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Electrochemical biosensor based on CdS nanostructure surfaces

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ABSTRACT

Well-defined hexangularly faced CdS nanorod arrays have been grown directly on a conductive ITO glass via a facile one-step and non-template hydrothermal approach. Gold nanoparticles were decorated onto the nanorods to enhance the electron transfer process of electrode. Glucose oxidase (GOD) was then immobilized on the CdS through crosslinking with chitosan (CS), which resulted in a glucose biosensor with high enzyme loading and excellent sensitivity. Such a chitosan-encapsulated GOD-based biosensor revealed a relatively rapid response time of less than 50 s, and an approximate linear detection range of glucose concentration, from 50 to 500 μmol L^{-1} with a detection limit of 38 μmol L^{-1} and an electrode sensitivity of 5.9 μA mM^{-1}.

1. Introduction

Enzymatic biosensor performance strongly depends on the electron transfer between the enzyme and electrode. Hence, proper immobilization of the enzyme on the electrode surface is critical to enzymatic biosensor development. It has been proved that nanomaterials are able to enhance the electron transfer process due to their unique properties at nanometer size scale, such as large specific surface area, high surface energy, excellent biocompatibility, and strong adsorption ability. Thus, kinds of nanomaterials have attracted much interest in the field of sensors and catalysis [1–6]. Among these nanomaterials, nanostructured cadmium sulfide (CdS) is a promising one for its better electrical properties [7–14]. Dai et al. constructed a novel nitrite biosensor based on the direct electron transfer of hemoglobin (Hb) immobilized on CdS hollow nanospheres (HS-CdS) modified glassy carbon electrode [10]. Marin and Merkoci developed a potentiometric sensor for DNA hybridization detection using CdS nanoparticles label [14].

Glucose, as the major energy source in cellular metabolism, plays an important role in the natural growth of cells. Hence, the monitoring of glucose levels in blood with faster and more accurate methods has become an increasingly active area of research. Considerable efforts have been made to obtain biosensors for fast and reliable quantification of glucose [15–20]. In the report of Yang et al. [15], Pt nanoparticles were electromodified on the aligned carbon nanotubes (ACNTs), greatly increasing the electrode surface area and its electronic transfer ability. Then, glucose oxidase mixed with chitosan and CdS nanoparticles were electrochemically coated on the above electrode surface. The resulted CS–GOD–CdS/ACNTs–Pt_{nano} electrode offered sensitive response to glucose.

In our work, the CdS nanorod arrays were grown directly on a conductive ITO glass to ensure excellent electrical contact between CdS nanostructure and the electrode. Besides, the CdS nanostructures are used to incorporate with GOD for realizing the direct electron transfer, as CdS has been found to be able to efficiently increase the electron transfer reactivity of GOD [21]. Then, Au nanoparticles are electrochemically deposited onto the surface of CdS nanorod arrays to enhance the electron transfer process [22]. In addition, it is well known that chitosan is an efficient redox mediator which has an unusual combination of properties [23], including excellent membrane-forming ability, high permeability toward water, good adhesion, high mechanical strength, biocompatibility, nontoxicity, and so on. So it can not only be used to immobilize enzyme, but also be used as membrane material of electrode for the preparation of chemical sensors and biosensors [24]. We thus use chitosan film to encapsulate GOD onto the electrode, and the modified CdS nanorods electrode is directly used as the working electrode for detection of glucose.

2. Experimental section

2.1. Materials and apparatus

Cadmium nitrate $Cd(NO_3)_2{\cdot}4H_2O$ was obtained from Shanghai Jinshan Tingxin Chemical Reagent Factory. Thiourea was from Chengdu Kelong Chemical Reagent Factory. Glutathione, $HAuCl_4$ (99.9%), D-glucose, and glucose oxidase (GOD) were purchased from

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Sigma, and chitosan was from Aladdin Co., Ltd. All the chemicals were used without further treatments. Ultrapure water used in the experiment was purified with the Aquapro water-purification system (ASW1-0501-U). Tin-doped indium oxide (ITO) glass was purchased from Shenzhen CSG Display Technology Co., Ltd.

