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Cleavage of Metal-Ion-Induced DNAzymes Released from Nanolabels for Highly Sensitive and Specific Immunoassay

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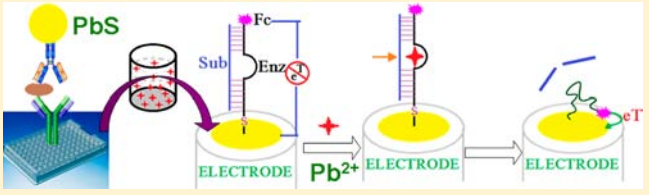
Supporting Information

ABSTRACT: This work reports a novel electrochemical immunoassay protocol with signal amplification for determination of low-abundance protein (free prostate-specific antigen, PSA, used as a model) with high sensitivity and high selectivity by coupling metal sulfide (PbS)-based nanolabels with cleavage of the corresponding lead ion-induced DNAzymes. The assay mainly consists of an antigen−antibody immunoreaction with metal nanolabel in a transparent 96-well polystyrene microplate, the release of metal ions from the nanolabel, and cleavage of metal ion-induced DNAzyme. The signal is amplified by the labeled redox tag (ferrocene) on the DNAzyme-based sensor. In the presence of target analyte, the sandwiched immunocomplex can be formed between the primary antibody on the microplate and the corresponding metal sulfide nanolabel. The carried nanolabel can release numerous metal ions by acid, and induce the cleavage of the corresponding DNAzyme, thus resulting in the change of electrochemical signal. Under optimal conditions, the DNAzyme-based immunoassay presents an obvious electrochemical response for the detection of PSA, and allows detection of PSA at a concentration as low as 0.1 pg mL^{-1}. Intra-assay and interassay coefficients of variation (CV) were less than 9.5% and 10%, respectively. No significant differences at the 0.05 significance level were encountered in the analysis of 12 clinical serum specimens between the developed immunoassay and a commercially available enzyme-linked immunosorbent assay (ELISA).



INTRODUCTION

Sensitive and specific detection of disease-related biomarkers is essential in biological studies, clinical diagnostics, and treatment.[1,2] Typically, the assay can be implemented by using certain affinity ligands including the corresponding aptamers and antibodies that specifically interact with the biomolecules and thus mediate a target-responsive signal transduction cascade.[3,4] Enzyme labels and fluorophore labels are usually employed for detectable signal amplification. Unfortunately, the conventional enzyme labels often exhibit low sensitivity, since there is usually a ratio of 1:1 for enzymes and biomolecules used for steric reasons. Hence, greatest attention has been recently focused on nonenzymatic signal amplification strategies, e.g., by employing nanolabels and molecular biological methods comprising polymerase chain reaction (PCR), rolling circle amplification (RCA), and hybridization chain reaction (HCR).[5,6]

Metal sulfide nanoparticles (ZnS, CdS, PbS, and CuS) have shown great potential in the fluorescence and electrochemical immunoassays owing to their unique merits: nanoscale size similar to proteins, broad excitation spectra for multicolor imaging, robust, narrowband emission, and versatility in surface modification.[7−9] Hansen's group utilized metal sulfide nanoparticle as labels to design a new electrochemical method that provided detection capabilities down to 100 attomol of target DNA.[7] The nanostructures exhibited sharp and well-resolved stripping voltammetric signals due to the well-defined oxidation potentials of metal components.[10,11] Unfavorably, metal ions of interest are electroplated on an *in situ* formed mercury drop electrode during a deposition step and oxidized from the electrode during the stripping step.[12,13] Moreover, the stripping process usually consists of a 1.0-min pretreatment and 2.0-min accumulation at high potential. Possible interfering materials might be adsorbed on the electrode during the accumulation, thus affecting the electrochemical response signal. To tackle this issue, our motivation in this work is to exploit a novel signal-transduction method by coupling the nanolabels with molecular biological techniques for signal amplification.

DNAzymes that can catalyze a broad range of chemical and biological reactions such RNA cleavage have been isolated in test tubes.[14,15] Most require certain metal ions as the cofactors for structure and function, and many DNAzymes show high metal-binding affinity and specificity.[16−18] For practical applications, DNAzymes are cost-effective to produce, much more stable than protein-based enzymes, and can be denatured and renatured many times without losing activity or binding ability.[19] DNAzymes were first used in lead-ion dependent RNA cleaving, and the catalytic action of them was 100-fold that of the noncatalyzed process.[20−22] These intrinsic advantages of DNAzymes make them many potential

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applications as components for DNA logic gates and nanomachines, DNA computing circuits, proofreading units in nanomaterial assembly, and high-efficiency tools for metal ion monitoring.[23−25] To the best of our knowledge, however, there is no report focusing on the cleavage method of metal ion-induced DNAzymes for development of electrochemical immunoassays by coupling with metal sulfide nanolabels.

