxian Ju*[,†]

[†]Key Laboratory of Analytical Chemistry for Life Science (Ministry of Education of China), Department of Chemistry, Nanjing University, Nanjing 210093, P.R. China

[‡]Jiangsu Institute of Cancer Prevention and Cure, Nanjing 210009, P.R. China

ABSTRACT: A novel ultrasensitive multiplexed immunoassay method was developed by combining alkaline phosphatase (ALP)-labeled antibody functionalized gold nanoparticles (ALP-Ab/Au NPs) and enzyme-Au NP catalyzed deposition of silver nanoparticles at a disposable immunosensor array. The immunosensor array was prepared by covalently immobilizing capture antibodies on chitosan modified screen-printed carbon electrodes. After sandwich-type immunoreactions, the ALP-Ab/Au NPs were captured on an immunosensor surface to catalyze the hydrolysis of 3-indoxyl phosphate, which produced an indoxyl intermediate to reduce Ag^+. The silver deposition process was catalyzed by both ALP and Au NPs, which amplified the detection signal. The deposited silver was then measured by anodic stripping analysis in KCl solution. Using human and mouse IgG as model analytes, this multiplexed immunoassay method showed wide linear ranges over 4 orders of magnitude with the detection limits down to 4.8 and 6.1 pg/mL, respectively. Acceptable assay results for practical samples could be obtained. The newly designed strategy avoided cross talk and the need of deoxygenation for the electrochemical immunoassay and, thus, provided a promising potential in clinical applications.

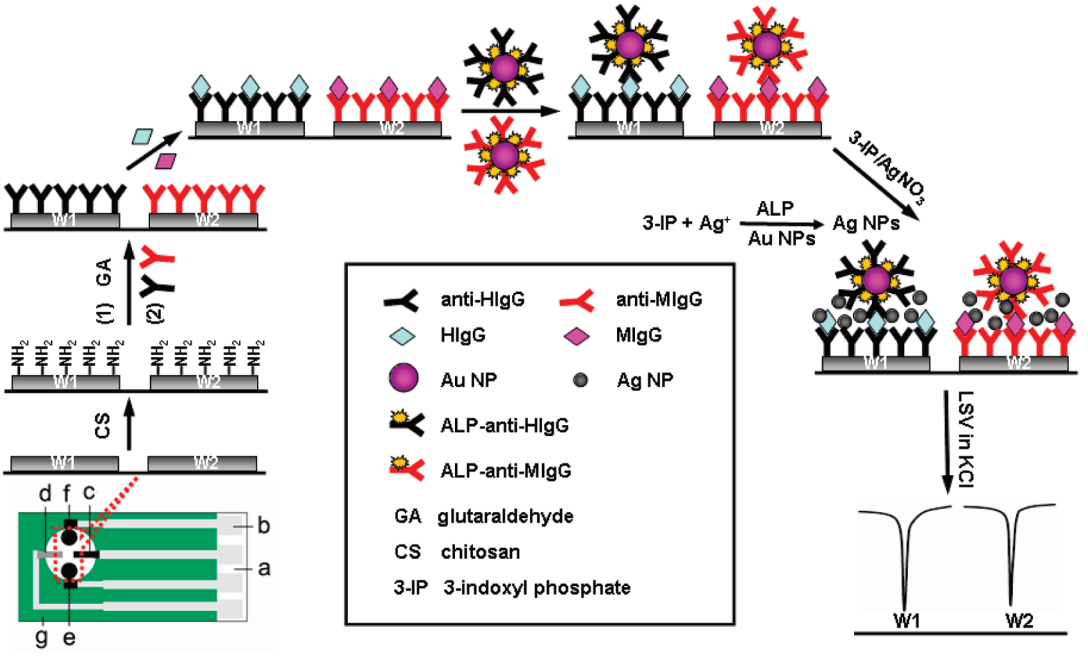


Recently, ultrasensitive immunosensors for detection of low-abundant biomarkers have gained increasing interest in the clinical cancer screening and early diagnostic application.[1−3] Various nanomaterials including colloidal gold nanoparticles (Au NPs),[4−6] carbon nanotubes,[1,2,7,8] bionanospheres,[9−11] and magnetic beads[12] have been used as excellent carriers for preparation of labels by loading numerous signal tags such as enzymes,[1,2,5−9,12] quantum dots,[10] oligonucleotide,[4] and dyes[11] on these carriers. The prepared labels can greatly amplify the transduction signal of a recognition event in bioassays; thus, they have been used not only in single-analyte detection but also in multiplexed immunoassay.[8]

