Cite this: DOI: 10.1039/c0xx00000x
www.rsc.org/xxxxxx

ARTICLE TYPE

A Phosphorylation-Sensitive Tyrosine-Tailored Magnetic Particle for Electrochemically Probing Free Organophosphates in Blood

Yanmei Si, Ning Zhang, Zongzhao Sun, Shuai Li, Liyang Zhao, Rui Li, Hua Wang*

Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX
DOI: 10.1039/b000000x

A simple, rapid, sensitive, selective, and field-deployable detection protocol has been initially proposed for the early warning and diagnosis of the exposures to organophosphates (OPs) by electrochemically monitoring the direct biomarkers of free OPs in blood. Phosphorylation-sensitive tyrosine (Tyr), which was tested with unique electroactivity, was bound onto Fe_3O_4 particles mediated by the mussel-inspired dopamine to form Fe_3O_4@Tyr particles with well-defined shape and well-retained Tyr electroactivities, as characterized separately by electron microscopes and electrochemical measurements. A "lab-on-a-particle"-based detection procedure combined with a magnetic electrode was thus developed by employing Fe_3O_4@Tyr particles as capturing probes for detecting free OPs in blood, dimethyl-dichloro-vinyl phosphate (DDVP) as an example. A significant difference in electrochemical responses could be obtained for Fe_3O_4@Tyr particles before and after DDVP exposures, based on the phosphorylation-induced inhibition of electroactivities of Tyr loaded. Investigation results indicate that highly specific and sensitive phosphorylation for the inhibition of Tyr electroactivities by sensitive electrochemical outputs could endow the OP detections with high selectivity and sensitivity (i.e., down to about 0.16 nM DDVP in blood). Moreover, strong and stable Tyr-OP bindings especially irreversible electrochemical oxidization of Tyr probe could facilitate the OP evaluation with high reproducibility and stability over time. In particular, the simple "lab-on-a-particle"-based detection procedure equipped with portable electrochemical transducer can be tailored for the field-deployable or on-site monitoring of the exposures to various nerve agents and pesticides.

1. Introduction

Neurotoxic organophosphates (OPs) including pesticides and chemical nerve agents have been widely used in agricultural industry and chemical war.[1,2] According to statistics, there are at least 13 types of OPs and hundreds of OP compounds in use, which are derivatives of phosphoric, phosphonic, or phosphinic acids.[3] As a result, there is a potential exposure for human and animals. Moreover, after a number of terrorist attacks such as the Tokyo subway attack in 1995 and the tragic events of September 11, 2001, the fatal damage of nerve agents has been realized. The need for more effective methods for early warning of potential terrorist attacks and for the rapid screening of OPs in air, water, soil, and food has thus become increasingly urgent.[3-5]

It is generally recognized that selecting suitable biomarkers of OPs exposure is of central importance for developing an efficient strategy to prove the use of a chemical agent and find application in diagnosis to ensure that appropriate medical countermeasures are administered. The exposure of OP agents with proteins and enzymes such as butyrylcholinesterase (BChE) and acetycholinesterase (AChE) in the biological matrix will produce four types of different kinds of biomarkers, including free OP, inhibited cholinesterase (ChE), phosphorylated adducts and their hydrolyzed metabolites.[6] Obviously, free OPs in blood is the most direct and accurate biomarkers for OP identification for warning the exposures of pesticides and chemical warfare agents. Up to date, many methods, such as liquid chromatography (LC), gas chromatography (GC), mass spectrometry (MS), have been employed for the determination of free OPs and multiple analogues with high sensitivity and specificity.[7-10] However, they may be limited by the need of expensive and complex analysis settings, well-trained personnel, and inconvenience for rapid field applications. Alternatively, recent years have witnessed the detection of phosphorylated adducts and enzyme (i.e., BChE and AChE) activities as the indicators of OPs exposure.[11] Nevertheless, the majority of current detection methods of OPs exposures might encounter with some formidable disadvantages. For example, phosphorylated adducts like enzymes were examined as the indicators of OP exposures, a challenge might lie in the interferences of complicated sample backgrounds and unavailability of appropriate recognition elements or receptors (i.e., antibodies) for targeting the phosphorylated adducts.[12] As a hot choice, numerous efforts have been devoted to the biologically-monitoring approaches based on the OP inhibition of catalysis activities of enzymes, most known as blood cholinesterase of BChE and AChE.[13] Gracefully successive as these methods are, the detections of low-level exposure may still require individual baseline measurements before meaningful changes of enzyme activities can be measured; especially some of the results are non-specific for the cause of cholinesterase inhibition in occupational health monitoring.[14] In addition, these OP-exposure valuation methods may suffer from a long analysis time and expensive sample preparation and multiple washing steps.[15,16] Hence, the development of simple, sensitive, selective, and field-deployable tools is highly desired for biomonitoring and diagnostic evaluation of OP exposures, especially to enhance our response to a sudden emergency and our ability to medically counteract the effects.

