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Multicolor biosensor for fish freshness assessment with the naked eye

Zhitao Chen[a,b,1], Yue Lin[a,1], Xiaoming Ma[a], Longhua Guo[a,*], Bin Qiu[a], Guonan Chen[a], Zhenyu Lin[a,*]

[a] MOE Key Laboratory of Analytical Science for Food Safety and Biology, Fujian Provincial Key Laboratory of Analysis and Detection Technology for Food Safety, Institute of Nanomedicine and Nanobiosensing, College of Chemistry, Fuzhou University, Fuzhou, Fujian 350116, China
[b] Fuqing Entry-Exit Inspection & Quarantine Bureau of P. R. C, Fuqing, 350300, China

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ABSTRACT

Fish product is one of the main seafood with the disadvantage of easy to perish. Various indexes and diverse methods have been developed to evaluate the fish freshness, but most of them are complex and cannot be applied for on-field detection. In this study, we reported a simple, visual and economical multicolor biosensor based on the etching of gold nanorods (GNRs) to evaluate the fish freshness with the naked eye. Hypoxanthine (Hx) is chosen as the index of fish freshness and it react with the dissolved oxygen to produce H_2O_2 at the presence of xanthine oxidase (XOD). Then GNRs are etched quickly by the H_2O_2 in the presence of Fe^{2+}. Correspondingly, the GNRs surface plasmon resonance (SPR) is regulated and results in a distinctly color change of the system, which can be easily distinguished with the naked eye. Therefore, the concentration of Hx in the fish samples can be semi-quantitatively analyzed with the naked eye. The proposed multicolor Hx sensor has been successfully applied to the detection of Hx in fish samples.

1. Introduction

Freshness of the fish meat is essential in food safety since it's essential for the high quality production [1]. After the death of fish, a series of complicated chemical reactions including proteolysis, lycolysis and lipolysis are initiated immediately and affected by handling methods, storage temperature, bleeding condition, the post-processing time and the type of fish species. Some substances, such as instant trimethyl amine [2], volatile amine [3], histamine [4,5], and H_2S [6], was applied to assess the freshness of fish. Hypoxanthine (Hx) has been regarded as the major catabolite of adenosine triphosphate (ATP) which is known to accumulate right after the animal slaughtered [7–9]. An excellent agreement existed between the amount of Hx and the total volatile basic nitrogen in fish extract was found [10], when the concentration of Hx was higher than 102 mg/kg, the fish started to deteriorate, and the fish was deteriorated completely when the concentration of Hx was over 144 mg/kg. So the concentration of Hx has been used as the index to assess the fish freshness frequently.

Many classical methods, such as high performance liquid chromatographic (HPLC) [11], near infrared spectroscopy [12], spectrophotometric [13,14], chemiluminescence [15], capillary electrophoresis[16], and electrochemical approaches [8,17,18], had been developed to detect Hx with high sensitivity and selectivity. But most of these methods need relative expensive equipment and skilled person to perform the experiments, which are difficult to be used in on-field applications. Therefore, it is necessary to develop some convenient methods for Hx determination and used to judge the fish freshness by an untrained person. Colorimetric detection has received much attention since it has the advantages of simplicity, cost-effectiveness and visualization. Colorimetric method had been developed for Hx determination with high selectivity since long time ago [19]. But only two different colors are presented in these colorimetric systems, and the concentration of Hx was determined by the intensity of the color, which is difficult to read by the naked eye. Thus these methods can only be used for the qualitative detection of Hx. And an expensive microplate reader is needed for quantitative detection of Hx, which limits its on-site application.

Human eyes can distinguish as many as 10 million of different colors [20], the accuracy of visual inspection can be greatly improved if plenty of colors are presented in the presence of different amount of target molecules. The optical properties of gold nanorods (GNRs) extremely depending on the size and shape. Different optical signals and vivid colors can be acquired by simply

* Corresponding authors.
E-mail addresses: guolh@fzu.edu.cn (L.H. Guo), zylin@fzu.edu.cn (Z.Y. Lin).
[1] These authors contributed equally to this work.

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adjusting the aspect ratio of GNRs, this character had been applied to develop colorimetric biosensors frequently [21]. For example, a Cr(VI) sensor based on the etching of GNRs has been fabricated since the presence of Cr(VI) can accelerate the etching of GNRs within 30 min at 50 °C [22]. Cu(II) also can be a catalyst to promote the oxidation of GNRs, while the reaction temperature need to reach 75 °C, which is not suitble for some rapid detection [23]. A new regent 3,3′,5,5′-tetramethylbenzidine(II) (TMB^{2+}) has been applied to etch GNRs to realize multicolor biosensor, which has been used in some disease markers (such as carcino-embryonic antigen, prostate specific antigen) in our previous work [24]. These results clearly indicate that the etching of GNRs can be used to develop sensitive multicolor biosensors. Previous report also showed that H_2O_2 can be used to oxidase GNRs, but long reaction time (up to 5 h) and high concentration of H_2O_2 are needed [25]. So, it can not be applied in real application conviniently. Our early study indicated that the present of Fe^{2+} in the react system can significantly accelerate the oxidation of GNRs by H_2O_2 at room temperature, and the concentration of H_2O_2 could be rather low [26]. This mechanism had been used to develop simple multicolor biosensors for diverse targets.

