SNP genotyping by DNA photoligation: application to SNP detection of genes from food crops

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Abstract
We describe a simple and inexpensive single-nucleotide polymorphism (SNP) typing method, using DNA photoligation with 5-carboxyvinyl-2′-deoxyuridine and two fluorophores. This SNP-typing method facilitates qualitative determination of genes from indica and japonica rice, and showed a high degree of single nucleotide specificity up to 10 000. This method can be used in the SNP typing of actual genomic DNA samples from food crops.

Keywords: biosensors, DNA recognition, ligation reactions, photochemistry, food crops

(Some figures in this article are in colour only in the electronic version)

1. Introduction

Detection of single-nucleotide polymorphisms (SNPs) in a large number of genes would enable estimation of the number of genes having different alleles in a population, and may furthermore contribute to identification of SNPs responsible for different phenotypes among cultivars [1]. The nucleotide sequence of the japonica rice genome has been published by the International Consortium on Rice Genome Sequencing [2]. The Beijing Genomics Institute has sequenced the genome of indica rice using a shotgun approach [3]. Comparison between the nucleotide sequence of the japonica rice genome and that of the indica rice genome has revealed a large number of SNPs [4, 5]. The availability of extensive genome sequences of the two rice subspecies offers a unique and rich comparative genomics opportunity. In the context of food safety concerns, there is an increasing demand for detecting tools for foreign DNA in complex samples. Thus, there is an ever-increasing need for a reliable, low-cost, high-throughput standardized assay that can quickly and accurately reveal the presence of any undesired DNA. Most methods of DNA detection involve hybridization by an oligodeoxynucleotide (ODN) probe to its complementary single-strand nucleic acid target, leading to signal generation [6, 7]. In as far as the detection relies on hybridization events [8–11], however, such ODN probes have inherent limitations in their selectivity. In our previous studies, we developed an SNP-typing method with high selectivity and sensitivity by introducing a form of photochemical ligation with 5-carboxyvinyl-2′-deoxyuridine (CVU) into an existing allele-specific hybridization [12–17]. In our recent study, we developed the SNP-typing method with CVU and two fluorophores and demonstrated that this method facilitates the SNP typing of a single strand of the target sequence such as the human aldehyde dehydrogenase 2 (ALDH2) gene [18]. In this report, we show the SNP typing of the duplex of the target sequence such as genes from indica and japonica rice and report the use of this method to detect contamination of indica...
2. Experimental details

2.1. Apparatus

Mass spectra were recorded using Voyager-DE PRO-SF, Applied Biosystems. Irradiation was supplied by a 25 W transilluminator (FUNAKOSHI, TFL-40, 366 nm, 5700 µW cm⁻²). High-performance liquid chromatography (HPLC) was performed on a Chemcobond 5-ODS-H column (10 × 150 mm, 4.6 × 150 mm) or a Chemcosorb 5-ODS-H column (4.6 × 150 mm) with a JASCO PU-980, HG-980-31, DG-980-50 system equipped with a JASCO UV 970 detector at 260 nm.

2.2. Oligodeoxynucleotide synthesis

ODN sequences were synthesized by the conventional phosphoramidite method using an Applied Biosystems 3400 DNA synthesizer, with coupling efficiency monitored using a trityl monitor. To capture the strand synthesis, 5'-amino group-modified ODNs were synthesized using the 5'-amino group-modifier C6 TFA as the final coupling monomer. For Cy3- and Cy5-labeled ODN syntheses, the 3'-PT-aminol modified C6 controlled pore glass (CPG) was used to generate 3'-amino-derived ODNs, and the 3'-PT-aminol modified C6 CPG was used to generate ODNs bearing a free amino group at the 3' end. The coupling time of the crude mixture of 5-carboxyvinyl-2'-deoxyuridine was 999 s. Cleavage and deprotection were carried out under mild conditions (concentrated NH₂OH at 55 °C, 12 h). ODNs were purified by reverse phase HPLC. 3'-amino-derived ODNs were further coupled with a Cy3 NHS ester or a Cy5 NHS ester in 100 mM tetraborate buffer (pH 8.5) at room temperature for 1 h to obtain Cy3- or Cy5-labeled ODNs. They were also purified by reverse phase HPLC. The preparation of oligonucleotides was confirmed by matrix-assisted laser desorption/ionization time-of-flight mass spectroscopy (MALDI-TOF-MS).