Moreover, the exposure of organophosphorus nerve agents for human or animals as aforementioned, their biochemical targets involve some special proteins (i.e., serum albumin) and enzymes such as BChE, AChE, serine esterase, trypsin, and chymotrypsin.[17-21] The phosphorylated sites of them in vivo generally include serine (Ser), threonine (Thr) residues, and tyrosine (Tyr) residues.[22-24] For example, the abundant blood protein albumin can be easily phosphorylated at the Tyr residue.[14,22] These evidences indicate that the Tyr should conduct a strong interaction with the OPs, serving as an ideal receptor for free OPs in blood for early warning of OP exposures.

Over the past decades, electrochemical assays have developed rapidly with high analysis selectivity and sensitivity, especially combined with various nanoscale materials.[25-29] More importantly, the merits of simple operation, fast detection, and miniaturized analysis instruments of these detection devices meet the need of rapid field detection applications. In this work, we seek to develop a novel electrochemical sensing method to probe the free OPs in human blood based on the special interactions of OPs with the meaningful amino acids (Ser, Thr and Tyr), aiming to circumvent the current drawbacks of the detection of phosphorylated targets for warning OP exposures. Herein, the electrochemical activities of the amino acids above have been screened, and only Tyr showed the electro-activity. A difference in electrochemical responses can be expected for Tyr before and after the OP inhibition. Moreover, considering the interferences of the complex components in blood samples, magnetic particles were employed as the carriers for loading Tyr, resulting in Fe_3O_4@Tyr particles. A "lab-on-a-particle"-based detection protocol has thereby proposed with a magnetic electrode for probing Ops in blood, dimethyl-dichloro-vinyl phosphate (DDVP) as an example, based on the phosphorylation-induced inhibition of electroactivities of Tyr. The detailed electrochemical detection procedure is illustrated in **Scheme 1**. To the best of our knowledge, this is the first report of a "lab-on-a-particle"-based sensing method by the phosphorylation-induced inhibition of electrochemical activities of Tyr on magnetic particles for the early warning of OP exposures through monitoring the direct biomarkers of free phosphorus agents in blood.

2. Experimental

2.1 Reagents and apparatus

Tyrosine (Tyr), serine (Ser), threonine (Thr), dopamine (DA), vitamin C (Vc), alanine (Ala) , glycine (Gly), arginine (Arg), phenylalanine (Phe), aspartic acid (Asa), and human serum albumin (HSA) were purchased from Sigma-Aldrich (Beijing, China). Tri-hydroxymethyl aminomethane (Tris) was obtained from Sinopharm Chemical Reagent Co. (China). Organophosphorus (OP) agents of dimethyl-dichloro-vinyl phosphate (DDVP), methidathion (Met), paraoxon (Par) were provided by Dibai Reagents (Shandong, China). Ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), glycol, sodium acetate and all other reagents were of analytical grade. Deionized water (>18 Mohm) used was obtained from an Ultra-pure water system (Pall, USA). The OP stock solutions, DDVP as a representative example, were simply dissolved into acetone and diluted to different concentrations.

Electrochemical measurements were conducted with an electrochemical workstation CHI760D (CH Instrument, Shanghai, China) connecting to a personal computer. Three-electrode system was applied consisting of glassy carbon working electrode with magnetic core (Incole Union Technology, Tianjin, China), a Pt wire counter electrode, and an Ag/AgCl reference electrode. Characterizations of the as-prepared materials were performed by using scanning electron microscopy (SEM, Hitachi E-1010, Japan) and transmission electron microscopy (TEM, FEI Tecnai G20, USA).

2.2 Synthesis and characterization of magnetic Fe_3O_4@Tyr particles

Magnetic Fe_3O_4 particles were prepared according to a modified synthesis procedure reported previously.[30] Briefly, 5.40 g of $FeCl_3 \cdot 6H_2O$ was dissolved in 40 mL of glycol to form a clear solution. Following that, 3.28 g of anhydrous sodium acetate was added to be vigorously mixed by ultra-sonication to give a homogeneous solution. Furthermore, the mixture was transferred into a Teflon-lined stainless steel autoclave for hydrothermal treatment at 200 °C for 12 h. Subsequently, the autoclave was cooled down to room temperature and the precipitate was magnetically collected and washed for several times with water and ethanol by sonication. The so obtained Fe_3O_4 particles were then dried under vacuum to be stored.