Herein, we report the proof-of-concept of a novel and powerful electrochemical immunoassay of free prostate-specific antigen (PSA, as a model protein) in biological fluids by coupling the signal amplification strategy of metal sulfide nanolabels with highly selective lead-specific DNAzymes. The assay protocol mainly consists of (i) the antigen−antibody reaction with the nanolabels in a transparent 96-well polystyrene microplate, (ii) the release of lead ions from the nanolabels, (iii) cleavage of Pb^{2+}-induced DNAzymes on the lead-specific DNAzyme sensor, and (iv) electrochemical measurement.

EXPERIMENTAL PROCEDURES

Materials. Mouse antihuman monoclonal prostate-specific antibody (anti-PSA, designated as mAb_1) was purchased from Amyjet Scientific Inc. (Abgent products, Wuhan, China, 0.1 mg mL^{-1}). Rabbit antihuman polyclonal anti-PSA antibody (designated Ab_2) and PSA standards were obtained from Biocell Biotechnol. Co., Ltd. (Zhengzhou, China). Transparent 96-well polystyrene microplates were obtained from Greiner (Frickenhausen, Germany). Diethylene glycol (DEG, 99 wt %), thiourea (TU, 99 wt %), *N*-hydroxysulfosuccinimid sodium salt (NHS), *N*-(3-dimethylaminopropyl)-*N*′-ethyl-carbodiimide hydrochloride (EDC), and polyacrylic acid (PAA) were obtained from Alfa Aesar (Beijing, China). All other reagents were of analytical grade and were used without further purification. Ultrapure water obtained from a Millipore water purification system (18 MΩ, Milli-Q, Millipore) was used in all runs. Tris-acetic acid stock buffer solution was the product of Sigma-Aldrich (USA). Phosphate-buffered saline (PBS, 0.1 M) solutions with various pHs were prepared by mixing the stock solutions of 0.1 M NaH_2PO_4 and 0.1 M Na_2HPO_4, and 0.1 M KCl was added as the supporting electrolyte. Clinical serum samples were made available by Fujian Provincial Hospital, China.

All oligonucleotides used in the experiments were obtained from Sangon Biotech. Co., Ltd. (Shanghai, China). The sequences of oligonucleotides and ferrocene (Fc)-labeled probes are listed as follows: Catalytic strand: 5′-HS-$(CH_2)_6$-CATCTCTTCTCCGAGCCGGTCGAAATAGTGAGT-$(CH_2)_6$-Fc-3′. Substrate strand: 5′-ACTCACTAT**rA**GGA-AGAGATG-3′.

The design of DNAzyme molecular beacons was adapted from the literature.[26] In the molecular beacons, lead ion-specific catalytic strands (33 bases labeled with ferrocene) and substrate strands with a ribo-adenosine (rA) (19 bases) were designated as Pb-Enz and Pb-Sub, respectively.

Preparation and Bioconjugation of Water-Soluble, Carboxylated PbS Colloids. Water-soluble, carboxylated PbS nanoparticles were synthesized based on the high-temperature polyol-mediated reaction according to the literature with a slight modification.[27] Briefly, 8.0 mmol of thiourea was initially added into 20 mL diethylene glycol (99.9 wt %). After heating to 100 °C for 60 min under the protection of nitrogen atmosphere, the resulting mixture was cooled to room temperature (RT) for preparation of TU/DEG stock solution (A). Meanwhile, another mixture (B) containing 0.4 mmol Pb(II) acetate, 6.0 mmol PAA, and 6.0 mL DEG was prepared, which was heated to 215 °C under N_2. Following that, 8.0 mL of TU/DEG stock solution was injected rapidly into the hot mixture (B). Upon addition of solution (B), the mixture turned black quickly. Afterward, the mixture was heated to 210 °C for 15 min. Subsequently, the suspension was cooled to RT, and centrifuged 10 min at 11 000 *g*. Finally, the water-soluble and carboxylated PbS nanoparticles were obtained and dispersed in 2.0 mL of distilled water for further use.

