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ORIGINAL PAPER

Molecular beacon-based fluorescence biosensor for the detection of gene fragment and PCR amplification products related to chronic myelogenous leukemia

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Abstract A novel fluorescence method has been established for the determination of gene fragment and PCR amplification products related to chronic myelogenous leukemia (CML). A molecular beacon (MB) which comprises a DNA loop section, a pair of fluorophore (tetramethoxyl rhodamine, TAMRA), and a quencher (4-(2-methyl-on-amino-azobenzene) benzoate, DABCYL) was designed. The loop sequence of MB was designed according to the DNA sequence relating to CML (type b3a2) which contained a single-stranded oligonucleotide. Before hybridization, the fluorescence from the TAMRA had been quenched by the DABCYL. After hybridization with the complementary DNA, the quencher will become far away from the TAMRA, and the fluorescence intensity detected will increase. Changes in the fluorescence intensity have a linear relationship with the concentration of complementary DNA (C) in the range of 4.0×10^{-9}–3.2×10^{-8} mol/L, with a correlation coefficient of 0.9973; the detection limit was 6.0×10^{-10} mol/L (S/N=3). The developed method has high selectivity, which can be used to discriminate single-base mismatch sequence. The method has been applied to detect the short-stranded CML DNA fragment (278 bp) with high sensitivity. This approach is a promising method for the detection of CML in real samples for medical diagnostics.

Keywords Chronic myelogenous leukemia · Molecular beacon · Fluorescence spectrophotometry

Ailin Liu and Zhouliang Sun contributed equally to the present study.

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Introduction

Chronic myelogenous leukemia (CML) is a clonal myeloproliferative disorder resulted from the neoplastic transformation of the primitive hemopoietic stem cell [1–3]. Since CML patients do not show any observable symptoms in the early stage and the chronic course can last for 3–5 years, it is difficult to diagnose CML in the early stage. The Philadelphia chromosome translocation can be found in as much as 95% of CML cases [4, 5]. This reciprocal t(9;22)

(q34;q11.2) results in a genetic recombination between the c-ABL proto-oncogene on chromosome 9 and the breakpoint cluster region (BCR) gene on chromosome 22. The resulting BCR/ABL chimerical fusion gene encodes a cytoplasmatic hybrid protein which plays an important role in the pathogenesis of CML [6–9]. Type b3a2, one of the most common mutation types about BCR/ABL, has often been studied [10]. Thus, detection of the BCR/ABL gene will afford an early diagnosis and monitoring of the disease, which in turn improves the facility of detecting minimal residual leukemia cells in the CML patients, especially after bone marrow transplantation. In recent years, monitoring methods for the clinical diagnosis and prognosis of the fusion gene of CML, including chromosome analysis, Southern blot, fluorescence in situ hybridization (FISH), PCR [11–13], etc., had been developed, but there remain certain limitations. Chromosome analysis is time-consuming and taxing; furthermore, its sensitivity is low. The Southern blot approach is complicated, long-playing, and expensive, and it is also insensitive to sequences with low copies. The FISH method has high sensitivity, but it has poor precision in localization. PCR-based methods have been proven to be highly sensitive diagnostic techniques for cellular recognition, but in general, the detection of specific DNA sequences is performed using gel electrophoresis of DNA fragments amplified by PCR using primers that are sequence-specific for the chosen region of DNA. Although it is simple and effective for the detection of PCR products, it cannot discriminate whether the sequence of the amplified target DNA is the same as that intended by using gel electrophoresis. In addition to these disadvantages, ethidium bromide, which is commonly used as a staining agent, is a carcinogenic chemical causing environment contamination [14, 15]. Thus, it is necessary to establish a novel, convenient, rapid, accurate, and sensitive approach for detecting the CML gene and solving the actual problems in the early diagnosis and prognosis monitoring of CML.

Since Tayagi and Krammer first established molecular beacon (MB) probe in 1996, it has been widely used as a new type probe for in vitro RNA and DNA monitoring [16, 17], biosensors and biochips [18, 19], real-time monitoring of genes and gene expression in living systems [20, 21], and the next generation of molecular beacons that will be highly useful for studies with proteins, molecular beacon aptamers [22]. As a brand-new tool, there are many advantages of the use of MBs over traditional fluorescent probes for the detection of specific gene targets, such as single-base mismatch distinguishing capability [23], an inherent signal transduction mechanism for high sensitivity [24], and the ability to detect target hybridization without separation of the hybridized and non-hybridized probes [25]. Therefore, MB can serve as a highly sensitive, extremely selective, nonradioactive probe for monitoring DNA hybridization.

