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Short communication

Tyrosinase-encapsulated liposomes: Toward enzyme-induced *in situ* sensitization of semiconductor for sensitive photoelectrochemical immunoassay

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ABSTRACT

Liposomal photoelectrochemical (PEC) bioanalysis holds enormous potential for future sensitive PEC bioanalysis. With tyrosinase (Tyr) and TiO_2 as representative enzyme and electrode, respectively, this communication reports the elegant use of Tyr-loaded liposomes (TLL) toward *in situ* sensitization of the electrode and thereby the realization of ultrasensitive PEC immunoassay. Specifically, Tyr-encapsulated and detection antibody-functionalized liposomes were first prepared and used as the signal probe. The subsequent sandwich immunobinding could confine the functional liposomes, which were then lysed with surfactant to release the encapsulated Tyr. The free Tyr could then initiate the transformation of tyrosine to dopa, the latter could bind with the under-coordinated Ti sites, forming the stable dopa–Ti charge transfer complex and thus generating enhanced anodic photocurrent under visible light for signaling. Since different semiconductors and enzymes may be adapted into this format, this work is expected to stimulate more interest in the enzyme-induced activation of semiconductors for advanced liposomal PEC bioanalysis.

1. Introduction

Photoelectrochemical (PEC) bioanalysis is a newly emerged technique for advanced biomolecular detection due to its desirable properties such as simple instrumentation and high sensitivity (Hong et al., 2015; Tu et al., 2018; Zhao et al., 2015, 2017). During past years, plenty of efforts have been made to fabricate various semiconductor materials (Ge et al., 2019; Han et al., 2018), tailor specific biomolecule–semiconductor hybrid systems (Li et al., 2018; Wang et al., 2015), and developing innovative signaling strategies in corresponding bioanalytical applications (Wang et al., 2018; Zeng et al., 2018). In general, from the perspective of signal-on amplification, an effective strategy may involve reinforced light-harvesting, improved charge separation and transfer, accelerated surface redox reactions that allow for the enhanced photocurrent signaling (Wang et al., 2009; Zhao et al., 2013).

Among recent efforts, one of the latest trends is liposomal PEC bioanalysis, which holds enormous potential for future PEC bioanalysis due to its superior merits of high loading of functional species and amplification of photocurrent signals (Lin et al., 2017; Mei et al., 2017; Zhuang et al., 2017). In such a protocol, incorporation of numerous functional species in the liposomes and separation of biorecognition event from the signaling step could generally contribute to the sensitivity and minimize the undesirable effects to the maximum extent. Especially, the incorporation of enzymes with high biocatalytic activities and specificities could catalyze the efficient formation of particular signaling molecules and thus possess high capacities for high-performance bioanalysis (Zhang et al., 2017). Lately, using the alkaline phosphatase (ALP)-encapsulated liposomal label, Zhuang's group has

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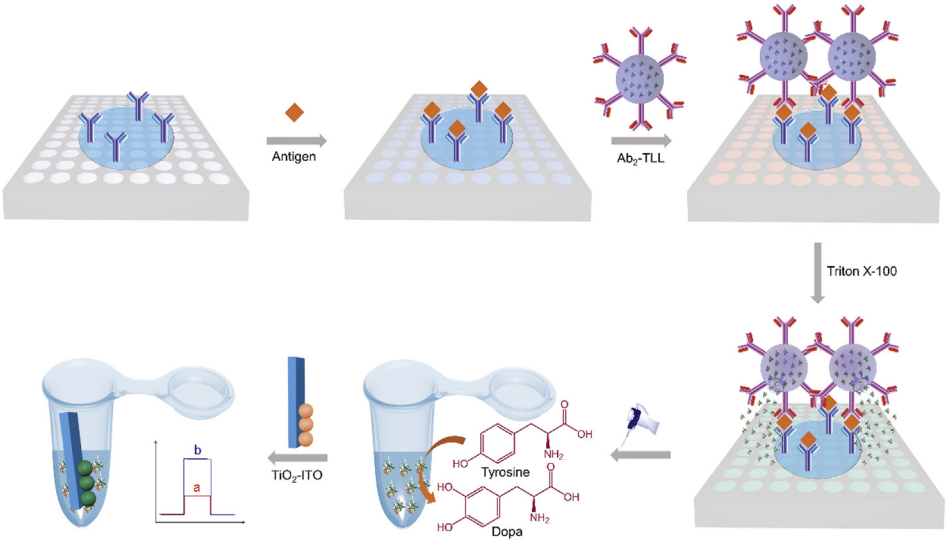

