Talanta 168 (2017) 62–66

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Nano-encapsulant of ascorbic acid-loaded apoferritin-assisted photoelectrochemical sensor for protease detection

Jiexia Chen[a,b], Guang-Chao Zhao[a,*]

[a] Anhui Key Laboratory of Chem-Biosensing, School of Chemistry and Materials Science, Anhui Normal University, Wuhu 241000, PR China
[b] Departement of Chemistry, Wannan Medical College, Wuhu 241002, PR China

ARTICLE INFO

Keywords:
Photoelectrochemistry
Trypsin sensing
Protease
Apoferritin

ABSTRACT

A signal-on photoelectrochemical (PEC) biosensor based on nano-encapsulant of ascorbic acid-loaded apoferritin-assisted for protease detection is described. The loaded ascorbic acid could be released from the central cavity of apoferritin into the buffer solution as sacrificial electron donor to capture the photo-generated holes of CdTe quantum dots when light is turned on. In this system, the biosensor relied on monitoring the photocurrent intensity as a result of enzymatic substrate proteolysis in the homogeneous aqueous solution. A low detection limit of 2.7 ng mL^{-1} for trypsin in the linear range from 30 to 450 ng mL^{-1} is achieved. We believe that such a sensing system not only holds the potential ability as a probe for trypsin activity assay, but also could be used for the corresponding inhibitor-screening. It might provide a potentially feasible alternative tool for determining trypsin in human serum in a clinical laboratory. The established method could open a different perspective for PEC enzyme detection and provide a new platform for future development of PEC analysis with other clinically important proteins.

1. Introduction

In the last years, special attention has been focused on detecting proteases for their crucial roles in numerous biological systems such as blood coagulation, digestion, fibrinolysis and complement. They can catalyze the hydrolysis of amide bonds at specific sites in peptides and proteins [1,2]. The most common methods for proteases detection include enzyme-linked immunosorbent assay (ELISA) [3,4], high performance liquid chromatography (HPLC) [5], gel electrophoresis [6], fluorescence [7,8] and electrochemical methods [9,10]. However, only a few reports utilized photoelectrochemical methods [11]. That may be because enzyme catalyzed reactions of the enzymatic hydrolysis process between enzyme solution and the substrate on the surface of the solid electrode were liquid-solid heterogeneous reactions, making them unsuitable for diagnostic applications. To overcome these difficulties, it is urgent to exploit a novel photoelectrochemical sensor for protease detection in a homogeneous system.

Photoelectrochemistry (PEC) sensing analysis has been proven to be a newly developed and promising analytical tool for tracing biomolecules determination for its unique advantages of both optical and electrochemical methods with high sensitivity and low background signals [12–14]. Its sensing principle was based on that upon illumination, electrons in semiconductor quantum dots (QDs) such as CdTe [15], CdS [16] and TiO_2/CdTe NPs [17] are excited, forming electron-hole pairs. If electron donors present in the surrounding solution, the transfer of electrons from the solution to the valence-band (VB) of the QDs and the ejection of electrons from the conduction-band (CB) to the electrode occurs, yield an anodic photocurrent. It was shown that the amplitude of the resulting photocurrent was determined by the concentration of the donor compounds in a certain range [18–21]. Among kinds of electron donor species, ascorbic acid (AA) was widely used as a nontoxic and efficient electron donor under mild solution medium [22] than other commonly used electron donors, such as Na_2SO_3 [23], Polysulfide (Na_2S and S) [24], and triethanolamine (TEA) [25]. In most of the previous works, the AA was added into the systems directly in advance [22]. However, we wanted to develop a templated synthesis strategy based on a protein cage to prepare AA encapsulation, and then enzymatic hydrolysis the encapsulation, in situ to release AA. In this system, the sensor relied on monitoring the photocurrent intensity as a result of enzymatic substrate proteolysis in the homogeneous aqueous solution. Thus, we may obtain a sensitive and specific enzyme detection in an indirect way, which could widen the range of sensor detection.

