Novel Aptamer Probe for D etcting Biphenol A Based on Fluorescence Resonance Energy Transfer

Xiaojing Li *
Fujian Provincial Key Laboratory of Inspection and Quarantine Technology
Technical Centre of Fujian Entry-Exit Inspection and Quarantine Bureau, Fuzhou 350001, China
e-mail: lxjciq@hotmail.com

Xi Tang
Fujian Provincial Key Laboratory of Inspection and Quarantine Technology
Technical Centre of Fujian Entry-Exit Inspection and Quarantine Bureau, Fuzhou 350001, China
e-mail: tangx@fjciq.gov.com

Minshi Chen
Technical Centre of Fujian Entry-Exit Inspection and Quarantine Bureau, Fuzhou 350001, China
e-mail: chenms@fjciq.gov.com

Zhexiang Zou
Fujian Provincial Key Laboratory of Inspection and Quarantine Technology
Technical Centre of Fujian Entry-Exit Inspection and Quarantine Bureau, Fuzhou 350001, China
e-mail: zouzx@fjciq.gov.com

Jinlan Dai
Fujian Provincial Key Laboratory of Inspection and Quarantine Technology
Technical Centre of Fujian Entry-Exit Inspection and Quarantine Bureau, Fuzhou 350001, China
e-mail: daijl@fjciq.gov.com

Siyuan Zheng
Fujian Provincial Key Laboratory of Inspection and Quarantine Technology
Technical Centre of Fujian Entry-Exit Inspection and Quarantine Bureau, Fuzhou 350001, China
e-mail: liuhj@fjciq.gov.com

Lijun Lu
Fujian Provincial Key Laboratory of Inspection and Quarantine Technology
Key Laboratory of Analysis and Detection Technology for Food Safety, Ministry of Education (Fuzhou University), Fuzhou, 350007
e-mail: 1038059276@qq.com

Huajian Liu
Fujian Provincial Key Laboratory of Inspection and Quarantine Technology
Technical Centre of Fujian Entry-Exit Inspection and Quarantine Bureau, Fuzhou 350001, China
e-mail: liuhj@fjciq.gov.com

Abstract—Fluorescence resonance energy transfer (FRET) is the radiation process which the donor molecules at the excited state transferred capacity to the acceptor molecules via remote dipole. Aptamers can specially recognize its target molecules. Based on these principles, a sensitive and selective fluorescent sensor for bisphenol A (BPA) has been designed. The fluorescence intensity of the sensing system increased gradually with the increasing of BPA concentration. △F has a linear relationship with the logarithm of BPA concentration in the range of 0.1 nM - 10 nM concentration with a correlation coefficient (R) of 0.9986 and the detection limit of 0.07 nM (defined as S/N=3). The proposed fluorescent sensor has good selectivity for BPA assay even in the presence of higher concentration of interferents.

Keywords-FRET;, aptame;, BPA; fluorescence; deteting

I. INTRODUCTION

Bisphenol A (BPA) is a small carcinogenic molecule, which has threaten to environment and humans[1]. BPA is currently regarded as an environmental endocrine disrupting chemical that is potentially estrogenic. Numberous studies have confirmed that BPA is present in plastic containers for food, baby bottles, water, food cans and food wrap, presenting a large number of routes for human exposure[2]. So there have been increasing needs for the detection of BPA. Until recently, BPA detection was done through chromatographic methods, such as gas

and liquid chromatography[3], or other conventional assay methods, such as immunoenzymebased assays[4].

Aptamers are molecular recognition probes with a high affinity for considerably different molecules, ranging from large targets such as proteins, peptides and complex molecules of drugs to organic small molecules or even metal ions[5-9]. Aptamers undergo conformational changes after binding with the targets[10, 11]. Aptamers are selected in vitro from a large library of nucleic acid sequences (1015) by the process known as systematic evolution of ligands by exponential enrichment (SELEX)[12]. It has generally been recognized that aptamer affinity is comparable to or even higher than that of antibodies[13, 14].