Field emission scanning electron microscopy (FESEM) images were taken on a UltraPlus (Zeiss) scanning electron microscope equipped with energy dispersive X-ray spectroscopy (EDX). Amperometric *i–t* curve and cyclic voltammograms (CVs) were taken on an electrochemical analyzer (CHI600B) with a three-electrode system.

2.2. Preparation of CdS nanorod arrays and modification with Au nanoparticles

In a typical reaction, the solution used for the preparation of CdS nanorod arrays was composed of 1 mmol L^{-1} of cadmium nitrate $Cd(NO_3)_2{\cdot}4H_2O$, 3 mmol L^{-1} of thiourea, and 0.6 mmol L^{-1} of glutathione [25]. The cleaned ITO glass was placed vertically in the Teflon-lined stainless-autoclave, which was then sealed and maintained at 200 °C for 3.5 h. After deposition, the ITO glass was rinsed with distilled water and dried naturally.

The coatings of the Au-NPs were performed according to the literature [26] with some modifications. Au nanoparticles were electrochemically deposited onto the surface of CdS nanorods using CV scanning on a computer-controlled electrochemical analyzer (CHI660B). It was obtained by scanning between 1.5 and −0.6 V in the acidic solution of 0.1 mmol L^{-1} H_2SO_4 containing 2.0 mmol L^{-1} of $HAuCl_4$ at a scan rate of 50 mV s^{-1} for 15 cycles. Then, the Au nanoparticles modified ITO–CdS nanorods electrode was rinsed with deionized water and applied for the further electrochemical studies.

2.3. Preparation of chitosan-encapsulated GOD electrodes

Chitosan is used for enzyme immobilization. First, chitosan of 50 mg was dissolved in the mixed solution of 9.9 mL pure water and 0.1 mL acetic acid with stirring for more than 1 h at room temperature to give a final concentration of 0.5 wt.%. Then, GOD of 5 mg was dissolved in the aforesaid chitosan solution of 0.5 mL and was stirred for 2 h at room temperature to give a final concentration of 1000–2500 units mL^{-1}. Finally, a 5 μL aliquot of the aforesaid solution was dropped onto the surface of our ITO–CdS–Au electrode (effective area of 30 mm^2) by a microsyringe. The aqueous solution was evaporated gradually overnight at 4 °C. All prepared enzyme electrodes were stored at 4 °C when not in use.

2.4. Electrochemical measurements

All the electrochemical measurements were carried out on an electrochemical analyzer (CHI600B) in a conventional three-2electrode configuration with a saturated calomel electrode (SCE) as the reference electrode with a Pt foil as the counter electrode and the modified CdS nanorod arrays/ITO electrode as the working electrode (effective area of 30 mm^2). The CVs were acquired from −0.6 to +0.6 V at a scan rate of 20 mV s^{-1} in 20 mL of 0.2 mmol L^{-1} PBS (pH 7.4). The amperometric response of the biosensor to glucose was recorded in a stirred PBS at 0.4 V vs. SCE reference electrode. All electrochemical experiments were performed at room temperature, 25 ± 2 °C.

3. Results and discussion

Firstly, the morphologies of ITO–CdS and ITO–CdS–Au electrode were characterized by FESEM. CdS nanorods were successfully grown on ITO glass substrates at 200 °C for 3.5 h (Fig. 1a). The nanorods are 100–200 nm in diameter and 400–500 nm in height. The SEM images of arrays at different locations and magnifications reveal that the whole ITO glass is covered with highly uniform CdS nanorods. After electrochemical deposition of Au nanoparticles on the surface of CdS nanorod arrays/ITO electrode, the typical SEM image is showed in Fig. 1b. The Au nanoparticles are uniformly dispersed on the CdS nanorod surface. From the corresponding high resolution (Fig. 1b, inset), we can find that the size of Au nanoparticles is in range of 10–80 nm in diameter. The EDX analysis (Fig. 1c) shows that the modified CdS nanorods consist of O, Si,

