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Fluorescent sensor assay for β-lactamase in milk based on a combination of aptamer and graphene oxide

Jing Qin [a], Xiping Cui [a], Panpan Wu [a], Zhengyun Jiang [a], Yingshan Chen [a], Ruili Yang [b], Qingqing Hu [a], Yuanming Sun [b], Suqing Zhao [a, *]

[a] Department of Pharmaceutical Engineering, Faculty of Chemical Engineering and Light Industry, Guangdong University of Technology, Guangzhou 510006, China

[b] Key Laboratory of Food Quality and Safety of Guangdong Province, College of Food Science, South China Agricultural University, Guangzhou 510642, China

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ABSTRACT

Fluorescent sensor assay (FSA) was developed by using a fluorescein-labeled aptamer assembled onto Graphene oxide (GO) in order to determine β-lactamase in milk. Under optimal conditions, FSA indicated a detection range from 1 to 46 U/mL with a limit of detection (LOD) of 0.5 U/mL ($R^2 = 0.999$, n = 3). In addition, commercial milk samples tainted with β-lactamase were detected by the established FSA with a recovery rate between 96.04 and 119.67%. Additionally, the reliability and sensitivity of FSA was validated by enzyme-linked immunoassay (ELISA) with a high correlation of 0.993. Thus, these data, combined with the ease and speed of the assay, suggest that the developed FSA may represent a promising method for monitoring β-lactamase contamination in milk.

1. Introduction

β-lactamase is produced by bacteria that hydrolyze β-lactam antibiotics (Xu et al., 2010), such as penicillin and cephalosporin (Bush, 2015; Singh et al., 2016). Under certain conditions, β-lactamase as an adulteration is added into food or raw materials for food, such as milk or raw milk, to mask the use of antibiotics. However, long-term consumption of β-lactamase increases the risk of bacterial resistance in humans (Cizman, 2003; Oteo, Perez-Vazquez, & Campos, 2010). Additionally, β-lactamase may reduce or even offset the curative effects of antibiotic drugs. And the breakdown of antibiotics may produce other hazardous substances, which could result in an allergic condition, such as urticaria, fever, or anaphylactic shock (Maslikowska et al., 2016). Since 2009, the China Food and Drug Regulation Administration (CFDA) has restricted the maximum allowed concentration of β-lactamase in milk to be 4 U/mL.

Presently, the main methods used to detect β-lactamase include the microorganism method (Gui, Zhuo, Chai, Xiang, & Yuan, 2015), the cups and saucers method (Thai et al., 2016), the iodometric method (S. H. Cui, Li, Hu, Jin, & Ma, 2007), the acidimetric method (Wintermans & Vandenbroucke-Grauls, 2016), the high performance liquid chromatography method (Lara, del Olmo-Iruela, Cruces-Blanco, Quesada-Molina, & Garcia-Campana, 2012) and the enzyme-linked immunoassay (ELISA) method (Wang et al., 2013). The majority of these methods are indirect colorimetric ones, with many disadvantages such as poor sensitivity and specificity, tedious sample preparation and expensive instruments to be needed. Although the ELISA method is sensitive, the preparation of the specific antibody is complex, and the reaction requires a long time (1–2 h) and several washing steps. Thus, it is necessary to develop new biological molecules to replace the standard antibody in the ELISA, in order to establish a more sensitive and quick method to monitor β-lactamase.

Aptamer is a short widowed nucleotide sequence *in vitro*, which can be efficiently combined with several target molecules (Ellington & Szostak, 1990; Li et al., 2014; Sefah, Shangguan, Xiong, O'Donoghue, & Tan, 2010; Tuerk & Gold, 1990). Compared with traditional antibodies, aptamer has the advantage of highly sensitivity, to be more specific, being easy to be obtained and highly stable. Without target, aptamer will be in the free straight chain state, while in presence of target, aptamer will change into the

* Corresponding author.
E-mail addresses: jingqin1002@163.com (J. Qin), cuixiping1989@163.com (X. Cui), wyuwpp@126.com (P. Wu), jkk928@163.com (Z. Jiang), chenysgdut@163.com (Y. Chen), rlyang77@scau.edu.cn (R. Yang), 18320408023@163.com (Q. Hu), ymsun@scau.edu.cn (Y. Sun), sqzhao@gdut.edu.cn (S. Zhao).

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tertiary structure, specifically binding to its target with unique structure (Duan et al., 2013). Aptamer has been widely used in biosensors (Qian, Shan, Chai, Chen, & Peng, 2015; S. Wu et al., 2015) and drug detection (Roushani & Shahdost-Fard, 2015).

Graphene oxide (GO) is the product of a reaction of graphite powder with strong chemical oxidants (X. H. Li, Wang, Li, Ma, & Zhu, 2015; Yu et al., 2015). GO has attracted significant attention in the fields of materials and biological studies for its unique properties, such as large surface area, electrical conductivity and dispersion in water, Due to its electrical properties (Y. W. Zhu et al., 2010), GO is a highly efficient fluorescence quencher (Ji, Qian, Wu, Zhang, & Cai, 2015; Sheng, Ren, Miao, Wang, & Wang, 2011; Wei et al., 2015), being used to construct the technique of fluorescence resonance energy transfer (FRET). In addition, GO can bind with single-stranded DNA (ssDNA) via hydrophobic and π-π stacking interactions between the ring structures in the nucleobases and the hexagonal cells of GO (Scheme 1) (Z. S. Lu, Chen, Wang, Zheng, & Li, 2015; Xing et al., 2012). However, GO rarely interacts with rigid double-stranded DNA (dsDNA) or aptamer and its target complexes (Bai et al., 2014). Furthermore, Zhu et al. (Y. Y. Zhu et al., 2015) and Lu et al. (C. H. Lu, Yang, Zhu, Chen, & Chen, 2009) have demonstrated that although GO can specifically adsorb and quench the labeled ssDNA probe, the fluorescence recovery as the probe breaks away from the GO surface. To the best of our knowledge, a sensitive and quick FSA to detect β-lactamase in milk is yet to be reported.