Compared with the traditional single-analyte immunoassay, the simultaneous multiplexed immunoassay is more efficient in clinical application since it can quantitatively detect a panel of biomarkers in a single run with improved diagnostic specificity. Moreover, the multiplexed immunoassay can shorten analytical time, enhance detection throughput, and decrease sampling volume and detection cost. Thus, it has been quickly developed using different measurement techniques. Among these techniques, electrochemical immunosensor arrays prepared by immobilizing capture antibodies or antigens on sputter-deposited[13−16] or screen-printed[8,17−20] electrode arrays have been successfully applied to multianalyte immunoassays using a single enzyme label for signal transduction. However, there are hitherto two challenges associated with this technique: (1) a deoxygenation process such that a nitrogen atmosphere is needed for excluding the interference of dissolved oxygen in electrochemical detection based on horseradish peroxidase label, the most popular label in enzyme-linked immunoassay, which limits its clinical application, particularly in point-of-care diagnosis; (2) cross talk due to the diffusion of electroactive product from one electrode to a neighboring electrode leads to the interference of detection signals, which limits the miniaturization of the arrays.[13−16] The second problem can be partly solved by immobilizing electron transfer mediators[17,18] or electroactive enzyme[19,20] on sensor surfaces to perform reagentless detection. Our previous work attempted to solve the first problem by designing a glucose oxidase functionalized nanocomposite as a trace label and coating a Prussia blue film on screen-printed carbon electrodes (SPCEs) for mediated enzymatic cycle.[8] After excluding the deoxygenation process, the developed method could detect biomarkers down to pg/mL. However, the detection involved the participation of dissolved oxygen; the fluctuation of dissolved oxygen content in solution may be a potential flaw. Thus, development of a nonoxygen detection protocol is very significant for completely eliminating

Received: December 17, 2010
Accepted: February 21, 2011
Published: March 03, 2011

Scheme 1. Schematic Representation of Preparation of Immunosensor Array and Detection Strategy by Sandwich-Type Immunoassay and Linear Sweep Voltammetric Stripping Analysis of Enzymatically Deposited Ag NPs[a]



[a] (a) Nylon sheet, (b) silver ink, (c) graphite auxiliary electrode, (d) Ag/AgCl reference electrode, (e) W1, (f) W2, and (g) insulating dielectric.

the effect of dissolved oxygen and improving the performance of the electrochemical immunoassay.

Alkaline phosphatase (ALP) is another trace enzyme commonly used in the bioassay. A detection signal generally arises from a redox process of the product produced from the enzymatic hydrolysis of an ALP substrate.[14−16,21] Although the interference of dissolved oxygen can be ignored due to the positive detection potential used, cross talk is a general problem in these detection systems.[14−16] Meanwhile, hydrolysis products of ALP substrates such as *p*-aminophenyl phosphate, 3-indoxyl phosphate (3-IP), and ascorbic acid 2-phosphate are known to be versatile reducing agents, and they can reduce silver cation to produce a silver deposition.[22−24] This character has been used for DNA hybridization assays[22,23] and immunosensing[24] by the stripping analysis of the deposited silver. This work used ALP-labeled antibody (ALP-Ab) to functionalize Au NPs for preparing the tracing signal antibody. After sandwich-type immunoreactions, both the enzyme and Au NPs in the newly designed ALP-Ab/Au NPs could catalyze the deposition of silver nanoparticles (Ag NPs). When the disposable immunosensor array was combined with electrochemically stripping detection of deposited Ag NPs, an ultrasensitive multiplexed immunoassay method (Scheme 1) was thus developed. The stripping analysis was performed at a positive potential range and, thus, completely avoided the interference of dissolved oxygen. Compared with the previous report,[24] the signal amplification by the high-content enzyme for a single recognition event and Au NP-accelerated silver deposition provided higher sensitivity and shorter analytical time. When used for simultaneous determination of panel of biomarkers, the designed method showed wide linear ranges over 4 orders of magnitude and avoided completely the interference of dissolved oxygen and cross talk. This assay approach possessed a great potential in multianalyte determination of low-abundant biomarkers for clinical diagnostics.

EXPERIMENTAL SECTION

Materials and Reagents. Human IgG (HIgG), mouse IgG (MIgG), polyclone goat antimouse IgG (anti-MIgG), ALP-labeled goat antihuman IgG (ALP-anti-HIgG), and ALP-labeled goat antimouse IgG (ALP-anti-MIgG) were purchased from Wuhan Boster Biological Technology Ltd. Polyclone goat antihuman IgG (anti-HIgG) was purchased from Beijing Dingguo Biological Technology Ltd. Chitosan (CS, ≥85% deacetylation), bovine serum albumin (BSA), and 3-indoxyl phosphate (3-IP) were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO). Glutaraldehyde (GA, 25% aqueous solution) was purchased from Alfa Aesar China Ltd. Chloroauric acid ($HAuCl_4 \cdot 4H_2O$), $AgNO_3$, and trisodium citrate were obtained from Shanghai Reagent Company (Shanghai, China). Ultrapure water obtained from a Millipore water purification system (≥18 MΩ, Milli-Q, Millipore) was used in all assays. The clinical serum samples were from The Second Hospital of Nanjing. All other reagents were of analytical grade and used as received.