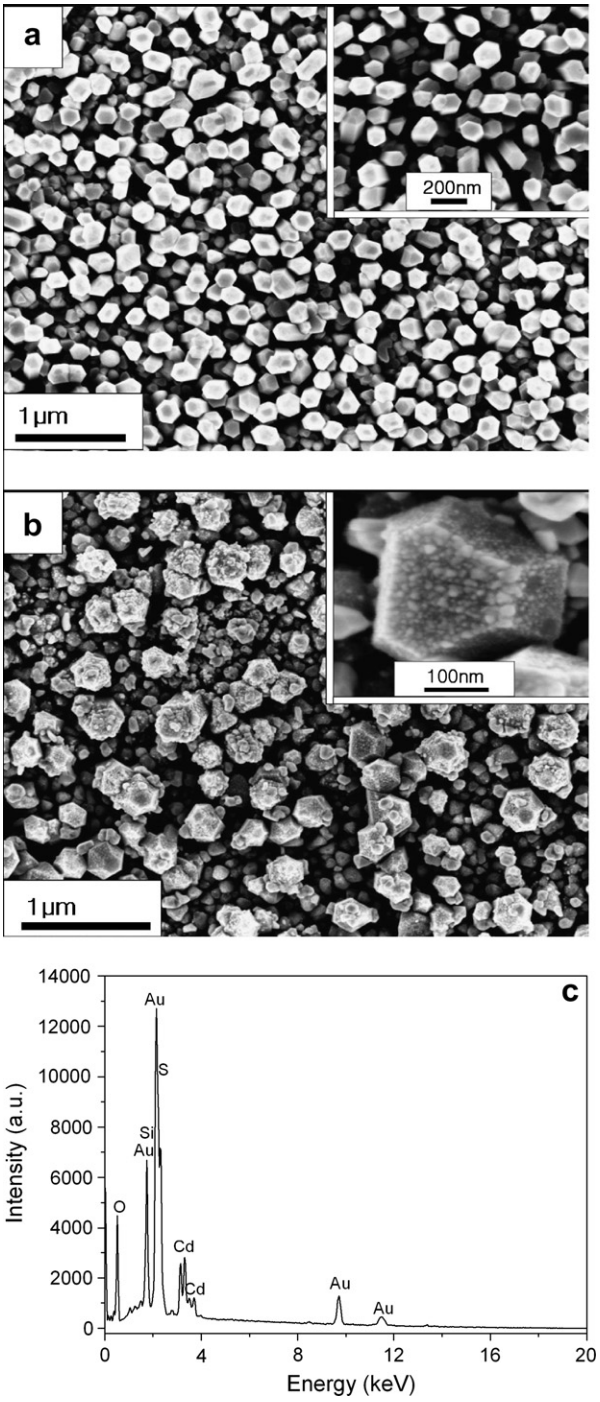

Fig. 1. (a) SEM image of the CdS nanorod arrays on ITO-coated glass substrates. (b) SEM image of the CdS nanorod arrays modified by Au nanoparticles. (c) EDX analysis indicating that the modified CdS nanorods consist of O, Si, Cd, S, and Au.

Cd, S, and Au, which indicates that the Au nanoparticles have been successfully deposited on the surface.

Cyclic voltammetry was then employed to characterize the two types of electrodes. Fig. 2 displays the performance of the ITO–CdS electrodes with (Fig. 2b) and without (Fig. 2a) Au nanoparticles modified. They were recorded in a solution with 2 mmol L^{-1} $K_3Fe(CN)_6$ and 1 mol L^{-1} $K(NO)_3$ at a scan rate of 50 mV s^{-1}. From the curves, it is found that a pair of well-defined oxidation and reduction peaks of the ITO–CdS–Au electrode are observed (Fig. 2b), which are more obvious than those of the ITO–CdS electrode (Fig. 2a). Besides, the ratio of anodic and cathodic peak currents (I_{pa}/I_{pc}) of the ITO–CdS–Au electrode (0.975) is close to 1, much better than that of the ITO–CdS electrode (0.635). The above results indicate that the ITO–CdS–Au electrode has better reversibility. Apparently, the Au nanoparticles deposited on the CdS nanorod arrays surface play a crucial role in the electron transfer. Therefore, the ITO–CdS–Au electrode was used for the detection of glucose after immobilization of GOD with chitosan.