In the present paper, we developed a rapid and novel molecular beacon-based assay for the fluorimetric detection of type b3a2 gene fragment and PCR-amplified real samples of CML. Compared with previous methods [11–13], the present approach is more sensitive, more rapid, simpler, and also has high specificity and selectivity. Therefore, this method is promising for the detection of CML in practical samples for medical diagnostics.

Experimental

Materials and chemicals

The 18-base synthetic oligonucleotides were purchased from Shanghai Sangon Biological Engineering Technology Services Reagents Co., Ltd. (China). The base sequences are as follows:

- Immobilized probe (molecular beacons): 5′-TAMRA-CGCTGC-AGA GTT CAA AAG CCC TTC-GCAGCG-DABCYL-3′. The 5′ end was modified with 6-carboxy-tetramethyl-rhodamine (TAMRA) as fluorophore and 3′ modified with 4-(4′-dioxane-amino-phenylazo) benzoic acid (DABCYL) as quencher.
- Complementary (18-base sequence S1): 5′-GAA GGG CTT TTG AAC TCT-3′
- Single-base mismatch (18-base sequence S2): 5′-GAA GGG CAT TTG AAC TCT-3′
- Non-complementary (18-base sequence S3): 5′-ACG TGG TCC CCA GCT CTC-3′

Two pairs of primers were purchased from Shanghai Sangon Biological Engineering Technology Services Reagents Co., Ltd. for the PCR amplification of the CML gene sample. Their base sequences are as below [26]:

- Primer 1 (21-base sequence): 5′-CGG GAG CAG CAG AAG AAG TGT-3′
- Primer 2 (21-base sequence): 5′-AAA GGT TGG GGT CAT TTT CAC-3′
- β-actin primer 1 (20-base sequence): 5′-CAT CTC TTG CTC GAA GCT CA-3′
- β-actin primer 2 (21-base sequence): 5′-ATC ATG TTT GAG ACC TTC AAC A-3′

DNA-Maker D was also purchased from Shanghai Sangon Biological Engineering Technology Services Reagents Co., Ltd. Ex Taq polymerase, which contained Ex Taq polymerase (5 U/μL), 10× Ex Taq buffer (Mg^{2+} Plus), dNTP mixture (2.5 mM, respectively), and 6× loading buffer, was obtained from TaKaRa Biotechnology Co., Ltd. (Dalian, China). Template cDNA of K562 and

real samples were kindly donated by the Fujian Institute of Hematology, the Affiliated Union Hospital of Fujian Medical University. All oligonucleotides stock solutions were prepared with Tris–HCl buffer (20 mM Tris–HCl, 10 mM $MgCl_2$, pH 8.00) and kept frozen. The results obtained from both the molecular beacon fluorimetric biosensor techniques and DNA agarose gel electrophoresis techniques were compared with each other. All chemicals were of analytical reagents grade and sterilized; deionized double distilled water was used throughout.

Apparatus

The spectra and intensity of fluorescence were measured by a 970-CRT spectrofluorometer (Shanghai Precision & Scientific Instruments Co., Ltd, Shanghai, China). PCR amplification was performed by a Gene Amp PCR system 2400 (Applied Biosystems, USA). Sequences of the PCR amplification products were determined by Shanghai Sangon Biological Engineering Technology Services Reagents Co., Ltd.

PCR amplification of DNA from blood samples

cDNA was obtained by applying a reverse transcription method from the total RNA which was extracted using a mini DNA-free total RNA extraction kit from venous blood, as described previously [27].

PCR amplification was performed on a Gene Amp PCR system 2400 thermal cycler using oligonucleotide primers (primer 1, primer 2, β-actin primer 1, and β-actin primer 2) as described by Elamaagacli et al. [26]. Briefly, 33.5 μL (or 34.5 μL) of double distilled water, 5 μL of 10× Ex Taq buffer solution (TakaRa), 4 μL of 2.5 mM dNTP (TakaRa), 1 μL of 10 pmol/μL primers 1 and 2 (or 0.5 μL of 10 pmol/μl β-actin primers 1 and 2), 5 μL of template cDNA of K562 or real samples, and 0.5 μL of Taq DNA polymerase (5 U/μL, TakaRa) were added into a 0.2-mL reaction tube, respectively. The reaction total volume was 50 μL. During the PCR process, DNA was denaturated at 94 °C for 5 min. Each of the 35 cycles of amplification consisted of annealing at 95 °C for 30 s, extension at 65 °C for 45 s, and denaturation at 72 °C for 60 s. Then, another extension at 72 °C for 7 min was carried out. Finally, the mixture was conserved at 4 °C before use. Amplification product detection was done by electrophoresis of the end products in a 1.5% agarose gel stained with ethidium bromide and visualized under ultraviolet light.