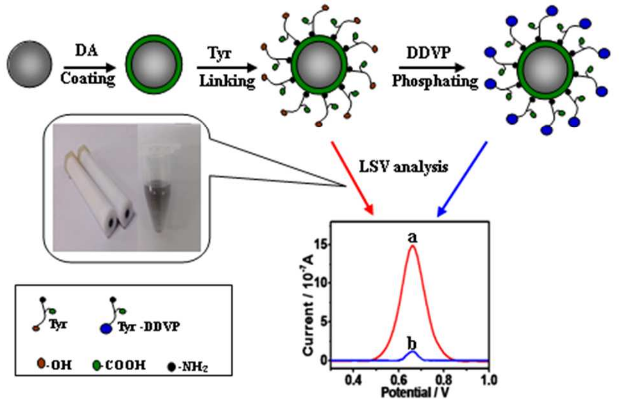
An aliquot of 5.0 mg Fe_3O_4 particles was added to 5.0 mL Tris-HCl buffer (pH 7.4) containing 20 mg DA to be mixed by sonication for 5.0 min and then incubated overnight. The so prepared DA-modified Fe_3O_4 particles were magnetically washed three times and then diluted to 4.5 mL with Tris-HCl buffer. After that, 0.5 mL 25 % glutaraldehyde and 18.0 mg Tyr (dissolved in NaOH) were added to the DA-modified Fe_3O_4 suspension to be further sonicated for 5.0 min. The resulting mixture was finally incubated for 2h, and then rinsed three times with Tris-HCl buffer to form the Fe_3O_4@Tyr particles, stored at 4 °C for future usage.

The so yielded Fe_3O_4@Tyr particles were sonicated for 30 min, and then characterized by SEM and TEM imaging. In addition, the electrochemical voltammetric characterizations of the stepping process for Fe_3O_4@Tyr setup was conducted using magnetic electrodes, which were performed following the electrochemical analysis procedure below.

2.3 Fe_3O_4@Tyr particles-based electrochemical measurements

An aliquot of Fe_3O_4@Tyr particles were added to the plastic tubes (0.5 mL), and OP samples with different concentrations were separately introduced to be reacted for 20 min. Then, each of the reaction suspensions was magnetically separated and washed for three times. An amount of 5.0 μL of the resulting magnetic mixture was added to the surface of magnetic working electrodes, which were regenerated after usage by polishing procedures. Electrochemical measurements were performed in Tris-HCl buffer for each of the as-modified electrodes by linear sweep voltammetry (LSV), scanning at a potential range of 0.2 - 1.0 V at a scanning speed of 100 mV s^{-1}. A baseline correction of the resulting voltammograms was performed with CHI software.

Besides, the control tests for different samples and the selectivity investigation of Fe_3O_4@Tyr particles-based electrochemical responses toward DDVP were conducted in the same way by comparing to Na^+, K^+, Ca^{2+}, Mg^{2+}, Fe^{2+}, Vc, and Ala, Gly, Arg, Phe, Asa, HSA, Met, Par.



Scheme 1 Schematic illustration of Fe_3O_4@Tyr particles-based electrochemical detection of free OPs using magnetic electrodes, based on the phosphorylation-induced inhibition of Tyr electro-activity on Fe_3O_4@Tyr particles.

3. Results and discussion

3.1 Comparison of electrochemical activities of OP-sensitive three amino acids

It is well established that the attacking sites of organic phosphorus for proteins (i.e., serum albumin) and enzymes (i.e., AChE and BChE) mainly include Ser, Thr and Tyr. In the present work, the electrochemical activities of free three amino acids in solutions were investigated (**Fig. 1**). **Fig. 1A** shows that Tyr had significant peak of oxidation current at about 0.67 V, in contrast to Ser and Thr with no obvious redox peaks. It is thought that the electro-oxidation of Tyr residues involves two-electron and two-proton transfers, of which the electrode process is similar to that of p-substituted phenols.[31-34] Ser and Thr, however, do not have relevant structure to obtain obvious electrochemical signals in the experiment. Further investigations indicate that the peak currents of Tyr could be reduced depending on different concentrations of organophosphorus exposed (**Fig. 1B**). Herein, organic phosphorus can react with phenol hydroxyl of Tyr,[14] that is, after OP exposure, the phosphorylated phenol group on Tyr would not be electrochemically oxidized to give out the corresponding signals. Such a finding suggests that Tyr may be used as the phosphorylation receptor for probing free OPs in blood by the electrochemical analysis.