Fig. 3a shows the cyclic voltammograms of ITO–CdS–Au–GOD electrode in PBS of pH 7.4 at 20, 50, 80, 100, 150, and 200 mV s^{-1} (from inner to outer). Fig. 3b is the plots of peak currents vs. scan rate v, showing that both the cathodic and anodic peak currents of the electrode have a linear relationship with the scan rates. This linear relationship indicates that the quasi-reversible reaction on the electrode is a surface-controlled process [27], and CdS and Au nanostructures on the ITO–CdS–Au–GOD electrode play an important part in this process as they can offer the protein direct electron transfer to the electrode [15].

The constructed sensor is sensitive to the concentration changes of glucose in PBS. Fig. 4 shows the cyclic voltammetric curves of

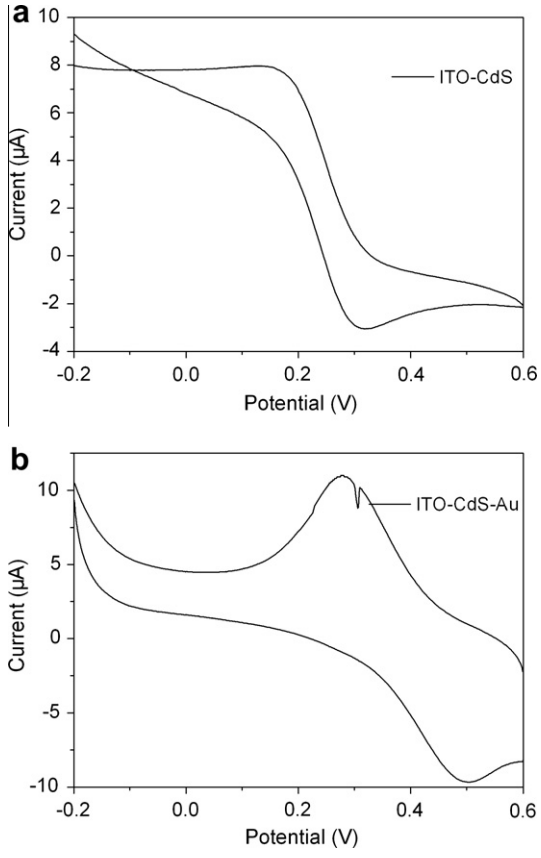

Fig. 2. Cyclic voltammograms of different electrodes. (a) ITO–CdS electrode, (b) Au NPs-modified ITO–CdS electrode. Supporting electrolyte: 2 mM $k_3Fe(CN)_6$ + 1 M KNO_3, scan rate: 50 mV s^{-1}.

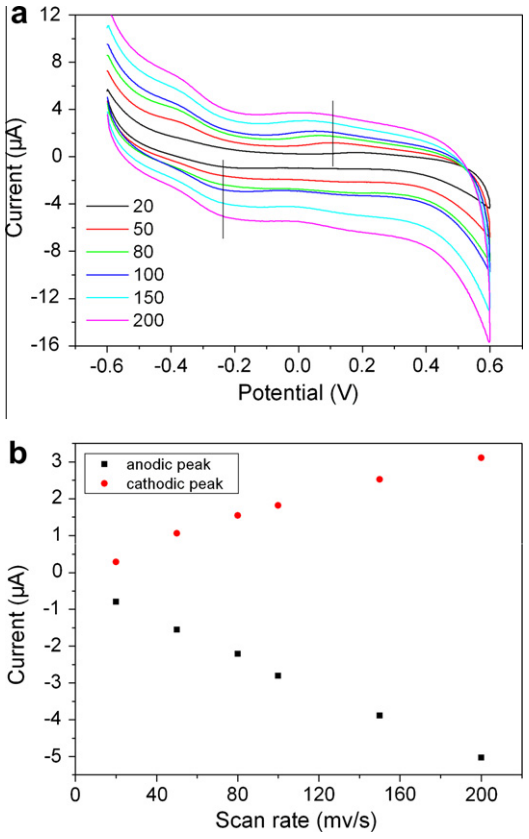

Fig. 3. (a) Cyclic voltammograms of ITO–CdS–Au–GOD electrode in PBS of pH 7.4 at 20, 50, 80, 100, 150, and 200 mV s^{-1} (from inner to outer). (b) Plots of peak currents vs. scan rate v.

