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DNA adducts as biomarkers for carcinogenesis analysed by capillary electrophoresis

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Table of contents Page

1. Reaction scheme 2

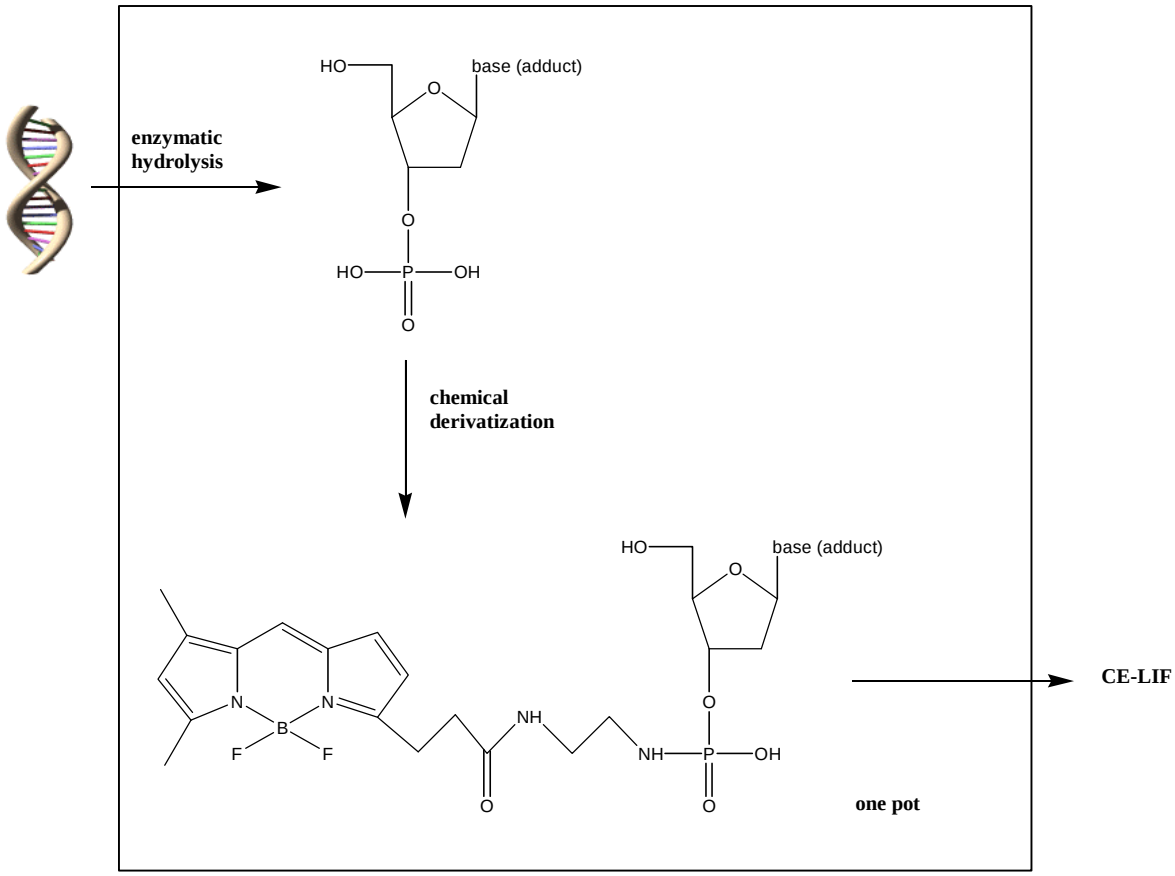
2. Modification of the DNA-hydrolysis 3

3. Synthesis and analysis of apurinic sites 5

1. Reaction scheme

Figure 1 Scheme of the DNA-adduct analysis.

After enzymatic hydrolysis of 10 µg DNA the modified and unmodified nucleotides were chemically derivatised with BODIPY® FL EDA and analyzed by capillary electrophoresis and laser-induced fluorescence detection. The minimum amount of DNA to determine the concentrations of dAMP, dGMP, dTMP, dCMP and 5-Me-dCMP is 100 ng.