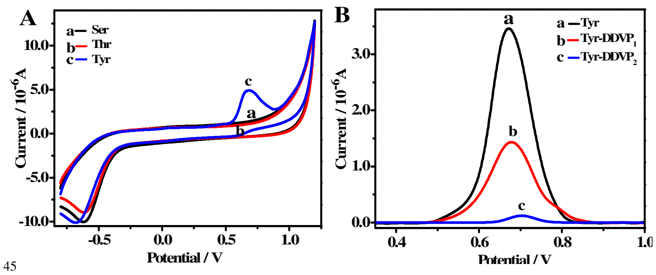


Fig. 1 Initial investigation of electrochemical activities of three OP-sensitive amine acids. (A) LSV responses of Ser, Thr, and Tyr of 1.0 mM in Tris-HCl buffer; (B) LSV responses of 80 μM Tyr (control) in the presence of 695 nM and 1135 nM DDVP in Tris-HCl buffer.

3.2 Characterization of Fe_3O_4@Tyr particles

The as-prepared Fe_3O_4@Tyr particles were systemically characterized (**Fig. 2**). The scanning electron microscope (SEM) image shows that Fe_3O_4@Tyr particles displayed a defined spherical shape but scabrous surface with average size of about 500 nm (**Fig. 2A**), which is also manifested in the transmission electron microscope (TEM) image (**Fig. 2B**).

Moreover, the electrochemical characterization of the stepping setup process for Fe_3O_4@Tyr particles was conducted comparably using magnetic electrodes, with results shown in **Fig. 2C**. One can find that the electrode with Fe_3O_4 particles present no electrochemical properties. A couple of redox peaks at 0.10 - 0.20 V were witnessed for the one with DA-modified Fe_3O_4 particles, indicating the stable coatings of DA onto magnetic particles. Importantly, the electrode with Fe_3O_4@Tyr particles could exhibit a well-defined irreversible oxidation peak at 0.67V of Tyr. It thus verifies that Tyr was successfully attached onto DA-modified Fe_3O_4 particles with well-retained electrochemical activity, presumably resulting from the mussel-inspired biological compatibility of DA coating particles. However, the redox peaks of DA might disappear after being activated by glutaraldehyde that could cause the chemical change of its electroactive groups. Furthermore, Fe_3O_4@Tyr particles were added into the DDVP solutions with different concentrations for phosphorylation, and further magnetically separated and attached onto the magnetic electrodes for electrochemical LSV measurements (**Fig. 2D**). It is noted that the peak current of Tyr on Fe_3O_4@Tyr particles decreased significantly with OP contents increased. Of note, the phosphorylation of Tyr residue is considerably stable with no aging,[14,22] and Tyr on Fe_3O_4@Tyr particles can be electrochemically oxidized with irreversible characteristics, as confirmed elsewhere.[31] Therefore, based on the OP inhibition of Tyr electroactivity of Fe_3O_4@Tyr particles, a "lab-on-a-particle" protocol can be established to facilitate the monitoring of OP exposures with high sensitivity, selectivity, and stability afterwards.

3.3 Electrochemical sensing procedure for OPs with Fe_3O_4@Tyr particles

The OP inhibition of Tyr electroactivities was employed in combination with electrochemical outputs to determine the content of free organic phosphorus in blood. The main detection procedure is illustrated in **Scheme 1**. Herein, considering the

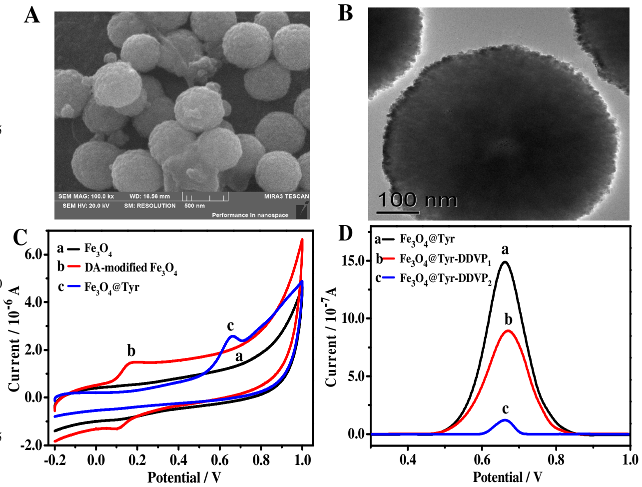


Fig. 2 Characterization of Fe_3O_4@Tyr particles by (A) SEM image, (B) TEM image, (C) electrochemical voltammetric responses of Fe_3O_4 particles, DA-modified Fe_3O_4 particles, and Fe_3O_4@Tyr particles of 1.67 mg mL^{-1}, and (D) LSV responses of Fe_3O_4@Tyr particles before and after treated with 2.5 nM and 500 nM DDVP for 20 min.