A Tris−HNO_3 buffer (0.1 M, pH 7.2) was prepared and used as working solution. The washing buffer was 0.1 M Tris−HNO_3 containing 0.05% (w/v) Tween-20. Tris−HNO_3 (0.1 M) containing 5% (w/v) BSA was used as blocking solution. A mixture solution of 5.0 mM 3-IP and 1.0 mM $AgNO_3$ was prepared daily for silver deposition in 0.1 M, pH 9.8 Tris−HNO_3 and 20 mM $Mg(NO_3)_2$ and stored in an opaque tube at 4 °C.

Apparatus. All electrochemical immunoassays were performed on a CHI 660B electrochemical workstation (Chenhua, Shanghai, China). The reference levels of the HIgG in the clinical human serum samples were obtained from the Hitachi automatic biochemical analyzer (Japan).

Preparation of ALP-Ab/Au NPs. Considering the loading of ALP-labeled antibody and the steric effect in immunoreaction, the colloidal Au NPs of 13 nm diameter were prepared.[25] Briefly,

dx.doi.org/10.1021/ac103283p |*Anal. Chem.* 2011, 83, 2726–2732

100 mL of 0.01% (w/v) $HAuCl_4$ solution was boiled with vigorous stirring, and 2.5 mL of 1% (w/v) trisodium citrate solution was quickly added to the boiling solution. When the solution turned deep red, indicating the formation of Au NPs, the solution was left stirring and cooling down. Then, 8.5 μg of ALP-anti-HIgG or ALP-anti-MIgG was added to 1.0 mL of colloidal Au NPs adjusted to pH 9.0 with 0.1 M K_2CO_3 and gently mixed at room temperature for 60 min. After centrifugation at 4800 rpm for 30 min, the supernatant was discarded and the soft sediment was washed with 0.1 M, pH 7.2 Tris−HNO_3. After another centrifugation and discarding the supernatant, the resulting ALP-Ab/Au NPs were finally resuspended in 1.0 mL of 0.1 M, pH 7.2 Tris−HNO_3 containing 1.0% BSA and 2 mM $Mg(NO_3)_2$ and stored at 4 °C.

Preparation of Immunosensor Array. The SPCEs containing two graphite working electrodes (W1 and W2, diameter: 2 mm), a Ag/AgCl reference, and a graphite auxiliary electrode were prepared with screen-printing technology according to our previous report.[17] The insulating layer printed around the working area constituted an electrochemical microcell.

The immunosensor array was constructed by immobilizing the corresponding capture antibodies on the working electrodes of SPCEs through CS coating and GA cross-linking. First, 1.0 μL of 0.25 mg/mL CS was coated on the working electrodes and dried at room temperature. After activating with 2.5% GA (in 50 mM, pH 7.4 phosphate buffer) for 2 h and washing with water, 1.0 μL of 0.5 mg/mL anti-HIgG and anti-MIgG were applied to the corresponding working electrodes W1 and W2, respectively, and reacted at room temperature for 60 min and then 4 °C overnight in a 100% moisture-saturated environment. Subsequently, excess antibodies were washed with washing buffer and pH 7.2 Tris−HNO_3, and a drop of 20 μL blocking solution was applied to the array and incubated for 60 min at room temperature to block possible remaining active sites against nonspecific adsorption. After another washing with washing buffer and pH 7.2 Tris−HNO_3, the resulting immunosensor array was obtained and stored at 4 °C in a dry environment prior to use.

Measurement Procedure. To carry out the immunoreaction and electrochemical measurement, the immunosensor array was first incubated in a 15 μL drop of the mixture of HIgG and MIgG standard solutions or serum samples for 40 min at room temperature, followed by washing with washing buffer and pH 7.2 Tris−HNO_3. It was then incubated in 15 μL of prepared ALP-Ab/Au NPs for 40 min at room temperature. After washing with washing buffer and Tris−HNO_3 again, 15 μL of silver deposition solution containing 5.0 mM 3-IP and 1.0 mM $AgNO_3$ was delivered to the electrochemical microcell for 10 min, which was protected from light. After silver deposition, the immunosensor array was rinsed with water, and linear sweep voltammetry (LSV) from −0.15 to 0.25 V at 50 mV s^{-1} was performed in a 1.0 M KCl solution to record the stripping currents at W1 and W2 for simultaneous detection of HIgG and MIgG.

RESULTS AND DISCUSSION

Immunoassay at Immunosensor Array. The preparation process of the immunosensor array and the detection strategy with sandwich-type electrochemical immunoassay are shown in Scheme 1. After sandwich-type immunoreactions, the ALP-Ab/Au NPs can be captured on the immunosensor surface by the formation of immunocomplex. After adding silver deposition solution onto the electrode surface, the attached ALP can catalyze the hydrolysis of 3-IP to produce an indoxyl intermediate of nitroblue tetrazolium, which reduces silver cation to metallic silver.[23] Finally, anodic stripping analysis of the quantitatively deposited silver on two immunosensors is performed for the multiplexed immunoassay.

