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A naked-eye liquid-phase colorimetric assay of simultaneous detect cysteine and lysine

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ABSTRACT

In this work, we proposed a naked-eye colorimetric strategy for simultaneous detection of cysteine and lysine based on 3,3′,5,5′-tetramethylbenzidine (TMB) system in a neutral condition (pH 7.0). Based of the fact that Ag^+ could oxidize 3,3′,5,5′-tetramethylbenzidine (TMB) to oxidized TMB (oxTMB) and induce a blue color solution corresponding to an absorption peak centered at 652 nm, thus the adding of cysteine or lysine to formation of different product results in the blue color fading to colorless quickly or change to scarlet, respectively. With this strategy, the limit of detection in experimental measurement for cysteine and lysine is 2.46 μM and 3.87 μM, respectively. And the selectivity can also be guaranteed among the other amino acid. The cost is quite low since the use of TMB and Ag^+. More importantly, the colorimetric detection operation is very simple without any further modification process and the reaction under physiological conditions is more meaningful for detection of amino acid.

1. Introduction

The amino acid is conducive to plants, mammals, and a variety of microscopic forms of life, the essential amino acid plays a vital role in biological and vegetal formation [1]. Cysteine (Cys) functions even in vegetal formation [2]. Cysteine plays a vital role in the human body in protein synthesis, detoxification, and metabolism [3–5]. As for lysine (Lys), is an important component of the human and animal diet which is closely related to the Krebs-Henseleit cycle and polyamine synthesis [6–8]. Therefore, an abnormal concentration in vivo may lead to many some congenital metabolic disorders like cystinuria or hyperlysinemia. Thus, the quantification of cysteine and lysine are of significant importance for human health. Especially, the simultaneous recognition like cysteine and lysine is valuable in the accurate predetermination and diagnosis of various diseases and disorders. On this direction, developing a simple and facile strategy is of significant importance.

To date, a great of efforts have been devoted to develop a highly sensitive and selective method to detect cysteine and lysine, including capillary electrophoresis [9,10], high-performance liquid chromatography [11,12], mass spectrometry [13,14], fluorescence analysis method [15–18], electrochemical voltammetry [19,20], and colorimetric methods [21–26], In some cases, the analytical methods usually require a complicated operation or an expensive cost. Among them, colorimetric sensors are especially promising due to their simple, naked-eye applications with less labor and less expensive equipment than other closely related methods. Moreover, to the best of our knowledge, there are only several works that have been reported for simultaneous detection of cysteine and lysine [27].

TMB is a benign and noncarcinogenic chromogenic substrate for colorimetric detection [28–32], typically used as a benign and non-carcinogenic color reagent in enzyme-linked immunosorbent assay (ELISA). Here, we report a simple colorimetric method for the simultaneous determination of cysteine and lysine in a system consisting of 3,3′,5,5′-tetramethylbenzidine (TMB) and silver nitrate ($AgNO_3$). In this reaction, TMB could be catalyzed by Ag^+ to oxTMB in acetate buffer (pH 7.0) and induce a blue color which is milder than other TMB reaction condition, on the basis of that, adding of cysteine or lysine, the blue color changes to colorless or scarlet, respectively. More interestingly, if we expand the concentration of the reaction, a lot of nanobelts precipitated out of the solution, after adding of cysteine or lysine, accompanied by precipitation of uniform small dot and well-proportioned schistose, respectively.

In short, we only need a basic reagent to achieve the simultaneous detection of cysteine and lysine by one step which also accompanies the

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transformation of nanostructures morphology. Besides, we use cyclic voltammetry (CV) to further testify the mechanism. And the test paper and the logic gate may also create new opportunities for on-site or point-of-care testing and design.

2. Material and methods

2.1. Chemicals and instrumentation

All chemicals were of analytical grade and used as received. Aqueous solutions used throughout were prepared with ultra-pure water (> 18.25 MΩ/cm) obtained from a Millipore system. 3,3′,5,5′-tetramethylbenzidine (TMB) and silver nitrate ($AgNO_3$) were purchased from Shanghai Aladdin Bio-chem Technology Co. Ltd. (Shanghai, China). All natural amino acids such as alanine (Ala), aspartic (Asp), cysteine (Cys), glutamic (Glu), glycine (Gly), histidine (His), leucine (Leu), lysine (Lys), phenylalanine (Phe), sarcosine (Sar), tryptophan (Trp), threonine (Thr), tyrosine (Tyr) and valine (Val) were supplied by Sigma Chemical Reagent Company. All other chemicals were of analytical grade and used as received. Sodium acetate buffer solution (NaAc, 0.2 mM, pH = 7) was prepared with sodium acetate (NaAc) and acetic acid (HAc). All experiments were performed at room temperature. The UV/vis experiments were performed using an Agilent UV-8453 spectrophotometer (Agilent Inc., Jpn). The acidity of the buffer solution was monitoring using a Metrohm 632 pH-meter. Scanning electron microscopy (SEM) was conducted using a Zeiss electron microscope (Zeiss, Oberkochen, Germany) equipped operated at an accelerating voltage of 5 kV. All electrochemical measurements and characterization were performed on a CHI660 electrochemical station (CHI Instruments Inc., USA) with a conventional threeelectrode system where bare and/or modified glassy carbon electrode (GCE, 3 mm in diameter) were used as working electrode, Pt wire and Ag/AgCl (saturated KCl) as auxiliary and reference electrodes, respectively.