Next, the as-synthesized PbS nanoparticles were utilized for the labeling of polyclonal rabbit antihuman PSA antibodies (Ab_2) by using the classical carbodiimide coupling method. Initially, 2.2 mg of NHS and 3.0 mg of EDC were dissolved into 500 μL of PbS colloids, followed by continuous stirring for 45 min at RT. Afterward, 100 μL of Ab_2 (1.0 mg mL^{-1}) was added into the mixture, and the mixture was gently stirred for 12 h to make the antibodies conjugate onto the PbS nanoparticles. The excess chemicals and antibodies were removed by centrifugation. Finally, the as-prepared Ab_2-PbS nanolabels were dispersed into 1.0 mL PBS (0.1 M, pH 7.4) for detection of PSA in the following experiments.

Fabrication of Lead-Specific DNAzyme-Based Sensor. A gold electrode (3 mm in diameter) was polished repeatedly with 0.3 and 0.05 μm alumina slurry, followed by successive sonication in bidistilled water and ethanol for 5 min and dried in air. Before modification, the gold electrode was cleaned with hot piranha solution (a 3:1 mixture of H_2SO_4 and H_2O_2. *Caution*!) for 10 min, and then continuously scanned within the potential range of −0.3 to 1.5 V in freshly prepared deoxygenated 0.5 M H_2SO_4 until a voltammogram characteristic of the clean gold electrode was established. After thorough rinsing with water and absolute ethanol, the cleaned gold electrode was immersed into a 0.2 M Tris-HCl buffer (pH 7.4) containing 2.0 OD Pb-Enz and 8.0 mM Tris-(2-carboxyethyl) phosphine hydrochloride (TCEP) (*Note*: The aim of using TCEP was to reduce the disulfide-bonded oligonucleotides) for 16 h. After rinsing with distilled water, the modified gold electrode was incubated with 1.0 mM 6-mercaptohexanol in 10 mM Tris-acetate buffer, pH 7.4, for 60 min. Following that, the Pb-Enz-modified electrode was dipped again into 2.0 OD substrate strand solution to make catalytic strands hybridize with substrate strands in an oven at 65 °C for 10 min. Afterward, the hybridization process was further carried out at RT for 12 h. Finally, the obtained DNAzyme sensor was stored at 4 °C when not in use.

Electrochemical Measurement. Route 2 in Scheme 1 displays the measurement process of the DNAzyme-based immunoassay. A high-binding polypropylene 96-well microtiter plate (ref. 655061, Greiner, Frickenhausen, Germany) was coated overnight at 4 °C with 50 μL per well of Ab_1 at a concentration of 10 μg mL^{-1} in 0.05 M sodium carbonate buffer (pH 9.6). The microplate was covered with adhesive plastic plate sealing film to prevent evaporation. On the following day, the plate was washed three times with pH 7.4 PBS, and then incubated with 300 μL per well of blocking buffer for 1 h at 37 °C with shaking. The plate was then washed as before. Following that, 50 μL of PSA standards or samples with various concentrations in pH 7.4 PBS were added into the microtiter plate, and incubated for 1 h at 37 °C under shaking. After washing, 50 μL of the above-prepared Ab_2-PbS suspension was added into the well and incubated for 1 h at 37 °C with shaking. After washing again, a 20 μL aliquot of 1.0

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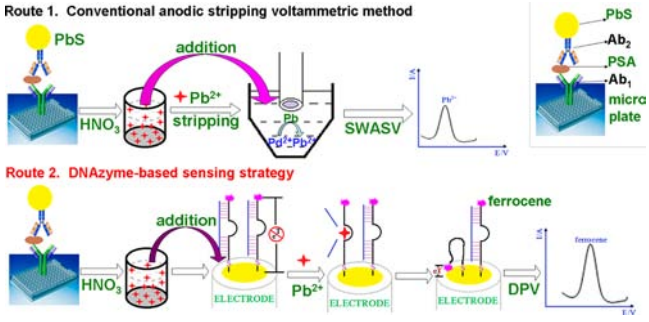
Scheme 1. Schematic Representation of Two Different Detection Protocols toward Metal Sulfide Nanoparticle-Labeled Probes[a]



[a]Route 1: Conventional anodic stripping voltammetric method. Route 2: DNAzyme-based immunosensing strategy.

M HNO_3 was added into each well to release lead ions from the Ab_2-PbS (~3 min). The resulting acid solution containing the dissolved Pb^{2+} ions was transferred onto the DNAzyme-modified sensor, and incubated for 60 min at RT. During this process, the liberated lead ion could induce the specific cleavage of substrate strand on the DNAzyme, thus resulting in the conformational change of catalytic strand. Meanwhile, the differential pulse voltammetric (DPV) measurement was implemented on a CHI 620D Electrochemical Workstation (Chenhua, Shanghai, China) with a conventional three-electrode system comprising a Pt-wire counter electrode, an Ag/AgCl reference electrode, and a modified gold working electrode. The DPV measurement was carried out after a 2-s rest period (without stirring) at an applied potential range of 0 to 0.4 V with a pulse amplitude of 20 mV and a pulse width of 50 ms. A baseline correction of the resulting voltammogram was performed with CHI 620D software (*Note*: The waste after each immunoassay should be collected in a bottle to be disposed of safety).

