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JMS Letters

Dear Sir,

Molecular Mass Tagging to One Strand of Polymerase Chain Reaction Products

Recently, electrospray ionization and matrix-assisted laser desorption/ionization (MALDI) mass spectrometry (MS) have been applied to polymerase chain reaction (PCR) products.[1-7] This combination is now providing promising new research methods in genetics, including the diagnosis of base mutations.[8,9] While the product of PCR is a double-stranded DNA, the resulting mutually complementary oligonucleotides, which are not covalently bound to each other, are separately detected in the mass spectrum measured under ordinary conditions. However, it is often the case that when these strands have nearly the same molecular size, their molecular ion signals are not clearly distinguishable owing to a wide distribution of the isotopic clusters comprising individual oligonucleotides or to the low resolution of measurement.[10] This problem is the focus of this letter.

The base compositions for the sense and antisense strands of a 50 base-pair (bp) region, presented at the top of Fig. 1, of human β globin gene exon 2 were A:11; T:11; G:12; C:16 and A:11; T:11; G:16; C:12, respectively, and PCR with *Taq* DNA polymerase generated a pair of oligonucleotides with molecular masses of 15 620 and 15 780, leaving single 3′ dA-nucleotide overhangs.[11] The difference of 160 units, or 1.0%, between these molecules was large enough to be resolved by a MALDI time-of-flight (TOF) measurement (Fig. 1(b)). The tops of the resolved peaks for individual strands were identified at m/z 15 660 and 15 815. These were presumed to be potassium-adducted species.

On the other hand, in the case of a PCR product from a 44 bp region of the L1CAM gene exon 22, the molecular masses calculated from the compositions A:12; T:6; G:13; C:14 for the sense and A:7; T:11; G:14; C:13 for the antisense strand including dA overhangs were 13 850 and 13 845, respectively. Their molecular ion signals, which differed by only five units or 0.04%, would not be clearly discriminated in the mass spectrum even when measured at a resolution of several thousand. As can be seen in the MALDI-TOF mass spectrum presented in Fig. 2(a), the peaks for individual strands were not resolved. The top of the fused signal was identified at m/z 13 890.

The aminohexyl linker is a commonly used arm, which is incorporated at the 5′-terminus of the oligonucleotides and can react to form oligonucleotide conjugates with a variety of substrates such as biotin, fluorescent dyes or alkaline phosphatase. This linker arm can be conveniently introduced in

5′ -TTAGTGATGGCCTGGCTCACCTGGACAACCTCAAGGGCACCTTTGCCACA-3′
3′ -AATCACTACCGGACCGAGTGGACCTGTTGGAGTTCCCGTGGAAACGGTGT-5′

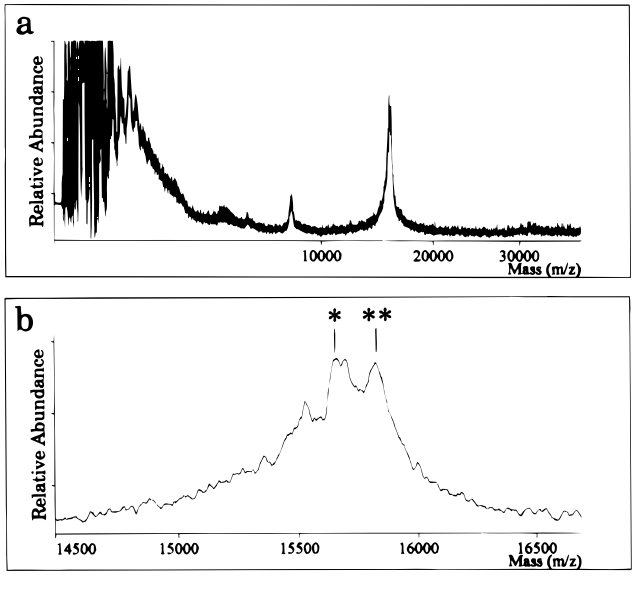

Figure 1. Positive-ion MALDI/TOF mass spectrum of a 50 bp PCR product from a β globin exon 2 region. PCR was carried out in 50 µl of solution containing 10 mM Tris–HCl at pH 9.0, 50 mM KCl, 0.1% Triton X-100, 1.6 mM $MgCl_2$, 0.2 mM each of dATP, dGTP, dCTP and dTTP, 0.4 µM each of primers, 1 µg of genomic DNA and 2 U of *rTaq* DNA polymerase. Primer sequences are underlined. After 30 cycles of amplification, the product DNA was extracted with phenol and then precipitated with ethanol in the presence of 2 M ammonium acetate. After washing with 70% ethanol, the pellet was dried and dissolved in 5 µl of distilled water. For MALDI, 0.3 µl of the sample solution was mixed with the same volume of a 30 mg ml^{-1} solution of 3-hydroxypicolinic acid on the probe tip of a Finnigan MAT Vision 2000 instrument (Thermo Bioanalysis, Hemel Hempstead, UK) equipped with a reflectron (5 keV ion source, 20 keV post-acceleration) and a nitrogen laser (337 nm). The sample amount loaded was estimated to be 0.5 pmol. (a) Full spectrum; (b) the molecular ion region for the amplified oligonucleotides. The sense (*) and antisense (**) strands were visualized separately.