interferences from the complex components in blood, magnetic separation has also been introduced by using magnetic particles as carriers to loading the OP-sensitive Tyr. DA was modified onto Fe_3O_4 particles, followed by the linking of Tyr through glutaraldehyde cross-binding chemistry. The so formed Fe_3O_4@Tyr particles were used to capture free OP, DDVP as a model, from the blood. The DDVP-induced phosphorylation of Tyr on Fe_3O_4@Tyr particles (Fe_3O_4@Tyr-DDVP) were then electrochemically measured comparing to original Fe_3O_4@Tyr particles with magnetic electrodes. An obvious difference in signals of linear sweep voltammetry (LSV) was obtained for the particles before and after DDVP treatment, thus profiling the "lab-on-a-particle" detection procedure for electrochemically monitoring OP exposures.

3.4 Optimization of detection conditions of Fe_3O_4@Tyr particles-based electrochemical assays for OP exposure

The detection conditions of Fe_3O_4@Tyr particles-based electrochemical assays for OP exposure are optimized (**Fig. 3**). The amounts of Fe_3O_4@Tyr particles were first investigated for the electrochemical Tyr responses to DDVP (**Fig. 3A**). As is shown in **Fig. 3A**, Tyr responses could increase with the increasing amounts of Fe_3O_4@Tyr particles. Interestingly, too high concentrations of Fe_3O_4@Tyr particles might lead to greatly decreased signals presumably due to too high density of nonconductive particles on the electrode surfaces. Accordingly, 1.67 mg mL^{-1} of Fe_3O_4@Tyr particles was chosen in the experiments. Moreover, the pH values can be another important parameter for the OP detections (**Fig. 3B**). One can see that the highest response was observed at pH 7.4. Herein, harsh acid or base solutions might induce either the instability of Fe_3O_4 particles (including their loadings) or cause the rapid decomposition of DDVP. Also, the temperature for phosphorylation reactions could exert an influence on the OP monitoring (**Fig. 3C**), and 25 °C is selected as the optimum reaction temperature. In addition, the interactions between DDVP

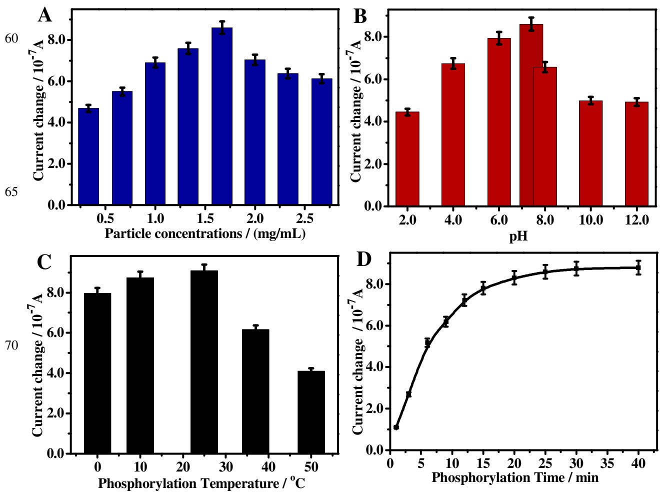


Fig. 3 Effects of experimental conditions on electrochemical responses of Fe_3O_4@Tyr particles to DDVP (15 nM) by using (A) amounts of Fe_3O_4@Tyr particles, (B) pH values, (C) phosphorylation temperature, and (D) phosphorylation time.

with Tyr on Fe_3O_4@Tyr particles could depend on the reaction time (**Fig. 3D**). Apparently, the current changes increased with the increasing reaction time and tended to be steady after 20 min to be stopped by magnetic separation. Such a reaction time is thus chosen to achieve the sufficient DDVP phosphorylation of Fe_3O_4@Tyr particles.

3.5 Detection selectivity and stability for electrochemical responses to DDVP with Fe_3O_4@Tyr particles

To determine the levels of OPs in complicated samples like blood, the potential interfering substances including common ions, vitamins, and amino acids with the similar levels in blood were electrochemically tested (**Fig. 4A**). Compared with the current changes for the OP solutions, no apparent current changes were observed for Na^+, K^+, Ca^+, Mg^{2+}, Fe^{2+}, Vc, HSA, and several kinds of amino acids indicated. Importantly, they could have no influence on the electrochemical responses to OP, even they co-exist with high concentrations (data not shown). Accordingly, these common components in blood might have negligible impacts on the detection of OPs. The high detection selectivity

