The use of capillary zone electrophoresis-nano-electrospray mass spectrometry in conjunction with sample stacking for the detection and identification of DNA adducts.

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1. Introduction

Benzo[a]pyrene is the prototypic member of the polycyclic aromatic hydrocarbons. This molecule is chemically relatively inert, but can be metabolically activated to *anti*-7,8,9,10-tetrahydrobenzo[a]pyrene-7,8-diol-9,10-epoxide (BPDE) and is capable of reaction with DNA to form DNA adducts, which are believed to play a role in the process of carcinogenesis. Mass spectrometry is a very powerful tool for the detection and identification of such low levels of formed adducts. Capillary zone electrophoresis (CZE) is able to separate the few modified and the many unmodified nucleotides and is very suited for the analysis of highly charged molecules. Sample stacking can be used to preconcentrate the sample as a compensation for the small injection volumes. Electrospray ionization (ESI) is currently the most used ionization technique for interfacing CZE separations to MS (1).

In our research, the utility of CZE-nano-ESI-MS and CZE-nano-ESI-MS/MS in conjunction with sample stacking for the detection and identification of DNA adducts, formed in vitro, was investigated. (-) ESI low-energy collision activated decomposition (CAD) spectra allowed the differentiation between phosphate-alkylation and base-alkylation. Further information on base-alkylation was obtained when (+) ESI low-energy CAD spectra were investigated.

2. Experimental

Synthesis of the BPDE DNA adducts:

Each (2'-deoxy)nucleotide was incubated with BPDE (Figure 1) at 37°C for 48 h. Unreacted BPDE was extracted from the mixture with chloroform (3x). The unmodified nucleotides as well as the other impurities were removed with solid phase extraction (SPE) (Chromabond HR-P extraction colums).

Calf thymus DNA was incubated with BPDE at 37°C for 48 h. DNA was isolated with ethanol precipitation and then hydrolyzed to deoxynucleotides with DNAase I, nuclease P1 and snake venom phosphodiesterase. The DNA-hydrolysate was subjected to the same SPE extraction.

CZE-ESI-MS and CZE-ESI-MS/MS conditions:

The CZE system (Lauerlab Prince, Lauerlabs, Emmen, The Netherlands) was equipped with a fused silica capillary of 1m x 50µm i.d. and directly coupled to a Q-TOF mass spectrometer (Micromass, Manchester, UK). The CZE-ESI interface was a nano-ESI source, which was equipped with a coaxial CZE-nano-ESI probe. The sheath flow consisted of 80/20 isopropanol/water (0.7 µl/min) (negative mode) or 50/50 methanol/water (positive mode). The electrophoresis buffer consisted of 20 mM ammonium acetate (pH 9.5). Sample introduction was carried out hydrodynamically for 1.5 min, applying 80 mbar injection pressure, and for 0.6 min (nucleotide adducts) or 1 min (DNA hydrolysate adducts) applying 100 mbar injection pressure in experiments prior to sample stacking. Electrophoresis was performed using a constant voltage of 23 kV. ESI was performed using an ionization voltage of –/+3.5 kV.

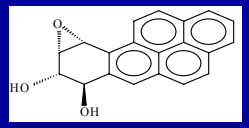

Figure 1 : Structure of *anti*-7,8,9,10-tetrahydro-benzo[a]pyrene-7,8-diol-9,10-epoxide

3. Results and discussion

CZE-(-)-nano-ESI-MS

In order to distinguish which adducts could be formed, each (2'-deoxy)nucleotide mixture was analyzed using CZE-nano-(-)-ESI-MS (with and without sample stacking). In the mixtures of BPDE with dAMP, dCMP or dGMP, one adduct was detected each time. In case of the reaction mixture with dCMP, sample stacking was necessary, because of the small amount of the adduct present in the sample. In the mixture with TMP, no adducts were found. The molecular masses (MM) of the three adducts correspond to the monoalkylated BPDE adducts with dAMP (MM of 633), dCMP (MM of 609) and dGMP (MM of 649). The binding occurs at the C-10 of BPDE. The dAMP-BPDE adduct eluted after about 10.3 min [$(M-H)^-$ at *m/z* 632]. The dCMP-BPDE adduct was found after about 10.4 min [$(M-H)^-$ at *m/z* 608]. The dGMP-BPDE adduct eluted after about 11.2 min [$(M-H)^-$ at *m/z* 648] (Figure 2).

