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PAPER

Dual color fluorescence quantitative detection of specific single-stranded DNA with molecular beacons and nucleic acid dye SYBR Green I

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We have developed a dual color fluorescence quantitative detection method for specific single-stranded DNA with molecular beacons (MBs) and nucleic acid dye SYBR Green I by synchronous scanning fluorescence spectrometry. It is demonstrated by a reverse-transcription oligonucleotide sequence (target DNA, 33 bases) of RNA fragment of human immunodeficiency virus (HIV) as a model system. In the absence of target DNA, the MBs are in the stem-closed state, the fluorescence of 5-carboxy-X-rhodamine (ROX) is quenched by black hole quencher-2 (BHQ-2), and the interaction between SYBR Green I and the MBs is very weak. At this time the fluorescence signals of ROX and SYBR Green I are all very weak. In the presence of target DNA, MBs hybridize with target DNA and form a double-strand structure, the fluorophore ROX is separated from the quencher BHQ-2, and the fluorescence of ROX recovers. At the same time, SYBR Green I binds to hybridized dsDNA, whose fluorescence intensity is significantly enhanced. Thus, dual color fluorescence quantitative detection for the target DNA can be realized by synchronous scanning fluorescence spectrometry. In this strategy, the fluorescence signal of SYBR Green I is far larger than that of ROX, so the quantitative analysis of target DNA with the fluorescence intensity of SYBR Green I can significantly improve the detection sensitivity. In addition, the false-positive signals of MBs do not affect the fluorescence signals of nucleic acid dye SYBR Green I. Thereby, in the analysis of complex samples, quantitative analysis of target DNA with SYBR Green I can avoid the false-positive signals of MBs and improve the detection accuracy.

1. Introduction

Selective detection of specific single-stranded DNA is gaining importance in the fields of clinical diagnostics, gene therapy, environmental research, food safety and a variety of biomedical studies.[1–6] Molecular beacons (MBs) are playing a very important role in the selective detection of specific single-stranded DNA (ssDNA) because they have been shown to have very high selectivity, sensitivity, and real-time detection capabilities.[7–12] In recent years, various techniques for the detection of specific single-stranded DNA with MBs have been developed.[13–16] And MBs have the potential to become a powerful tool in gene detection and quantification in living cells.[17] Unfortunately, so far the utility of traditional MBs for quantification in practical applications is limited because of two major limitations. On the one hand, the traditional MBs have background emission in the absence of the target DNA, and the residual fluorescence critically limits the detection sensitivity.[18] On the other hand, MBs are likely to produce false-positive signals once introduced into the complex fluids resulting from non-specific interactions, and the false-positive signals will greatly affect the accuracy of quantification detection.[19]

To address these two problems, we have developed a new method of dual color fluorescence quantitative detection of specific single-stranded DNA with MBs and nucleic acid dye SYBR Green I. It is demonstrated by a reverse-transcription oligonucleotide sequence (target DNA, 33 bases; for the sequences please see Section 2.1) of RNA fragment of human immunodeficiency virus (HIV) as a model system. The detection principle for target DNA is depicted in Fig. 1. SYBR Green I is an unsymmetrical cyanine dye, it has minimal intrinsic fluorescence and exhibits an 800- to 1000-fold fluorescence enhancement upon binding to double-stranded DNA (dsDNA).[20] In the absence of target DNA, the MBs are in the stem-closed state, fluorescence resonance energy transfer (FRET) will occur, and the fluorescence of 5-carboxy-X-rhodamine (ROX) is quenched by black hole quencher-2 (BHQ-2). Meanwhile, the interaction between SYBR Green I and the MBs is very weak. At this time the fluorescence signals of ROX and SYBR Green I are all very weak. In the presence of target DNA, MBs hybridize with target

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3.7. The linear correlation and the detection limit

Under the optimum conditions, the relationship between the fluorescence intensity of the two fluorescence dyes (ΔI) and the concentration of target DNA (C) is investigated. Fig. 8 shows the synchronous scanning fluorescence spectra in the presence of different concentrations of target DNA and the linear relationship between the fluorescence intensity (ΔI) of the two fluorescence dyes and the concentration of target DNA (C). A good linear relationship can be obtained under the optimum conditions. For the fluorescence intensity of SYBR Green I (ΔI_1) and the concentration of target DNA (C), the fitted regression equation is $\Delta I_1 = 0.7263C + 17.9803$ ($R^2 = 0.9988$) in the range from 80 to 8000 pM, and the detection limit (3σ, where σ is the standard deviation of a blank solution, $n = 11$) is 30 pM. For the fluorescence intensity of ROX (ΔI_2) and the concentration of target DNA (C), the fitted regression equation is $\Delta I_2 = 0.2379C + 4.4356$ ($R^2 = 0.9965$) in the range from 400 to 8000 pM, and the detection limit is 250 pM. Seven parallel measurements of 1 nM target DNA with SYBR Green I are used for estimating the precision, and the relative standard deviation (RSD) is 2.75%. In the two regression equations, the slope with SYBR Green I is far larger than that of ROX: this shows the detection sensitivity can be improved significantly with SYBR Green I. In this experiment, the ratio $(\Delta I_{\text{SYBR Green I}})/\Delta I_{\text{ROX}}$ remains basically unchanged in different concentrations of target DNA (Table 1): this shows the false-positive signals of MBs resulting from non-specific interactions in complex samples can be distinguished by the change of the ratio $(\Delta I_{\text{SYBR Green I}})/\Delta I_{\text{ROX}}$.

3.8. SNP analysis

In this study, we investigated the specificity of this method. The assay is challenged with different mismatched target DNA (for the DNA sequences, please see Section 2.1). Fig. 9 shows that the proposed method can markedly distinguish the perfectly complementary sequences from the single-base mismatched sequences. Therefore, this proposed method has a high selectivity, and it holds great promise to allow the analysis of single-nucleotide polymorphisms (SNPs).

3.9. Comparison of the determination results in different fluid

In order to examine the application of the proposed method in complex fluid, the target DNA is detected in the serum (the original serum is diluted five times by PB solution), and the determination results in the serum are compared with that in the PB solution. The results (Fig. 10A and B, Table 2) show that the fluorescence intensity of SYBR Green I remains basically unchanged in different fluids at the same concentration of target DNA, and that the fluorescence intensity of ROX in the serum is larger than that in the PB solution. The ratio $(\Delta I_{\text{SYBR Green I}})/\Delta I_{\text{ROX}}$ remains basically unchanged in the PB solution at different concentrations of target DNA; however, the ratio $(\Delta I_{\text{SYBR Green I}})/\Delta I_{\text{ROX}}$ has obvious changes in the serum. This means that the false-positive signals that the MBs introduced into complex fluids resulting from non-specific interactions can be distinguished by the change of the ratio $(\Delta I_{\text{SYBR Green I}})/\Delta I_{\text{ROX}}$, and quantitative analysis of target DNA with SYBR Green I can overcome the interference of the false-positive signals when MBs are introduced into the complex fluids.

4. Conclusions

In conclusion, we have developed a new strategy for dual fluorescence DNA detection with MBs and the nucleic acid dye SYBR Green I. This strategy displays high sensitivity and selectivity in quantification detection for target DNA with a low detection limit (Table 3). The proposed method has a good precision and can markedly distinguish perfectly complementary sequences from single-base mismatched sequences. In addition, the proposed method can effectively overcome the interference of false-positive signals that MBs introduce into complex fluids resulting from non-specific interactions.

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