International Congress Series 1288 (2006) 70–72

www.ics-elsevier.com

Simultaneous detection of DNA length and sequence variations by liquid chromatography electrospray ionization time-of-flight mass spectrometry

H. Oberacher [a,*], H. Niederstätter [a], B. Casetta [b], W. Parson [a]

[a] *Institute of Legal Medicine, Innsbruck Medical University, Muellerstrasse 44, 6020 Innsbruck, Austria*
[b] *Applied Biosystems, Monza, Italy*

Abstract. The applicability of liquid chromatography online hyphenated to electrospray ionization time-of-flight mass spectrometry to the characterization of length heteroplasmic samples was demonstrated. Two segments within the first and second hypervariable regions (HV1=16144...16237; HV2=220...381) of the mitochondrial DNA (mtDNA) control region were selected as targets. The two mtDNA regions were simultaneously amplified within a single PCR and analyzed. 90 maternally unrelated mother–offspring pairs served as Austrian population sample. The plausibility of ICEMS results was checked by sequencing.

Keywords: Mass spectrometry; Liquid chromatography; Electrospray ionization; Length heteroplasmy; Mitochondrial DNA

1. Introduction

The first (HV1) and the second hypervariable region (HV2) of the mitochondrial control region contain poly cytosine (C) tracts. These mononucleotide repeats are known as mutation hot spots. Slippage of the DNA polymerase during replication is considered to be responsible for the occurrence of multiple mitochondrial DNA species within one individual (=heteroplasmy) differing in the number of C's lined up. In HV1 the poly C stretch is located between position 16184 and 16193. The homopolymeric tract is interrupted by a thymine (T) residue at position 16189 and is stable (C_5TC_4). However, a T to C transition at position 16189 induces instability giving rise to length heteroplasmy.

* Corresponding author. Tel.: +43 512 507 3322; fax: +43 512 507 2764.
E-mail address: herbert.oberacher@uibk.ac.at (H. Oberacher).

0531-5131/
doi:10.1016/j.ics.2005.09.137

Under these circumstances, the mitochondrial genome of a given individual represents a mosaic of poly C tracts varying in length from 8 to 14 nts. In HV2 the poly C stretch is located between positions 303 and 315. According to the revised Cambridge reference sequence (rCRS), seven C's precede a T that is subsequently followed by five more C's (C_7TC_5). Almost every mtDNA haplotype observed so far carries one additional nucleotide in the second part of that C-stretch introducing a stable insertion with respect to the reference sequence (315.1C). Due to the presence of a 7 C's-long stretch, length heteroplasmy can occur even without the substitution of position 310.

Sequencing represents the most commonly applied technique for the characterization of length heteroplasmic samples. Electropherograms show a characteristic "out-of-phase" pattern downstream of the homopolymeric region. Interpretation is difficult and usually only the predominant type is identified. Cloning of PCR products and consecutive sequencing represents an alternative strategy, which enables the relative quantification of all present mitochondrial DNA species. Since cloning is costly and time-consuming, this approach does not represent a practicable strategy for routine analysis. Hence, we propose the use of liquid chromatography electrospray ionization time-of-flight mass spectrometry for the characterization of the poly C stretches.

2. Materials and methods

Duplex PCR amplification was performed on a Gene Amp PCR System 9700 (AB, Applied Biosystems, Foster City, CA) in a total volume of 20 μl containing 1× Advantage 2 SA buffer (BD Biosciences Clontech, Uppsala, Sweden), 200 μM each dNTP, 500 nM each of the primers F16144 (TGACCACCTGTAGTACATAA), R16237 (TGTGTGATAGTTGAGGGTTG), and R381 (GCTGGTGTTAGGGTTCTTTG), 1 μM primer F220 (TGCTTGTARGACATAATAAT), 1× BD Advantage 2 Polymerase Mix (BD Biosciences Clontech) and 1 μl total genomic DNA. The thermal cycler protocol consisted of initial denaturation at 95 °C for 1 min, 40 cycles of 95 °C for 15 s, 56 °C for 30 s, and 68 °C for 1 min. The final extension step was carried out at 68 °C for 20 min.

A detailed description of the chromatographic system can be found in [1]. ESI-MS was performed on a QSTAR XL mass spectrometer (Applied Biosystems).

3. Results and discussion

Two segments within HV1 (16144..16237) and HV2 (220..381) were selected as targets to demonstrate the applicability of ***i***on-pair reversed-phase high performance liquid ***c***hromatography online hyphenated to ***e***lectrospray ionization time-of-flight ***m***ass ***s***pectrometry (ICEMS) to the simultaneous characterization of length and nucleotide polymorphisms. The two targets were simultaneously amplified within a single PCR and analysed by ICEMS. According to the rCRS, amplicons had a length of 94 base pairs (bp) and 162 bp, respectively. The chromatographic separation enabled the highly efficient fractionation and purification of nucleic acids prior to their mass spectrometric analysis. Hence, sample preparation was limited to PCR only. As separations were performed at elevated temperatures (65–80 °C), liquid chromatography represented an elegant way for denaturing double-stranded nucleic acids into the corresponding single strands. The denaturation was advantageous because of the division in half of the masses of the detected species and the possibility to identify base substitutions, which would have been missed by measuring the nearly composition-independent molecular masses of double-stranded nucleic acids. Furthermore, as

Table 1
Characteristic mass differences indicating the presence of length variations and nucleotide polymorphisms

		Insertion/Deletion of			
		C	T	A	G
Mass difference		±289.18230	±304.1944	±313.2078	±329.2072
		Substituted by			
		C	T	A	G
Original base	C	0	15.0114	24.0248	40.0243
	T	−15.0114	0	9.0134	25.0129
	A	−24.0248	−9.0134	0	15.9994
	G	−40.0243	−25.0129	−15.9994	0

the allelic state derived from one single strand was confirmed by the result obtained from the complementary single strand, the reliability of the mass spectrometric genotyping assay was increased.

Taking advantage of the high mass spectrometric performance of the applied time-of-flight mass analyzer [1], the individual single strands present within the chromatographic fractions were completely separated from each other. For the classification of these species, measured molecular masses were compared with molecular masses calculated from the rCRS. Based on the obtained molecular mass differences, sequence variations were identified. Characteristic mass differences for the detection of nucleotide polymorphisms and/or length polymorphism are summarized in Table 1. Moreover, within heteroplasmic samples the relative contents of individual length variants were determined by measuring relative intensities of the signals corresponding to the molecular mass of each of the alleles in the deconvoluted mass spectrum.

ICEMS results obtained from the analysis of 90 maternally unrelated mother–offspring pairs correlated well with the sequencing results. Within samples with a single substitution, ICEMS clearly proved the presence of any of these nucleotide polymorphisms. Among samples with multiple substitutions, in 13 cases only parts of the present sequence variability was allocable. In all these cases, two sequence variations were present, which compensated each other due to molecular mass shifts of about the same value in opposite directions. Length heteroplasmy in HV1 was detected in 12 out of 90 mother–offspring pairs (13.3%). In HV2, 46 individuals of the mother population (52.9%) and 45 individuals of the offspring population (51.1%) showed length heteroplasmy.

In conclusion, the observed performance suggests that ICEMS represents an efficient method for the simultaneous detection of nucleotide polymorphisms and length variants.

Reference

[1] H. Oberacher, et al., Detection of DNA sequence variations in homo- and heterozygous samples via molecular mass measurements by electrospray ionization time-of-flight mass spectrometry, Anal. Chem. 77 (2005) 4999–5008.