2. Modification of the DNA-hydrolysis

Figure 2 Comparison of the new hydrolysis method with the standardised procedure.

Conditions: 10 µg DNA were diluted with 5.2 µL water and hydrolysed by incubation with 4.0 µL enzyme mixture (micrococcal nuclease, 150 $mUµL^{-1}$, and spleen phosphodiesterase, 2.5 $mUµL^{-1}$) and 0.80 µL buffer (250 mM sodium succinate (**A**) or 250 mM HEPES (**B**), 100 mM $CaCl_2$, pH 6.0) for 3 h at 37 °C. **1**: control with water (no DNA); **2** and **3**: 10 µg CT-DNA were hydrolysed by **A**; **4** and **5**: 10 µg dNMP (2.5 µg of each nucleotide) were hydrolyzed by **B**; **6** and **7**: 10 µg CT-DNA were hydrolyzed by **B**.

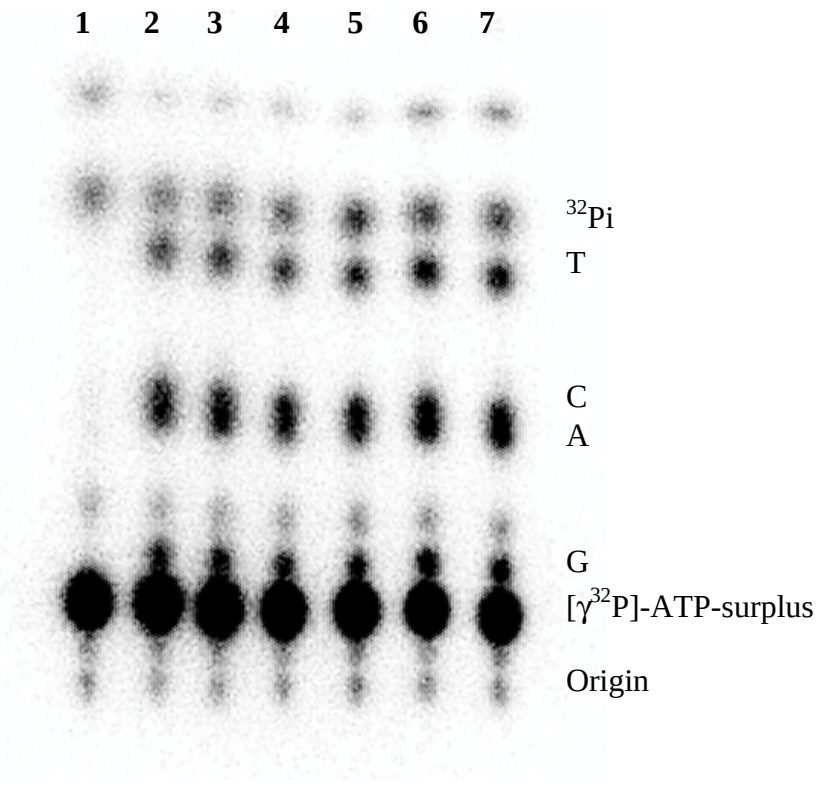


Table 1 Comparison of the new hydrolysis method with the standardised procedure.

	Hydrolysis procedure	Total radioactivity [cpm]
2	CT-DNA; 250 mM sodium succinate, pH 6.0	12804
3	CT-DNA; 250 mM sodium succinate, pH 6.0	13879
4	3'-dNMPs; 250 nM HEPES, pH 6.0	11872
5	3'-dNMPs; 250 nM HEPES, pH 6.0	11833
6	CT-DNA; 250 nM HEPES, pH 6.0	14500
7	CT-DNA; 250 nM HEPES, pH 6.0	13222

3. Synthesis and analysis of apurinic sites

Figure 3 Analysis of dGMP after treatment with water for 16 h. 10 µg dGMP were incubated with 200 µL water, pH 5.6 for 16 h at 37 °C, isolated by solid-phase extraction (eluted with methanol) and derivatised.

