G-quadruplex DNAzyme as the turn on switch for fluorimetric detection of genetically modified organisms†

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A novel fluorescent sensor for detection of genetically modified organisms was developed, and in the sensor G-quadruplex DNAzyme (G-quadruplex–hemin complex) was used as the turn on switch.

In recent years, more and more genetically modified food derived from genetically modified organisms (GMOs) has been put on the market.[1] However, there are still some controversial issues, for instance, environment risk, and biosafety.[2] For this purpose, many countries and areas have introduced labeling thresholds for GM foods,[3–5] for example, 0.9% in the European Union (EU),[6] 3% in Korea[7] and 5% in Japan.[8] In order to fulfil the labeling policy, DNA analysis for determining the modified genes in food is necessary. Polymerase chain reaction (PCR) based methods are the first choice in the detection of GMOs.[1,9,10] In spite of PCR being excellent in terms of high sensitivity and stability, it is criticized for some drawbacks such as complicated and labor-intensive procedures, expensive running costs, and being time-consuming.[11,12] Therefore, the development of simple, cheap and rapid detection methods for GM foods is necessary. Herein, we introduce a novel nucleic acid-based sensor, which uses G-hemin quadruplex DNAzyme as an effective fluorescence booster for rapid GMO DNA detection.

G-quadruplex produced by the folding of G-rich sequences is able to interact with hemin effectively even though their affinity is only submicromolar. Consequently, a DNAzyme similar to the horseradish peroxidases (HRP)[13–15] is formed. It is thought that the unusual four GGG repeats are possibly dividing the whole sequence into two parts.[16] Interestingly, the splitting strategy leads to an extraordinary selectivity for nucleic acid recognition.[17] For example, Nakayama and Sintim utilized the split G-quadruplex probes for nucleic acid sensing *via* probe re-modeling;[18] Kolpashchikov demonstrated a split DNA enzyme for visual single nucleotide polymorphism typing (SNP) with 3,3′-diaminobenzidine tetrahydrochloride (DAB).[19] In this context, we explored a new DNA probes with the split mode for GMO detection.

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The 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt ($ABTS^{2-}$) is commonly used as a chromogenic substrate. Its corresponding product after oxidation, $ABTS^{-}$, has a strong UV-visible absorption signal at 415 nm. However, several biomolecules such as cytochromes also absorb light in this region. The presence of these biomolecules significantly interfere with the use of ABTS as the probe in assays that use crude cell lysates or whole cells.[20] Most importantly, the analysis methodology depending on UV-visible absorbance usually has lower dynamic range and sensitivity compared to fluorescence. In addition, Nakayama and Sintim[20] proved that 2′,7′-dichlorodihydrofluorescein diacetate (H_2DCFDA) is a superior alternative to ABTS in bioanalyte detection. In this study, we thus employed H_2DCFDA as the reducing substrate in GMO detection.

The working principle of this GMO sensor is outlined in Fig. 1. The probes were dissolved in the tris-HCl buffer and there was no G-quadruplex DNA formed without an appropriate cation.[21] As the target DNA-T was added, it hybridized with the two probes. As a result, probes 1 and 2 were fixed and were getting closer to each other. Under these conditions, with the addition of hemin, NH_4^+ and K^+ to the solution, the G-quadruplex–hemin complex (DNAzyme) was formed. The complex is a very effective catalyst for H_2DCFDA oxidation with H_2O_2. The oxidized product (2′,7′-dichlorofluorescein) ultimately gives strong fluorescence intensity.

The effect of pH on enhancement rate ($\Delta F/F_R$) was investigated. The experimental results taking in the pH range of 6.0–8.4 were shown in Fig. S1 (see ESI† for details). It is obvious that $\Delta F/F_R$ increases as the pH of the solution is increased from pH 6.0 to 7.0. However, when the solution becomes more alkaline (pH > 7.0), $\Delta F/F_R$ was found to decrease sharply, which maybe because the alkaline

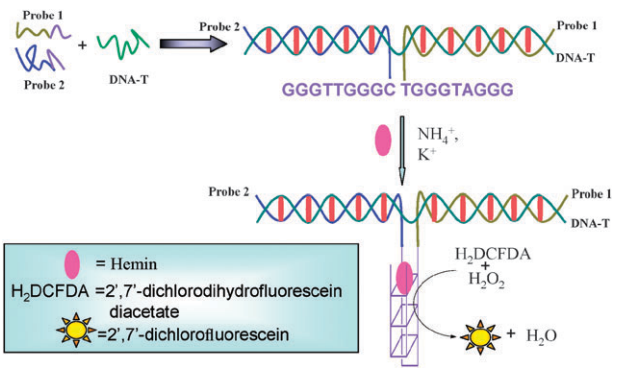

Fig. 1 Working principle of this GMO sensor.