For comparison, the liberated lead ion was also monitored by using conventional anodic stripping voltammetric method as follows (Route 1 in Scheme 1). Initially, the resulting acid solution containing the dissolved Pb^{2+} ion was transferred into 2 mL acetate buffer (0.2 M, pH 5.6) containing 10 μg mL^{-1} of mercury ion (from Hg(II)acetate). And then, the square wave anodic stripping voltammetric (SWASV) measurement was conducted on a CHI 620D Electrochemical Workstation (Chenhua, Shanghai, China) with a conventional three-electrode system comprising a Pt-wire counter electrode, an Ag/AgCl reference electrode, and an *in situ* formed mercury drop electrode (on a glassy carbon electrode surface, effective working area: 3.14 mm^2). The stripping process contained a 1.0-min pretreatment at +0.6 V and 2.0-min accumulation at −1.4 V. The SWASV measurement was carried out after a 15-s rest period (without stirring) at an applied potential range of −0.7 to −0.3 V with a potential step of 4 mV, a frequency of 25 Hz, and an amplitude of 25 mV.

RESULTS AND DISCUSSION

Principle and Characteristics of DNAzyme-Based Electrochemical Immunoassay. In this work, the signal is amplified by the labeled ferrocene tag on the DNAzyme-based sensor. In the presence of PSA, the sandwiched immunocomplex can be formed between mAb_1-coated microplate (mAb_1) and PbS-labeled Ab_2. The carried PbS nanoparticles can release numerous Pb^{2+} ions by acid, which can induce the cleavage of the corresponding DNAzyme, thus resulting in the amplification of electrochemical signal within the applied potentials owing to the conformational change of DNAzyme.

Figure 1A shows typical transmission electron microscope (TEM) of water-soluble, carboxylated PbS nanoparticles. The mean size was 20 nm, which exhibited the homogeneous dispersion in the distilled water. Using the carbodiimide coupling method, anti-PSA detection antibodies could be covalently conjugated onto the surface of PbS nanoparticles. As seen from inset in Figure 1A, the UV−vis absorption spectra of pure PbS colloids were complicated, and increased with the decreasing wavelength in the range 600−200 nm (curve *a*), while the as-synthesized Ab_2-PbS exhibited one absorption peak at 278 nm (curve *b*). The peak at 278 nm derived from the labeled anti-PSA antibodies.[28] The results indicated that anti-PSA detection antibody could be covalently conjugated on the PbS nanoparticle.

As mentioned above, the electrochemical signal was mainly from the cleavage of Pb^{2+}-induced DNAzyme. Logically, a question was whether lead ions could cause the current change of DNAzyme-based sensor. To clarify this issue, the DNAzyme-based sensor was used for determination of various concentrations of lead ions in pH 8.2 Tris-acetate buffer (50 mM, 0.5 M NaCl) by using differential pulse voltammetry (DPV). As indicated from Figure 1B, the peak current increased with increasing Pb^{2+} concentration. The relationship between peak current and Pb^{2+} level was i (nA) = 1.907 × $C_{[Pb]}$

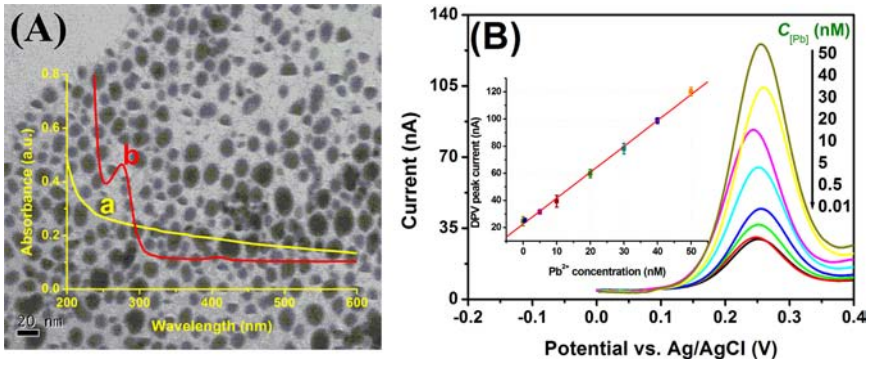


Figure 1. (A) TEM image of PbS nanoparticles [*Inset*: UV−vis absorption spectra of (a) PbS colloids and (b) Ab_2-PbS]. (B) DPV response of DNAzyme-based sensor toward Pb^{2+} ions with various concentrations in pH 8.2 Tris-acetate buffer (50 mM, 0.5 M NaCl) [*Inset*: the relationship between the peak currents and Pb^{2+} levels].