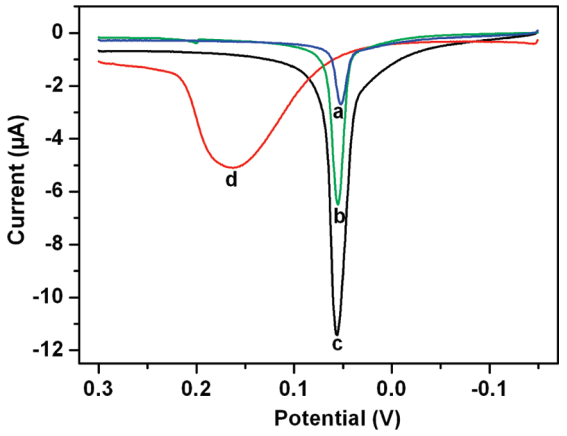


Figure 1. Linear sweep stripping voltammetric curves of Ag NPs deposited at HIgG immunosensors after incubation with 0 (a) and 100 ng/mL (b, c, d) HIgG and then ALP-labeled antibody (b) and ALP-labeled antibody/Au NPs (a, c, d) as trace tags at 50 mV s^{-1}. The detection solutions were 1.0 M KCl (a, b, c) and 0.1 M HNO_3 containing 0.6 M KNO_3 (d), respectively.

Figure 1 shows the linear sweep stripping voltammetric curves of Ag NPs at the immunosensor in 1.0 M KCl after sandwich-type immunoreactions and silver deposition. A small background response was observed at the immunosensor treated without target protein (curve a), while a sharp stripping peak with rapid increase of stripping current was obtained when the immunosensor was incubated with 100 ng/mL HIgG (curve c), indicating efficient association of ALP-Ab/Au NPs on the immunosensor surface, which led to the catalytic generation of the intermediate of nitroblue tetrazolium and then the reduction of silver cation to a silver deposition. The enzymatically catalytic deposition of silver could be easily measured by stripping analysis in KCl solution. In comparison with the stripping peak in acidic KNO_3 electrolyte (curves c and d),[24,26] the well-defined sharp silver stripping peak was favorable for high detection sensitivity due to the Ag/AgCl solid-state voltammetric process in KCl.[27] Furthermore, the stripping peak current using ALP-Ab/Au NPs as a trace label was almost 2-fold higher than that with commercial ALP-labeled antibody (curves b and c), which is attributable to the signal amplification from high-content enzyme on ALP-Ab/Au NPs. Hence, sensitive immunoassay could be performed at the immunosensor array with the proposed ALP-Ab/Au NPs signal amplification.

Optimization of Detection Conditions. The amount of deposited Ag NPs depended on the amount of indoxyl intermediate generated by enzymatic hydrolysis of 3-IP substrate. Thus, the effect of 3-IP concentration on the silver stripping current was first examined. As shown in Figure 2A, after silver deposition for 10 min in pH 9.8 Tris−HNO_3 solution containing 1.0 mM $AgNO_3$ and varying concentration of 3-IP, the anodic stripping current increased sharply with the 3-IP concentration up to 5.0 mM and then started to level off. This phenomenon indicated that 5.0 mM 3-IP corresponded to the maximum enzyme activity for this substrate, which was

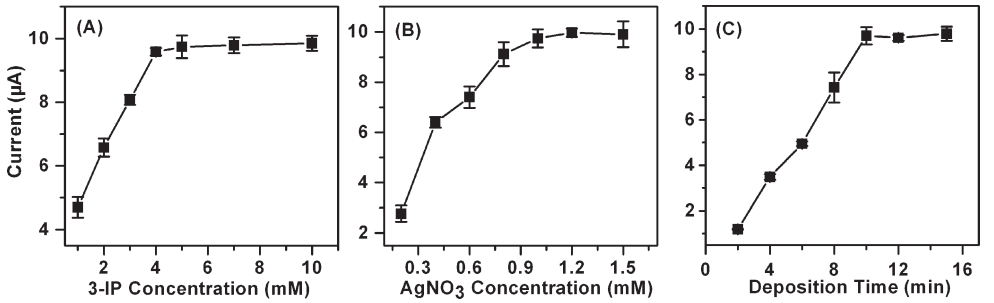


Figure 2. Effects of 3-IP concentration (A), $AgNO_3$ concentration (B), and deposition time (C) on stripping current of Ag NPs in 1.0 M KCl.