Scheme 1. Preparation and operation principle of the proposed liposomal PEC bioanalysis on the basis of Tyr-encapsulated liposomes.

exploited the first enzyme-supported liposomal PEC bioanalysis by steering ALP to catalyze the production of ascorbic acid (AA) as electron donor for the graphene/g-C_3N_4 photoelectrode (Zhuang et al., 2017). Despite the progress by this study, no other enzyme-supported liposomal PEC bioanalysis has been reported.

In this communication, with tyrosinase (Tyr) and TiO_2 as representative enzyme and electrode, respectively, we report the employment of Tyr-loaded liposomes (TLL) toward *in situ* sensitization of the electrode and thereby the realization of ultrasensitive PEC bioanalysis. Specifically, as illustrated in Scheme 1, TLL was fabricated further conjugated with antibodies as the signaling labels, while the TiO_2 nanoparticles (NPs) electrode was obtained from the liquid phase deposition (LPD) method. After the sandwich immunobinding, the confined TLL were destructed to release the Tyr, which could catalyze the oxidation of phenol derivatives to the respective catechol derivatives in the presence of O_2 (Yildiz et al., 2008). In present system, the Tyr can catalyze the transformation of tyrosine to dopa, which could coordinate with the undercoordinated Ti sites on the electrode, forming the stable dopa–Ti charge transfer complex and thus generating enhanced anodic photocurrent under visible light. Due to the target-dependent visible-light-responsibility, the low background of TiO_2 electrode, as well as the high efficiency of enzyme chemistry, an ultrasensitive liposomal PEC bioanalysis could be achieved which to our knowledge has not been reported. This work features the first enzyme-induced *in situ* sensitization of semiconductor for liposomal PEC bioanalysis. Because different semiconductors and many other enzymes may be utilized for similar sensitization reaction, this strategy might open up new horizons for liposomal PEC bioanalysis and biosensor design.

2. Results and discussion

2.1. Feasibility

The morphology information and optical properties of the as-prepared Tyr-loaded liposomes and LPD TiO_2 NPs electrode were characterized by transmission electron microscopy (TEM), scanning electron microscopy (SEM) and UV–vis absorption spectrum (See the Supporting Information for the experimental section). As shown in Fig. 1a and magnified image of Fig. S1, the TEM demonstrated that the size of TLL was ca. 45.0 nm, which was smaller than ca. 60.6 nm of the corresponding dynamic light scattering (DLS) results, as shown in Fig. 1a inset. The difference could be attributed to the shrinkage caused by the vacuum TEM condition. After one day and two months storage, Fig. 1b shows the corresponding zeta potentials of TLL measured to be −48 and −45 mV, respectively, indicating its highly stability. Besides, the Tyr-catalytic chemistry in this proposed system was also investigated using Tyrosinase activity assay kit with spectrophotometric method. Based on the activity of Tyr to catalyze L-dopa to generate Dopachrome with characteristic peak at 475 nm, as shown in Fig. 1c, no absorption peak at 475 nm was found in the absence of Tyr (curve a). In contrast, a distinct absorption peak at 475 nm was observed in the presence of 150 U mL^{-1} Tyr (curve b). As expected, the absorption peak at 475 nm was much weaker when catalytic reaction occurred between L-dopa and the supernatant containing free Tyr (curve c). According to these results, the concentration of the encapsulated Tyr can be estimated as 2.75×10^8 U L^{-1}, as calculated in the Supporting Information. Moreover, there was no absorption peak at 475 nm after the addition of Ab_2-TLL (curve d), indicating that hardly any Tyr was attached on the TLL surface. Fig. 1d exhibited the as-fabricated titania coating directly grown onto ITO with large roughness is composed of many self-assembled small TiO2 NPs. Such nanoporous morphology indicates the high specific surface area with plenty binding sites for constituting coordination bonds. To validate the feasibility of the system, the chronoamperometric i−t curves were then recorded from the stepwise transient photocurrent responses upon intermittent light irradiation. As shown in Fig. 1e, bare ITO substrate had no photocurrent response (curve a), while the TiO_2 NPs electrode had a weak photocurrent signal (curve b). After incubation with the dope-containing solution corresponding to 1 ng mL^{-1} antigen, the electrode exhibited much enhanced photocurrent intensity (curve c). Fig. 1f of the corresponding UV–vis diffuse reflectance spectra further confirmed the enhanced absorption after the incubation procedure, which due to the coordination between the undercoordinated Ti sites and dopa *in situ* forming the dopa–Ti charge transfer complex, as shown in Fig. 1f inset (Dimitrijevic et al., 2005; Rajh et al., 2002).