In this work, we exploited apoferritin (APO) as a protein cage. It has been reported that APO is a spherical hollow shell of the iron-storage protein ferritin. The hollow structure is a protein comprising of 24

* Corresponding author.
E-mail address: gczhao@mail.ahnu.edu.cn (G.-C. Zhao).

http://dx.doi.org/10.1016/j.talanta.2017.03.002
Received 13 December 2016; Received in revised form 28 February 2017; Accepted 2 March 2017
Available online 07 March 2017

polypeptide subunits and has an interior and exterior diameter of 8 nm and 12 nm, respectively [26–29]. The central cavity is empty in APO that can accommodate any molecules of interest. AA was used as an efficient electron donor in the experiment. The encapsulation of AA inside the APO cavity (formation of APOAA) is realized in the fact that the assembly-disassembly mechanism of the APO protein cage undergoes pH dependent [29]. Interestingly, when APOAA was mixed into detection buffer including trypsin, the APOAA could be catalytically hydrolyzed by trypsin and the loaded AA could be released from the central cavity of APO as sacrificial electron donor to capture the photo-generated holes of CdTe QDs, leading to an increased photocurrent response. Thus, using trypsin, which has been considered as a biomarker for pancreatic diseases and health conditions as a model [30–32], we can build a simple and signal-on enzyme biosensor, which can be used for low concentration detection of trypsin. To the best of our knowledge, no reports have focused on the enzyme-catalyzed in situ release of AA for detection of enzyme in a homogeneous system for PEC biosensor. The established method could open a different perspective for PEC enzyme detection in the future.

2. Experimental section

2.1. Chemicals and materials

Sodium metaborate ($NaBH_4$), sodium hydroxide (NaOH), and cadmium chloride ($CdCl_2$•$2.5H_2O$) were all obtained from Shanghai Chemical Reagent Co. (China). Poly (diallyldimethylammonium chloride) PDDA (20%, w/w in water, molecular weight 200 000–350 000), ascorbic acid (AA), 3-Mercaptopropionic acid (MPA), Te power were purchased from Aladdin Chemistry Co. Ltd (Shanghai, China). APO was bought from Sigma-Aldrich (America). Phosphate buffer (PBS, pH 7.8, 10 mM) was used for incubating trypsin and the photocurrent measurement. All other reagents were of analytical grade and used as received. All aqueous solutions were prepared using deionized water (18 MΩ·cm^{-1}), which was obtained from a Milli-Q water purification system.

2.2. Preparation of ascorbic acid-loaded APO nano-encapsulant (APOAA)

The APOAA was prepared using a modified version of previous report [33] with minor modifications and the procedure was shown in Scheme 1A. In detail, a 100 μL APO solution (50 mg mL^{-1}, equine spleen, from Sigma-Aldrich) was diluted with 1 mL 0.1 M PBS (pH 7.4). Then 0.1 M HCl solution was slowly added and the solution pH was adjusted to 2.0 with continuous stirring. The solution was further stirred for 30 min to make the APO completely disassociated. Then, 100 μL AA solutions with different concentrations were added into APO solution dropwise with stirring to allow AA to diffuse around the subunits of the APO. And then, the solution pH was adjusted to 8.5 with 0.1 M NaOH. The obtained mixture was stirred for another 3 h at the room temperature to allow AA to enter the core of the APO. Subsequently, a dialysis for 36 h was realized to remove free AA using MD10 dialysis bag (Union Carbide Corporation, USA) with a molecular weight cutoff (MWCO) of 8000–14000 against 0.1 M PBS (pH 7.8). The dialysis process was repeated for three times. The absorbance of free AA, which caused by the incomplete encapsulation, and then removed to the solution using dialysis, is tested by UV–Vis Spectrophotometer. The obtained APOAA solution was stored in amber glass bottles at 4 °C for further use.