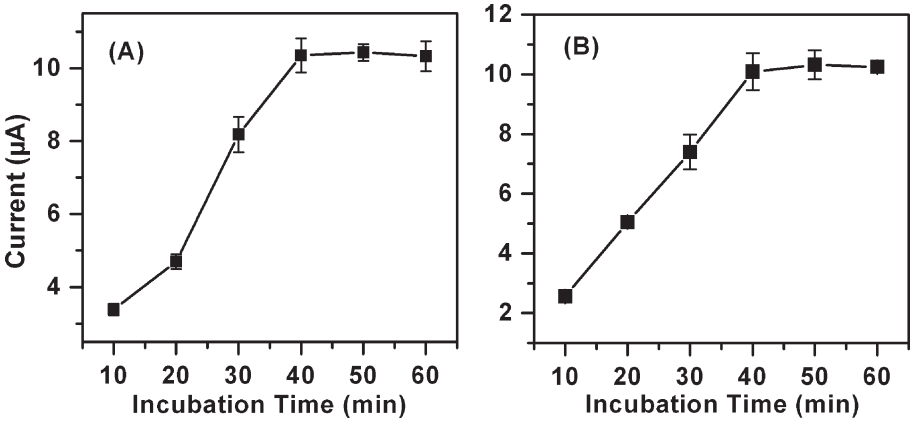


Figure 3. Effect of incubation time on stripping current of Ag NPs in 1.0 M KCl for 100 ng/mL HIgG (A) and MIgG (B).

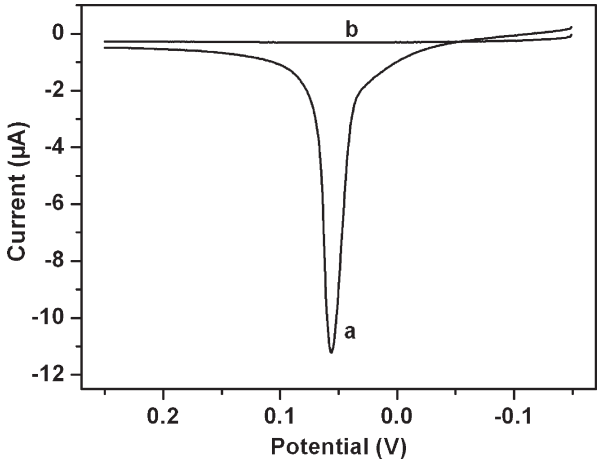


Figure 4. Linear sweep stripping voltammetric curves of Ag NPs deposited at the HIgG immunosensor (a) and neighboring bare electrode (b) in 1.0 M KCl after sandwich-type immunoreactions with 100 ng/mL HIgG and enzymatic silver deposition.

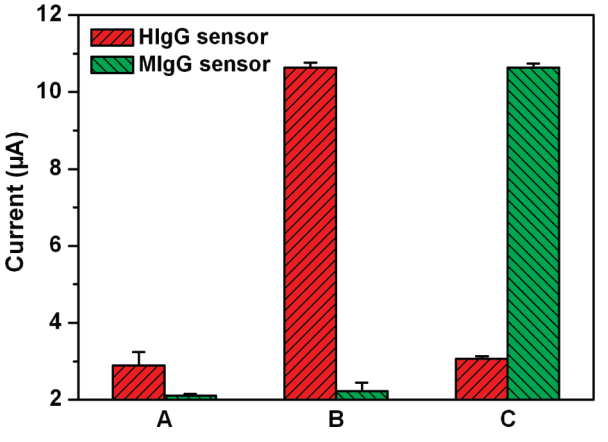


Figure 5. Linear sweep stripping voltammetric responses of the HIgG and MIgG immunosensor array for blank control (A), 100 ng/mL HIgG (B), and 100 ng/mL MIgG (C).

sufficient for the ALP-induced silver deposition. As a result, a 3-IP concentration of 5.0 mM was adopted in the subsequent study.

In order to maximize the deposition rate and detection sensitivity, sufficient silver cation should be added to the substrate solution. At a deposition time of 10 min in pH 9.8 Tris−HNO_3 solution containing 5.0 mM 3-IP and varying concentration of $AgNO_3$, the anodic stripping peak current increased with the increasing concentration of silver cation and then trended to a constant value at 1.0 mM (Figure 2B), which was used in subsequent experiments.

Apparently, the amount of deposited silver at the electrode surface was related to the deposition time. As shown in Figure 2C, the stripping peak current increased rapidly with the deposition time until 10 min at the 3-IP and silver cation concentrations of 5.0 and 1.0 mM, respectively. This phenomenon resulted from the limited total amount of $AgNO_3$ spread on the immunosensor surface. The deposition time of 10 min was much shorter than 30[26] and 35 min[24] used in the previous report for ALP-induced silver deposition due to the limited total amount of $AgNO_3$ and