Hybridization detection in synthetic oligonucleotides

A certain amount of molecular beacon was added into a 1-cm quartz cell which contained the molecular beacon hybridization buffer. The fluorescence intensity of the solution was detected at the determined excitation and emission wavelengths (λ_{ex}=521 nm, λ_{em}=586 nm, respectively) in the fluorescence spectrophotometer. Then, synthetic target oligonucleotides were added into the molecular beacon hybridization buffer. Under optimum conditions, the molecular beacon hybridized with the target sequence for 30 min. After that, the fluorescence intensity of the mixed solution under the same conditions was detected again to compare changes in fluorescence intensities before and after the hybridization.

Hybridization detection in PCR-amplified real samples

Hybridization with PCR products was performed by immersing into a denatured PCR product solution. The diluted sample was then denatured by heating in a water bath at 95 °C for 10 min and then freezing the sample in an ice bath for 5 min. Hybridization was allowed to proceed for 30 min and then the fluorescence intensity detected.

Results and discussion

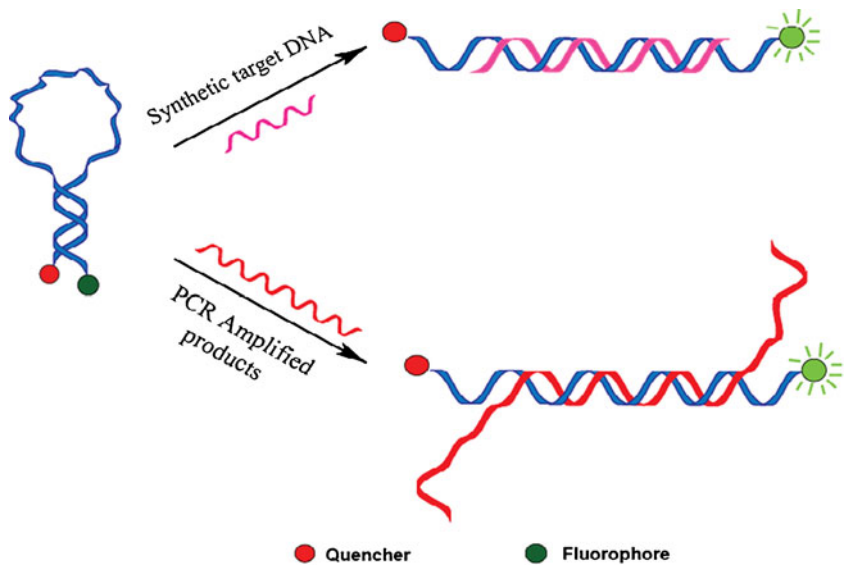
Principle of the biosensor

The scheme of the biosensor is shown in Scheme 1. In this approach, a new MB which comprised a DNA loop section, a pair of fluorophore (tetramethoxyl rhodamine, TAMRA), and a quencher (4-(2-methyl-on-amino-azobenzene) benzoate, DABCYL) was designed. As a DNA probe, the loop sequence of MB, which was designed according to the DNA sequences relating to chronic myelogenous leukemia (CML, type b3a2), contained a single-stranded oligonucleotide. The ends of the stem–loop structure were modified with the fluorophore and quencher, respectively. Before hybridization, the stem maintains a close proximity of the fluorophore and quencher, causing fluorescence to be quenched by energy transfer, and lower fluorescence intensities can be detected. If the MB is hybridized with its complementary DNA, it undergoes a spontaneous conformational reorganization to the opening state, leading to fluorescence restoration. Changes in fluorimetric behavior before and after hybridization have a relationship with the concentration of complementary DNA (C), which can be applied to detect the concentration of complementary DNA (C) with high sensitivity and selectivity.