2.3. Preparation of modified electrodes

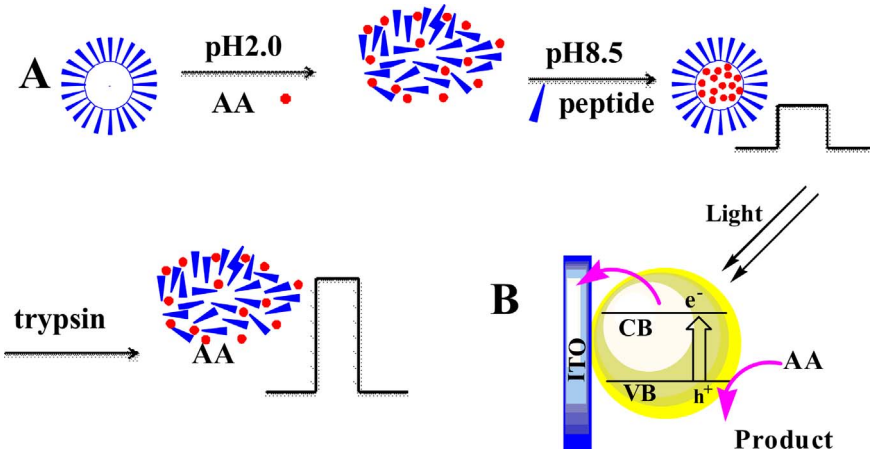
CdTe QDs functionalized with MPA were prepared according to the previous protocol [34]. The indium-tin oxide (ITO) slices were sonicated in sequence with acetone, water, ethanol, water, and NaOH (1 M) in 1:1 (v/v) ethanol/water for about 15 min each, respectively. The preparation process of modified electrodes by the layer-by-layer self-assembly technology followed the previously described procedure [34]. By alternately immersing the ITO slices into a solution of 2% PDDA including 0.5 M NaCl and the QDs solution for 15 min, respectively for repeating three times, we obtained simple QDs-modified ITO electrodes. After each dipping step, the ITO slices were washed with deionized water carefully. The obtained ITO slices were used as the working electrodes and stored at 4 °C in a dark environment before use.

2.4. Trypsin detection

The measurement mechanism of the sensing design was shown in Scheme 1A. For detection of trypsin, the working electrodes were immersed into detection buffer (0.1 M PBS, pH 7.8) containing APOAA showed a baseline photocurrent. When adding different concentrations of trypsin into detection buffer and incubated for some minutes, the loaded AA could be released from the cavity of APO into the detection buffer as sacrificial electron donor to capture the photo-generated hole of CdTe QDs, leading to a prominently increased photocurrent response. Thus, a simple and signal-on enzyme biosensor which can be used for trypsin determination was obtained.

2.5. Apparatus

A 500 W Xe lamp equipped with a monochromator was used as the visible light source. The PEC measurements were performed on a 660b



Scheme 1. Schematic diagram of the fabrication process (A) and electron transfer mechanism of the PEC enzyme biosensor (B).

electrochemical workstation (CHI, Shanghai, China) with a conventional three-electrode system: a CdTe QDs-modified ITO electrode with an area of 0.25 cm^2 as the working electrode, a platinum wire and a saturated Ag/AgCl electrode as the auxiliary and reference electrodes.. All the photocurrent measurements were performed at a constant potential of −0.5 V with detection buffer containing 4×10^{-9} M APOAA as the supporting electrolyte. The UV–Vis absorption spectra were performed with a 757 CRT UV–Vis Spectrophotometer (Shanghai, China).

3. Results and discussion

3.1. UV–Vis characterization of APOAA

APOAA solutions encapsulated with different concentrations of AA were observed. The absorbance of free AA, which caused by the incomplete encapsulation, and then removed to the solution using dialysis was tested by UV–Vis absorption spectra. More AA was used for encapsulation reaction with increasing concentrations, leading to the increased absorbent intensity. According to calculation results of Bouguer-Lambert-Beer law, the amount of encapsulated AA and disrobed AA, which was still remaining in the dialysis solution of the sample of APOAA even after the purification by dialysis, was displayed in the Fig. S1. It was examined that the encapsulation yield increased with the increasing applied AA concentration. When joining the maximum amount of AA, by calculation, a molecular APOAA can hold about 4000 molecules AA. Although all these APOAA could use for the detection of trypsin, we chose the highest AA content APOAA for use in the subsequent experiments.