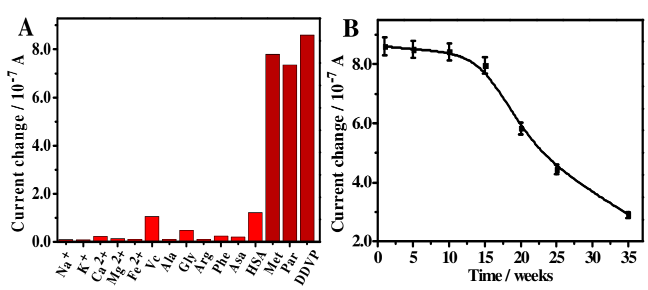


Fig. 4 (A) The detection selectivity of Fe_3O_4@Tyr particles-based electrochemical responses toward OPs (including Met, Par, and DDVP of 15 nM), Na^+, K^+, Ca^{2+}, Mg^{2+}, Fe^{2+}, Vc, Ala, Gly, Arg, Phe, Asa, and HSA, each with the concentrations similar to the levels in blood; (B) the detection stability of electrochemical responses to DDVP for the electrodes modified with Fe_3O_4@Tyr particles stored over time.

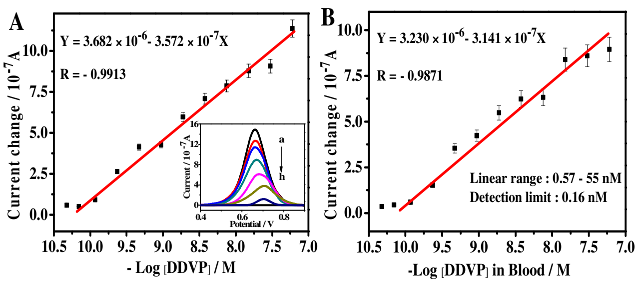


Fig. 5 Calibration curves of the relationships between the Fe_3O_4@Tyr particles-based electrochemical responses and DDVP of different concentrations in (A) water (insert: real-time LSV response profiles), and (B) blood, where the inserted a-g curves correspond to 0.0, 0.235, 0.470, 1.88, 15.00, 60.00, and 500 nM DDVP, and h curve refers to the electrode baseline.

for OP exposures can thereby be expected, presumably resulting from the specific and strong phosphorylation between Tyr and OPs,[14,22] which in turn could induce the unique inhibition of Tyr electro-activities. Moreover, as shown in **Fig. 4A**, three kinds of OPs (Met, Par, and DDVP) could display approximately high electrochemical responses, suggesting that the proposed method can determine the total amounts of OPs in various media.

Moreover, the detection stability of electrochemical responses to DDVP was evaluated for the electrodes with Fe_3O_4@Tyr particles (**Fig. 4B**). Here, Fe_3O_4@Tyr particles were stored over time in a refrigerator at 4 °C to be taken out at different time intervals for DDVP treatments and further electrochemical tests. As can be seen from **Fig. 4B**, no significant change of electrochemical signals was monitored up to ten weeks. Such a high stability for the electrochemical DDVP detections might be related to the considerably strong interaction between DDVP and Tyr on Fe_3O_4@Tyr particles, as well as its unique merit of irreversible electrochemical oxidization. The above results indicate that the prepared Fe_3O_4@Tyr electrodes could present high detection stability for free OPs.

3.6 Electrochemical detections of DDVP samples with magnetic Fe_3O_4@Tyr electrodes

Under the optimized conditions, DDVP samples with different concentrations in Tris-HCl buffer were examined by the "lab-on-a-particle"-based detection method (**Fig. 5A**). It can be observed that the current responses could decrease with the increasing of DDVP concentrations, as shown in the insert in **Fig. 5A**. A linear relationship was obtained for the electrochemical responses over –log [DDVP] of DDVP concentrations ranging from 0.12 - 60 nM (R = 0.9913), with the detection limit of about 0.056 nM, estimated according to 3σ rule. The detection linearity range and limit of the developed method were compared with those of the electrochemical assays for DDVP reported elsewhere (**Table 1**), showing the better or comparable detection sensitivity. Herein, the high sensitivity and reproducibility of DDVP detections might presumably result from the highly-sensitive and stable phosphorylation of Tyr on Fe_3O_4@Tyr particles, in addition to highly sensitive electrochemical LSV outputs as aforementioned.

Moreover, the Fe_3O_4@Tyr particles-based method was used to probe the levels of DDVP samples spiked in blood (**Fig. 5B**). Herein, Fe_3O_4@Tyr particles were added to the diluted blood samples containing different concentrations of DDVP, and then magnetically immobilized on the magnetic electrodes for electrochemical measurements under the optimal conditions. The linear detection relationship between the electrochemical responses and –log [DDVP] in blood was obtained over the DDVP concentration range of 0.57 - 55 nM (R = 0.9871), with the detection limit of about 0.16 nM. Therefore, the application feasibility of the developed "lab-on-a-particle"-based detection strategy for the early warning and diagnosis of OP exposures was demonstrated by probing the direct biomarkers of free OPs in blood with high sensitivity and reproducibility.

