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Short communication

Robust hybrid enzyme nanoreactor mediated plasmonic sensing strategy for ultrasensitive screening of anti-diabetic drug

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ABSTRACT

Enzyme inhibition based drug screening strategy has been widely employed for new drug discovery. But this strategy faces some challenges in practical application especially for the trace active compound screening from natural products such as the stability of enzyme and the sensitivity of screening approach. Inspired by the above, we for the first time demonstrate the self-assembly of α-glucosidase (GAA) and glucose oxidase (GOx) into one multi-enzymes-inorganic nanoreactor with hierarchical structure (flower shape). The hybrid enzyme nanoreactor enjoys the merits including the character of assembly line, the enhanced enzymatic activity and robust stability. The flower shape of enzyme nanoreactor possessed a bigger specific surface area, facilitating the trace GAA inhibitor detection. Based on the above, we proposed an enzyme nanoreactor mediated plasmonic sensing strategy for anti-diabetic drug screening. First, maltose was chosen as the substrate for GAA and the generated glucose were immediately utilized by GOx to generate H_2O_2, and finally, H_2O_2 etched the Ag nanoprism to round nanodiscs, resulting in the blue shift of surface plasmon resonance (SPR) absorption band. With the aid of hybrid enzyme nanoreactor guided SPR, the ultrasensitive screening of GAA inhibitor (i.e. anti-diabetic drug) can be realized with the detection limit of 5 nM for acarbose. The proposed approach was successfully utilized for GAA inhibitor screening from natural products. We anticipate that the proposed sensing method may provide new insights and inspirations in the enzyme inhibition based drug discovery and clinical diagnosis.

1. Introduction

Diabetes mellitus is a group of metabolic diseases in which there are high blood sugar levels over a prolonged period (Kitabchi et al., 2009). Past decades have witnessed a rapid rise in the prevalence of diabetes mellitus and an estimated 415 million people worldwide have diabetes and the figure is projected to reach 642 million by 2040 (Zone and Guide, 2017). Diabetes can cause many complications including heart disease, stroke, chronic kidney failure, foot ulcers, damage to the eyes and so on (Association, 2010). Due to the obvious side effects of the existed chemical anti-diabetic drugs, more and more attentions have focused on the screening of active compounds with low side effects from natural products. The GAA inhibition based detection model is the first choice for anti-diabetic drug screening with the advantages of satisfactory conveniences and rapidity. However, the approach for GAA inhibitor detection remains poorly investigated. The mostly used method is based on the GAA hydrolysis of 4-nitrophenyl-α-d-glucopyranoside to produce 4-nitrophenol and then monitoring of ultraviolet absorption of 4-nitrophenol. This method suffers from high consumption of the tested compounds, and the limited sensitivity and selectivity. Recently, we developed several methods for GAA inhibitor detection, in which carbon dots combining with inner filter effect (IFE) and fluorescence resonance energy transfer (FRET) strategy were developed for fluorescent sensing of GAA inhibitor (Kong et al., 2017; Li et al., 2016). Although satisfactory sensitivity and selectivity were obtained, the problem of GAA instability in conventional and long-term storage conditions and the limited detection model have been the challenges for the inhibitor screening. Thus, the screening methods with excellent stability, applicability and sensitivity are still highly desirable in the fields of clinical research and drug discovery.

Recently, biomineralization has provoked some interests because the embedded proteins may present both higher stability and activity compared to their soluble counterparts (Jesionowski et al., 2014; Jia et al., 2014). For example, Ge's group reported a method for creating

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hybrid organic–inorganic nanoflowers using copper (II) ions as the inorganic component and proteins as the organic component (Ge et al., 2012). Park and Champion demonstrated colloidal assembly of hierarchically structured porous supraparticles from flower-shaped protein–inorganic hybrid nanoparticles. The prepared protein–inorganic supraparticles are protein compatible, have large surface area, and provide specific affinity recognition for protein immobilization, indicating a variety of functional proteins could be immobilized to the porous supraparticles (Park and Champion, 2016). Therefore, enhancement of stability or biological activity of immobilized proteins by biomineralization could be beneficial for many applications.

Furthermore, nanomaterial technologies provide an important strategy for the detection of specific analytes (Bastus et al., 2014). Among the nanomaterials, silver-based nanomaterials have attracted intensive attention due to the excellent physical and chemical properties (Gliga et al., 2014). Moreover, silver nanomaterials has extensive application prospect in chemical, biological sensing and bioimaging (Logeswari et al., 2015). In the previous works, different shapes of silver nanostructures have been synthesized, including nanowires, nanorods, nanoprisms, nanoplates, nanodisks and nanocubes (Choi et al., 2013; Walkey et al., 2014). Silver nanoparticles possess strong surface plasmon resonance (SPR) absorption from the visible to near-infrared region. Furthermore, the SPR absorption is extremely sensitive to their size, shape, composition, distance, and the surrounding media, based on which various colorimetric sensors can be realized (Huang et al., 2014).