As far as we know, AA readily undergoes oxidation reduction reaction as soon as it is in contact with other solvents. Oxidized AA would produce a bad effect on the experimental results. In order to investigate whether the AA remains unoxidized when it being loaded during nano-encapsulation, a control experiment was performed under the same measuring conditions. The result was shown in Fig. S2. The concentration of released AA from APOAA is about 4.02×10^{-5} M. Under this concentration, the change of the photocurrent intensity is 62 ± 2 nA. That is consistent with the result of the control experiment, in which the photocurrent was measured under the same concentration of fresh prepared AA solution. This result confirmed that the AA remains unoxidized when being loaded during nano-encapsulation. It also revealed that the photocurrent was relative to AA released from APOAA.

3.2. Characterization of the fabrication process of the biosensor

The entire constructed strategy of the biosensor was characterized by PEC measurements. As shown in Fig. 1A, after successful assembly of $(PDDA/QDs)_3$ on the surface of the ITO electrode, the photocurrent varied slightly (~20 nA) in 0.1 M PBS (pH 7.8) without AA (curve b) when compared with the bare ITO electrode (curve a), indicating that although CdTe QDs have excellent light response, photocurrent was very small when there was no electron donor. Then the prepared modified electrodes were immersed in the solution of APO or APOAA and incubated for certain time, the photocurrent increased a very small value (~5 nA) (curves c and d) and there was no obvious difference between them. But after adding the same concentration of trypsin to the mixture, photocurrent response of APO and APOAA showed a very big difference (~56 nA) (curves e and f). When adding trypsin into detection buffer, the APO and APOAA both could be catalytically hydrolyzed by trypsin. In contrast to the APO, the loaded AA could be released from APOAA into the detection buffer as a sacrificial electron donor, yielding the improved photocurrent [35]. This photocurrent generation mechanism can be described as in Scheme 1B. The PEC measurements further demonstrated the successful construction of the PEC enzyme biosensor.

3.3. Mechanism for the detection of trypsin

Usually, the enzyme reaction of a certain enzyme concentration is dependent on the reaction time under certain experimental conditions [36]. Therefore we researched the trypsin-catalyzed hydrolysis reaction as a function of time so as to display the applicability of using APOAA based test for a real-time trypsin activity monitoring. Fig. 1B showed the correlation between the increase of photocurrent intensity of CdTe QDs and time based on adding different concentrations of trypsin over the range of 30–450 ng mL^{-1} in the tests. All the tests demonstrated photocurrent increased at different rates and up to different levels throughout the whole time period. Generally speaking, the higher concentration of the enzyme, the faster the reaction proceeded and the higher photocurrent intensity was achieved by the test. It is remarkable to point out that the test showed the process is enzymatic catalysis. The mechanism for the detection of trypsin relied on monitoring the photocurrent intensity as a result of enzymatic hydrolysis of APOAA [36]. These results further demonstrated the successful construction of the PEC biosensor for trypsin detection from another aspect.

3.4. Detection of trypsin

Under the optimal conditions in PBS buffer containing different concentrations of trypsin with incubation time of 60 min (Fig. S3D), incubation temperature of 37 °C (Fig. S3C) and the applied potential of −0.5 V (Fig. S3B), the photocurrent variation of the new PEC biosensor to the analyte was demonstrated (Fig. 2) with the excitation wavelength of 400 nm (Fig. S3A). The photocurrent intensity gradually increased with increasing trypsin concentrations. A good linear relationship presenting the photocurrent increases versus trypsin concentration in logarithmic scale ranging from 30 to 450 ng mL^{-1} was observed. The regression equation was $\Delta I = -40.6+39.8\lg C_{trypsin}$ (ng mL^{-1}) with a correlation coefficient of 0.997, where ΔI is the increased photocurrent responses of the PEC biosensor after and before incubating in trypsin of elevated concentrations, respectively, and C is the concentration of trypsin. The detection limit was calculated to be 2.7 ng mL^{-1}. These results showed that the proposed strategy might provide a potentially feasible alternative tool for determining trypsin in human serum in a clinical laboratory.