2.2. Preparation for colorimetric assay by UV-vis spectral measurements characterization and apparatus

Deionized water was used to prepare a stock solution (1×10^{-2} M) of Ala, Asp, Cys, Glu, Gly, His, Leu, Lys, Phe, Sar, Trp, Thr, Tyr, and Val. The proposed colorimetric method controlled by a system consisting of 3,3′,5,5′-tetramethylbenzidine (TMB) and silver nitrate ($AgNO_3$) solution in liquid phase media. Control solutions containing 0.16 mM TMB indicator solution and 0.24 mM Ag^+ in 0.2 M acetic acid buffer solution at pH 7.0 were prepared, meantime the color of the whole solution obviously changed from colorless to blue.

2.3. Preparation of test paper and image capture for Cys and Lys determination

Colorimetric test paper for Cys and Lys was prepared by immersing filter paper in 0.2 M acetic acid buffer solution (pH 7.0) that containing TMB (0.48 mM) and silver ions (0.72 mM) for 30 min and then dried. And then, test paper was immersed into the samples that containing a different concentration of Cys and Lys, and color changes of the test papers can be observed for the aqueous solutions containing Cys and Lys.

3. Results and discussion

3.1. Design of the proposed colorimetric sensor

TMB as a common redox mediator has been widely used in biosensor applications, such as UV–vis [31], electrochemical assays [33], biological detection [34] and fluorescence techniques [35]. There into, colorimetric analytical strategies have attracted considerable attention on account of advantages of simple, low cost and no need of any complicated instruments. TMB is the most common chromogenic substrate, especially in enzyme-linked immunosorbent assay (ELISA), which requires multiple steps, complex operation and time-consuming. The oxidation of TMB by enzyme/enzyme-like or catalyst usually in acetate buffer (pH 4.0) with H_2O_2 produces a blue color, with major absorbance peaks at 370 and 652 nm [28,31,36]. The reaction condition has some limitations for biological detection. Besides, enzymatic reaction needs more harsh reaction condition, such as pH control, catalyst and reaction temperature which is infaust to the reaction. Based on the concept, if we use enzyme-like reagent which have the similar property to oxidize TMB, we will get better results. As we know that some metal ion also have the catalytic effect, such as Ag^+ has probably played a significant part in the development of plasmonics, and its properties make it suitable for most of the next generation plasmonic technologies [37,38], so we chooses silver ion to oxidize TMB not only as silver ion is more stable than enzyme but also didn't need complicated process. Ag^+ could oxidize 3,3′,5,5′-tetramethylbenzidine (TMB) to oxidized TMB(ox TMB) induce a color change from colorless to blue, based on the fact, we use a more mild pH value (pH 7.0) which is approach to a physiological environment and we didn't use H_2O_2 anymore which is different from the previous reports. In addition, we use this system for simultaneous detect of cysteine and lysine by one step and the color of the solution is a change from blue to colorless and from blue to scarlet, respectively. Fig. 1 shows representative absorbance spectra of the oxTMB blue solution and two reaction mixtures with distinctively different colors (colorless and scarlet). It showed the optical behavior of our colorimetric assay by using UV-Vis technology. As clearly, oxidized TMB has an absorption peak centered at 652 nm (curve a), with the addition of lysine, the absorbance at 652 nm is slowly decreased and exhibited a typical reaction product absorption at 495 nm (curve b), and there is no obvious peak can be observed (curve c) upon the addition of cysteine.

3.2. Mechanistic investigation on colorimetric sensing of Cys and Lys

A plausible mechanism for simultaneous detection of lysine and cysteine is schematically presented in Scheme 1, according to our experimental results and foregoing reports of TMB system, TMB can be oxidized into its free radical form cationic free radical via single

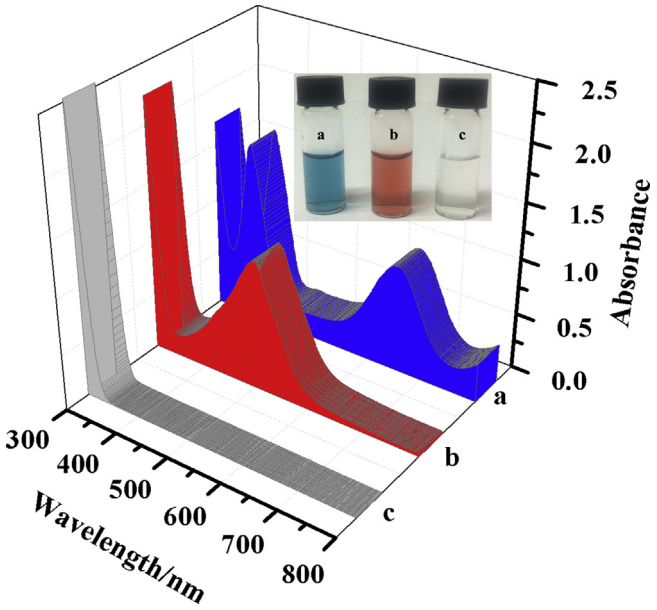

Fig. 1. The UV-vis absorption spectra of different solution: (a) 0.16 mM TMB + 0.24 mM Ag^+ solution; (b) a + 1 mM of lysine; (c) a + 0.29 mM of cysteine (Inset is the photography of the corresponding solution).

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