In the present study, we innovatively present a facile, robust and effective approach for ultrasensitive screening of anti-diabetic drug based on the hybrid enzyme nanoreactor mediated plasmonic sensing strategy. Frist, GAA and GOx were co-precipitated into one multi-enzymes-inorganic nanoreactor by self-assembly to form a hierarchical structure with flower shape. Maltose was chosen as the initial substrate for enzyme nanoreactor. Second, Ag nanoprism was chosen as the signal output. With the operation of the hybrid enzyme nanoreactor, H_2O_2 was released to etch the Ag nanoprism to round nanodiscs, resulting in surface plasmon resonance (SPR) blue shift. Finally, the hybrid enzyme nanoreactor mediated plasmonic sensing strategy was achieved. When inhibitor was introduced into the hybrid enzyme nanoreactor, the discharge of H_2O_2 was hindered and then the blue shift of SPR was inhibited. Based on these principles, a novel sensing strategy for GAA inhibitor detection was achieved readily. By integration of the merits the hybrid enzyme nanoreactor (e.g. the character of assembly line, the enhanced enzymatic activity and robust stability) and the inherent sensitivity of plasmonic Ag nanoprism, an ultra-low detection limit of 5 nM was obtained for acarbose (famous anti-diabetes drug). In addition, the proposed sensing method was successfully utilized for GAA inhibitor screening from natural products. The proposed novel biosensor was proven to enjoy many merits including high sensitivity, good selectivity and excellent stability, which provides new insights and inspirations to design the multi-enzyme sensors in clinical diagnosis and drug discovery.

2. Experimental

2.1. Reagents and apparatus

All the details of materials and apparatus are shown in Supplementary material.

2.2. Construction of the hybrid enzyme nanoreactor and synthesis of silver nanoplates

The detailed information for synthesis is presented in Supplementary material.

2.3. α-glucosidase inhibitor investigation

The substrate solutions including the hybrid enzyme nanoreactor (100 μL) and 50 μL of acarbose solutions with different concentrations (0.05, 0.5, 1, 5, 10, 15, 20, 40, 60, 80 and 100 μM) were mixed in PBS buffer (pH = 6.8) and shocked gently for 5 min. Then, 200 μL of 10^{-3} M maltose solutions were added into mixed solutions. After 40 min incubation time at 38 °C, the mixed solutions were centrifuged at 12,000 rpm for 3 min. The supernatant was collected and added into 500 μL of Ag nanoplates solutions, then sufficiently shaken for 10 min. The UV–vis spectra were recorded, and the SPR peak shift (Δλ) was used for quantitative analysis.

2.4. Application to screen α-glucosidase inhibitor from natural products

The inhibiting ability of the extracts from traditional Chinese medicines was also screened by the proposed sensing method. The detailed analysis process was shown in Supplementary material.

3. Results and discussions

3.1. Morphology characterizations

The morphology, size and microstructure of the hybrid enzyme nanoreactor and the triangular silver nanoplates were respectively characterized by scanning electron microscope (SEM) and transmission electron microscopy (TEM). The SEM of nanoreactor was showed in Fig. 1A. As shown in Fig. 1A1, flower shaped nanoparticles were successfully synthesized with the diameter of 6–8 μm. Fig. 1A2 showed the individual nanosphere and their clear surface structure. Obviously, the nanospheres have highly porous structures and the surface structure as the blooming petal, which not only endows a high surface-to-volume ratio but also enhances the stability of the enzymes.

As shown in Fig. 1B, the silver nanoparticles showed a triangular shape with a length of 70 nm. The inset showed the high-resolution TEM (HRTEM) image of silver nanoplates, the spacing of the adjacent lattice planes for random nanoplates was 2.5 Å, corresponding to the {111} plane of face-centered cubic silver metal. This result indicated that the as-prepared silver nanoplates were {111}-oriented single crystals. This special triangular microstructure has highly reactive sharp edges/tips and large surface-to-volume ratio, which greatly improved the sensitivity of the detection of targeted analytes. Fig. 1B2 showed the etched silver nanoplates with 10^{-4} M H_2O_2, and the structure of the silver nanomaterials was changed to roundness, leading to the blue shift of SPR absorption band.

3.2. Properties of the hybrid enzyme nanoreactor

In order to verify the properties of the as-synthesized hybrid enzyme nanoreactor, a series of control experiments were designed and explored. The activities of the enzyme nanoreactor were measured by using 100 μL of 5 mM maltose as the substrates and 500 μL of Ag nanoplates as the signal response in PBS solution. The free GAA and GOx systems in solution with the same concentrations were used as the control. As shown in Fig. 2A, compared to the free enzyme systems, the enzyme activity of enzyme nanoreactor was significantly increased by about 148%. This significant increase in enzymatic activity is presumed to be due to the below characters of enzyme nanoreactor. (1) Flower shaped enzyme nanoreactor with abundant porous nanostructure not only offers a wide reaction platform, but also can strongly adsorb the substrate on the surface to improve the catalytic efficiency. And the interaction between copper ions in nanocrystals and the incorporated metalloenzymes resulted in a higher enzymatic activity. (2) The integration of two enzymes (GAA and GOx) in one nanoreactor endowed the character of the assembly line work (i.e. reduction of