Optimization of the conditions

Effect of temperature

In order to keep the function of molecular beacons optimal under a given set of assay conditions, it is important to



Scheme 1 Scheme of molecular beacon-based fluorescence biosensor

understand how their fluorescence changes with temperature in the presence and in the absence of their targets. Therefore, the effect of hybridization temperature was studied. The hybridization of MB with its complementary DNA had been performed at different temperatures; the results were shown in Fig. 1A. The intensities increase with increasing temperature and reach their maximum at 45 °C; after this, the intensities decrease with the further increase of temperature. The reason may be that the molecular beacons hybridized with the target sequence completely at 45 °C, and with further increase of hybridization temperature, the denaturation of dsDNA would accelerate and the

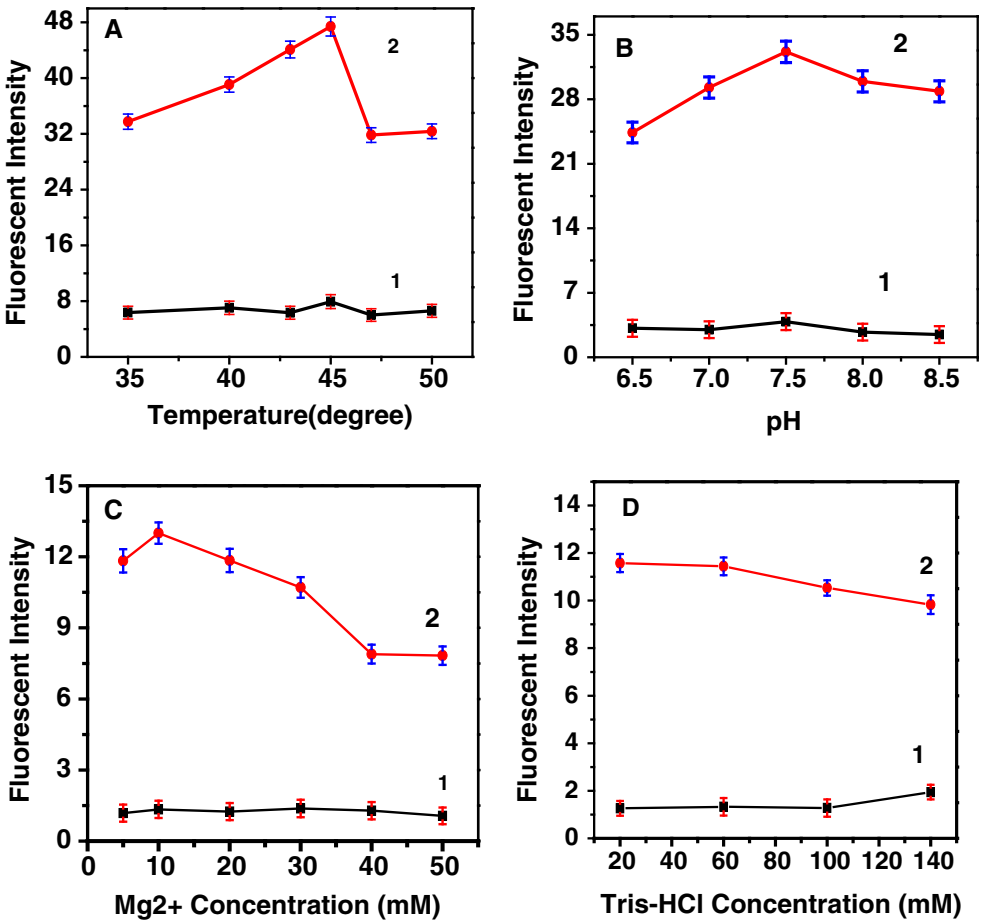


Fig. 1 **A** Fluorescent graph of the molecular beacon and MB/complementary DNA hybrid. The temperature range is from 35 °C to 50 °C. **B** Fluorescent graph of the molecular beacon and MB/complementary DNA hybrid. The pH range was from 6.5 to 8.5. **C** Fluorescent graph of the molecular beacon and MB/complementary DNA hybrid. The concentration of Mg^{2+} ranges from 5 to 50 mM. **D** Fluorescent graph of the molecular beacon and MB/complementary DNA hybrid. The concentration of Tris–HCl ranges from 20 to 140 mM. *1* MB, *2* MB/complementary DNA hybrid

absolute hybridization number would decrease [3]. Therefore, in the following measurement, 45 °C was used for the hybridization of molecular beacons with complementary DNA.

Effect of medium

The power of hydrogen plays an important role in the hybridization of DNA. The effect of hybridization buffer pH values (6.5–8.5) on the fluorescence intensity at 586 nm was shown in Fig. 1B. The maximum fluorescence intensity showed at pH 7.5. This result suggests that at pH 7.5, the probe–target hybrids formed completely, separating the fluorophore from the quencher and restoring fluorescence. Therefore, pH 7.5 was chosen as the optimized condition.