dx.doi.org/10.1021/ac103283p |*Anal. Chem.* 2011, 83, 2726–2732

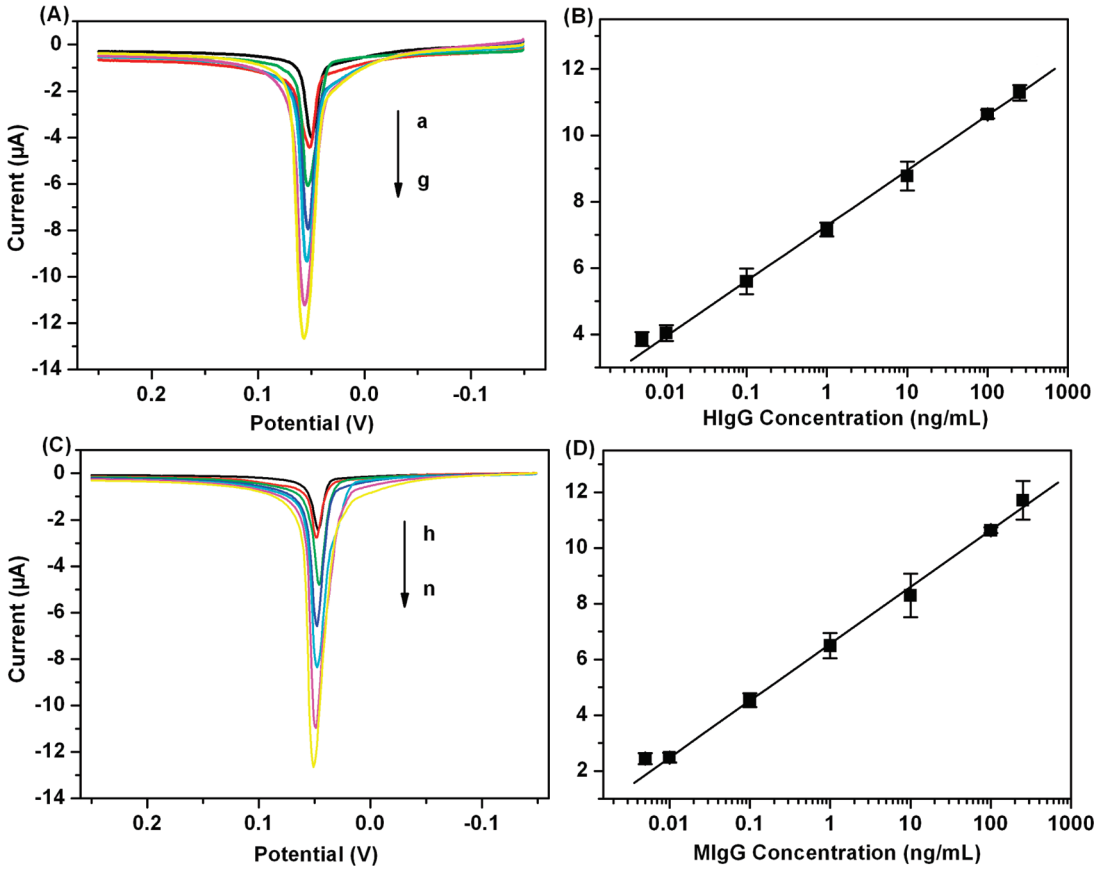


Figure 6. Linear sweep stripping voltammetric curves of Ag NPs deposited at immunosensors (A, C) and calibration curves (B, D) for simultaneous multiplexed detection of HIgG (A, B) and MIgG (C, D) using the proposed strategy. Curves a−g and h−n are for 5 pg/mL to 250 ng/mL HIgG and MIgG at W1 and W2, respectively.

the acceleration of silver deposition by the captured Au NPs on the immunosensor surface.[28,29] The catalysis of Au NPs toward the silver deposition not only shortened the analytical time but also improved the sensitivity of immunoassay.

The incubation time is an important parameter affecting the analytical performance of immunoassay. At room temperature, the stripping voltammetric responses for HIgG and MIgG increased with the increasing incubation time used in sandwich-type immunoassay and then tended to constant values after 40 min (Figure 3), which showed the saturated binding both between the analyte and the capture antibody and between the complexed analyte and signal antibody on electrode surface. Therefore, an incubation time of 40 min was selected for the sandwich-type immunoassay.

Evaluation of Cross Talk and Cross-Reactivity. An excellent immunosensor array must exclude cross talk, which generally results from the diffusion of electroactive indicator produced on one electrode to neighboring electrodes. In this work, the conjugated ALP could catalyze the hydrolysis of 3-IP substrate to indoxyl intermediate on the electrode surface. Once the indoxyl intermediate was generated in the enzymatic reaction, it would be quickly oxidized by sufficient silver cation surrounding the working electrode to produce silver deposition on the corresponding immunosensor surface. Because the ALP-labeled antibodies were immobilized on Au NPs, the enzymatic reduction product of silver cation to silver deposition by ALP would only accumulate on the Au NPs, resulting in a localized stripping reaction at the individual electrode. Hence, the possible cross talk could be well avoided, which was confirmed by the results shown in Figure 4. After an electrode array containing one HIgG immunosensor and a bare electrode was incubated with HIgG and then ALP-Ab/Au NPs, the silver deposition process and then the stripping analysis were performed. Only the immunosensor showed a stripping peak, while no obvious response was observed at the bare electrode. This result indicated the potential cross talk between the electrodes could be completely avoided at the immunosensor array.

