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Colorimetric sensing strategy for β-lactamase inhibitor using generation of 2-amino-3-mercapto-3-methylbutanoic acid via enzyme catalysis and Cu(II)-mediated redox

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ABSTRACT

A colorimetric sensing method has been proposed for the detection of β-lactamase (β-Lac) activity and the screening of the inhibitors in this work. The method is based on the aggregation of AuNPs induced by 2-amino-3-mercapto-3-methylbutanoic acid (AMMBA) through the formation of Au-S bond and electrostatic interaction. Since AMMBA can form through β-Lac catalyzed hydrolysis and Cu(II)-mediated redox with penicillin as enzyme substrate, β-Lac activity is related to the extent of AuNPs aggregation. The enzyme activity can be determined at levels as low as 0.556 U/mL and over a linear detection range as wide as from 0 U/mL to 30 U/mL. Furthermore, the inhibitory effects of clavulanic acid and sulbactam on the activity of β-Lac have also been tested and given IC_{50} values of 0.92 μM and 0.06 μM, respectively. The proposed method can be not only used for the detection of β-Lac activity but also the screening of the inhibitor.

1. Introduction

There are high proportions of antibiotic resistance in bacteria that cause common infections in all regions of the world. Antimicrobial resistance threatens the effective prevention and treatment of an ever-increasing range of infections caused by bacteria, parasites, viruses, and fungi. World Health Organization (WHO) has developed a draft global action plan to combat antimicrobial resistance, which has been submitted to the sixty-eighth World Health Assembly, taking place in May 2015. Since the introduction of β-lactam antibiotics, many organisms previously susceptible to the penicillins have developed resistance. This resistance is often due to the presence of β-lactamase (β-Lac), an enzyme that is capable of hydrolyzing the β-lactam ring, thereby destroying the antibiotic activity [1]. One way to restore the susceptibility of resistant strains of bacteria to the antibiotic is to coadminister β-Lac inhibitor [2]. Consequently, it is of the importance to detect the enzyme activity and screen its inhibitors.

Currently, different kinds of methods have been exploited for the determination of β-Lac activity and the screening of the inhibitors, and these methods include fluorescence spectrometry [3], colorimetric method [4], and titration method [5]. For the colorimetric analysis of β-Lac, the substrate containing a linker which can induce gold nanoparticles (AuNPs) aggregation [6] and a methoxyimino cephalosporin derivative with a pair of fluorescence resonance energy transfer fluorophores [7] have been synthesized. The procedure of synthesis is relatively complicated. So, it is highly hopeful to fabricate a simple and economic method for the detection of β-Lac activity and the screening of the inhibitors.

Due to their good optical properties, for example, high molar absorption coefficient and surface plasmon resonance that can cause the red shift of resonance wavelength, AuNPs have been widely used as sensitive probe for the DNA hybridization [8,9], carbohydrate sensing [10,11], and monitoring special enzyme activities such as alkaline phosphatases, kinases, proteases, glucosidase, etc. [9,12–14]. It has been well confirmed that cysteine can induce the transformation of AuNPs agglomeration states from dispersion to aggregation through the formation of Au-S bond and electrostatic interaction [15]. Similar to cysteine, 2-amino-3-mercapto-3-methylbutanoic acid (AMMBA) also contains a mercapto group, an amino group, and a carboxylic group, so it can be well predicted that the compound can lead to AuNPs aggregation. Penicillin (Pen) can be converted into AMMBA via β-Lac

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catalysis and Cu(II) mediated ordox with the formation of mediate product, penicilloic acid [16,17]. Therefore, a simple and easily operational colorimetric method can be exploited for the assay of β-Lac activity and the screening of the inhibitors. For one thing, Pen, an enzyme substrate, does not need to synthesize intricately and can be obtained commercially. For another, without the modification of AuNPs, enzymes can freely react with substrates in the detection system with fast enzyme kinetics.

2. Materials and methods

2.1. Materials and reagents

Sulfate copper, gold acid chloride trihydrate, citric acid trisodium salt dehydrate, clavulanic acid, and sulbactam were purchased from Sigma-Aldrich Co. (Shanghai, China). Pen and β-Lac (Blas, EC 3.5.2.6, 1000 U/mg) were obtained from Aladdin Co. (Shanghai, China). All buffers and aqueous solutions were prepared with ultrapure water which was purified with a Millipore Milli-Q water purification system (Branstead, USA) to a specific resistance of 18 MΩ cm.

2.2. Synthesis of AuNPs

AuNPs were synthesized according to a published procedure [18]. Briefly, 10 mL trisodium citrate (38.8 mM) was added to a boiling solution of $HAuCl_4$ (100 mL, 1 mM), and the resulting solution were kept continuously boiling for another 30 min to give a wine red mixture which was cooled to room temperature. Then the mixture was filtrated through a Millipore syringe (0.45 μm) to remove the precipitate and the filtrate were stored in a refrigerator at 4 °C for use.