The ionic strength of the reaction buffer had a critical role in the formation of the molecular beacon. Therefore, the effect of the concentration of Mg^{2+} in the reaction buffer on the response of the probe hybridized with the target sequence has been investigated also. As shown in Fig. 1C, when Mg^{2+} concentration was 10 mM, the fluorescence signal of the hybridization mixture showed the strongest one, indicating that the hybridization proceeds more completely in this ionic concentration as compared with others. Therefore, the concentration of Mg^{2+} used in the hybridization buffer was set to 10 mM in this study.

The effect of Tris–HCl concentration was also studied. When the Tris–HCl concentration was 20 mM, the fluorescence intensity of the molecular beacons was at a minimum. When the concentrations changed from 40 to 140 mM, the fluorescence signal of the molecular beacons showed an upward trend. However, at 20 mM, the fluorescence signal change of the hybridization mixture was the strongest one. From Fig. 1D, the result indicates the concentrations of Tris–HCl in which molecular beacons exist in a closed state without the target sequence, whereas the probe–target hybrids form spontaneously and are stable in the same concentrations of Tris–HCl when there are target sequences in the hybridization buffer. Therefore, 20 mM Tris–HCl was selected.

Investigation of specificity

The fluorescence spectra of molecular beacons hybridized with complementary sequence (S1), single-base mismatched sequence (S2), and non-complementary sequence (S3) were recorded (Fig. 2). When the molecular beacons interacted with the complementary sequence in the solution, the fluorescence increased significantly. This increase clearly showed that the molecular beacon had successfully hybridized with its complementary sequence and that it had undergone a

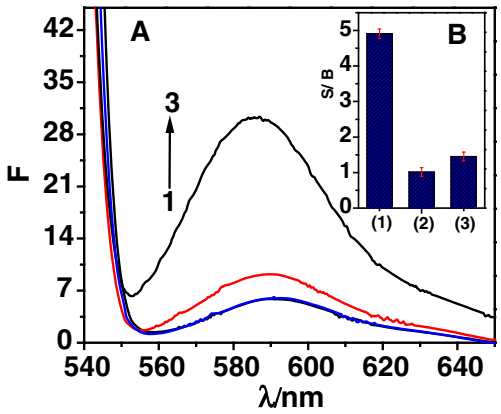


Fig. 2 **A** Fluorescent spectra of MB hybridizing with different gene fragments. *1–3* Samples are MB, complete mismatch DNA, single-base mismatch DNA, and complementary DNA. **B** Signal-to-background ratio (*S/B*) bar graph of MB hybridizing with different gene fragments. *1–3* Samples are complementary DNA, complete mismatch DNA, and single-base mismatch DNA

spontaneous conformational reorganization with the opening of the stem, leading to fluorescence restoration. As expected, there was no significant difference in fluorescence intensity for the molecular beacon and its hybridization with a non-complementary sequence. This means that the properties of the molecular beacon do not change after its interaction with the non-complementary sequence and that the fluorophore and the quencher are still held in close proximity to each other by the hairpin stem. In the presence of oligonucleotide containing a single-base mismatched sequence, a significantly decreased fluorescence signal was obtained as compared with the complementary sequence. This difference indicates that complete hybridization was not accomplished due to the base mismatch. In other words, no successful hybridization occurred in this case due to the sequence mismatch. These results demonstrate that the present approach has high specificity.

Calibration curve

Under optimal conditions, the fluorescence spectra after the hybridization of MB with different concentrations of the complementary DNA (complementary DNA concentrations were 4, 8, 16, 24, and 32 nmol/L) were recorded (shown in Fig. 3). The peak intensity increases with the concentration of complementary DNA. It was found that the relationship between the signal-to-background ratio (S/B) ratio and the concentration of complementary DNA was linear in the range of 4.0×10^{-9}–3.2×10^{-8} mol/L, with a correlation coefficient of 0.9973. The detection limit (S/N=3) is 6.0×10^{-10} mol/L (Fig. 4, inset). The regression equation was Y (S/B)=4.0354X(C)+3.1754. The result showed that this novel biosensor achieved an even higher gain than the previously reported method [11].

