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A portable chemical sensor for histidine based on the strategy of click chemistry

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ABSTRACT

A novel portable chemical sensor is developed in combination of the personal glucose meters (PGM) with click chemistry for sensitive and selective determination of histidine. Invertase-labeled alkynyl-DNA can be modified onto the surfaces of Streptavidin Magnespheres Paramagnetic Particles (PMPs) through copper(I) catalyzed azide–alkyne cycloaddition (CuAAC) reaction and formed invertase-functionalized PMPs, which can be separated easily. The presence of invertase can convert sucrose to glucose and can be monitored by the PGM easily. The presence of histidine can inhibit the CuAAC, so the read-out signal of PGM decreased. The difference in signals from the PGM before and after addition of histidine has a good linear correlation with the logarithm of the histidine concentrations in the range of 0.01 ~ 100 μM with a detection limit of 3.4 nM, which is lower than those of many other chemical sensors. Moreover, the assay of histidine in milk samples is demonstrated with satisfactory results.

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1. Introduction

Histidine, one of the 20 natural amino acids, plays an essential role in the growth, repair of tissues and mammalian central-nervous system. Additionally, it participates in the action of controlling the transport of metal ions in biologically important bases (Chen et al., 1999) and minimizing internal bleeding from microtrauma (Horrobin et al., 1979). High levels of histidine could result in symptoms of intoxication (Liao et al., 2004), causing stress and psychological disorders (Jones et al., 2005). Whereas, lack of histidine often induces a lot of diseases, such as epilepsy, Parkinson's disease, nerve deafness, and so on (Leebeek et al., 1989; Rao et al., 1993; Ma et al., 2008). People usually need to take in histidine from food, such as milk and banana. Therefore, the monitoring of histidine in the food samples (such as milk) has become significant and indispensable.

Many analytical techniques have been utilized to detect histidine with high sensitivity and selectivity, including chromatography (Ruta et al., 2007), mass-spectrometry (Miyagi and Nakazawa, 2008), fluorescence (Kong et al., 2011; Liu et al., 2013), electrochemistry (Prasad et al., 2011; Li et al., 2011), colorimetry (Lata et al., 2005; Bae et al., 2009), capillary electrophoresis (Zhang and Sun, 2004; Zhou et al., 2010) and so on. However, these methods either require laboratory-based instruments, well-trained personnel and complicated sample pretreatments, or they are not cost effective or commercially available to the general public. Also some colorimetric methods do not need any instrument, it is difficult to distinguish the color change at low concentration and is only semi-quantitative.

Click chemistry, first proposed by Sharpless and coworkers in 2001 (Kolb et al., 2001), owns great advantages such as outstanding selectivity, high yield, high purity, mild reaction conditions and good reaction kinetics. Copper(I) catalyzed azide–alkyne cycloaddition (CuAAC) is a typical example of click reaction (Rostovtsev et al., 2002; Lutz, 2008; Qiu et al., 2013). In order to initiate the reaction, copper(I) species can be obtained from the reduction of copper(II) by sodium ascorbate (SA) or ascorbic acid (AA) in situ, and at almost the same time quantitatively catalyzes the azides and alkynes cycloaddition reaction.

The personal glucose meters (PGM), one of the most successful examples of portable sensors, have been widely accessible to the public worldwide due to the portable "pocket" size, cost effectiveness, reliable quantitative results and easy operation (Carroll et al., 2007). However, this successful portable sensor is still limited in its single target (glucose). Lu's group (Xiang and Lu, 2011, 2012a, 2012b, 2013) expanded the range of targets to a series of non-glucose targets such as recreational drug, toxic metal ions, important biological cofactor, disease marker by taking advantage of invertase-labeled functional DNAs and antibodies. Furthermore, Yan et al. (Yan et al., 2013) reported a target-responsive "sweet" hydrogel combined with PGM for non-glucose targets detection, such as cocaine and ATP. Su and coworkers (Su et al., 2013) published a sensitive

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copper(II) sensor which combined the merits of PGM and click chemistry.

Early reports show that the presence of histidine can inhibit the reduction of Cu(II) to Cu(I) by the ascorbate (Scarpa et al., 1996; Somasundrum et al., 1996; Hasebe and Gu, 2005), which in turn inhibits the CuAAC reaction. Based on this principle, a fluorescent sensor for detection of histidine had been developed with high sensitivity and selectivity (Qiu et al., 2013). In this study, a convenient histidine detection method by using PGM as read-out has been developed. The azido-DNA is immobilized on Streptavidin Magnespheres Paramagnetic Particles (SA-PMPs) and the alkynyl-DNA has been conjugated to an invertase firstly. Cu(II) can be reduced to Cu(I) by sodium ascorbate and in turn induces the CuAAC between the azide modified PMPs and alkynyl-DNA, resulting in the attachment of invertase on the PMPs. Then PMPs with invertase are separated from the mixture by a magnet easily. Subsequently, the invertase can catalyze the hydrolysis of sucrose to produce a large amount of glucose, which is quantitatively readout using the PGM. However, if histidine had been added into the above mentioned solution, which could inhibit the CuAAC reaction, results in the read-out signal reduced obviously. Based on this principle, a portable sensor for histidine can be developed. The signal from PGM has a linear relationship with the logarithm of histidine concentration in the range of $0.01 \sim 100\ \mu M$ with a detection limit of 3.4 nM. The proposed sensor has been applied to assay histidine in the milk samples with satisfying results and recoveries.