In this study, a novel, rapid, and sensitive fluorescent sensor assay (FSA) has been developed by using a fluorescein-labeled aptamer assembled onto GO in order to determine the broad spectrum of β-lactamase in milk.

2. Materials and methods

2.1. Reagents and apparatus

β-lactamase was obtained from Aladdin Co. Ltd. (Shanghai, China). Graphite powder, ethylene diamine tetraacetic acid (EDTA) and trisbase were purchased from Tianjin Damao Chemical Reagent Co. Ltd. (Tianjin, China). *Anti*-β-lactamase aptamer was synthesized by Sangon Biotechnology Co. Ltd. (Shanghai, China). β-lactamase ELISA Kit was purchased from Shanghai Enzyme Linked Biological Technology Co. Ltd. (Shanghai, China). All chemicals were of analytical reagent grade and ultrapure water was used throughout the experiment. The sequence of β-lactamase specific aptamer [fluorescein amidite (FAM)-ssDNA, FDNA] reported before was 5′-FAM-CCAAACTCGGG-3' (Fast & Sutton, 2013).

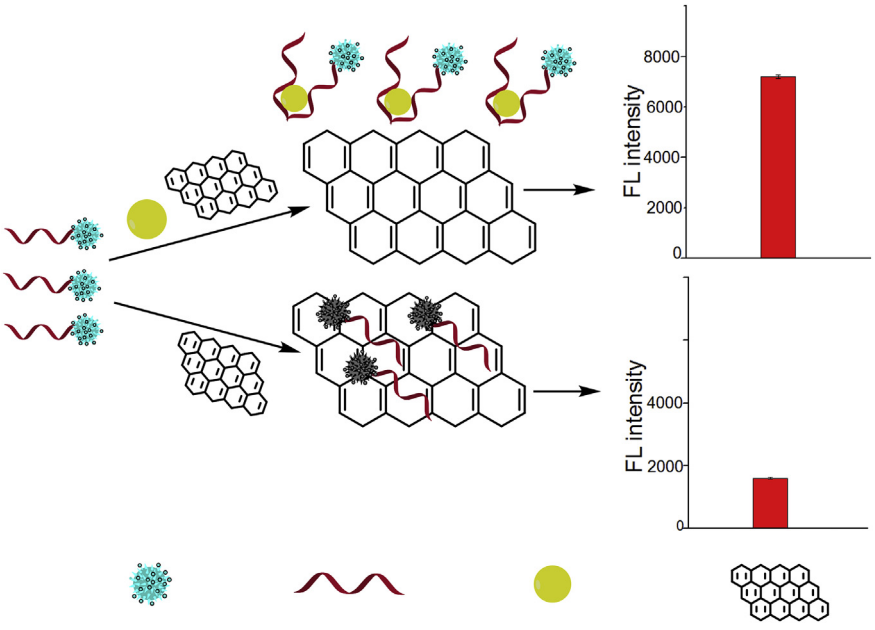
TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.2), sodium phosphate buffer (10 mM, pH 7.2), and potassium phosphate buffer (10 mM, pH 7.2) were made in our laboratory. The 96-well black plates were purchased from Corning (USA). Millipore Milli-Q ultrapure (18 MΩ.cm) water was used throughout the experiment.

Ultraviolet visible spectrophotometer (Lambda 25, Perkin Elmer, USA), Fourier Transform Infrared Spectrometers (Nicolet 6700, Thermo Fisher, USA), UV Laser Raman Spectroscopy (LabRAM HR 800, HORIBA JOBIN YVON, France), Ultra high resolution field emission scanning electron microscope (MAIA 3 LMH, TESCAN, Czech), Infinite F200 Multimodel Plate Reader (TECAN, Austria). The excitation wavelength was $\lambda = 485$ nm, and the emission wavelength was $\lambda = 535$ nm.

2.2. Preparation of GO

The GO sheets were prepared from graphite powder according to the Hummers method (Hummers & Offeman, 1958; Marcano et al., 2010). Firstly, 3 g of graphite powder was added into 70 mL of concentrated sulfuric acid in a beaker with stirring in an ice water bath. Next, 2 g of $NaNO_3$ and 9 g of $KMnO_4$ were slowly added to the mixture followed by heating at 35 °C for 30 min to obtain a dark green solution. Lastly, 138 mL of H_2O was added to the reaction system. After stirring for 15 min, the reaction was terminated by adding 120 mL of 60 °C H_2O_2 (3%) until there were no obvious bubbles. The reaction color changed from brown to brown to yellow finally. The yellow solution was centrifuged and washed in HCl (5%) until the supernatant pH value was 4–6. Finally, the product was washed in water and SO_4^{2-} was removed with $BaCl_2$.

The mixture solution was dried in the oven to obtain the GO sheets. The product was dissolved in water by ultrasonic dissolution for 1 h (Hu, Song, & Lopez-Valdivieso, 2015).



Scheme 1. Schematic illustration of the FSA for β-lactamase based on GO and β-lactamase binding aptamer.