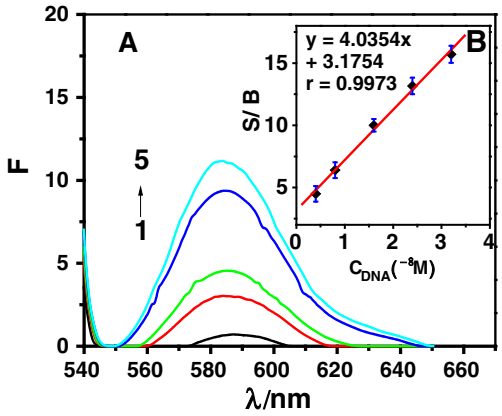


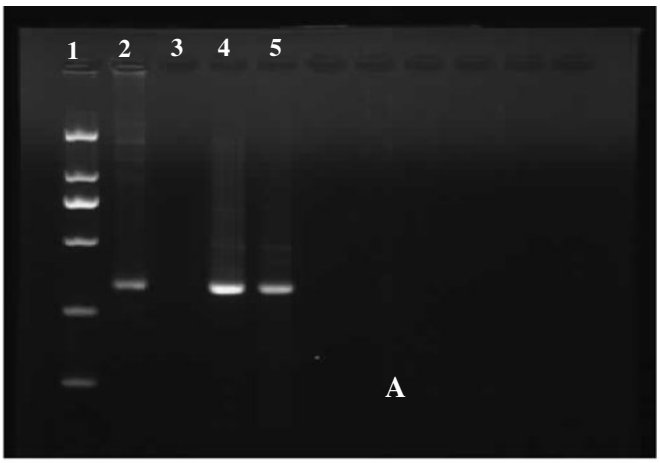
Fig. 3 **A** Fluorescence spectra of the MB hybridizing with different concentrations of DNA. Complementary DNA concentrations were 4 nmol/L (*1*), 8 nmol/L (*2*), 16 nmol/L (*3*), 24 nmol/L (*4*), and 32 nmol/L (*5*). **B** Signal-to-background ratio (*S/B*) calibration curve

Detection of electrophoresis of PCR products and gene sequencing

Figure4A shows the result of the electrophoresis of the PCR products. The PCR amplification products from the positive real sample and the K562 cell (Fig. 4A, lanes 4 and 5) showed bright bands in 1.5% agarose gel. However, the PCR amplification products from the negative real sample (Fig. 4a, lane 3) did not present any bands in the gel. In order to examine the quality of template cDNA from the negative sample, β-actin primers for PCR amplification were carried out. The bright band in lane 2 in Fig. 4A showed that the quality of the template cDNA from the negative sample is very well. As is known, K562 cell is the positive cell strain of leukecythemia which contains t(9;22)(q34;q11.2), and the PCR amplification band from the positive real sample consisted of the band from the K562 cell. Thus, we can extrapolate that the primers have an amplified specific band to discriminate a positive real sample and a negative real sample, and this PCR amplification product from the positive real sample may contain the target gene fragment we need.

After purification, the sequence of the PCR amplification products from the positive real sample was shown as follows:

CGTTCTCTGATCCGTGGAGCTGCAGATGCTGAC
CAACTCGTGTGTGAACTCCAGACTGTCCACAGCAT
TCCGCTGACCATCAATAAGGAAGATGATGAGTCTC



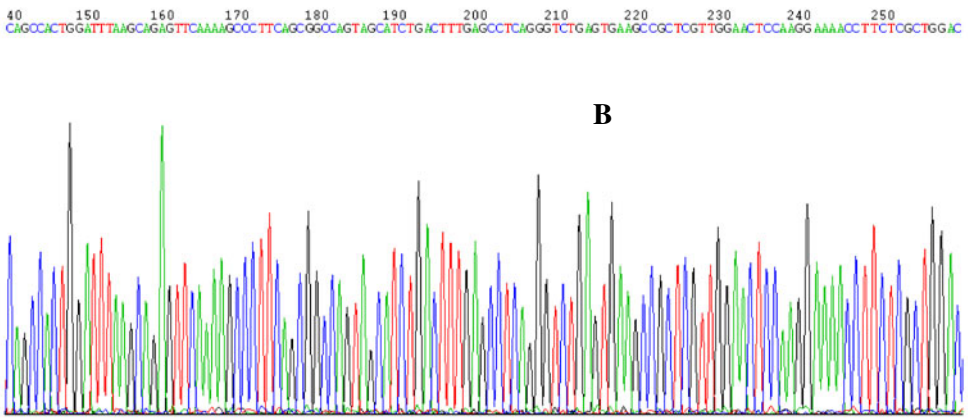


Fig. 4 **A** Result of the electrophoresis of PCR products. *Lane 1* DL2000 DNA marker (the bands from *top* to *bottom*: 2,000, 1,000, 750, 500, 250, and 100 bp). *Lane 2* β-actin amplification products. *Lane 3* Negative PCR-amplified real sample. *Lane 4* Positive PCR-amplified real sample. *Lane 5* K562 cell. **B** Result of gene sequencing. The gene fragment from 47 to 250 bp, which contained the same sequence as the MB probe we designed, was consistent with the gene fragment from 207 to 410 bp of BCR-ABL mRNA b3a2, which was checked in GeneBank