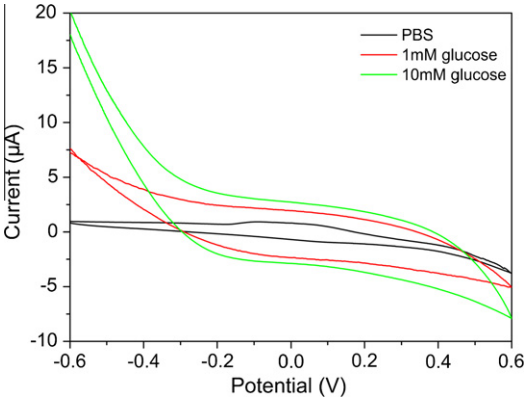

Fig. 4. Cyclic voltammograms of ITO–CdS–Au–GOD electrode in the same PBS (pH 7.4) in the absence (black) and the presence of 1 mM (red), 10 mM (green) glucose. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

ITO–CdS–Au–GOD electrode in an unstirred 0.2 mol L^{-1} PBS with 1 mmol L^{-1} glucose (red), 10 mmol L^{-1} glucose (green), or without glucose (black) at the scan rate of 20 mV s^{-1}, respectively. It can be seen that with the increase of glucose concentrations, the redox currents increase correspondingly, indicating the good response performance to glucose. The relatively minor increase of anodic currents could be attributed to the inherent electroconductivity of the CdS nanostructure [28], which has been improved partly by the electrochemically deposited Au nanoparticles.

Fig. 5 shows a typical amperometric response of the ITO–CdS–Au–GOD electrode to successive addition of glucose into an air-

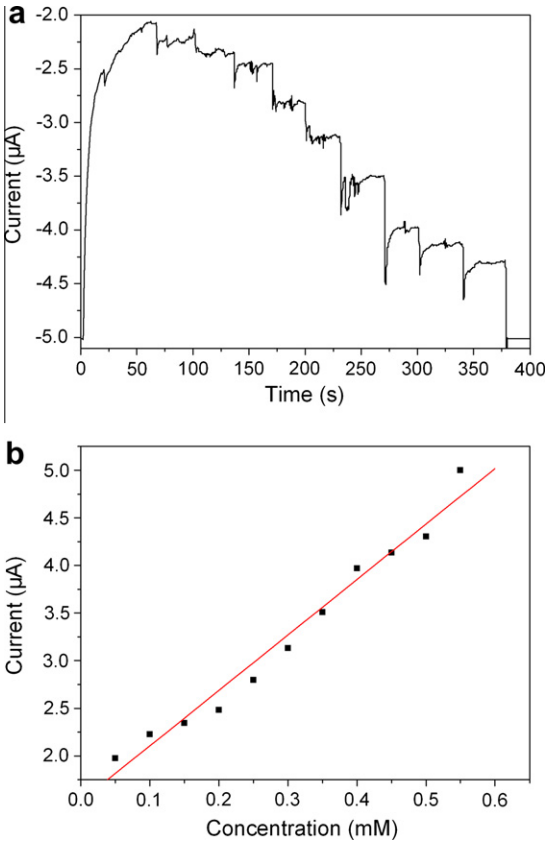

Fig. 5. (a) Typical amperometric response curve of ITO–CdS–Au–GOD electrode for increasing glucose concentration in 50 μM per step with successive addition of glucose to the 0. 2 M pH 7.4 PBS under stirring. The applied potential was +0.4 V vs. SCE. (b) Calibration curve: anodic currents of the biosensor vs. solutions of different concentrations of glucose.

saturated and stirred PBS by increasing the glucose concentration at 50 μmol L^{-1} per step. The applied potential was 0.4 V vs. SCE reference electrode. Each successive injection of glucose resulted in a decrease of the steady-state current. For the ITO–CdS–Au–GOD biosensor, 90% of the steady-state current was achieved at less than 15 s. The corresponding calibration curve of the electrode is shown as Fig. 5b. A linear relationship between the current and the concentration of glucose is obtained from 50 to 500 μmol L^{-1} with a correlation coefficient of 0.9875. The electrode exhibits an extremely high sensitivity of 5.9 μA mM^{-1} and a limit of detection of 38 μmol L^{-1}. The amperometric response of the ITO–CdS–Au–GOD electrode is much better than that of many others [15,16,29]. The relative standard deviation of the electrode response to 500 μM glucose was 3.4% for five consecutive measurements. Such fast response and low limited detection may be attributed to the large surface area of the nanorod arrays and good electron transfer capacity of Au nanoparticles. The large surface area of the nanorod array made it possible to absorb large amount of enzymes, while the ordered orientation of the nanorod array makes the enzymes spatially patterned, improving significantly the performance of the resulting biosensor. Furthermore, the chitosan-based crosslinking in enzyme immobilization could improve the stability. More importantly, the semiconductor nanostructure, i.e., CdS nanorods, might help to incorporate with GOD for realizing the direct electron transfer from GOD to electrode, as CdS has been found efficiently to enhance the electron transfer reactivity of GOD [21].