Table 1 Comparison of detection performances among different electrochemical assays for DDVP detections

Detection methods	Linear ranges (M)	Detection limits (M)	Refs.
Amperometry	ND	1.0×10^{-10}	35
Amperometry	ND	7.0×10^{-12}	36
Amperometry	4.52×10^{-11}-4.52×10^{-8}	1.13×10^{-11}	37
Amperometry	Up to 8×10^{-6}	6.0×10^{-8}	38
LSV	1.2×10^{-10}- 6.0×10^{-8}	5.6×10^{-11}	This study

4. Conclusions

Electrochemically active Tyr was successfully bound onto Fe_3O_4 particles to form Fe_3O_4@Tyr particles as capturing probes for detecting free DDVP in blood by electrochemical outputs. The sensitive and specific phosphorylation-induced inhibition of the electro-activities of Tyr on Fe_3O_4@Tyr particles could allow for the electrochemical detections of free OP with high sensitivity and selectivity. Also, the strong interactions between Tyr and OPs especially irreversible electrochemical oxidization of Tyr probe could facilitate the OP evaluation with high stability over time. Moreover, the magnetic separation-based detection of the direct phosphorylation biomarkers of free OPs in complicated media could be achieved without any sample purification. Particularly, this simple and rapid detection procedure with portable electrochemical transducer device can allow for the field-deployable or on-site OP monitoring. Therefore, such a "lab-on-a-particle" detection strategy may find wide applications for the early warning or diagnosis of the exposures to OPs in environment (i.e. pesticides), warfield (i.e., nerve agents), and clinical laboratories.

Acknowledgments

This work is supported by the National Natural Science Foundations of China (No. 21375075, 21302109, and 21302110), the Taishan Scholar Foundation of Shandong Province, and the Natural Science Foundation of Shandong Province (ZR2013BQ017 and ZR2013BM007), P. R. China.

Notes and references

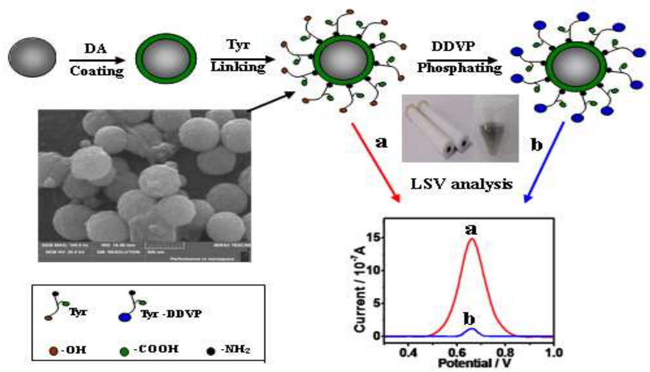
Shandong Province Key Laboratory of Life-Organic Analysis, School of Chemistry and Chemical Engineering, Qufu Normal University, Qufu City, Shandong Province 273165, P. R. China.