2.3. β-Lac activity assay

β-Lac solution were obtained by dissolving into the phosphate buffer solution (10 mM, pH 7.4). 10 μL β-Lac solution at different concentrations (2.5 to 40 U/mL) was mixed with 20 μL Pen aqueous solution (20 mM) and the resulting solution was kept at 30 °C. Subsequently, 20 μL sulfate copper aqueous solution (7 mM) was further added into the mixture and continuously incubated for 30 min, followed by the addition of AuNPs solution (50 μL). After 10 min, the reaction solution were photographed with a digital camera (NET-3NL, SONY, Japan) and used for UV–vis spectroscopic measurements (Shimadzu Co., Kyoto, Japan).

2.4. Inhibition efficiency evaluation

For the inhibition assay, clavulanic acid or sulbactam with different concentrations (5 μL) were firstly premixed with β-Lac (5 μL) for 10 min at 30 °C. Then Pen (20 μL, 20 mM) was added into the mixed solution and continuously incubated at 30 °C. Subsequently, 20 μL sulfate copper (7 mM) was added into the resulting mixture and successively reacted for 30 min, followed by the addition of AuNPs solution (50 μL). After 10 min, the reaction solution was photographed and the absorbance of each sample was recorded using UV–vis spectroscopy with the IC_{50} value calculated. The inhibitory ratio (%) of clavulanic acid or sulbactam on β-Lac was expressed as follows:

$$\text{Inhibitory ratio}\,(\%) = \frac{A_{620}/A_{530} - A^{*}_{620}/A^{*}_{530}}{A^{0}_{620}/A^{0}_{530} - A^{*}_{620}/A^{*}_{530}} \times 100$$

where A_{620}/A_{530} was the ratio of the absorbance value at 620 nm to that at 530 nm in the presence of both the inhibitor and the enzyme, A^{0}_{620}/A^{0}_{530} was the ratio of the absorbance value at 620 nm to that at 530 nm without the enzyme and inhibitor, A^{*}_{620}/A^{*}_{530} was the ratio of the absorbance value at 620 nm to that at 530 nm in the presence of enzyme only.

3. Results and discussions

The mechanism of the proposed method for the assay of β-Lac activity and the screening of the inhibitor is illustrated in Scheme 1. Under β-Lac catalysis, Pen can be hydrolyzed into penicilloic acid which has a strong reduction capability [16]. (Scheme 1(a)). In the presence of Cu(II), penicilloic acid can convert to the intermediate compound which can reorganize to render the 2-hydroxy-3-phenyl-6-methylpyrazine. With the sulfide group as the probable oxidation site, the compound can subsequently react into the final reaction species including Cu(I) complex and AMMBA (Scheme 1(a)) [17]. Due to the reason that it contains a mercapto group, an amino group, and a carboxylic group (Scheme 1(a)), AMMBA can induce the dispersive AuNPs aggregate through the formation of Au-S bond and the electrostatic interaction (Scheme 1(b)). Thus, when Pen, β-Lac, and Cu(II) coexist in the detection system, the aggregation of AuNPs will happen and the color of testing solution will transfer from red to blue owing to the formation of AMMBA, a hydrolyzed product of Pen (Scheme 1(c)). On the contrary, in the presence of inhibitor, β-Lac activity will be inhibited and Pen cannot convert into penicilloic acid, so AuNPs will remain dispersive state and the testing solution will keep red as a result of no occurrence of AMMBA in the detection system (Scheme 1(c)).

3.1. Mechanism investigation for β-Lac activity assay

As depicted in Fig. 1, upon adding PBS buffer to the AuNPs as a control experiment, the red color of the testing solution remains unchanged and the solution exhibits a maximum absorption at 522 nm (vial 1, blank curve), representing the feature of well-dispersive AuNPs [18]. With the addition of Pen into the AuNPs solution, the initial color and UV–vis spectra of the testing solution also keep unchanged (vial 2, red curve). It indicates that Pen alone does not induce the aggregation of AuNPs. When β-Lac (vial 3, blue curve) and Cu(II) (vial 4, green curve) are separately mixed with Pen followed by the addition of AuNPs, the two resultant solution also keep initial red and exhibit similar UV–vis spectra. The former suggests that penicilloic acid, the hydrolyzed product, has no evident impact on AuNPs stability. The later indicates that simultaneous addition of Pen and Cu(II) can not lead to change of AuNPs states.

Nevertheless, it can be noticed that the addition of these three compounds including Pen, β-Lac, and Cu(II) may essentially trigger the aggregation of AuNPs, resulting in a change from red to blue and the production of a new absorption peak at 620 nm (vial 5, pink curve). Typically, a change in the color of the AuNPs solution is caused by particle aggregation (or agglomeration) and light scattering assisted surface plasmons [14]. The phenomena can be explained by the formation of AMMBA which can induce AuNPs aggregation through the formation of Au-S bond and electrostatic interaction (Scheme 1(c)) [15].

3.2. Optimization of colorimetric assay

In the presence of β-Lac, Pen can be hydrolyzed into penicilloic acid and Cu(II) with oxidization ability can react with the hydrolyzed product, penicilloic acid, to form AMMBA which can lead to AuNPs aggregation. So the increased amount of Pen and Cu(II) can benefit the occurrence of AuNPs aggregation so as to improve the sensitivity and linear range of the proposed method. On the other hand, high concentrations of Pen and Cu(II) in the