3.5. The screen of inhibitor

To further confirm the analytic performance of the exploited biosensor, the inhibition experiments were carried out in the presence of a series of different concentrations of the Bowman-Birk inhibitor (BBI) [11]. As a broad-spectrum trypsin inhibitor, the BBI can inhibit the cleavage of arginines by inactivating trypsin. Consequently, photocurrent would not increase when the corresponding inhibitors of trypsin are present in the solution. As shown in Fig. 3, with the increasing of inhibitor concentration, the inhibition efficiency of the BBI gradually rose. The value of the half-inhibitory concentration IC_{50} (the inhibitor concentration required to inhabit 50% of the enzyme activity) of BBI toward trypsin was estimated to be 140 ng mL^{-1}. This IC_{50} value of BBI is lower than that previously reported [37]. All these results obviously indicated that the PEC biosensor not only holds the potential ability as a probe for trypsin activity assay, but also could be used for the corresponding inhibitor-screening.

3.6. Selectivity, repeatability and stability of the biosensor

The selectivity of this biosensor for trypsin at 450 ng mL^{-1} was evaluated over other enzymes. Contrast experiments were performed for 450 ng mL^{-1} collagenase, lysozyme and thrombin. As can be seen in Fig. 4, the photocurrent responses from these protein molecules were much lower than that of the target enzyme, indicating that the PEC analysis method has satisfactory selectivity. Moreover, the assay

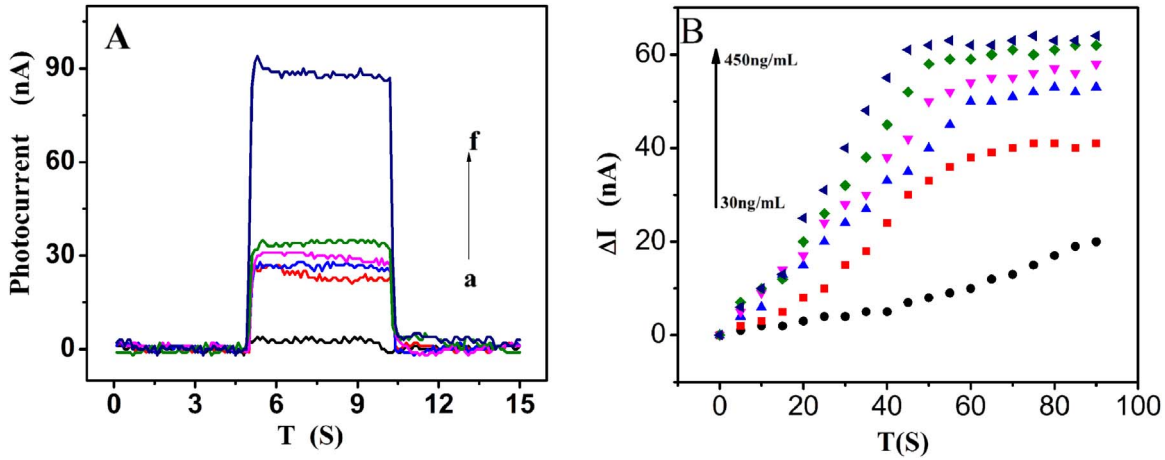


Fig. 1. (A) The photocurrent variation during the modified ITO electrodes assembly process (*a*-f): bare ITO, ITO/QDs, ITO/QDs/APO, ITO/QDs/APOAA, ITO/QDs/APO/trypsin (300 ng mL^{-1}), and ITO/QDs/APOAA/trypsin (300 ng mL^{-1}). (B) Photocurrent intensity changes of the signal-on PEC assays after the addition of different concentrations of trypsin (30, 75, 150, 225, 300 and 450 ng mL^{-1}). The photocurrent measurements were carried out in 0.1 M PBS (pH 7.8) containing 4×10^{-9} M APOAA with −0.5 V working potential and 400 nm light on and off.