2.2. Performance

Since the extent of signal increase depended upon the PSA concentration, a TLL-supported PEC immunoassay was tailored. Fig. 2a shows the increment of photocurrent after reaction with lysis solution corresponding to different antigen concentrations. Fig. 2b shows the corresponding linear range from 0.5 pg mL^{-1} to 100 ng mL^{-1} and the lowest detection limit of PSA was experimentally determined as 0.5 pg mL^{-1}, which was comparable to some recent reports of other detection methods as listed in Table S1. Essentially, upon the increased

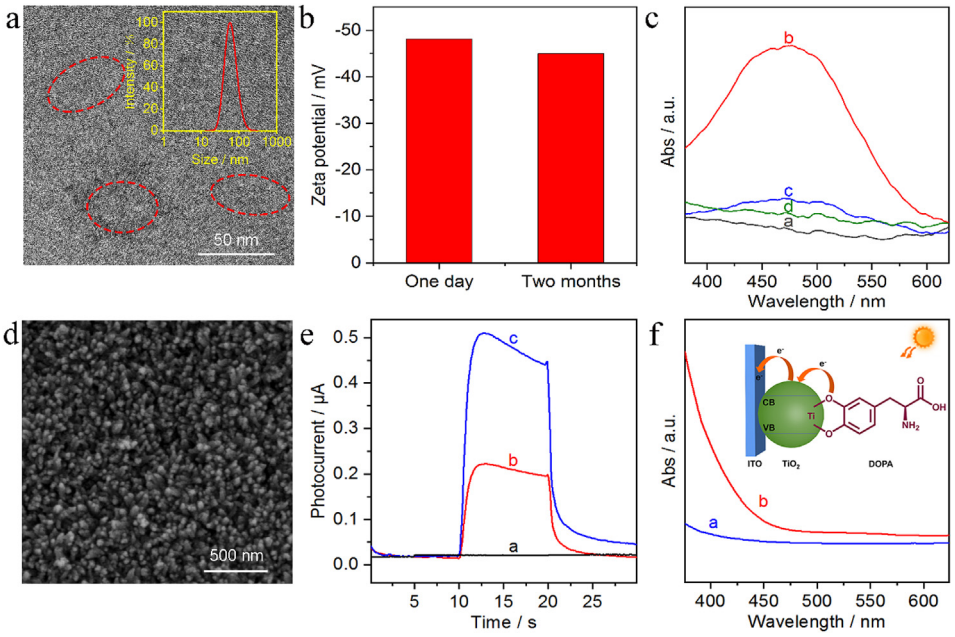

Fig. 1. (a) TEM image of Tyr-loaded liposomes. Inset: DLS of Tyr-loaded liposomes. (b) Zeta potentials of Tyr-loaded liposomes after one day and two months storage. (c) UV–vis spectra of Dopachrome after the reaction of L-dopa with blank (curve a), Tyr (curve b), supernatant containing free Tyr (curve c) and Ab_2-TLL (curve d). (d) SEM image of TiO_2 NPs electrode. (e) Photocurrent responses of bare ITO substrate (curve a), after TiO_2 NPs deposition (curve b), after incubation with the lysis solution corresponding to 1 ng mL^{-1} antigen (curve c). (f) UV–vis diffuse reflectance spectra of TiO_2 (curve a) and TiO_2/DOPA complex (curve b). Inset: schematic diagram of surface Ti atom chelated by dopa and the resultant charge transfer processes upon light excitation.