The cross-reactivity between analytes and noncognate antibodies was also investigated. At the immunosensor array, two different capture antibodies for HIgG and MIgG were immobilized on W1 and W2 separately. The cross-reactivity was evaluated by comparing the silver stripping currents at the immunosensor arrays incubated with blank solution, 100 ng/mL HIgG, or 100 ng/mL MIgG. As expected, only the immunosensors prepared with corresponding capture antibodies showed obvious stripping responses (Figure 5). Obviously, the cross-reactivity between the two analytes and noncognate antibodies was negligible. Thus, simultaneous multianalyte immunoassay could be performed in a single run using the designed disposable immunosensor array.

Analytical Performance. Under the optimum conditions, the stripping peak currents of the immunosensor array for simultaneous detection of HIgG and MIgG increased with increasing concentration of analytes (Figure 6). Both calibration plots

Table 1. Comparison of HIgG Determinations Using the Proposed and Reference Methods and Recovery Tests of MIgG in Human Serum Samples

detection of HIgG (mg/mL)	sample no.	1	2	3
	proposed method	12.1	18.3	14.8
	reference method	13.1	17.6	15.3
	relative error (%)	−7.6	4.0	−3.3
detection of MIgG (ng/mL)	added	5.00	25.00	50.00
	found	5.31	26.14	51.10
	recovery (%)	106	105	102

showed a good linear relationship between the peak currents and the logarithm values of the analyte concentrations in the ranges from 0.01 to 250 ng/mL. The correlation coefficients were 0.9996 and 0.9998 for HIgG and MIgG, respectively. The limits of detection for HIgG and MIgG were 4.8 and 6.1 pg/mL at a signal-to-noise ratio of 3, respectively, which were much lower than 40 pg/mL,[5] 3 ng/mL,[16] and 1.1 ng/mL[30] for HIgG and 2 ng/mL,[14] 3 ng/mL,[16] and 0.3 ng/mL[31] for MIgG reported in previous studies. The wide linear ranges over 4 orders of magnitude for two analytes were also very significant for practical application.

The interassay precision of the immunosensor array was examined with two panels of proteins at different concentrations. Each panel was measured five times using five arrays. The coefficients of variation were 3.5% and 6.3% for 10 ng/mL HIgG and MIgG and 3.0% and 3.2% for 100 ng/mL HIgG and MIgG, respectively. In addition, the immunosensor array could be stored in dry conditions at 4 °C. In this way, over 90% of the initial responses remained after 2 weeks of storage for both HIgG and MIgG. These results indicated the immunosensor array had acceptable stability and reproducibility.

Application in Analysis of Serum Samples. To evaluate the analytical reliability and application potential of the designed immunosensing method, the assay results of HIgG in human serum samples using the proposed method were compared with the reference values obtained by the commercial turbidimetric immunoassay after appropriate dilution. In addition, different amounts of MIgG were added into human serum sample for recovery tests. The results are listed in Table 1, which showed acceptable results with relative errors less than 7.6% for HIgG detection and recoveries between 102% and 106% for the MIgG recovery experiment, indicating good accuracy of the proposed method for sample detection.

CONCLUSION

A novel ultrasensitive multiplexed immunoassay was developed by combining newly designed ALP-Ab/Au NPs with stripping analysis of deposited silver at a disposable immunosensor array. The ALP-Ab/Au NPs not only provided a high-content ALP to the immunosensor surface for signal amplification but also accelerated in situ enzyme-induced silver deposition due to the catalysis of Au NPs. The selected KCl electrolyte gave a sharp stripping peak. Thus, the proposed method showed short detection time and ultrahigh sensitivity. The hydrolysis product of ALP substrate could be quickly oxidized by sufficient silver cation surrounding the working electrode to produce silver deposition, which excluded completely the cross talk between adjacent immunosensors. The proposed electrochemical method also avoided the need of deoxygenation and showed a wide linear range. The immunosensor array had acceptable stability, reproducibility, and accuracy. The convenient operation and ultrahigh sensitivity of the proposed multianalyte immunoassay method provided a promising potential in clinical applications.

AUTHOR INFORMATION

Corresponding Author

*Phone/fax: +86-25-83593593. E-mail: hxju@nju.edu.cn.

ACKNOWLEDGMENT

This work was financially supported by National Basic Research Program of China (2010CB732400), the National Science Fund for Creative Research Groups (20821063), and the projects (2087-5044, 21075055) from National Natural Science Foundation of China and Natural Science Foundation of Jiangsu (BK2008014).