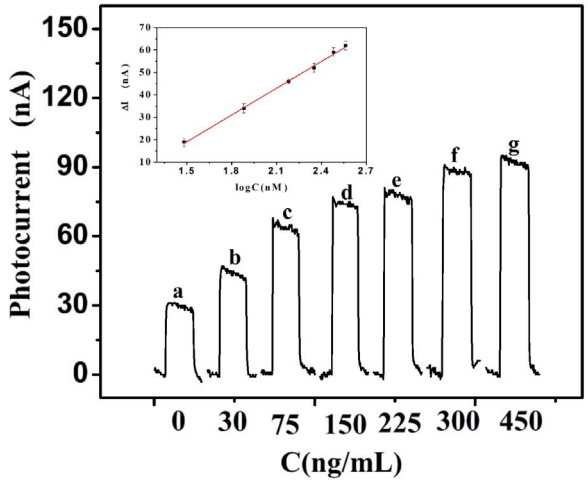


Fig. 2. PEC response of the biosensor to a–g: 0, 30, 75, 150, 225, 300, and 450 ng mL^{-1} trypsin, respectively. Inset: The calibration curve of the photocurrent increment versus lg [trypsin]. The photocurrent measurements were carried out in 0.1 M PBS (pH 7.8) containing 4×10^{-9} M APOAA with −0.5 V working potential and 400 nm light on and off.

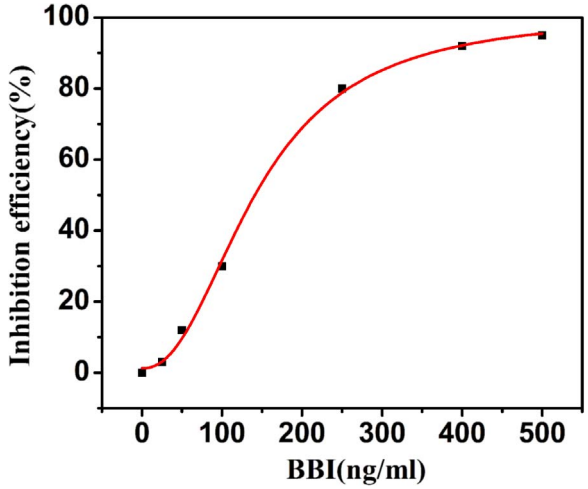


Fig. 3. The plot of inhibition efficiency vs. the concentration of BBI.

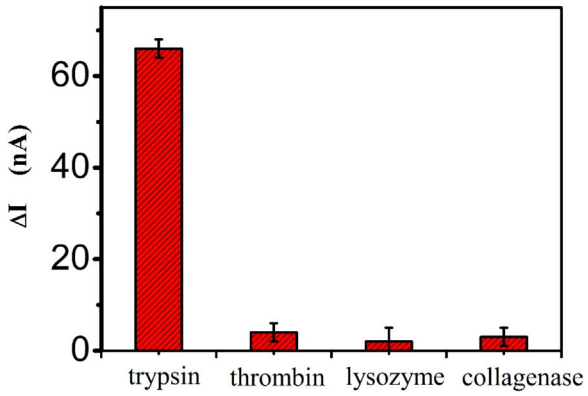


Fig. 4. Selectivity of the designed biosensor at 450 ng mL^{-1} of collagenase, lysozyme and thrombin and the photocurrent change value of trypsin. Error bars show the four times the standard deviation of the measurement results.

precision of biosensor was also measured by freshly prepared modified ITO electrodes. With trypsin at concentrations of 75 and 150 ng mL^{-1}, the PEC biosensor showed good repeatability with relative standard deviations of 5.5% and 2.8%, respectively. These results indicated that the method had good fabrication reproducibility. The long-term stability of the biosensor was also examined. After the modified electrodes were stored at 4 °C for two week, the photocurrent intensity didn't show any obvious changes because of its simple production process via layer-by-layer assembly. These results demonstrated that the electrodes possessed high stability, which displayed that the electrodes were suitable for the fabrication of PEC biosensors.

