Identification of benzo[a]pyrene diol epoxide DNA-adducts by capillary zone electrophoresis-electrospray mass spectrometry (CZE-ES-MS/MS).

GHENT UNIVERSITY

A. Willems[1], D. Deforce[2], K. Clauwaert[1], W. Lambert[3], E. Van den Eeckhout[2], C. Van Peteghem[3] and J. Van Bocxlaer[1]

[1]Laboratory of Medical Biochemistry, [2]Laboratory of Pharmaceutical Biotechnology, [3]Laboratory of Toxicology, Ghent University, Harelbekestraat 72, B-9000 Ghent, Belgium

1. Introduction

Polycyclic aromatic hydrocarbons are a class of environmental carcinogens. Benzo[a]pyrene is the prototypic member of this class of compounds. When metabolized to anti-7,8,9,10-tetrahydrobenzo[a]pyrene-7,8-diol-9,10-epoxide (BPDE) (Figure 1), it may react with DNA. Mass spectrometry is a very powerful tool for the detection and identification of such low levels of formed adducts (1). Capillary zone electrophoresis is often coupled with mass spectrometry for the analysis of DNA adducts. This separation technique is not only fast, but is also inexpensive, and easy to use (1). Here, a CZE-electrospray mass spectrometric (ES-MS) method for the analysis of DNA adducts with BPDE is presented.

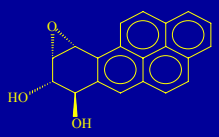

Figure 1 : Structure of anti-7,8,9,10-tetrahydro-benzo[a]pyrene-7,8-diol-9,10-epoxide

3. Results and discussion

The molecular masses of the compounds present in the DNA hydrolysate samples which reacted for 48 h with BPDE were determined under ES(-) using single MS. The following compounds could be identified: a BPDE-dCMP adduct [$(M-H)^-$ at m/z 608, t_R = 10 min], a BPDE-dAMP adduct [$(M-H)^-$ at m/z 632, t_R = 9.9 min] and a BPDE-dGMP adduct [$(M-H)^-$ at m/z 648, t_R = 11 min] (Figure 2). To obtain more structural information, low energy collision-activated dissociation product ion spectra of these products were obtained from the $(M-H)^-$ ions (Figures 3-5). A proposed fragmentation pathway for the BPDE-dGMP adduct is described in the scheme below. There appear to be three main fragmentations: across the 5'C-O bond of the ribose (1), between the ribose and the nucleobase (2), and between the nucleobase and the adduct (3). These cleavages are often accompanied by hydrogen transfers. In the three MS/MS spectra, a product ion at m/z 195 was observed. The presence of this ion is typical for the alkylation on the heterocyclic moiety.

2. Materials and methods

Synthesis of the BPDE DNA adducts:

Calf thymus DNA was incubated with BPDE at 37°C for 48 h. Unreacted BPDE was extracted from the mixture with chloroform (3x). DNA was isolated with ethanol precipitation and then hydrolyzed to deoxynucleotides with DNAase I, nuclease P1 and snake venom phosphodiesterase (2). The unmodified nucleotides as well as the other impurities were removed with solid phase extraction (Chromabond HR-P extraction colums).

CZE-ES-MS Conditions:

The CZE system (Lauerlab Prince) was coupled to the Q-TOF mass spectrometer (Micromass) with a fused silica capillary of 1m x 75µm i.d. The sheath flow consisted of 80/20 isopropanol/water (0.7 µl/min). The buffer system used to perform electrophoresis was 25 mM ammonium acetate (pH 9.5). Electrophoresis was performed using a constant voltage of 23 kV. Negative ES ionization was performed using an ionization voltage of –3.5 kV. The cone voltage was set at 46 V. The source temperature was kept at 80°C.

4. Conclusion

In the DNA hydrolysate, the adducts between BPDE and dGMP, dAMP and dCMP were detected and identified. So, the use of CZE-ES-MS/MS allowed for the detection and identification of the adducts found between BPDE and calf thymus DNA.

5. Acknowledgements

We wish to thank Ing. S. Vande Casteele for all her help with the analysis of the samples. This work was supported by grant GOA99-120501-99 (Eigen OnderZoeksFonds).

6. References

(1) Barry, J.P., Norwood, C., and Vouros, P. Analytical chemistry, 68, 1432-1438 (1996).

(2) Deforce, D.L.D., Lemière, F., Hoes, I., Millecamps, R.E.M., Esmans, E.L., De Leenheer, A., and Van den Eeckhout, E.G. Carcinogenesis, 19 (6), 1077-1086 (1998).

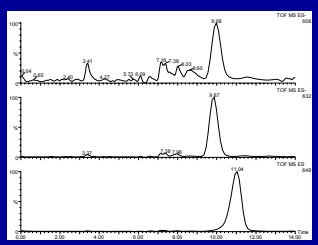

Figure 2: Chromatogram of the DNA hydrolysate

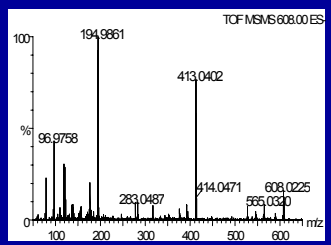

Figure 3: MS/MS spectrum of the BPDE-dCMP adduct

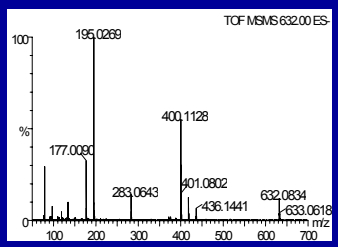

Figure 4: MS/MS spectrum of the BPDE-dAMP adduct

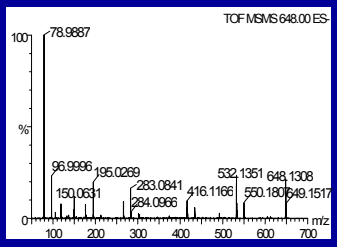

Figure 5: MS/MS spectrum of the BPDE-dGMP adduct