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+ 22.63 (nM) (R^2 = 0.997). In this case, each DNAzyme consisted of a catalytic strand and a substrate strand. Upon addition of Pb^{2+} ion, substrate DNA segment in the DNAzyme was catalytically cleaved. The binding reaction disrupts the conformational structure of DNAzyme, and liberates a flexible, single-stranded element to strike the electrode, leading to an increase in the current due to the approached ferrocene tag. Based on the results, we might conclude that the designed DNAzyme sensor could be used for detection of lead ions, which provided the facilitation for PbS-based immunoassay.

Comparison of Electrochemical Responses of Various Strategies. Typically, anodic stripping voltammetry is utilized as a signal-transduction method in the metal sulfide-based electrochemical immunoassays. To further elucidate the signal amplification efficiency of using lead-specific DNAzyme sensor, the square wave anodic stripping voltammetric (SWASV) method were also used for detection of PSA (0.1 ng mL^{-1} uses as an example) by using a mercury drop electrode. The judgment was based on the change in the peak current relative to zero analyte. As seen from Figure 2, the immunoassay of

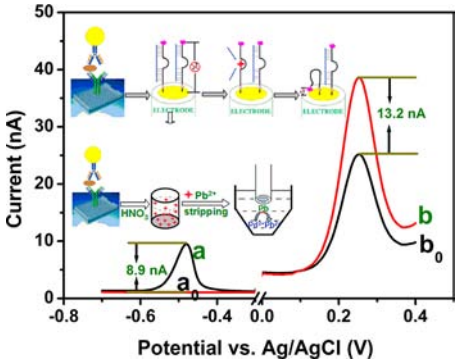

Figure 2. Electrochemical responses of the PbS-based immunoassay toward (a_0,b_0) zero analyte and (a,b) 100 pg mL^{-1} PSA in the Tris-acetate buffer (50 mM, pH 8.2) containing 0.5 M NaCl using (a,a_0) conventional anodic stripping voltammetric method and (b,b_0) DNAzyme-based sensing strategy.

using the SWASV strategy exhibited a weak current response (8.9 nA, curve *a* vs curve a_0). In contrast, a high peak current was achieved by using lead-specific DNAzyme sensor (13.2 nA, curve *b* vs curve b_0). Therefore, the DNAzyme-based sensing strategy could cause a 148.3 ± 16.2% signal increase of the conventional SWASV method. This is most likely a consequence of the fact that the labeled ferrocene, as a good electron mediator, was greatly conducive to electron transfer between the supporting electrolyte and the base electrode. Meanwhile, not all of the stripping lead ions from the PbS nanolabels were accumulated on mercury drop electrode during a deposition step. Hence, lead-specific DNAzyme sensor could be considered as the signal-transduction strategy for highly sensitive electrochemical detection of PSA by coupling with the PbS-labeling strategy.

Analytical Performance of the DNAzyme-Based Immunoassay. To acquire an optimal electrochemical response signal, we also monitored the effect of various experimental conditions on the current of the developed immunoassay. As seen from Figures S2–S3 (see Supporting Information), the optimal parameters were 30 min for the formation of sandwiched immunocomplex in the microplate and 60 min for the cleavage of Pb^{2+}-induced DNAzyme on the sensor.

Under optimal conditions, the sensitivity and quantitative range of the electrochemical immunoassay were studied by assaying routine samples with different PSA concentrations based on the developed protocol. The currents increased with the increasing PSA concentration in the sample (Figure 3). A linear dependence between the peak currents and the logarithm of PSA levels was obtained in the range from 0.1 pg mL^{-1} to 20 ng mL^{-1}. The detection limit (LOD) was 0.1 pg mL^{-1} at $3s_B$. For comparison, we also investigated the analytical properties of the immunoassay using conventional anodic stripping voltammetric method. The linear range and LOD were 10 pg mL^{-1} to 20 ng mL^{-1} and 5.0 pg mL^{-1} PSA, respectively. Additionally, the analytical performance of the developed immunoassay was compared with other PSA assay methods. As seen from Table S1 (see Supporting Information), the DNAzyme-based immunosensing strategy possessed a wide linear range and a low LOD. More importantly, the sensitivity of the developed immunoassay was 220-fold lower than that of commercially available free PSA ELISA kit (0.022 ng mL^{-1}, Catalogue No.: EL10050, Anogen, Canda).[29] The results further demonstrated the amplified efficiency of DNAzyme-based sensing strategy.