REFERENCES

(1) Yu, X.; Munge, B.; Patel, V.; Jensen, G.; Bhirde, A.; Gong, J. D.; Kim, S. N.; Gillespie, J.; Gutkind, J. S.; Papadimitrakopoulos, F.; Rusling, J. F. *J. Am. Chem. Soc.* **2006**, *128*, 11199–11205.
(2) Malhotra, R.; Patel, V.; Vaqué, J. P.; Gutkind, J. S.; Rusling, J. F. *Anal. Chem.* **2010**, *82*, 3118–3123.
(3) Rusling, J. F.; Kumar, C. V.; Gutkinde, J. S.; Patel, V. *Analyst* **2010**, *135*, 2496–2511.
(4) Nam, J. M.; Thaxton, C. S.; Mirkin, C. A. *Science* **2003**, *301*, 1884–1886.
(5) Cui, R.; Huang, H.; Yin, Z.; Gao, D.; Zhu, J. J. *Biosens. Bioelectron.* **2008**, *23*, 1666–1673.
(6) Bi, S.; Yan, Y.; Yang, X.; Zhang, S. S. *Chem.—Eur. J.* **2009**, *15*, 4704–4709.
(7) Wang, J.; Liu, G.; Jan, M. R. *J. Am. Chem. Soc.* **2004**, *126* 3010–3011.
(8) Lai, G. S.; Yan, F.; Ju, H. X. *Anal. Chem.* **2009**, *81*, 9730–9736.
(9) Du, D.; Zou, Z.; Shin, Y.; Wang, J.; Wu, H.; Engelhard, M. H.; Liu, J.; Aksay, I. A.; Lin, Y. *Anal. Chem.* **2010**, *82*, 2989–2995.
(10) Chen, L.; Chen, C.; Li, R.; Li, Y.; Liu, S. Q. *Chem. Commun.* **2009**, 2670–2672.
(11) Cai, W.; Gentle, I. R.; Lu, G. Q.; Zhu, J. J.; Yu, A. *Anal. Chem.* **2008**, *80*, 5401–5406.
(12) Mani, V.; Chikkaveeraiah, B. V.; Patel, V.; Gutkind, J. S.; Rusling, J. F. *ACS Nano* **2009**, *3*, 585–594.
(13) Kojima, K.; Hiratsuka, A.; Suzuki, H.; Yano, K.; Ikebukuro, K.; Karube, I. *Anal. Chem.* **2003**, *75*, 1116–1122.
(14) Wilson, M. S. *Anal. Chem.* **2005**, *77*, 1496–1502.
(15) Wilson, M. S.; Nie, W. *Anal. Chem.* **2006**, *78*, 6476–6483.
(16) Wilson, M. S.; Nie, W. *Anal. Chem.* **2006**, *78*, 2507–2513.
(17) Wu, J.; Zhang, Z. J.; Fu, Z. F.; Ju, H. X. *Biosens. Bioelectron.* **2007**, *23*, 114–120.
(18) Wu, J.; Yan, F.; Tang, J. H.; Zhai, C.; Ju, H. X. *Clin. Chem.* **2007**, *53*, 1495–1502.
(19) Wu, J.; Yan, F.; Zhang, X. Q.; Yan, Y. T.; Tang, J. H.; Ju, H. X. *Clin. Chem.* **2008**, *54*, 1481–1488.
(20) Wu, J.; Yan, Y. T.; Yan, F.; Ju, H. X. *Anal. Chem.* **2008**, *80*, 6072–6077.
(21) Preechaworapun, A.; Dai, Z.; Xiang, Y.; Chailapakul, O.; Wang, J. *Talanta* **2008**, *76*, 424–431.
(22) Hwang, S.; Kim, E.; Kwak, J. *Anal. Chem.* **2005**, *77*, 579–584.
(23) Fanjul-Bolado, P.; Hernández-Santos, D.; González-García, M. B.; Costa-García, A. *Anal. Chem.* **2007**, *79*, 5272–5277.
(24) Qu, B.; Chu, X.; Shen, G. L.; Yu, R. Q. *Talanta* **2008**, *76* 785–790.
(25) Ambrosi, A.; Castañeda, M. T.; Killard, A. J.; Smyth, M. R.; Alegret, S.; Merkoçi, A. *Anal. Chem.* **2007**, *79*, 5232–5240.
(26) Qu, B.; Guo, L.; Chu, X.; Wu, D. H.; Shen, G. L.; Yu, R. Q. *Anal. Chim. Acta* **2010**, *663*, 147–152.

(27) Ting, B. P.; Zhang, J.; Khan, M.; Yang, Y. Y.; Ying, J. Y. *Chem. Commun.* **2009**, 6231–6233.
(28) Basnar, B.; Weizmann, Y.; Cheglakov, Z.; Willner, I. *Adv. Mater.* **2006**, *18*, 713–718.
(29) Wu, J.; Chumbimuni-Torres, K. Y.; Galik, M.; Thammakhet, C.; Haake, D. A.; Wang, J. *Anal. Chem.* **2009**, *81*, 10007–10012.
(30) Leng, C.; Lai, G. S.; Yan, F.; Ju, H. X. *Anal. Chim. Acta* **2010**, *666*, 97–101.
(31) Preechaworapun, A.; Ivandini, T. A.; Suzuki, A.; Fujishima, A.; Chailapakul, O.; Einaga, Y. *Anal. Chem.* **2008**, *80*, 2077–2083.