immunobinding, more sandwich immunocomplexes could be introduced and more Tyr would be released for catalyzing the oxidation of Tyr to generate dopa. The signal enhancement over 100 ng mL^{-1} became placid which should be attributed to the near saturation of the undercoordinated Ti sites on the electrode. In addition, the effect of different Tyr concentrations to photocurrent responses was also investigated. As shown in Fig. 2c, the photocurrent increment increased with increasing Tyr concentrations. Fig. 2d shows the corresponding linear range from 0.1 U L^{-1} to 100 U L^{-1} with detection limit of ca. 0.1 U L^{-1}. Fig. 2e showed the signal response of the DOPA/TiO_2/ITO upon irradiation repeated every 10 s. The irradiation process was repeated 20 times over 400 s and no obvious variation could be observed, indicating the good stability of the assay. To verify the selectivity, as shown in Fig. 2f, immunoglobulin G (IgG), cardiac troponin I (cTnI), p53, carcinoembryonic antigen (CEA), immunoglobulin A (IgA) were investigated as interference. And the photocurrent responses of interfering agents with the addition of 100-fold excess in comparison with the target PSA were very close to the blank test, indicating the good selectivity of the assay.

3. Conclusion

In summary, this work communicated the possibility of integrating the enzyme-induced sensitization process into the liposomal PEC platform to exploit innovative signaling mechanism for PEC bioanalysis. By the aid of TLL, the free Tyr from the sandwich immunocomplex could initiated the transformation of tyrosine to dopa, which could bind with the undercoordinated Ti sites to form the stable dopa–Ti charge transfer complex and thus generate enhanced anodic photocurrent under visible light for signaling. In the immunoassay, the photocurrent enhancement from the sensitization effect could be correlated to the PSA concentration, and the as developed immunoassay exhibited good performance in terms of high sensitivity and selectivity, good reproducibility and stability with a linear range from 0.5 pg mL^{-1} to 100 ng mL^{-1}. This work underlay a novel and general strategy for ultrasensitive liposomal PEC bioanalysis and is expected to inspire more interest to the development and implementation of enzyme-involved liposomal PEC bioanalysis.