The reproducibility and precision of the electrochemical immunoassay were evaluated by using the variation coefficients (CVs) of intra- and interassays with high–middle–low 3 PSA levels. Experimental results that the CVs of the intra-assay with

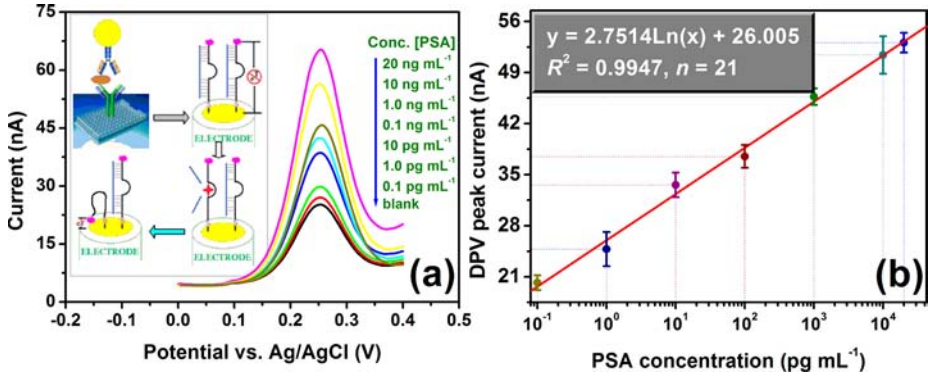

Figure 3. (a) Typical DPV response curves of the DNAzyme-based immunoassay toward different PSA standards, and (b) the relationship between DPV peak currents and PSA concentrations. Measurements were performed in the Tris-acetate buffer (50 mM, pH 8.2) containing 0.5 M NaCl. Each data point represents the average.

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the same batch were 5.2%, 9.3%, and 7.5% at 0.001, 0.1, and 10 ng mL^{-1} PSA, respectively. Similarly, the interassay CVs with various batches were 7.6%, 9.8%, and 8.4% at the above-mentioned levels, respectively. Thus the developed immunoassay could be used repeatedly, and further verified the possibility of batch preparation.

The specificity of the immunoassay were also investigated by challenging the system with other several possible components in human serum, e.g., AFP, CEA, CA 125, CA 15−3, CA 19−9, and IgG. As shown from Figure 4, no obvious currents were

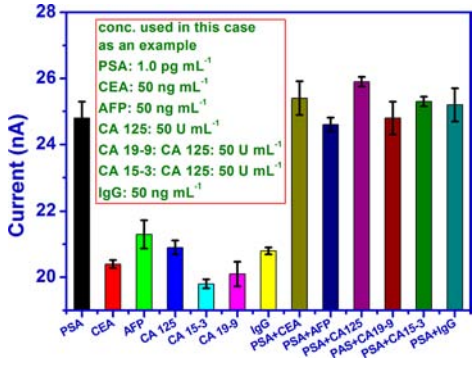

Figure 4. Specificity of the DNAzyme-based electrochemical immunoassay. Initially, the human serum sample used for spiking was assayed by using the DNAzyme-based immunoassay method, and various sample matrices were then spiked into the human serum. Following that, the resulting mixtures were determined by using the same method.

obtained toward these components alone. Significantly, the coexistence of PSA target with these interfering components did not obviously affect the change in the current relative to target analyte alone. The results manifested the high specificity of the electrochemical immunoassays.

Analysis of Real Samples and Validation of Method Accuracy. To monitor the analytical reliability and applicable potential of the electrochemical immunoassay for testing real samples, we collected 12 human serum specimens with various PSA levels from Fujian Provincial Hospital of China according to the rules of the local ethical committee. Prior to measurement, these samples were gently shaken at RT (*Note*: all handling and processing were performed carefully, and all tools in contact with patient specimens and immunoreagents were disinfected after use), and then evaluated by using the electrochemical immunoassay. The assay results were compared with those obtained by using the commercially available free PSA ELISA kit. The assay results are listed in Table 1 and Figure S4 (see Supporting Information for details). Statistical analysis of the experimental results was performed using a *t*-test and linear regression analysis between two methods. As seen from Table 1, the t_{exp} values in all samples were less than t_{crit} ($t_{crit[4,\ 0.05]}$ = 2.77). Moreover, the slope and intercept of the regression equation between two methods were close to 1 and 0, respectively, thereby revealing a good agreement between both analytical methods.