CGGGGCTCTATGGGTTTCTGAATGTCATCGTCCAC
TCAGCCACTGGATTTAAGCAGAGTTCAAAAGCCCT
TCAGCGGCCAGTAGCATCTGACTTTGAGCCTCAG
GGTCTGAGTGAAGCCGCTCGTTGGAACTCCAAGG
AAAACCTTCTCGCTGGACCCAGTGAAAATGACCC
CCA

Among the results, the gene segment from 47 to 250 bp was consistent with the gene segment from 207 to 410 bp of the BCR-ABL mRNA b3a2, which was checked in the GeneBank; the patterns of gene sequencing were shown as Fig. 4B.

Fluorimetric detection of PCR-amplified real samples

Figure 5 represents the fluorescence spectra after MB hybridized with PCR-amplified real samples. A series of three repetitive measurements resulted in reproducible results. The MB fluorescence intensity gave an averaged fluorescence intensity of 3.578 with a relative standard deviation (RSD) value of 2.1%. The fluorescence signals obtained from the hybridization of the positive and negative PCR-amplified real samples gave averaged signals of 16.344, with a RSD value of 7.2%, and 5.674, with a RSD value of 6.3%, respectively. The fluorescence intensity of the MB after hybridization with the positive real sample was significantly higher than that of the MB after hybridization with the negative PCR-amplified real sample. The obvious increase in the magnitude of the fluorescence intensity was obtained with positive PCR-amplified real samples. The increase in fluorescence signals showed that before hybridization, the structure of the DNA probe kept hairpin. At this time, the efficiency of fluorescence quenching played a key role. Therefore, the fluorescence signal of the DNA probe was small. After hybridization with the complementary DNA, with the hybridization of DNA and the change of the hairpin structure, the fluorescence intensity increased. If the blood sample, from which DNA was extracted, was negative, the negative PCR-amplified real sample would not contain a target sequence complementary to the MB. Interaction between these negative PCR-amplified real samples and the MB did not lead to hybridization; thus, the magnitude of fluorescence intensity was nearly as high as the MB signal. Therefore, the results obtained from the fluorimetric detection were in good agreement with the ones obtained from gel electrophoresis. In order to increase the fluorescent signal, reduce the detection limited, and get the larger measurement range, the molecular beacon probes would be modified onto nanogold particles in future research. Through optimizing different parameters, such as the molecular beacon design, nanogold particle size, molecular beacon–nanoparticle conjugation protocol, and molecular beacon loading, this method would become a better way in the diagnostic procedure of CML patients.

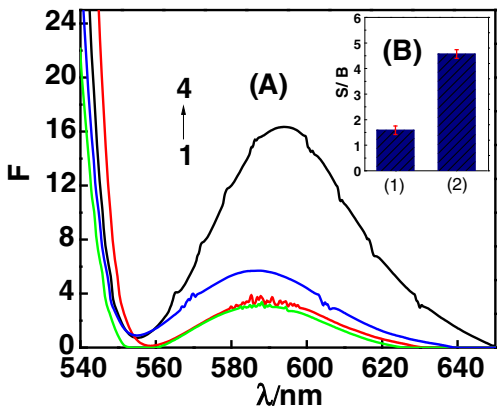


Fig. 5 **A** Fluorescent spectra of MB hybridizing with PCR amplification products of positive and negative PCR-amplified real samples. *1–4* Samples are MB before hybridizing with positive PCR-amplified real samples, MB before hybridizing with negative PCR-amplified real samples, MB after hybridizing with the negative PCR-amplified real sample, and the positive PCR-amplified real sample. **B** Signal-to-background ratio (*S/B*) bar graph of MB hybridizing with positive and negative PCR amplification products