In the presence of xanthine oxidase (XOD), Hx can quantitatively react with the dissolved O_2 to generate H_2O_2 [27]. In this study, by coupling with the high selectivity of the enzymatic reaction and the colorful color display of GNRs, a novel multicolor colorimetric biosensor for Hx detection has been proposed. The produced H_2O_2 from the enzymatic reaction can be quickly reduced to •OH through Fenton reaction by Fe^{2+} at room temperature, which can be used to quantitatively etch the GNRs quickly and vivid color changes can be observed with the naked eye. The shift of longitudinal surface plasmon resonance (LSPR) has a relationship with the Hx concentration, which can be used to quantitative detection of Hx concentration. Furthermore, the color of the system can be used to semi-quantitative detection of Hx concentration with the naked eyes easily, so the freshness of the fish can be evaluated through the colors of system (not through the intensity of the same color) just like a pH test paper. The proposed biosensor has been applied to detect Hx and to assess the freshness of the fish samples with high satisfaction.

2. Material and methods

2.1. Reagents and equipment

The cetyltrimethyl ammonium bromide (CTAB) was purchased from J&K Chemical Technology (Beijing, China) and the ascorbic acid (AA) was acquired from Fu Chen Chemistry (Tianjin, China). Xanthine oxidase (XOD) was bought from Sigma-Aldrich (USA), while amino acids, $NaBH_4$, $AgNO_3$, and H_2O_2 were purchased from Sinopharm (Shanghai, China). Other chemicals, such as Hx, $FeSO_4$, HCl, $HAuCl_4$, lactic acid (LA) and uric acid (UA) were acquired from Aladdin (Shanghai, China). Fish samples were purchased fChemistry (Tianjin, China). Xanthine oxidase (XOD) was bought from Sigma-Aldrich (USA), while amino acids, $NaBH_4$, $AgNO_3$, and H_2O_2 were purchased from Sinopharm (Shanghai, China). Other chemicals, such as Hx, $FeSO_4$, HCl, $HAuCl_4$, lactic acid (LA) and uric acid (UA) were acquired from Aladdin (Shanghai, China). Fish samples were purchased from local supermarket. All solutions were prepared with ultrapure water (18.2 MΩ Cm) from Direct-Q3 UV system (Millipore).The gold nanorods had been synthesized according to early reported literature[24,26,28].

Ultraviolet-visible (UV–vis) absorption spectra were acquired by UV–vis absorption spectroscopy (1102UV-Vis spectrophotometer, Techcomp, China) and Multiskan spectrum microplate spectrophotometer (Thermo, USA).

2.2. Fabrication of standard color card for hypoxanthine

Firstly, CTAB on the surface of GNRs was removed by centrifugation twice and stored in a brown glass bottle. Then the resulting concentrated GNRs solution was mixed with CTAB at a volume ratio of 1:4, and the final concentration of CTAB was 0.14 M 50 μL of different concentrations of Hx was added into 96-well plates followed by mixed respectively with XOD (20 μL, 0.01 mg/mL). After the enzymatic reaction, HCl (25 μL, 2 M) and $FeSO_4$(25 μL, 10 mM) were added into the above wells with sufficient mixing, the ferrous ion must be dissolved directly in acid because it was prone to autooxidation at neutral pH [29], and HCl was used here to provide an acid environment. Eventually, 100 μL of GNRs-CTAB mixture was introduced into solution above mentioned with sufficient oscillation. Various vivid colors appeared according to the concentrations of Hx, the color of the system has been recorded by a Canon digital camera. Furthermore, extinction spectra of GNRs solutions were measured using a Microplate Spectrophotometer (Multiskan GO, Thermo Scientific, USA)

2.3. Fish samples treatment

Miichthys miiuy and whitebait were chosen as test samples and had been purchased from the local supermarket. The fish was scaled and removed the intestine, and then cut off a piece of fish where the meat is thick. These fish samples were minced by meat grinder and then stored at −20 °C before testing in lab. Trichloroacetic acid (10 mL, 2 M) was added into 5 g of fish samples followed by ultrasonic cleaned for 15 min and then centrifuged (10000 r/min, 10 min). Supernatant liquor was taken out and the value of pH was adjusted to 6.0 with 1 M of NaOH. Afterwards, 50 μL of fish samples solution were introduced into 96-well plates and the next steps were the same as fabrication of standard color card for Hx. Finally, the resulting etched–GNRs solution was compared with the standard color card followed by recorded with normal camera and Multiskan spectrum microplate spectrophotometer. For the reference, the fish samples also were detected by Hx assay kit purchased from Sigma-Aldrich (USA).

3. Results and discussion

3.1. Principle of the proposed multicolor biosensor for hypoxanthine

The principle of proposed multicolor biosensor for Hx based on the etching of GNRs is shown in Fig. 1A. Hx reacts with the dissolved oxygen to form H_2O_2 under the catalysis of XOD through the following reactions,

$$Hx + O_2 \xrightarrow{XOD} Xanthine + H_2O_2$$

$$Xanthine + O_2 \xrightarrow{XOD} UricAcid + H_2O_2$$

The amount of H_2O_2 produced has a direct relationship with the Hx concentration. In the presence of Fe^{2+}, the classical Fenton reaction can be proceed under the following reaction [30],

$$Fe^{2+} + H_2O_2 + H^+ \rightarrow Fe^{3+} + H_2O + {}^{\circ}OH$$

Since •OH has much stronger oxidability than that of H_2O_2, which can promote the etching reaction from the tips of GNRs quickly. So the aspect ratio of GNRs will change and accompanied by vivid colors changing of the solution, which can be discerned by the naked eyes easily. Different concentration of Hx will result in different colors of the solution observed to realize semi-quantitative determination of Hx. Furthermore, the shift of LSPR has a direct relationship with Hx concentration and this can be used to futher