CONCLUSIONS

In summary, this work describes a novel electrochemical immunoassay protocol for detection of free PSA with high sensitivity and high selectivity by using PbS nanoparticle as the label and lead ion-specific DNAzyme sensor. Highlight of this work is to avoid the use of anodic stripping voltammetric method and the accumulation of metal ions on the mercury drop electrode. Although the present system is focused on the analysis of target PSA, it can be readily extended for testing other low-abundance proteins by controlling the target antibody.

Table 1. Comparison of the Assay Results for Clinical Serum Specimens Using the Developed Immunoassay and the Referenced ELISA Method

	method; concentration [mean ± SD (RSD), n = 3, ng mL^{-1}]		
sample no.[a]	DNAzyme-based immunoassay	ELISA	t_{exp}
1	6.73 ± 0.21	6.54 ± 0.43	0.69
2	4.89 ± 0.43	5.11 ± 0.32	0.71
3	9.87 ± 0.12	9.56 ± 0.43	1.21
4	12.5 ± 0.91	13.2 ± 0.23	1.29
5	17.8 ± 1.2	16.9 ± 0.78	1.09
6	8.43 ± 0.92	8.56 ± 0.35	0.23
7	1.23 ± 0.13	1.46 ± 0.11	2.34
8	3.21 ± 0.19	3.54 ± 0.13	2.48
9	0.43 ± 0.09	0.37 ± 0.05	1.01
10	0.21 ± 0.07	0.18 ± 0.05	0.61
11	0.012 ± 0.01	No detection	No application
12	0.005 ± 0.01	No detection	No application

[a]Samples 1−6 were clinical serum specimens, and samples 7−12 were the diluted samples by using newborn cattle serum.

ASSOCIATED CONTENT

Supporting Information

Characteristics of DNAzyme-based sensor and experimental optimization. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Xue, Q., Wang, Z., Wang, L., and Jiang, W. (2012) Sensitive detection of proteins using assembled cascade fluorescent DNA nanotags based on rolling circle amplification. *Bioconjugate Chem.* 23, 734−739.

(2) Minami, K., Ihara, M., Kuroda, S., Tsuzuki, H., and Ueda, H. (2012) Open-sandwich molecular imprinting: Making a recognition matrix with antigen-imprinted antibody fragments. *Bioconjugate Chem.* 23, 1463−1469.

(3) Liu, Y., Zhou, S., Tu, D., Chen, Z., Huang, M., Zhu, H., Ma, E., and Chen, X. (2012) Amine-functionalized lanthanide-doped zirconia

