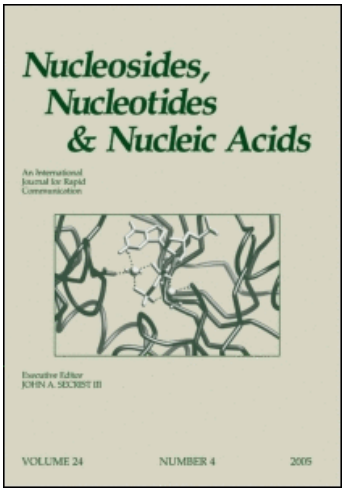

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Low Level, Detection of Modified DNA Bases and Nucleosides by FAB MS/MS

E. De Pauw[a]; E. Marafante[a]; J. Riego[a]; C. Leuratti[a]
[a] Radiochemistry Division, Environment Institute, Commission of the European Comrrunities, Joint Research Centre, ISPRA (VA), Italy

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LOW LEVEL DETECTION OF MODIFIED DNA BASES AND NUCLEOSIDES BY FAB MS/MS

E. DE PAUW, E. MARAFANTE, J. RIEGO, C. LEURATTI
Commission of the European Communities, Joint Research Centre,
Radiochemistry Division, Environment Institute
21020 ISPRA (VA) Italy

General context and approach

The identification and quantitation of DNA adducts formed by reaction of genotoxic chemicals with DNA may provide direct evidence of exposure to mutagens and carcinogens and may make possible a beginning of risk estimation based on Molecular Dosimetry approach.

This needs the development of innovative analytical-biochemical methods able to monitor subnanogram levels of modified nucleotides in human DNA samples. Immunochemical and ^{32}P-Postlabelling assays meet the required sensitivity and specificity, with detection limits estimated at fmol adduct/ug DNA (1).

Classical gas chromatography coupled with mass spectrometric detection has been used to reach this level (2). It requires however the derivatization of the analyte to allow vaporization and electron ionization. On the other hand, the Soft Ionisation methods allow the analysis of fragile molecules without derivatization. They are however considered by far less sensitive.

We present here a strategy designed to optimize the experimental conditions of Fast Atom Bombardment Mass Spectrometry. To avoid the interferences encountered in FAB it includes the use of Tandem Mass Spectrometry (MS/MS).

Experimental

The mass spectra were recorded on a VG 70 SEQ hybrid mass spectrometer. FAB Spectra were produced using a 8keV Xenon beam. The samples were prepared by dissolving the analyte in 01N.HCl and 20

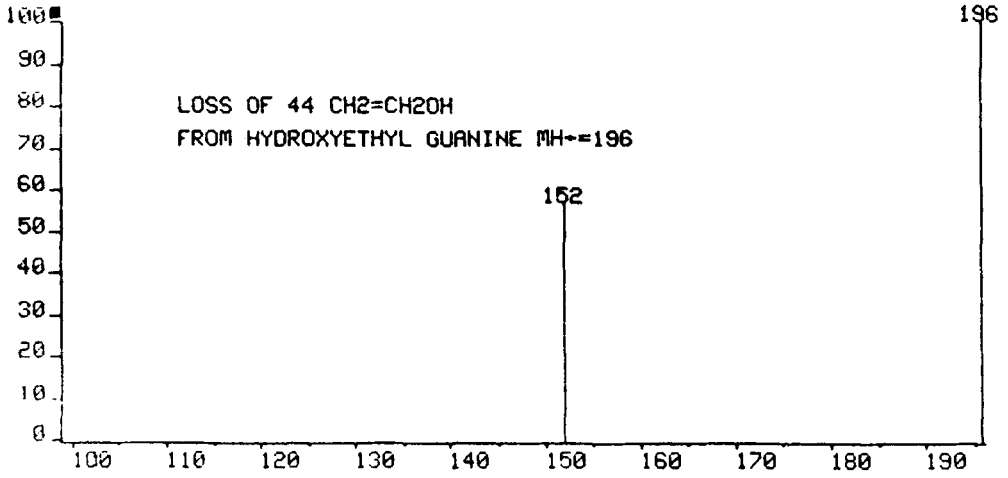

Fig. 1 - Daughter ion spectrum of hydroxyethyl guanine.

