Novel approach for SNP genotyping based on site-selective RNA scission

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ABSTRACT
Novel genotyping method for single nucleotide polymorphisms (SNPs), based on site-selective RNA scission, has been developed. A substrate RNA is activated at two sites by complementary acridine-modified DNA having two acridine residues, and is site-selectively cleaved by metal ion catalyst to produce short RNA fragment containing the SNP site. Genotype of the substrate is accurately and easily determined by mass analysis of the fragment.

INTRODUCTION
High-throughput, accurate, and low-cost SNP genotyping method is crucial for future design of tailor-made medicine (1). The most accurate discrimination of SNP allele is realized by direct observation of the mass number of genomic samples. Matrix-assisted laser desorption / ionization time of flight mass spectroscopy (MALDI-TOF MS) is suitable for the purpose. To make the best use of its accuracy, the samples must be shortened appropriately.

Recently, we prepared non-covalent catalytic systems for site-selective RNA scission by combining free lanthanide(III) ion with acridine-modified DNA (2). The acridine residue in the modified DNA site-selectively activates the target phosphodiester linkages in complementary RNA, so that the catalysis of lanthanide(III) ion is enormously promoted only there.

In this study, we successfully applied this scission technique to reliable genotyping of SNPs in exon of human apolipoprotein E gene (APOE, Figure 1). An acridine-modified DNA, in which two acridine residues are incorporated, simultaneously activates two target sites in the substrate RNA. The following cleavage reaction with lanthanide(III) ion clips short RNA fragment between these two target sites. The mass number of this fragment is then measured by MALDI-TOF MS, and genotype of the substrate is accurately determined.

Figure 1. Schematic representation of novel genotyping method utilizing site-selective RNA scission by acridine-modified DNA and lanthanide(III) ion. The A-allele and G-allele in A-G transition is discriminated by mass difference of 16. The acridines are shown as ellipses.
RESULTS AND DISCUSSION

All the oligonucleotides used in this study (Figure 2) were synthesized with standard phosphoramidite chemistry. The 40-mer RNA substrates have the sequence of antisense strand of APOE around a SNP site (amino acid position 112), and 1-g and 1-a correspond to its G- and A-allele, respectively (3). The acridine-modified DNA 2 is complementary to the substrates, except that two acridine residues (X) are substituted for the opposite nucleotides of the two target sites. Moreover, nucleotide analogue P, which makes stable base pair both with G and with A, is introduced in the SNP site for analyses of heterozygous samples. Each product was purified by PAGE and RP-HPLC, and identified by MALDI-TOF MS. RNA cleavage reactions were performed at pH 7.5 (10 mM Tris buffer), 37 °C in the presence of 200 mM NaCl. After a predetermined time, the reaction mixture was desalted by micropipette tip containing C18 media at the end (ZipTip® from Millipore Co.), and directly applied to mass analysis.

A typical mass spectrum is shown in Figure 3. Here, 1:1 mixture of both the substrates 1-a and 1-g, which simulates heterozygous sample, is activated by 2, and cleaved by 150 μM of Lu(III). Expected two fragments are clearly detected, and discriminated by theoretical mass difference of 16. Accordingly, closely placed acridine residues do not inhibit each other, and efficiently activate their target sites. The signal a (observed M/z = 4494.1) corresponds to the fragment derived from 1-g (calculated mass; M-H = 4512.2). Cleavage reaction with single substrate gives only one fragment as expected (data not shown). The present reliable SNP genotyping method is highly promising for future practical applications.

REFERENCES