2.3. DNA probe immobilization and DNA photoligation on DNA chip

The capture strand was diluted to 10 µM in 100 mM sodium cacodylate buffer (pH 7.0), and spotting was carried out using 3 µl aliquots with a standard micropipette. The capture strand was allowed to bind to the glass surface over a period of 12 h at room temperature inside a desiccator. Following probe immobilization, the glass surface was rinsed with 0.1% aqueous sodium dodecyl sulfate (SDS) solution and deionized water, and the surface was deactivated with a solution of NaBH₄ (3.75 mg) in phosphate-buffered saline (PBS, 1.5 ml) and ethanol (375 µl) over a period of 5 min. The surface was subsequently washed with deionized water and dried.

A glass chip spotted with a solution (5 µl) containing 1 µM target ODN (target sequence for indica rice), 2 µM Cy3-labeled ODN and 2 µM Cy5-labeled ODN in 50 mM sodium cacodylate buffer (pH 7.0) and 100 mM sodium chloride was irradiated at 366 nm for 15 min at room temperature. After the chip was washed with deionized water at 98 °C for 30 s, fluorescence intensity was measured on a microarray scanner CRBIO II e (Hitachi) equipped with 532 nm and 635 nm lasers. The target sequence for japonica rice was detected with the same method, using a combination of Cy3-labeled ODN and Cy5-labeled ODN.

2.4. PCR amplification of genomic DNA

Polymerase chain reaction (PCR) amplification was performed in 50 µl of Tris-HCl buffer (pH 8.0) with KCl, 2.0 mM MgCl₂, 200 µM deoxyribonucleotide triphosphate (dNTP), 0.4 µM forward and reverse primers, and genomic DNA. The following primers were used: forward, 5'-TGCTTGTGTTTCTGGTGTGC-3'; reverse, 5'-TTTCCAGCCCAACCTTAC-3'. Amplification was achieved by thermal cycling for 35 cycles at 95 °C for 30 s, 60 °C for 30 s, 72 °C for 60 s and a final extension at 72 °C for 2 min.

3. Results and discussion

Rice is one of the most important global food crops and it feeds over half the world population. In Japan, two cultivated species are grown, namely, japonica and indica rice. We detected SNPs in the genomic sequences of indica and japonica rice via photoligation. The synthesized ODNs are summarized in table 1. We determined the feasibility.
Figure 2. The effect of DNA photoligation on specificity. The capture strands were attached to the surface, and targets (left: ODN (indica rice), 3 × 3 spots; right: ODN (japonica rice), 3 × 3 spots) were applied. (a) Use of both hybridization specificity and photoligation specificity and washing with deionized water at 98 °C for 30 s. (b) Fluorescence images.

Table 1. ODNs used in this study.

| Sequencesa,b | ODN(IND)c | ODN(JPN)c | ODN(Cy3) | ODN(Cy5) |
|--------------|-----------|-----------|-----------|-----------|
| Capture      | 5'-NH2-(S)-ATCAGGAAGAACATC-3' | 5'-GCATGTGATCGATCTGAATAAG | 5'-CVUGCAAGGTACy3-3' | 5'-CVUGCAACTICA-Cy5-3' |
| ODN(IND)c    | AGGGGAAACAAGAATTATAAACA | AGGGGAAACAAGAATTATAAACA | AAGCA-3' |
| ODN(JPN)c    | 5'-GCATGTGATCGATCTGAATAAG | 5'-GCATGTGATCGATCTGAATAAG | AAGCA-3' |
| ODN(Cy3)     | 5'-CVUGCAAGGTACy3-3' | 5'-CVUGCAAGGTACy3-3' | |
| ODN(Cy5)     | 5'-CVUGCAACTICA-Cy5-3' | 5'-CVUGCAACTICA-Cy5-3' | |

a The underlined letters indicate a mutation point.
b S corresponds to a hexa (ethylene glycol) linker fragment.
c IND and JPN represent indica and japonica rice, respectively.

d of the photoligation through ODN containing CVU on a DNA chip. Ligations were carried out on the DNA chip by 366-nm irradiation at room temperature using ODN(Cy3) and ODN(Cy5), and on the single strand of the target sequence in sodium cacodylate buffer. After the chip was washed with deionized water at 98 °C for 30 s, surface fluorescence intensity was measured on a CRBIO IIe microarray scanner. As shown in figure 2, we observed a strong fluorescence signal of a photoligated product with the completely complementary case. Therefore, the present method using a combination of ODN(Cy3) and ODN(Cy5) constitutes a very powerful tool for SNP typing.