In order to shed light on the mechanism of how CdS and Au nanostructures influence the electrode performance, we further

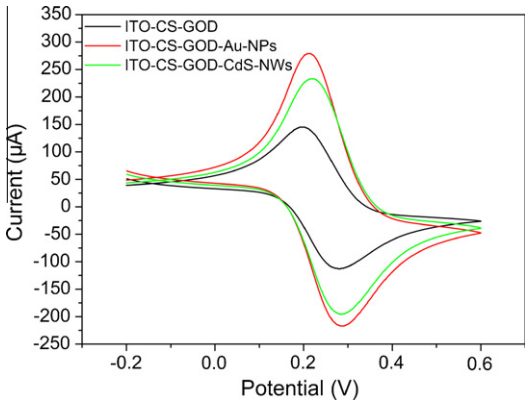

Fig. 6. The CVs recorded in a solution of 2 mmol L^{-1} $K_3Fe(CN)_6$ and 1 mol L^{-1} $K(NO)_3$. (black: ITO–CS–GOD; green: ITO–CS–GOD–CdS–NWs; red: ITO–CS–GOD–Au–NPs). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

designed a set of experiments. Firstly, we configurated the following three mixed solutions. The first solution contains 0.5 wt.% CS and 10 mg mL^{-1} GOD; the second one contains 0.5 wt.% CS, 10 mg mL^{-1} GOD and 0.067 mg mL^{-1} CdS-NWs; the last one contains 0.5 wt.% CS, 10 mg mL^{-1} GOD and 0.067 mg mL^{-1} Au-NPs. Then, a 5 μL aliquot of the aforesaid three solutions was dropped onto the surface of cleaned ITO electrode, respectively, and corresponding three kind of electrodes (ITO–CS–GOD, ITO–CS–GOD–CdS-NWs, and ITO–CS–GOD–Au-NPs) were obtained. Fig. 6 shows the CVs recorded in a solution of 2 mmol L^{-1} $K_3Fe(CN)_6$ and 1 mol L^{-1} $K(NO)_3$. From the Fig. 6, we can find that the CdS-NWs and Au nanoparticles can improve the electrode performance effectively for their good electron transfer capacity. It is a powerful explanation for our high performance of the resulting glucose sensors.

The repeatability and selectivity of the electrodes were also tested. The glucose concentration of 1 mM was measured continuously for 10 times, and a low relative standard deviation (RSD) was obtained. And after being stored at 4 °C for three weeks, the performance of the electrodes retained around 75% of the initial response, which is due to the enzymatic stability and activity. Moreover, there was no obvious interference when adding ascorbic acid and dopamine of 1 mmol L^{-1} into the same concentration of glucose solution at the applied potential of 0.4 V (not shown here).

4. Conclusions

In summary, we have demonstrated a novel glucose sensor based on the highly efficient immobilization of GOD with chitosan on a Au nanoparticles modified ITO–CdS nanorods surface. The encapsulated GOD maintains its bioactivity and electrochemical activity. Our prepared sensor displays a good precision and a linear range from 50 to 500 μmol L^{-1} for glucose determination, and a detection limit of 38 μmol L^{-1}. This satisfactory performance of our biosensor is owing to the large surface area of the nanorod arrays, the inherent electroconductivity of the CdS and Au nanostructure as well as the chitosan-based crosslinking in enzyme immobilization. Due to its simplicity, low cost, and high stability, this glucose biosensor may have great potential applications in clinical diagnosis, food science, and industry.

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