2. Experimental section

2.1. Materials and reagents

Sodium ascorbate, copper sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$), L-histidine, sucrose and other reagents were obtained from Alfa Aesar China (Tianjin) Co. Ltd. 4-(N-Maleimidomethyl)cyclohexane-1-carboxylic acid 3-sulfo-N-hydroxysuccinimide ester sodium salt (Sulfo-SMCC), 6-mercaptohexanol Tris(2-carboxyethyl)phosphine hydrochloride (TCEP), invertase (β-fructosidase, from baker's yeast) and Tween-20 were achieved from Sigma-Aldrich (St. Louis, MO, USA). NaH_2PO_4 and Na_2HPO_4 were bought from Shanghai Chemical Reagent Company (Shanghai, China). Streptavidin Magnespheres Paramagnetic Particles (PMPs) was purchased from Promega Corporation (Madison, USA). A commercially available glucose meter (ACCU-CHEK Avia) and test strips were received from Roche Diagnostics India Pvt. Ltd. (Mannheim, Germany) and used at room temperature. The photographs were taken with a Panasonic DMC-FX35 digital camera (Panasonic, Japan). The following oligonucleotides DNA (left to right: 5′–3′) were synthesized by Shanghai Sangon Biotech. Co. Ltd. (Shanghai, China):

5′-biotin modified DNA (azido-DNA):

Biotin–AAAAAAAAAAAATCACAGATGAGTAGT–N_3

3′-thiol group modified DNA for invertase conjugation (alkynyl-DNA):

CH≡CH–TGTCCGTAGCTAAAAAAAAAAAAA–SH

All solutions were prepared using Milli-Q reagent water (Milli-Q, Millipore, 18.2-MΩ resistivity). Buffer solution used in this study:

Buffer A: 0.1 M NaCl, 0.1 M sodium phosphate buffer solution, pH 7.3, 0.05% Tween-20.

Buffer B: 0.1 M NaCl, 0.1 M sodium phosphate buffer solution, pH 7.3.

The sucrose solution (1.0 M) is prepared in the buffer A solution (pH 7.3) and stored at 4 °C until used. All materials and chemical reagents were of analytical grade or better and used directly without special indication. The milk samples were purchased from the local supermarket and the samples were diluted by the buffer A solution for 100 times before detection.

2.2. Synthesis of alkynyl-DNA-invertase conjugate

The invertase-labeled DNA sequence (DNA-invertase) was prepared according to the previous report (Hermanson, 2008) with a slight modification. 30 μL of 10 μM Thiol-DNA which was dissolved in Millipore water, 2 μL of 0.3 mM TCEP and 2 μL of 10 mM sodium phosphate buffer at pH 5.5 were added into the Millipore water, then mixed and incubated for 1 h at room temperature. Finally, the mixture dialyzed against buffer B solution to obtain the thiol-DNA. For invertase conjugate, 710 μL of 11.29 mg/mL invertase solution and 1 mg of Sulfo-SMCC were added into buffer B solution, after incubated and vibrated for 5 min, the mixture was mixed on a shaker for 1 h at room temperature. Noted that the mixture was then centrifuged and separated the insoluble excess Sulfo-SMCC. The clear solution of Sulfo-SMCC-activated invertase was then mixed with the above solution of thiol-DNA and incubated at room temperature for 48 h. To remove unreacted thiol-DNA, the solution was dialyzed against buffer B solution to obtain alkynyl-DNA-invertase conjugate.

2.3. Immobilization of biotin modified azido-DNA onto Streptavidin Magnespheres Paramagnetic Particles

A portion of 0.6 mL of 1 mg/mL Streptavidin Magnespheres Paramagnetic Particles (SA-PMPs) was washed three times by buffer A solution and then dispersed in 1 mL of buffer A solution. Biotin modified azido-DNA was added into the solution to achieve a final concentration of 0.6 μM, and the mixture was placed on a shaker for 1 h at room temperature. Under these conditions, the specific combination of streptavidin with biotin (Weber et al., 1989) allowed the combination of PMPs with biotin modified azido-DNA. After that, PMPs with azido modified DNA were formed and can be separated from the mixture by a magnet for the paramagnetic property of PMPs. Finally, they were washed by buffer A solution three times and resuspended in buffer A solution. PMPs with azido modified DNA (azido-DNA-PMPs) were formed and stored at 4 °C for the later use.

2.4. Procedures for histidine detection

Azido-DNA-PMPs, alkynyl-DNA-invertase, 10 nM $CuSO_4$ and various concentrations of histidine were added into the buffer A solution (pH 7.3); then, ascorbate sodium was added into the above solution and incubated for 1.5 h in a dark drawer at room temperature to initiate the reaction. After that, PMPs with invertase were separated from the mixture using a magnet to remove the unreacted reactants. The PMPs with invertase were further washed with buffer A solution (50 μL) for three times and eventually dispersed in 10 μL buffer A solution. Subsequently, 20 μL of the sucrose solution (1.0 M) was added into the chemical sensor carefully and incubated at room temperature for 20 min. The enzymatic reaction generates glucose and it can be detected by the glucose meters (PGM). The data was collected as the difference of the signals from the PGM with and without addition of histidine, and used for the quantitative analysis. All measurements were repeated three times and the standard deviation was calculated as the error analysis.

2.5. Conditional experimental conditions

The compositions of the mixtures at different conditions were shown below:

Condition a: buffer A solution (as the blank); condition b: condition a+PMPs; condition c: condition a+azido-DNA-PMPs; condition

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