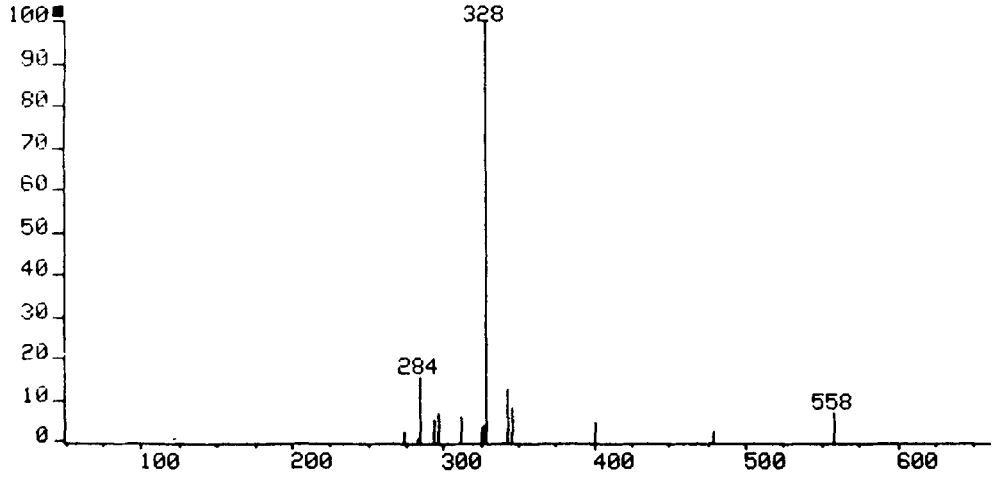

Fig. 2. - Constant Neutral Loss spectrum (132) of modified bases mixture.

microliters of the solution were mixed to the same volume of FAB matrix. Glycerol was doped with a surfactant acid (camphorsulfonic acid).

Modified bases were a gift from Dr. R. Baan, Medical Biological Laboratory, TNO, Rijswijk, NL. O^6-Ethylguanine, O^6-Ethylguanosine and deuterated analogs were synthetized at the JRC Ispra.

Results

Full scan FAB spectra of a set of modified guanine and guanosine were recorded. The general sensitivity lies in the microgram range. Moreover, trace amounts of inorganic salts strongly suppress their signal. It is important to note that this low sensitivity is mainly due to the intense background noise coming from the matrix. The addition of a surfactant acid lowers this chemical noise and brings the detection limit to 50 nanograms. At this level, the signal of the analyte is however hardly separated from matrix peaks and does not allow unambiguous detection.

To pick up the structurally significant ions avoiding matrix interferences, the daughter ion spectra of the analytes were taken. The spectra of the modified bases (ethylated, methylated, hydroxyethylated) show an intense peak corresponding to the loss of the side chain with hydrogen retention. The spectra of the corresponding nucleosides show only the loss of the sugar moiety. The spectrum of hydroxyethyl guanine is shown in Fig. 1. Particular compounds such as open ring analogs exhibit in addition to the above peaks the signals corresponding to the loss of water. From the daughter ion spectra, tables of transitions were constructed. The main limitation of the daughter ion scan is that it applies only when the precursor ion is defined. This scan is then not applicable for screening purposes.

Comparing transition tables, one observes the presence of transition specific for a given analyte. For example, the loss of water is specific of the open ring compounds. The loss of sugar is observed for all the sugar containing moieties. The loss of the alkyl group is restricted to the modified bases.

Such specificities allow us to design a screening strategy based on the Constant Neutral Loss scan in which the mass spectrometer is scanned in such a way that only the precursors able to lose well defined neutral molecules are detected. This scan gives us the MS/MS improvement in signal to noise without restricting the analysis to a well defined target molecule. In Fig. 2 one finds the sugar loss spectra of the ethyl and hydroxyethylguanosine mixture.

Sensitivities in the low (2 to 5) nanograms range have been obtained (3). This allows us to analyse off line fractions of DNA treated in vitro by ethylating agents. The monitoring of solutions containing the modified base or nucleotide as well as an internal isotopically labelled compound allows quantitative analysis.

Conclusions and prospects

Limits of detection similar to those obtained in full scan GC/MS have been reached without going through a derivatization step. The next development will now be the evaluation of the multiple reaction monitoring technique in which specific transitions and not full scan neutral loss spectra are monitored. A gain comparable to the one obtained when passing from full scan to single or multiple ion monitoring GCMS can be expected. The full power of the method will be reached once the direct on line HPLC coupling, using continuous Flow FAB will be optimised.

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