II. Materials and Methods

A Reaents and Apparatus

BPA, BPA aptamer and its complementary strand were synthesized by Sangon Inc. (Shanghai, China) and their sequences were shown as follows:

BPA aptamer (D1):

5'-tggtcgttggtcgttcgcgtttctggattt tttatttctggggttcagtt ctttttgt-3' ;

Complementary strand 1 of BPA aptamer on the one end added FAM (T1):

3'-AGCAAGCGCA AAGACCTA-FAM -5';

Complementary strand 2 of BPA aptamer on the one end added TAMRA (T2):

3'-TAMRA-TAAAGACCCCAAGTCAA-5'.

All other chemicals were of analytical grade. Milli-Q reagent water (Milli-Q, Millipore, 18.2-MΩ resistivity) was used throughtout the whole process.

Cary Eclipse Fluorescence Spectrophotometer (Varian Corporation, USA) was used for the measurements. The apparatus parameters were set as follows: λex= 485 nm (slit 10 nm), λem= 500~650 nm (slit 10 nm), Voltage = 700 V.

B Fluorescence spectroscopic measurements

BPA aptamer (D1) , Complementary strand 1 of BPA aptamer (T1) and Complementary strand 2 of BPA aptamer (T2) were added into the britton robinson burrer (BR) solution (50 mM, pH 7.4), incubated for 2 h at room temperature. And then BPA was added into the solution, reacted for 10 h. The data of fluorescence emission spectra were collected at the excitation wavelength of 485 nm in BR buffer solution (50 mM, pH 7.4). The fluorescence increase factor ($\triangle F= F-F_0$) had been used for quantative analysis, where F_0 represented the ratio of the fluorescence intensity at 520 nm and 580 nm of T1 and T2 hybridize with D1 and F represented the ratio of the fluorescence intensity at 520 nm and 580 nm after BPA was added in to the solutio of T1, T2 and D1.

Fluorescence resonance energy transfer (FRET) is the radiation process which the donor molecules at the excited state transferred capacity to the acceptor molecules via remote dipole[15]. In this study, we chose 6-carboxyfluorescein (FAM) which was added on the one end of the complementary strand of BPA aptamer to be FRET donor and 6-carboxytetramethylrhodamine (TAMRA) to be FRET acceptor which was added on the one end of the other complementary strand of BPA aptamer. When the distance between FAM and TAMRA is shorter than about 10 nm, FRET happened and the signal of fluorescence is weak. As the distance between FAM and TAMRA become farther, the signal of fluorescence is strong. We make advantage of this rule to detect BPA.

III. Results and Discussions

A Optimization of the reaction system

The effect of the pH of the BR buffer on the fluorescence intensity had been studied. As DNA is more stable under the neutral or a little alkaline condition, we chose pH 6.0, 6.5, 7.1, 7.4, 7.8, 8.2 and 8.5 to be studied in this experiment. As shown in Fig .1, $\triangle F$ increased with the increasing of pH from about pH 6.0 to 7.0, and reached a plateau from about pH 7.0 to 8.0. Then $\triangle F$ decreased if pH was higher than 8.0. So pH 7.4 had been chosen as the optimized reaction pH.

The effect of the concentration of the BR buffer on the fluorescence intensity had been studied. We chose 5 mM, 25 mM, 50 mM, 75 mM and 100 mM to be studied in this experiment. As shown in Fig .2, $\triangle F$ increased with the increasing of the concentration of the BR buffer and then decreased if the concentration was higher than 50 mM. So 50 mM had been chosen as the optimized concentration.

The effect of the reaction time between BPA and its aptamer on the fluorescence intensity had been studied. We chose 2 h, 4 h, 6 h, 8 h, 10 h and 12 h to be studied. As shown in Fig .3, $\triangle F$ increased with the increasing of the reaction time and reached a plateau as the time was more than 10 h. So 10 h had been chosen as the optimized reaction time.

