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Short communication

Rational surface silane modification for immobilizing glucose oxidase

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ABSTRACT

Glucose oxidase (GOx) has many significant applications in biosensor and biocatalysis. In this study, we firstly quantitatively analyzed the binding efficiency of (3-aminopropyl) trimethoxysilane (APTES) modified onto the surface of GOx. It was found that the contents of the grafted silane did not significantly influence the relative activities and tertiary structures of all surface modified GOxs. Immobilization ratio and relative activity of all instances of APTES modified GOx increased, compared with those of native enzyme. However, good stability of immobilized GOx at extreme pH and high temperature could only be obtained when modified protein with low binding silane content. At pH 2.0, the immobilized GOx with low binding content showed a more than 600% activity, compared to the free enzyme. Therefore, rational surface modification would be beneficial to improving the activity and stability of immobilized enzyme as well as increasing loading amount.

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1. Introduction

Glucose oxidase (β-D-glucose:oxygen 1-oxidoreductase, EC 1.1.2.3.4, GOx) is an oxido-reductase that catalyses the oxidation of glucose to hydrogen peroxide and D-glucono-δ-lactone by utilizing molecular oxygen as an electron acceptor [1]. GOx has many significant applications, including in biosensor and biocatalysis. To enhance the enzymatic activity, lifetime, and stability of GOx, various new nanoscale materials and technologies have been used to immobilize enzymes. Compared with free enzymes, the immobilized GOx performs much better in a variety of batch or continuous processes, such as rapid termination of reactions, controlled product formation, ease of enzyme removal from the reaction mixture and adaptability to various engineering designs [2–5]. Immobilized GOx on various nanoparticles, including gold [2], ZnO [3,4], NiO and silica [5–7], has been reported in the development of glucose biosensor.

Nanoparticles can offer many advantages, such as large surface-to-volume ratio, high surface activity, and strong adsorption ability for the immobilization of desired biomacromolecules [8–10]. Silica nanoparticles, as one of the most studied inorganic materials, are used to immobilize enzymes by covalent or non-covalent methods. Although the non-covalent (including adsorption and entrapment) methods provide many advantages, the vital disadvantage of the non-covalent method is protein leakage over time, which results in activity losses. Covalent binding to insoluble silica nanoparticles can provide a much more efficient enzyme retention with materials with open structures that present minimal mass transfer resistance to substrates [10].

In most reported researches, GOx was usually immobilized onto the pre-made silica nanopartices modified by (3-aminopropyl) trimethoxysilane (APTES) [11–13]. However, low loading ratio of GOx immobilized by silica nanoparticles usually happened, owing to diffusion limit and steric hindrance of enzyme molecules [14,15]. Furthermore, the dissociation of the enzyme subunits would lead to the inactivation of the immobilized GOx. Although multipoint attachment is a strategy well-suited for the immobilization of multimeric enzymes, this random immobilization is not enough to involve the maximum amount of enzyme subunits [16,17]. Moreover, the enzyme will be fully dispersed on the support after immobilization, which will prevent aggregation or other inactivation phenomena [18]. However, binding efficiency of APTES onto the surface of enzyme proteins may influence catalysis and stability of immobilized GOx. It is very difficult to rationally control the bonding ratio of APTES when using pre-modified silica nanoparticles to immobilize GOx. In this study, GOx was firstly modified with APTES and then immobilized by a one-pot reversible tetraethyl orthosilicate (TEOS) hydrolysis method. Furthermore, we quantitatively analyzed the binding efficiency of APTES modified on the surface of GOx. We studied changes of catalysis activity and protein tertiary structure of GOx after modified with different concentration of APTES. Effects of surface modification on loading amount,

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enzyme activity, re-usability, pH and thermostability of immobilized GOx were also investigated.

2. Materials and methods

2.1. Materials

GOx (EC 1.1.3.4, 160 kDa), HRP (EC 1.11.1.7, 44 kDa), tetraethyl orthosilicate (TEOS), APTES, and Triton X–100 were purchased from Sigma–Aldrich, USA. Cyclohexane, *n*-hexanol, acetone, ammonia solution, and hydrogen peroxide (30%) were obtained from HEOWNS Company, Tianjin, China. EDC·HCl and NHS were obtained from GL Biochem (Shanghai) Ltd. Glucose and 2-Azinobis (3-ehtylbenzothiazolin-6-sulfnic acid) diammonium salt (ABTS) were obtained from Aladdin, Shanghai, China.