nanoparticles: optical spectroscopy, time-resolved fluorescence resonance energy transfer biodetection, and targeted imaging. *J. Am. Chem. Soc. 134*, 15083−15090.
(4) Cheng, T., Chuang, K., Chen, B., and Roffler, S. (2012) Analytical measurement of PEGylated molecules. *Bioconjugate Chem. 23*, 881−899.
(5) Zhou, J., Xu, M., Tang, D., Gao, Z., Tang, J., and Chen, G. (2012) Nanogold-based bio-bar codes for label-free immunosensing of proteins coupling with an in situ DNA-based hybridization chain reaction. *Chem. Commun. 48*, 12207−12209.
(6) Zhang, B., Liu, B., Tang, D., Niessner, R., Chen, G., and Knopp, D. (2012) DNA-based hybridization chain reaction for amplified bioelectronic signal and ultrasensitive detection of proteins. *Anal. Chem. 84*, 5392−5399.
(7) Hansen, J., Mukhopahyay, R., Hansen, J., and Gothelf, K. (2006) Femtomolar electrochemical detection of DNA targets using metal sulfide nanoparticles. *J. Am. Chem. Soc. 128*, 3860−3861.
(8) Wang, J., Liu, G., and Merkoci, A. (2003) Electrochemical coding technology for simultaneous detection of multiple DNA targets. *J. Am. Chem. Soc. 125*, 3214−3215.
(9) Freeman, R., and Willner, I. (2012) Optical molecular sensing with semiconductor quantum dots (QDs). *Chem. Soc. Rev. 41*, 4067−4085.
(10) Golub, E., Pelossof, G., Freeman, R., Zhang, H., and Willner, I. (2009) Electrochemical, photoelectrochemical, and surface plasmon resonance detection of cocaine using supramolecular aptamer complexes and metallic or semiconductor nanoparticles. *Anal. Chem. 81*, 9291−9298.
(11) Freeman, R., Finder, T., and Willner, I. (2009) Multiplexed analysis of Hg^{2+} and Ag^{+} ions by nucleic acid functionalized CdSe/ZnS quantum dots and their use for logic gate operations. *Angew. Chem., Int. Ed. 48*, 7818−1821.
(12) Zhang, B., Tang, D., Goryacheva, I., Niessner, R., and Knopp, D. (2013) Anodic stripping voltammetric immunoassay for ultrasensitive detection of low-abundance-proteins using quantum dots-aggregated hollow microspheres. *Chem.—Eur. J. 19*, 2496−2503.
(13) Izadyar, A., Kim, Y., Ward, M., and Amemiya, S. (2012) Double-polymer-modified pencil lead for stripping voltammetry of perchlorate in drinking water. *J. Chem. Educ. 89*, 1323−1326.
(14) Zhu, L., Li, C., Zhu, Z., Liu, D., Zou, Y., Wang, C., Fu, H., and Yang, C. (2012) In vitro selection of highly efficient G-quadruplex-based DNAzymes. *Anal. Chem. 84*, 8383−8390.
(15) Golub, E., Freeman, R., and Willner, I. (2011) A hemin/G-quadruplex acts as an NADH oxidase and NADH peroxidase mimicking DNAzyme. *Angew. Chem., Int. Ed. 50*, 11710−11714.
(16) Kong, R., Zhang, X., Chen, Z., Meng, H., Song, Z., Tan, W., Shen, G., and Yu, R. (2011) Unimolecular catalytic DNA biosensor for amplified detection of L-histidine via an enzymatic recycling cleavage strategy. *Anal. Chem. 83*, 7603−7607.
(17) Xiang, Y., and Lu, Y. (2011) Using personal glucose meters and functional DNA sensors to quantify a variety of analytical targets. *Nat. Chem. 3*, 697−703.
(18) Xiang, Y., Tong, A., and Lu, Y. (2009) Abasic site-containing DNAzyme and aptamer for label-free fluorescent detection of Pb^{2+} and adenosine with high sensitivity, selectivity, and tunable dynamic range. *J. Am. Chem. Soc. 131*, 15352−15357.
(19) Ali, M., Aguirre, S., Lazim, H., and Li, Y. (2011) Fluorogenic DNAzyme probes as bacterial indicators. *Angew. Chem., Int. Ed. 50*, 3751−3754.
(20) Wang, F., Elbaz, J., Teller, C., and Willner, I. (2011) Amplified Detection of DNA through an Autocatalytic and Catabolic DNAzyme-Mediated Process. *Angew. Chem., Int. Ed. 50*, 295−299.
(21) Li, C., Liu, K., Lin, Y., and Chang, H. (2011) Fluorescence detection of lead(II) ions through their induced catalytic activity of DNAzymes. *Anal. Chem. 83*, 225−230.
(22) Monsur Ali, M., and Li, Y. (2009) Colorimetric sensing by using allosteric-DNAzyme-coupled rolling circle amplification and a peptide nucleic acid−organic dye probe. *Angew. Chem., Int. Ed. 48*, 3512−3515.
(23) Kong, D., Xu, J., and Shen, H. (2010) Positive effects of ATP on G-quadruplex-hemin DNAzyme-mediated reactions. *Anal. Chem. 82*, 6148−6153.
(24) Xiang, Y., Wang, Z., Xing, H., Wong, N., and Lu, Y. (2010) Label-free fluorescent functional DNA sensors using unmodified DNA: a vacant site approach. *Anal. Chem. 82*, 4122−4129.
(25) Young, D., Lively, M., and Deiters, A. (2010) Activation and deactivation of DNAzyme and antisense function with light for the photochemical regulation of gene expression in mammalian cells. *J. Am. Chem. Soc. 132*, 6183−6193.
(26) Xiao, Y., Rowe, A., and Plaxco, K. (2007) Electrochemical detection of parts-per-billion lead via an electrode-bound DNAzyme assembly. *J. Am. Chem. Soc. 129*, 262−263.
(27) Li, C., Zhao, Y., Li, F., Shi, Z., and Feng, S. (2010) Near-infrared absorption of monodisperse water-soluble PbS colloidal nanocrystal clusters. *Chem. Mater. 22*, 1901−1907.
(28) Tang, D., Su, B., Tang, J., Ren, J., and Chen, G. (2010) Nanoparticle-based sandwich electrochemical immunoassay for carbohydrate antigen 125 with signal enhancement using enzyme-coated nanometer-sized enzyme-doped silica beads. *Anal. Chem. 82*, 1527−1534.
(29) http://www.anogen.ca/datasheets/ELISA%20kits.