5′ -TTCAGGCCACCACCAAAGAGGGCCCTGGTGAAGCCATCGTACGG-3′
3′ -AAGTCCGGTGGTGGTTTCTCCCGGGACCACTTCGGTAGCATGCC-5′

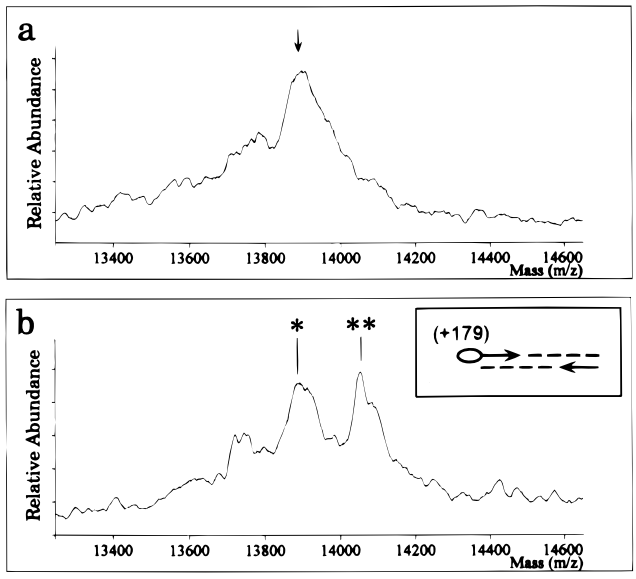

Figure 2. Positive-ion MALDI/TOF mass spectra of a 44 bp PCR product from the L1CAM exon 22 region. The DNA region, in exon 22, presented at the top was amplified by nested PCR. The first PCR was carried out at a region involving exons 21 and 22 of L1CAM by using a primer set of 5′-TGGGCGAGGACC-TACCTGCCACTC-3′ and 5′-CAGGTCATTCCTCCAGCTTAC-3′ and genomic DNA as a template. Subsequently, nested PCR was performed using 1 µl of the first PCR solution. The primer sequences for the second PCR are underlined. An unmodified (a) or an aminohexyl-modified (b) sense primer was paired with an unmodified antisense primer. The volume scales of PCR and succeeding procedures were the same as in Fig. 1. The individual ion signals for the component strands of the amplified DNA were not resolved when unmodified primers were used (arrow). This modification allowed the sense strand (**) to be distinguished from the antisense strand (*). The strategy is explained in the inset.

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the final sequence on the automated DNA synthesizer by reaction with (trifluoroacetylamino)hexylmethyl (*N*,*N*-diisopropyl)phosphoramidite (Aminolink-2, Perkin-Elmer). An L1CAM sense primer modified with this arm was prepared, and the PCR was run using a pair of modified sense and unmodified antisense primers (see inset in Fig. 2(b)). The efficiency of amplification was comparable to that achieved using an unmodified pair. As can be seen in Fig. 2(b), MALDI/TOF-MS of the product gave two major peaks corresponding to the individual strands; the sense strand was detected at m/z 14 055 with an additional aminohexyl residue of 179 Da at the 5′-terminus, while the antisense was at m/z 13 880. These modified primers are expected to be useful in a combination of PCR and MS, when the individual peaks or isotopic clusters for the amplified strands overlap.

In this study, positive ions of oligonucleotides were measured by MALDI/TOF-MS. Unexpectedly, the positive ion mode provided more favorable conditions than the negative-ion mode, especially for PCR products derived from a simple purification procedure involving phenol extraction and ethanol precipitation. Details of this observation will be discussed elsewhere. In addition, it is clear that the sensitivity of oligonucleotides was not affected by the introduction of an aliphatic primary amine moiety, when the signal intensities for sense and antisense strands are compared (Fig. 2(b)). Introduction of electrophores to the aminohexyl linker did not improve the sensitivity of a 17-mer synthetic oligonucleotide in the study of Britt *et al.*,[12] who did not investigate PCR or the positive ion mode.

Yours,

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