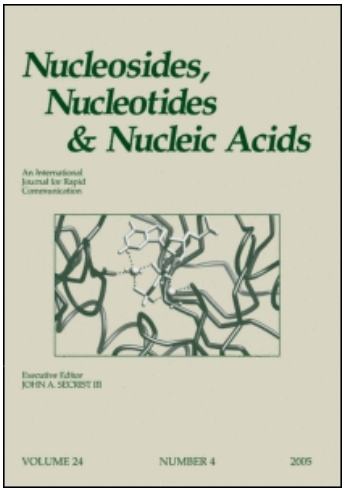

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MALDI-TOF Mass Spectrometry as a Powerful Tool to Study Enzymatic Processing of DNA Lesions Inserted into Oligonucleotides

D. Gasparutto[ab]; C. Saint-Pierre[a]; M. Jaquinod[a]; A. Favier[a]; J. Cadet[a]
[a] Laboratoire des Lésions des Acides Nucléiques, Service Chimie Inorganique Biologique-UMR 5046, Grenoble Cedex, France [b] CEA Grenoble, Grenoble, France

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MALDI-TOF Mass Spectrometry as a Powerful Tool to Study Enzymatic Processing of DNA Lesions Inserted into Oligonucleotides

D. Gasparutto,* C. Saint-Pierre, M. Jaquinod, A. Favier, and J. Cadet

Laboratoire des Lésions des Acides Nucléiques, Service Chimie Inorganique Biologique–UMR 5046, DRFMC, CEA-Grenoble, Grenoble Cedex, France

ABSTRACT

MALDI-TOF mass spectrometry measurements, coupled with either exonuclease or DNA *N*-glycosylases digestions of lesion-containing oligonucleotides, were used to assess biochemical features of several oxidative DNA damage. The latter analytical approach was shown to be an informative and efficient alternative technique to conventional electrophoresis and chromatographic analyses.

Key Words: DNA lesions; Synthetic oligonucleotides; Enzymatic processing; MALDI-TOF.

Cellular DNA is constantly assaulted by both endogenous and exogenous agents leading to hydrolysis, deamination, oxidation, alkylation and formation of cross-links. The resulting damage has been implicated in numerous diseases including cancer. With the aim to further delineate the biological significance of a defined DNA lesion (in terms of enzymatic degradation, repair and replication), site-specifically

*Correspondence: D. Gasparutto, CEA Grenoble, LAN/SCIB/DRFMC, 17, Avenue des Martyrs, Grenoble F-38054, France; Fax: +33 4 3878 5090; E-mail: dgasparutto@drfmc.ceng.cea.fr.

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modified oligodeoxynucleotides (ODNs) have been shown to be excellent tools that may be used as probes or substrates.[1a–d] Then, to assess the biochemical features, mass spectrometry analysis of enzymatic reaction products from DNA oligomers offers a rapid and sensitive alternative to usual chromatographic and electrophoretic techniques. Recently, we have applied matrix-assisted laser desorption/ionization – time-of-flight (MALDI-TOF) mass spectrometry to the analysis of enzymatic hydrolysates by exonucleases and DNA *N*-glycosylases of base lesion-containing DNA fragments. We reported herein the methodologies developed and used in these enzymatic assays together with the main MALDI-TOF/MS results obtained recently by using a set of site-specifically modified oligonucleotides that contain oxidized nucleobases.

Monitoring of the Base Lesions Digestion by Exonucleases Within DNA Fragments

Synthetic DNA fragments containing nucleobase lesions were submitted to partial digestion by exonucleases, namely calf spleen phosphodiesterase ($5' > 3'$ nuclease activity) and snake venom phosphodiesterase ($3' > 5'$ nuclease activity), and then analyzed by MALDI-TOF/MS. This technique allows the determination of the complete oligonucleotide sequence, confirming the location and the integrity of the modified base within the DNA oligomer.[2] Interestingly relevant information on the resistance of several lesions toward the digestion by nucleases was gained from such experiments (TABLE 1). The latter observation is highly indicative of a phosphodiester bond distortion, which may cause misreading of nucleotide sequences by DNA polymerases, leading to toxicity and mutagenicity within cells.

Table 1. Resistance of several oxidative DNA base lesions to exonucleases digestion.