Ligations were carried out on the DNA chip by 366-nm irradiation at room temperature with ODN(Cy3) and ODN(Cy5), and the duplex of the target sequence, in sodium cacodylate buffer. ODN(Cy3) and ODN(Cy5) were mixed with a sample solution of the target sequences at various ratios between indica and japonica rice. As shown in figure 3, the ratio of the fluorescence intensity of Cy3 to that of Cy5 corresponded to the ratio of the content of indica rice to that of japonica rice. Therefore, the method developed constitutes a very powerful tool for distinguishing between indica and japonica rice species. Photoligation-coupled SNP typing has enabled detection of attomolar levels of single-nucleotide mismatches, as reported previously [15].

To show that the method developed is applicable to real samples, the PCR products of genomic DNA were detected under optimized conditions. A gel electrophoresis image of the two PCR products confirmed the length of these two sequences as 169 bp. As a result of the photoligation with the PCR product of the genomic DNA for indica rice, a strong fluorescence intensity of Cy3 was obtained, whereas that of Cy5 was negligible (figure 4). In contrast, for a sample solution containing the PCR product of the genomic DNA.
Figure 4. (a) Fluorescence intensity and (b) images acquired on microarray scanner for SNP detection of PCR product of actual genomic DNA samples.

Figure 5. (a) Fluorescence intensity and (b) images acquired on microarray scanner for SNP detection of extracted genomic DNA samples from plants of japonica rice. In the control experiment, we used no target DNA sequence for SNP typing.

for japonica rice, Cy5 showed a strong fluorescence intensity, whereas a very weak fluorescence was observed for Cy3. Furthermore, the extracted genomic DNA samples from plants of japonica rice were examined under optimized condition. As shown in figure 5, we detected a strong fluorescence intensity of the photoligated product with the extracted genomic DNA sequence of japonica rice. The results suggest that this method is cost- and time-effective for high-throughput SNP detection of genes from food crops. Therefore, these results showed that this method enables detection of SNPs of actual genomic DNA samples.

4. Conclusions

In summary, we described an approach for the SNP detection of the duplex of the target sequences such as genes from indica and japonica rice. The results are reproducible, and the colour-based screening facilitates qualitative determination. In addition, this method facilitates the SNP typing of actual genomic DNA samples. The results suggest that this method is reliable, flexible, and cost- and time-effective for high-throughput SNP detection of genes from food crops.

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References

[1] Han B and Xue Y 2003 Curr. Opin. Plant Biol. 6 134
[2] International Rice Genome Sequencing Project 2005 Nature 436 793
[3] Yu J et al 2002 Science 296 79
[4] Feltus F A, Wan J, Schulze S R, Estill J C, Jiang N and Paterson A H 2004 Genome Res. 14 1812
[5] Shen Y J et al 2004 Plant Physiol. 135 1198
[6] Drummond T G, Hill M G and Barton J K 2003 Nat. Biotechnol. 21 1192
[7] Nakatani K 2004 ChemBiochem 5 1623
[8] Guo Z, Liu Q and Smith L M 1997 Nat. Biotechnol. 15 331
[9] Frutos A G, Pal S, Quesada M and Lahiri J 2002 J. Am. Chem. Soc. 124 2396
[10] Ali M F, Kirby R, Goodey A P, Rodriguez M D, Ellington A D, Neikirk D P and McDevitt J T 2003 Anal. Chem. 75 4732
[11] Ketomäki K, Hakala H, Kuronen O and Lönnberg H 2003 Bioconjug. Chem. 14 811
[12] Fujimoto K, Matsuda S, Takahashi N and Saito I 2000 J. Am. Chem. Soc. 122 5646
[13] Ogasawara S and Fujimoto K 2006 Angew. Chem., Int. Ed. Engl. 45 4512
[14] Yoshimura Y, Noguchi Y, Sato H and Fujimoto K 2006 ChemBiochem 7 598
[15] Yoshimura Y, Okamura D, Ogino M and Fujimoto K 2006 Org. Lett. 8 5049
[16] Yoshimura Y, Noguchi Y and Fujimoto K 2007 Org. Biomol. Chem. 5 139
[17] Ami T, Ito K, Yoshimura Y and Fujimoto K 2007 Org. Biomol. Chem. 5 2583
[18] Ami T, Ozaki G, Yoshimura Y and Fujimoto K 2008 Chem. Lett. 37 134