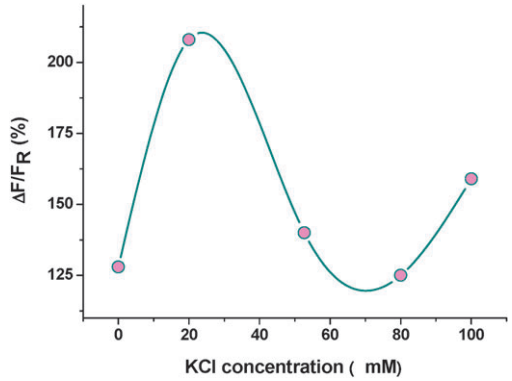

Fig. 2 Effect of KCl concentration on $\Delta F/F_R$ in 50 mmol L^{-1} Tris-HCl containing 150 mmol L^{-1} NH_4Ac (pH 7.0) with probes 5×10^{-7} mol L^{-1}, DNA-T 3.0×10^{-7} mol L^{-1} and hemin 5.0×10^{-7} mol L^{-1}.

environment reduced the catalytic activity of the DNAzyme. The results indicate that pH = 7.0 is the optimum condition for this study.

It is known that potassium ions show a higher affinity for the G-quadruplex. Nevertheless, the DNAzymes (G-quadruplex–hemin complex) in this study are prepared using ammonium cations, which possess higher catalytic activity.[18] The effect of KCl concentration in buffer on $\Delta F/F_R$ was therefore studied. As shown in Fig. 2, the enhancement rate increases with increasing KCl concentration. When the KCl concentration is higher than 20 mmol L^{-1}, $\Delta F/F_R$ decreases remarkably. The results are in agreement with the findings reported by Sen *et al.*[21] In this study, 20 mmol L^{-1} of KCl was used as the optimized concentration for GMO detection.

Although the complex produced from hemin and G-quadruplex has a superior catalytic activity,[22] the unreacted hemin in solution causes background noise.[20] Hence, the effect of hemin concentration is also an important factor for investigation. Fig. S2 (see ESI† for details) shows the relationship between $\Delta F/F_R$ and hemin concentration. The highest $\Delta F/F_R$ value was obtained when the hemin concentration was 1.0×10^{-7} mol L^{-1}. Therefore, the optimized concentration of hemin was found to be 1.0×10^{-7} mol L^{-1}.

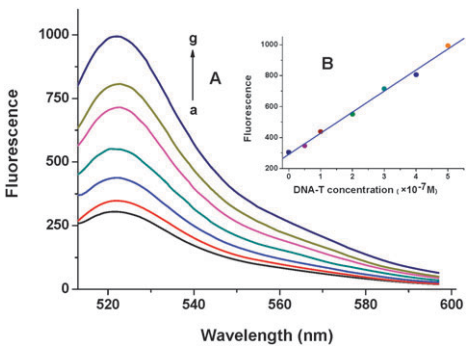

Fig. 3 Calibration curve for DNA-T in 50 mmol L^{-1} Tris-HCl containing 150 mmol L^{-1} NH_4Ac and 20 mmol L^{-1} KCl (pH 7.0) with probes 5×10^{-7} mol L^{-1} and hemin 1.0×10^{-7} mol L^{-1}. A: fluorescence curve with various concentration (mol L^{-1}), (a) 0.0, (b) 5.0×10^{-8}, (c) 1.0×10^{-7}, (d) 2.0×10^{-7}, (e) 3.0×10^{-7}, (f) 4.0×10^{-7}, (g) 5.0×10^{-7}. B: linear relationship between fluorescence intensity and DNA-T concentration. The PMT detector voltage is 700 V.

Fluorescence detection of target DNA-T utilizing the nucleic acid probes was carried out in a H_2DCFDA–H_2O_2 solution at room temperature. The results are shown in Fig. S3 (see ESI† for details). The addition of control sequence DNA-R shows no significant enhancement in the fluorescence intensity (curve b), whereas DNA-T enhances the fluorescence intensity more than 3-fold compared to DNA-R. These observations suggests that the probes are able to detect DNA-T effectively and sensitively with respect to the fluorescence changes.

A calibration curve was established for the detection of target DNA-T in a tris-HCl buffer containing 150 mmol L^{-1} NH_4Ac and 20 mmol L^{-1} KCl under optimal conditions. As shown in Fig. 3, the fluorescence intensity is linearly proportional to the concentration of DNA-T in the range of 5.0×10^{-8} to 5.0×10^{-7} mol L^{-1}. A linear regression eqn (1) was set up:

$$F = 292.32 + 136.03C_{\mathrm{DNA-T}} \ (R = 0.9972) \qquad (1)$$

where F is the fluorescence intensity at 525 nm, $C_{\mathrm{DNA-T}}$ represents the concentration of DNA-T, and R is the regression coefficient. The detection limit is estimated to be 7.0×10^{-9} mol L^{-1} (defined as three times the concentration corresponding to the standard deviation of the blank).

In summary, a novel and simple sensor using binary probes for detection of GMO with good selectivity and sensitivity was successfully developed. The sensor is able to fold into a G-quadruplex structure in the presence of DNA-T and give a G-quadruplex–hemin complex *via* complexation with hemin. This unique complex was found to effectively catalyze H_2O_2 oxidation of H_2DCFDA, and thus turned on fluorescence in GMO detection. Based on the changes of fluorescence intensity, the DNA-T is able to quantify in the range of 5.0×10^{-8} to 5.0×10^{-7} mol L^{-1} with a detection limit of 7.0×10^{-9} mol L^{-1}.

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