Lesions	Resistance to SVPDE	Resistance to CSPDE	References
Formylamine	+	+	2a
5-OH-cytosine	−	−	2b
Cyanuric acid	+	+	2c
Oxazolone	−	−	2d
Oxaluric acid	−	−	2e
5-OH-hydantoin	+	++	2f
5-OH-5-Me-hydantoin	+	++	2g
Spiroiminodihydantoin	+	++	2h
Cyclopyrimidines	+	++	2i,2j
Cyclopurines	+	++	2k

−: total cleavage.
+: resistance to cleavage at the lesion level.
++: resistance to cleavage one base before the lesion.

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Mapping of the Specificity and the Mechanism of Action of DNA *N*-Glycosylases Toward Damaged Nucleobases

Another interesting application of MALDI-TOF/MS in the DNA lesion research field consists in the direct mapping of the specificity and the mechanism of action of several DNA *N*-glycosylases, implicated in the Base Excision Repair (BER) machinery of different damaged nucleobases in DNA. Thus, we have shown that the latter sensitive and resolutive mass spectrometry technique was efficient to determine the mechanism of action of DNA *N*-glycosylases toward damaged ODNs (*N*-glycosylase activity/endonuclease activity/β,δ-elimination/hydrolysis, ...). For this purpose, the excision reaction mixtures were analyzed by MALDI-TOF/MS to gain insights into mechanistic aspects of ODNs cleavage by repair enzymes. Earlier studies concluded to a β,δ-elimination mechanism for Fapy DNA *N*-glycosylase (Fpg). Under the present experimental conditions, Fpg, when acting on 5-OHC-, DHT-, oxazolone- oxaluric acid-, guanidino-hydantoin-, 5-OH-hydantoin derivative-, thymine glycol- or formylamine-containing oligonucleotides, gave rise to fragments with molecular weights corresponding to the expected products of a β,δ-elimination mechanism (Fig. 1). [1c,2b] Further work is in progress to extend this study to other DNA *N*-glycosylases implicated in the Base Excision Repair process.

In conclusion, MALDI-TOF mass spectrometry, appears to be an efficient alternative technique to conventional electrophoresis and chromatographic analyses

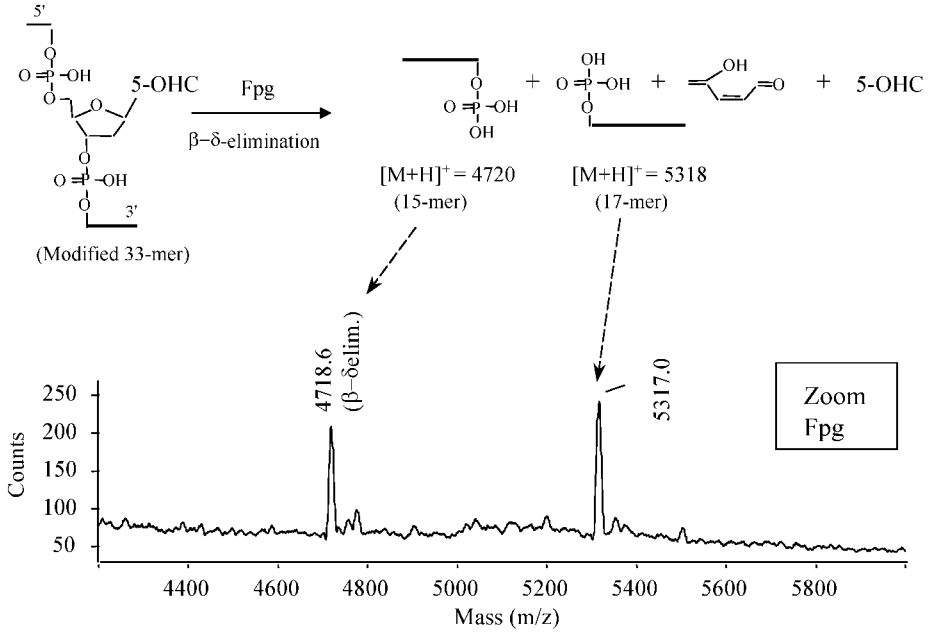

Figure 1. MALDI-TOF/MS analysis of the 5-OH-cytosine excision from a 33-mer DNA fragment by Fpg repair enzyme.

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to locate the lesion into DNA sequence and to assess the mechanistic pathway of its processing by nucleases, DNA *N*-glycosylases and other lesion-interfering enzymes.

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