3.7. Trypsin detection in biological samples

The feasibility of the proposed biosensor for clinical application was investigated on several biological samples of healthy human serum. The samples were prepared according to the previous protocol [38] by adding certain amount of trypsin into serum that was provided by Center Blood Station of Wuhu. The results were shown in Table 1. It was found that the recoveries were in the range from 89% to 102%, which indicated that the precision of the proposed method was satisfactory. These results demonstrated the potential applicability of the developed PEC biosensor for detecting trypsin in serum samples.

Table 1
Analytical results of trypsin in biological samples.

Sample	Trypsin added (ng mL^{-1})	Trypsin found (ng mL^{-1})[a]	Recovery (%)
serum 1	0	–	–
serum 2	30	29 ± 0.1	97
serum 3	75	73 ± 0.3	97
serum 4	150	134 ± 0.3	90
serum 5	225	200 ± 0.4	89
serum 6	300	305 ± 0.5	102

[a] Mean of three detections.

4. Conclusions

In summary, it is possible to obtain a novel signal-on PEC biosensor for the sensitive determination of trypsin using AA, which in situ release by protease enzymolysis of APOAA as the sacrificial electron donor. The APOAA could be catalytically hydrolyzed by trypsin and the loaded AA could be released from the cavity into the detection buffer as sacrificial electron donor to arrest the photo-generated hole of CdTe QDs, resulting in an increased photocurrent response. This PEC biosensor has the potential ability not only could be used as a probe for trypsin activity assay, but also for the corresponding inhibitor-screening. It might provide a potentially feasible alternative tool for determining trypsin in human serum in a clinical laboratory. To the best of our knowledge, no reports have focused on the enzyme-catalyzed in situ releasing of AA for detection of enzyme in a homogeneous system for PEC biosensor. The strategy of employing in situ enzymaticlysis to detect the enzyme opens up new avenues for PEC sensing.

Acknowledgments

We gratefully acknowledge the National Natural Science Foundation of China (20975001 and 21645006) and the Young Program of Wannan Medical College (No. WK201510 and 201319).

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.talanta.2017.03.002.