Conclusion

In this paper, we developed a rapid and novel molecular beacon-based assay for the fluorimetric detection of type b3a2 relating to chronic myelogenous leukemia in gene fragment and PCR amplification products. As a fluorescent DNA probe, a new MB which comprised a DNA loop section and a pair of fluorophore (TAMRA) and quencher (DABCYL) was designed. Under optimal conditions, we studied the changes of fluorimetric behaviors of MB before and after hybridization. The relationship between the signal-to-background ratio (S/B) and the concentration of complementary DNA was linear in the range of 4.0×10^{-9}–3.2×10^{-8} mol/L, with a correlation coefficient of 0.9973. Hybridization of MB with non-complementary or single-base mismatched DNA sequence resulted in lower fluorescence intensity than hybridization with complementary DNA. It showed that this novel method has high specificity. The difference between the responses of hybridization with PCR amplifications from the negative real sample and the positive real sample suggested that the molecular beacon could be conveniently used to monitor DNA hybridization with high sensitivity. The results obtained from the fluorimetric detection were in good agreement with the ones obtained from gel electrophoresis. Thus, compared with other methods, this approach is more sensitive, more rapid, simpler, and with high specificity and selectivity. With the abundant literature about MB, it seems clear that molecular beacons for selective DNA sequence detection

have a very promising future. While the concept in this study has been demonstrated in connection with PCR real samples for the detection of CML, further improvements may be achieved to quantitatively detect target sequences from real samples, solving the actual problems of the early diagnosis and prognosis monitoring of CML and other diseases.

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References

1. Fialkow PJ, Gartler SM, Yoshida A (1967) Proc Natl Acad Sci U S A 58:1468–1471
2. Kantarjian HM, Deisseroth A, Kurzrock R, Estrov Z, Talpaz M (1993) Blood 82:691–701
3. Lin XH, Wu P, Chen W, Zhang YF, Xia XH (2007) Talanta 72:468–471
4. Sawyers CL (1999) New Engl J Med 340:1330–1340
5. Faderl S, Talpaz M, Estrov Z, O'Brien S, Kurzrock R, Kantarjian HM (1999) New Engl J Med 341:164–172
6. Konopka JB, Watanabe SM, Singer JW, Collins SJ, Witte ON (1985) Proc Natl Acad Sci U S A 82:1810–1814
7. Daley GQ, Vanetten RA, Baltimore D (1990) Science 247:824–830
8. Gargallo PM, Cuello MT, Aranguren PN, Larripa IB (2003) Cancer Genet Cytogen 143:140–144
9. Goh HG, Hwang JY, Kim SH, Lee YH, Kim YL, Kim DW (2006) Transl Res 148:249–256
10. OtazúI B, Zalcberg I, Tabak DG, Seuánez HN (1999) Leukemia Res 23:185–190
11. Cortes J, Talpaz M, Kantarjian H (1996) Am J Med 100:555–570
12. Müller C, Hennig E, Franke C, Krahl R, Leiblein S, Niederwieser D, Deininger MWN (2002) Cancer Genet Cytogenet 136:149–150
13. Zhou R, Stolt MF, Kronenwett U, Gruber A, Liliemark J, Liliemark E (2002) Leukemia Res 26:487–494
14. Meric B, Kerman K, Ozkan D, Kara P, Erensoy S, Akarca US, Mascini M, Ozsoz M (2002) Talanta 56:837–846
15. Kai E, Sawata S, Ikebukuro K, Iida T, Honda T, Karube I (1999) Anal Chem 71:796–800
16. Tyagi S, Bratu DP, Kramer FR (1996) Nat Biotechnol 14:303–308
17. Yao J, Liu Z, Ko LS, Pan G, Jiang Y (2005) J Virol Methods 129:40–46
18. Zheleznaya LA, Kopein DS, Rogulin EA, Gubanov SI, Matvienko NI (2006) Anal Biochem 348:123–126
19. Strohsahl CM, Du H, Miller BL, Krauss TD (2005) Talanta 67:479–485
20. Culha M, Stokes DL, Griffin GD, Vo-Dinh T (2004) Biosens Bioelectron 19:1007–1012
21. Zhao J, He D, He H, Li L, Zhang LL, Wang XY (2007) Urology 70:60–64
22. Xu Y, Yu RMK, Zhang X, Murphy MB, Giesy JP, Lam MHW, Lam PKS, Wu RSS, Yu H (2006) Chemosphere 63:772–784
23. Hianik T, Ostatná V, Sonlajtnerova M, Grman I (2007) Bioelectrochemistry 70:127–133
24. Bonnet G, Tyagi S, Libchaber A, Kramer FR (1999) Proc Natl Acad Sci U S A 96:6171–6176
25. Bratu DP, Cha BJ, Mhlanga MM, Kramer FR, Tyagi S (2003) Proc Natl Acad Sci U S A 100:13308–13313
26. Elamaagacli AH, Bleen DW, Opalka B, Seeber S, Schaefer UW (2000) Ann Hematol 79:424–431
27. He R, Liu B, Yang C, Yang RC, Tobelem G, Han ZC (2003) Cancer Gene Ther 10:879–886