2.2. Surface modification of GOx with APTES

GOx was modified with APTES by the conventional EDC/NHS method. Firstly, 10 mg GOx was dissolved with 3 mL of 50 mM phosphate buffer (pH 7.5). Then 1 mL aqueous solution containing 50 mg EDC·HCl and 50 mg NHS and certain volume of APTES were added. The mixture was stirred for 24 h. The APTES-modified GOx was purified on a G-25 column (GE, USA) and concentrated by ultrafiltration with Millipore Amicon Ultra-50 filters. The content of APTES grafting on GOx was calculated by determining the content of silica using ICP method [19]. Circular dichroism (CD) measurements were carried out between 190 and 250 nm for measuring the tertiary structure of GOx.

2.3. Covalent encapsulation of GOx in silica NPs

Native and modified GOx were immobilized by a one-pot traditional reversible microemulsion TEOS hydrolysis method. Firstly, cyclohexane (7.2 mL), *n*-hexanol (1.5 mL), Triton X–100 (1.5 mL) and GOx solution (0.5 mL) were mixed together and stirred about 10 min for preparing a water-in-oil microemulsion. And 0.15 mL TEOS was added into the water-in-oil microemulsion. For initiating hydrolysis and polycondensation, 50 μL of 25% ammonia was added and stride for 24 h. Immobilized GOx nanoparticles were obtained by adding 10 mL acetone and centrifuging 10 min at 15,000 rpm for precipitating silica nanoparticles. The loading amount of enzyme protein was measured by subtracting the residual protein content in the solution from the initial protein content using Bradford method. Scanning electron microscope (SEM, HITACHI, S4700) and transmission electron microscope (TEM, JEM 200CX, Japan) were used to study the morphology of the immobilized GOx nanoparticles. Fourier transform infrared spectroscopy (FTIR) spectra were obtained by using a FT-IR spectrophotometer, Nicolet170SX (Hitachi, Tokyo, Japan).

2.4. Free and immobilized enzyme activities assay

According to the reported method, free and immobilized GOx activity was assayed at 420 nm by a standard oxidase assay with ABTS as chromogen [20].

3. Results and discussion

APTES was usually integrated onto silica nanoparticles, and then the amino group of APTES reacted with the carboxyl groups on the surface of GOx [21–23]. In this research, GOx was firstly modified with APTES and then immobilized by inorganic sol–gel physical entrapment. The procedure of the modification and immobilization of GOx is shown in Fig. 1. The γ and β carboxyl groups of glutamic acid (Glu) and aspartic acid (Asp) (Fig. 2a) and C-terminal carboxyl groups on the surface of GOx could react with APTES by the EDC/NHS method. Different with common crosslinking agents, EDC and NHS introduce "zero length" amide cross-links between carboxylic groups from enzymes and amino groups from APTES [24]. Moreover, the bioactivity of enzymes would be mostly maintained because the modification by the EDC/NHS method was usually in a mild condition. However, a molecular GOx could be modified by several molecules of silanes when adding different amount of APTES into the reaction system. Therefore, we firstly attempted to study effect of binding APTES contents onto the GOx on the enzyme catalysis activity. According to the results of Table 1, it was found that the contents of the silanes increased with the increase of addition of APTES. However, the relative activities of all modified GOxs decreased a little compared with that of native enzyme. The CD data of modified GOxs are very similar to those of native GOx (see Table 2 in Ref. [25]). It indicated that there was no measurable dif-

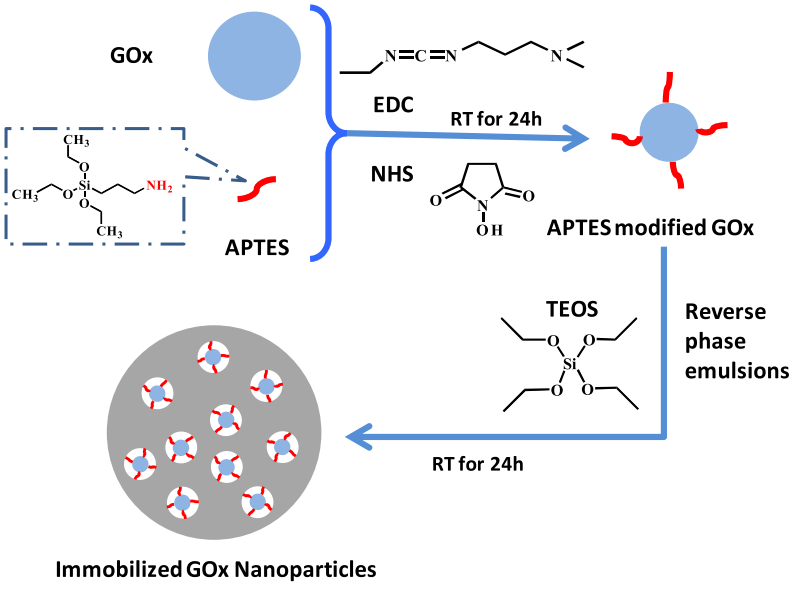

Fig. 1. Preparation of immobilized GOx nanoparticles after silane modification.

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