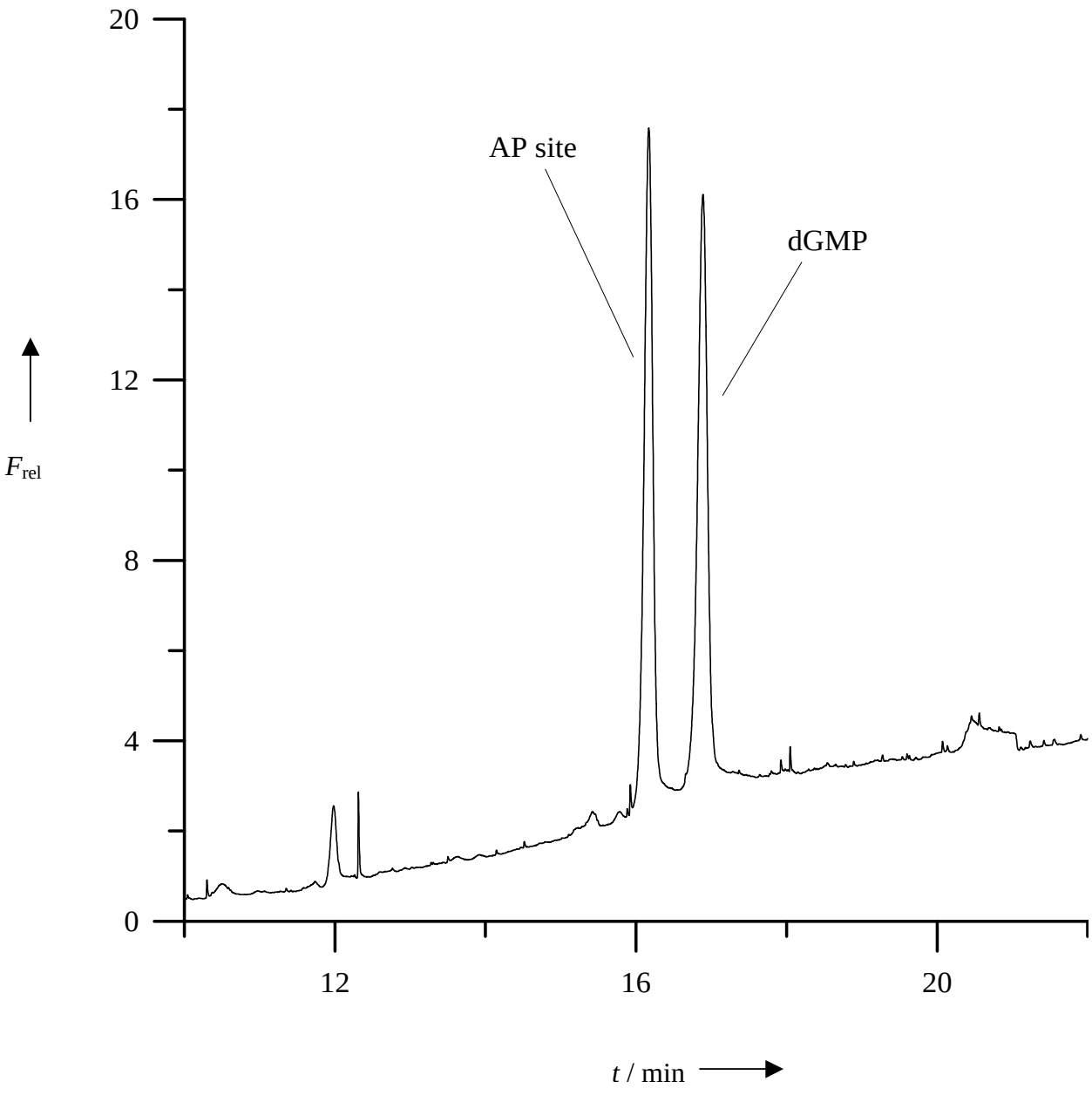


Figure 4 Analysis of an oligonucleotide after treatment with HCl for 16 h. 10 µg of an oligonucleotide were incubated with 5 µL 30 µM HCl for 16 h at 37 °C, isolated by solid-phase extraction (eluted with methanol), hydrolysed and derivatised.