CZE-nano-(-)-ESI-MS in conjunction with sample stacking was performed to search for DNA adducts in the DNA hydrolysate. Based on the comparison of the MM and the retention times with those of the reference compounds, the same adducts could be identified: the dAMP-BPDE adduct [$(M-H)^-$ at *m/z* 632], eluting after about 11 min, the dCMP-BPDE adduct [$(M-H)^-$ at *m/z* 608], found after about 11.2 min, and the dGMP-BPDE adduct [$(M-H)^-$ at *m/z* 648] after about 11.9 min.

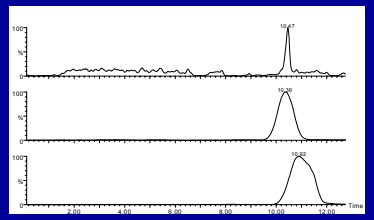

Figure 2. Reconstructed mass chromatogram of the $[M-H]^-$ ions of the DNA adducts found in the 2'-deoxy-nucleotide reaction mixtures: (A) BPDE-dCMP adduct at *m/z* 608. (B) BPDE-dAMP adduct at *m/z* 632. (C) BPDE-dGMP adduct at *m/z* 648.

CZE-(-)-nano-ESI-MS/MS

To gain more structural information, each adduct was analyzed by CZE-(-)-nano-ESI-MS/MS. The product ion spectra of the respective precursor $[M-H]^-$ ions of the found 2'-deoxynucleotide-BPDE adducts allowed the differentiation between phosphate- and base-alkylation of the 2'-deoxynucleotide. Alkylation of the 5'-phosphate group could be recognized by the presence of a product ion at *m/z* 399, representing the phosphate group with BPDE bound to it. Alkylation of the heterocyclic moiety of the nucleobase could be recognized by the presence of a product ion at *m/z* 195, representing the phosphorylated sugar (2).

In each MS/MS spectrum (nucleotide mixtures and DNA hydrolysate), a product ion at *m/z* 195 was present, meaning that the adduct is alkylated at the base moiety of the 2'-deoxynucleotide (Figure 3). In case of the BPDE-dAMP adduct, the alkylation can occur at the N1 or the N^6 of the nucleobase adenine. The alkylation of BPDE-dCMP can happen at the N3 or the N^4 of cytosine. The BPDE-dGMP adduct can be alkylated at the N^2 or the N7 of guanine (3).

CZE-(+)-nano-ESI-MS/MS

In a next step, CZE-(+)-ESI-MS/MS was performed to obtain further information on base-alkylation. In each CAD spectrum, no loss of NH_3 from the nucleobase is noticed (Figure 4). Loss of this neutral molecule points to a non-alkylated exocyclic NH_2 position of the nucleobase (4). Based on these findings, one can conclude that the three adducts, found in the nucleotide mixtures and in the DNA hydrolysate, are alkylated on the exocyclic amine group of the nucleobase and can be identified as: BPDE-N^6-dAMP, BPDE-N^4-dCMP and BPDE-N^2-dGMP.

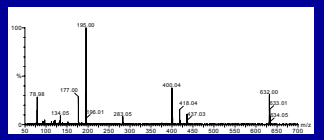

Figure 3: Low energy CAD spectrum of the $[M-H]^-$ ion at *m/z* 632 of the BPDE-dAMP adduct present in the reaction mixture of dAMP with BPDE.

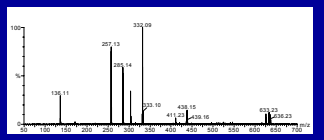

Figure 4: Low energy CAD spectrum of the $[M+H]^+$ ion at *m/z* 634 of the BPDE-dAMP adduct present in the DNA hydrolysate with BPDE.

4. Conclusion

The developed CZE-nano-ESI-MS method was able to detected three monoalkylated BPDE-DNA adducts formed in vitro: the BPDE-dCMP adduct, BPDE-dAMP adduct and BPDE-dGMP adduct. By examination of the low energy (-) CAD spectra, we could identify the adducts as alkylated at the nucleobase. Low energy (+) CAD spectra showed that the three adducts were alkylated on the exocyclic amine of the nucleobase. The use of CZE, if needed with sample stacking, proved an advantageous approach to the analysis of DNA adducts for its separation efficiency and seemless coupling to MS as an informative and sensitive detection technique.

5. Acknowledgements

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6. References

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