References

[1] O.D. Ratnoff, Perspect. Biol. Med. 31 (1987) 4–33.
[2] C. Angeletti, V. Khomitch, R. Halaban, D.L. Rimm, Diagn. Cytopathol. 31 (2004) 33–37.
[3] Z.D. Zhang, P.J. Zhang, Z.L. Mo, C.L. Wang, Y. Yu, Acta Oceanol. Sin. 24 (2005) 155–161.
[4] I.E. Tothill, Semin. Cell Dev. Biol. 20 (2009) 55–62.
[5] N.H. Schebb, T. Vielhaber, A. Jousset, U. Karst, J. Chromatogr. A 1216 (2009) 4407–4415.
[6] S.D. Lamore, S. Qiao, D. Horn, G.T. Wondrak, Photochem. Photobiol. 86 (2010) 1307–1317.
[7] H. Ao, Z. Qian, Y. Zhu, M. Zhao, C. Tang, Y. Huang, H. Feng, A. Wang, Biosens. Bioelectron. 86 (2016) 542–547.
[8] P.M. Shih, T.K. Liu, K.T. Tan, Chem. Commun. 49 (2013) 6212–6214.
[9] K.L. Gemene, M.E. Meyerhoff, Anal. Biochem. 416 (2011) 67–73.
[10] N. Xia, P. Peng, S. Wang, J. Du, G. Zhu, W. Du, L. Liu, Sens. Actuators B 232 (2016) 557–563.
[11] Y.F. Liu, J.X. Chen, M.Q. Xu, G.C. Zhao, Int. J. Electrochem. Sci. 9 (2014) 4014–4023.
[12] Y. Li, L. Sun, Q. Liu, E. Han, N. Hao, L. Zhang, S. Wang, J. Cai, K. Wang, Talanta 161 (2016) 211–218.
[13] G. Wen, H. Ju, Talanta 134 (2015) 496–500.
[14] Y. Guo, Y. Sun, S. Zhang, Chem. Commun. 47 (2011) 1595–1597.
[15] H. Feng, L. Zhou, J. Li, T. Tran.T, N. Wang, L. Yuan, Z. Yan, Q. Cai, Analyst 138 (2013) 5726–5733.
[16] E. Golub, A. Niazov, R. Freeman, M. Zatsepin, I. Willner, J. Phys. Chem. C 116 (2012) 13827–13834.
[17] Y.J. Li, M.J. Ma, J.J. Zhu, Anal. Chem. 84 (2012) 10492–10499.
[18] W. Tu, Y. Dong, J. Lei, H. Ju, Anal. Chem. 82 (2010) 8711–8716.
[19] K. Wang, J. Wu, Q. Liu, Y. Jin, J. Yan, J. Cai, Anal. Chim. Acta 745 (2012) 131–136.
[20] Q. Hao, P. Wang, X. Ma, M. Su, J. Lei, H. Ju, Electrochem. Commun. 21 (2012) 39–41.
[21] G.L. Wang, H.J. Jiao, K.L. Liu, X.M. Wu, Y.M. Dong, Z.J. Li, C. Zhang, Electrochem. Commun. 41 (2014) 47–50.
[22] G.L. Wang, P.P. Yu, J.J. Xu, H.Y. Chen, J. Phys. Chem. C 113 (2009) 11142–11148.
[23] S.G. Hickey, D.J. Riley, J. Phys. Chem. B 103 (1999) 4599–4602.
[24] A.B. Ellis, S.W. Kaiser, M.S. Wrighton, J. Am. Chem. Soc. 98 (1976) 6855–6866.
[25] L. Sheeney-Haj-Ichia, B. Basnar, I. Willner, Angew. Chem. Int. Ed. 44 (2005) 78–83.
[26] I. Blazkova, H.V. Nguyen, S. Dostalova, P. Kopel, M. Stanisavljevic, M. Vaculovicova, M. Stiborova, T. Eckschlager, R. Kizek, V. Adam, Int. J. Mol. Sci. 14 (2013) 13391–13402.
[27] X. Liu, W. Wei, C. Wang, H. Yue, D. Ma, C. Zhu, G. Ma, Y. Du, J. Mater. Chem. 21 (2011) 7105–7110.
[28] Y. Takezawa, P. Böckmann, N. Sugi, Z. Wang, S. Abe, T. Murakami, T. Hikage, G. Erker, Y. Watanabe, S. Kitagawa, T. Ueno, Dalton Trans. 40 (2011) 2190–2195.
[29] M. Kim, Y. Rho, K.S. Jin, B. Ahn, S. Jung, H. Kim, M. Ree, Biomacromolecules 12 (2011) 1629–1640.
[30] H. Rinderknecht, Dig. Dis. Sci. 31 (1986) 314–321.
[31] S. Park, H. Yang, Analyst 139 (2014) 4051–4055.
[32] J.V. Olsen, S.E. Ong, M. Mann, Mol. Cell. Proteom. 3 (2004) 608–614.
[33] H. Yin, M. Wang, Y. Zhou, X. Zhang, B. Sun, G. Wang, S. Ai, Biosens. Bioelectron. 53 (2014) 175–181.
[34] J. Chen, Y. Liu, G.C. Zhao, Sensors 16 (135) (2016) 1–11.
[35] W.W. Zhao, J. Wang, J.J. Xu, H.Y. Chen, Chem. Commun. 47 (2011) 10990–10992.
[36] F.F. Zheng, J.F. Wu, G.C. Zhao, Anal. Methods 4 (2012) 3932–3936.
[37] R. Liu, Y. Tan, C. Zhang, J. Wu, L. Mei, Y. Jiang, C. Tan, J. Mater. Chem. B 1 (2013) 1402–1405.
[38] X. Wang, Y. Wang, H. Rao, Z. Shan, J. Braz. Chem. Soc. 23 (2012) 2011–2015.