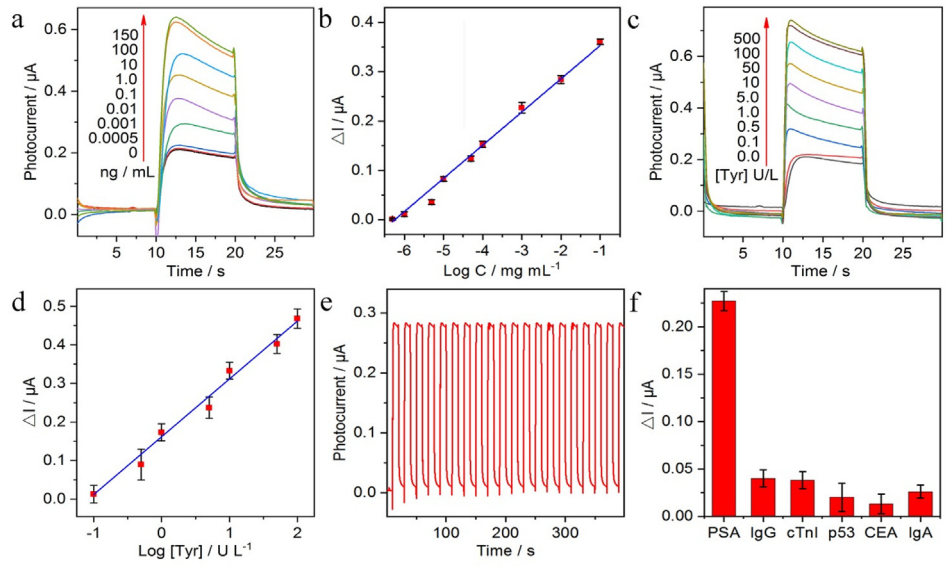

Fig. 2. (a) Photocurrent responses of PSA with different concentrations. (b) The corresponding calibration curve for (a). (c) Photocurrent responses of Tyr with different concentrations. (d) The corresponding calibration curve for (c). (e) Operational stability of DOPA/TiO_2/ITO by repeated on/off illumination cycles. (f) Selectivity of the proposed immunoassay to PSA with 1 ng mL^{-1} by comparing to the interfering proteins at 100 ng mL^{-1} level: IgG, cTnI, p53, CEA, and IgA. ΔI is the photocurrent increment corresponding to the various antigen concentrations. The PEC tests were performed in 0.1 M PBS solution (pH 7.0) with 0.0 V applied voltage and 410 nm excitation light.

Declaration of interest statement

We declare that we have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bios.2019.04.037.

References

Dimitrijevic, N.M., Saponjic, Z.V., Rabatic, B.M., Rajh, T., 2005. J. Am. Chem. Soc. 127, 1344–1345.
Ge, L., Xu, Y., Ding, L., You, F., Liu, Q., Wang, K., 2019. Biosens. Bioelectron. 124–125, 33–39.
Han, Q., Wang, R., Xing, B., Zhang, T., Khan, M.S., Wu, D., Wei, Q., 2018. Biosens. Bioelectron. 99, 493–499.
Hong, Z., Jing, L., Zhang, S., 2015. TrAC Trends Anal. Chem. 67, 56–73.
Li, Y., Chen, F., Luan, Z., Zhang, X., 2018. Biosens. Bioelectron. 119, 63–69.
Lin, Y., Zhou, Q., Tang, D., 2017. Anal. Chem. 97, 11803–11820.
Mei, L.P., Liu, F., Pan, J.B., Zhao, W.W., Xu, J.J., Chen, H.Y., 2017. Anal. Chem. 89, 6300–6304.
Rajh, T., Chen, L.X., Lukas, K., Liu, T., Thurnauer, M.C., Tiede, D.M., 2002. J. Phys. Chem. B 106, 10543–10552.
Tu, W.W., Wang, Z., Dai, Z.H., 2018. TrAC Trends Anal. Chem. 105, 470–483.
Wang, G.L., Xu, J.J., Chen, H.Y., 2009. Biosens. Bioelectron. 24, 2494–2498.
Wang, M., Yang, Z., Guo, Y., Wang, X., Yin, H., Ai, S., 2015. Microchim. Acta 182, 241–248.
Wang, B., Mei, L.P., Ma, Y., Xu, Y.T., Ren, S.W., Cao, J.T., Liu, Y.M., Zhao, W.W., 2018. Anal. Chem. 90, 12347–12351.
Yildiz, H.B., Freeman, R., Gill, R., Willner, I., 2008. Anal. Chem. 80, 2811–2816.
Zeng, R., Su, L., Luo, Z., Zhang, L., Lu, M., Tang, D.P., 2018. Anal. Chim. Acta 1038, 21–28.
Zhang, K., Lv, S., Lin, Z., Tang, D.P., 2017. Biosens. Bioelectron. 95, 34–40.
Zhao, W.W., Ma, Z.Y., Xu, J.J., Chen, H.Y., 2013. Anal. Chem. 85, 8503–8506.
Zhao, W.W., Xu, J.J., Chen, H.Y., 2015. Chem. Soc. Rev. 44, 729–741.
Zhao, W.W., Xu, J.J., Chen, H.Y., 2017. Biosens. Bioelectron. 92, 294–304.
Zhuang, J., Han, B., Liu, W., Zhou, J., Liu, K., Yang, D., Tang, D.P., 2017. Biosens. Bioelectron. 99, 230–236.