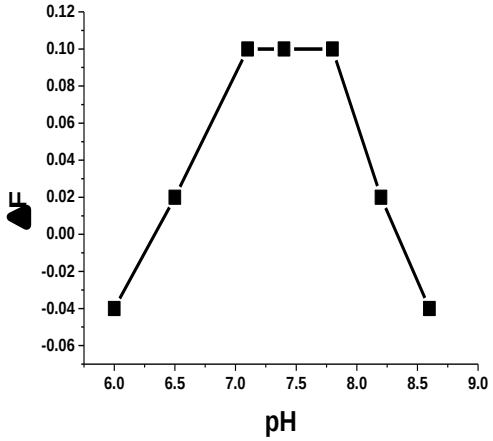

Figure 1. The effect of pH of the buffer on the fluorescence intensity of the system

△F=F-F_0: F_0 is the ratio of the fluorescence intensity at 520 nm and 580 nm of T1 and T2 hybridize with D1; F is the ratio of the fluorescence intensity at 520 nm and 580 nm after BPA was added in to the solutio of T1, T2 and D1.

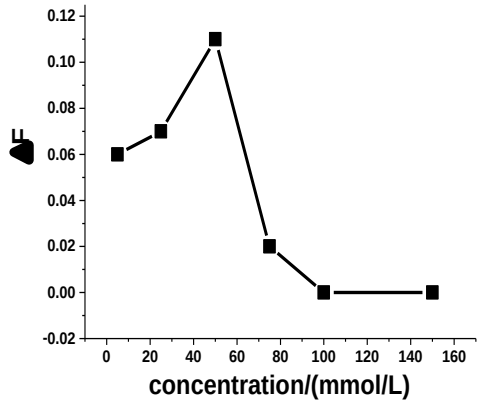

Figure 2. The effect of the concentration of the buffer on the fluorescence intensity of the system

△F=F-F_0: F_0 is the ratio of the fluorescence intensity at 520 nm and 580 nm of T1 and T2 hybridize with D1; F is the ratio of the fluorescence intensity at 520 nm and 580 nm after BPA was added in to the solutio of T1, T2 and D1.

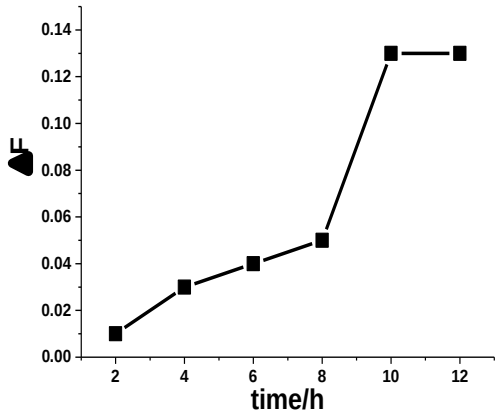

Figure 3. The effect of the reaction time on the fluorescence intensity of the system

△F=F-F_0: F_0 is the ratio of the fluorescence intensity at 520 nm and 580 nm of T1 and T2 hybridize with D1; F is the ratio of the fluorescence intensity at 520 nm and 580 nm after BPA was added in to the solutio of T1, T2 and D1.

B Linear relationship and the detection limit

The fluorescence intensity of the sensing system increased gradually with the increasing of BPA concentration. △F has a linear relationship with the logarithm of BPA concentration in the range of 0.1 nM - 10 nM concentration with a correlation coefficient (R) of 0.9986 (shown in Fig .3). The regression equation is $\triangle F = 0.2493 + 0.0203 \log C/\mathrm{M}$. The detection limit was calculated to be 0.07 nM (defined as S/N=3), which is lower than the dissolubility of the national standard requirement of carbonic acid ester resin and the phenol in moulded which is 0.05 mg/kg.

C Specificity of the sensor

The potential interferences such as some derivatives of phenol and metal ions have been investigated. The concentration of BPA was settled as 10 nM and the other interferents were 1.0 mM. The results were shown in Fig .4. The result indicated that the interferents did not cause interference for BPA determination and the proposed method exhibited high selectivity.

IV. CONCLUSIONS

Taking advantage of FRET and aptamers specially recognize its target molecules, a simple fluorescent method for BPA determination has been developed. This proposed method has high sensitivity and selectivity.

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