1. S. Young, L. Balluz and J. Malilay, *Sci. Total Environ*., 2004, 322, 3-20.
2. R. G. Hendrickson and J. R. Hedges, *Crit Care Clin.*, 2005, 21, 641-652.
3. G. Liu, J. Wang, R. Barry, C. Petersen, C. Timchalk, P. L. Gassman and Y. Lin, *Chem.-A Eur. J.*, 2008, 14, 9951-9959.
4. H. Wang, J. Wang, C. Timchalk and Y. Lin, *Anal. Chem.*, 2008, 80, 8477-8484.
5. M. Liang, K. Fan, Y. Pan, H. Jiang, F. Wang, D. Yang, D. Lu, J. Feng, J. Zhao, L. Yang and X. Yan, *Anal. Chem.*, 2013, 85, 308-312.
6. X. Zhang, H. Wang, C. Yang, D. Du and Y. Lin, *Biosens. Bioelectron.*, 2013, 41, 669-674.
7. D. Noort, H. P. Benschop and R. M. Black, *Toxicol. Appl. Pharm.*, 2002, 184, 116-126.
8. R. W. Read and R. M. Black, *J. Chromatogr. A.*, 1999, 862, 169-177.
9. A. Fidder, A. G. Hulst, D. Noort, R. De Ruiter, M. J. Van der Schans, H. P. Benschop and J. P. Langenberg, *Chem. Res. Toxicol.*, 2002, 15, 582-590.
10. M. Polhuijs, J. P. Langenberg and H. P. Benschop, *Toxicol. Appl. Pharm.*, 1997, 146, 156-161.
11. A. Gahlaut, A. Gothwal, A. K. Chhillar and V. Hooda, *Open J. Appl. Biosens.* 2012, 1, 1-8.
12. D. Du, J. Wang, J. N. Smith, C. Timchalk and Y. Lin, *Anal. Chem.*, 2009, 81, 9314-9320.
13. O. A. Sadik, W. H. Land and J. Wang, *Electroanalysis*, 2003, 15, 1149-1159.
14. N. H. Williams, J. M. Harrison, R. W. Read and R. M. Black, *Arch. Toxicol.*, 2007, 81, 627-639.
15. H. Li, J. Li, Z. Yang, Q. Xu and X. Hu, *Anal. Chem.*, 2011, 83, 5290-5295.
16. J. Cocker, H. J. Mason, S. J. Garfitt and K. Jones, *Toxicol. Lett.*, 2002, 134, 97-103.
17. A. J. J. Ooms and C. Van Dijk, *Biochem. Pharmacol*., 1966, 15, 1361-1377.
18. H. L. Boter and A. J. J. Ooms, *Biochem. Pharmacol.*, 1967, 16, 1563-1569.
19. D. J. Ecobichon and A. M. Comeau, *Toxicol. Appl. Pharm.*, 1973, 24, 92-100.
20. M. K. Johnson, *Arch. Toxicol.*, 1975, 34, 259-288.
21. M. K. Johnson and P. Glynn, *Toxicol. Lett.*, 1995, 82, 459-463.
22. R. M. Black, J. M. Harrison and R. W. Read, *Arch. Toxicol.*, 1999, 73, 123-126.
23. N. Qu, B. Wan and L. H. Guo, *Analyst*, 2008, 133, 1246-1249.
24. K. Kerman, M. Vestergaard, M. Chikae, S. Yamamura and E. Tamiya, *Electrochem. Commun.*, 2007, 9, 976-980.
25. G. A. Evtugyn, H. C. Budnikov and E. B. Nikolskaya, *Talanta*, 1998, 46, 465-484.
26. G. Liu, Y. Lin, J. Wang, H. Wu, C. M. Wai and Y. Lin, *Anal. Chem.*, 2007, 79, 7644-7653.
27. M. Pumera, S. Sanchez, I. Ichinose and J. Tang, *Sensor. Actuat. B-Chem.*, 2007, 123, 1195-1205.
28. G. Liu and Y. Lin, *Talanta*, 2007, 74, 308-317.
29. J. Wang, *Analyst*, 2005, 130, 421-426.
30. S. Liu, R. Xing, F. Lu, R. K. Rana and J. Zhu, *The J. Phys. Chem. C.*, 2009, 113, 201042-201047.
31. V. Brabec and V. Mornstein, *Biophys. Chem.*, 1980, 12, 159-165.
32. N. C. Reynolds, B. M. Kissela and L. H. Fleming, *Electroanalysis*, 1995, 7, 1177-1181.
33. J. A. Reynaud, B. Malfoy and A. Bere, *J. Electroanal. Chem. Interfac.*, 1980, 116, 595-606.
34. H. P. Permentier, U. Jurva, B. Barroso and A. P. Bruins, *Rapid commun. Mass spectrom: RCM*, 2003, 17, 1585-1592.
35. A. Vakurov, C. E. Simpson, C. L. Daly, T. D. Gibson and P. A. Millner, *Biosens. Bioelectron.*, 2004, 20, 1118-1125.
36. G. Valdés-Ramírez, D. Fournier, M. T. Ramírez-Silva and J.-L. Marty, *Talanta*, 2008, 74, 741-746.
37. S. Sotiropoulou, D. Fournier and N. A. Chaniotakis, *Biosens. Bioelectron.*, 2005, 20, 2347-2352.
38. J. C. Vidal, S. Esteban, J. Gil and J. R. Castillo, *Talanta*, 2006, 68, 791-799.

The Table of Contents:

A Phosphorylation-Sensitive Tyrosine-Tailored Magnetic Particle for Electrochemically Probing Free Organophosphates in Blood

Yanmei Si, Ning Zhang, Zongzhao Sun, Shuai Li, Liyang Zhao, Rui Li, Hua Wang *



Phosphorylation-sensitive tyrosine was coated onto Fe_3O_4 particles, resulting in a "lab-on-a-particle"-based electrochemical detection protocol for probing free organophosphates in blood.