Simulation of metabolic processes of polycyclic aromatic hydrocarbons using electrochemistry/mass spectrometry

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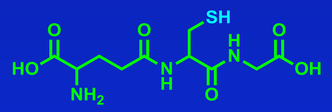
Introduction

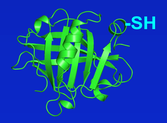
- Polycyclic aromatic hydrocarbons (PAHs) are a group of ubiquitous environmental contaminants
- Responsible for their toxic effects are reactive metabolites: epoxides, radical cations and quinones
- Pyrene was investigated as a PAH model compound (Figure 1)
- Electrochemistry in combination with liquid chromatography and mass spectrometry (EC-LC-MS) was used to study the generation of metabolites and their reactivity towards thiol groups (SH groups)

Pyrene

Figure 1: Structure of pyrene



Glutathione



ß-Lactoglobulin A

Figure 2: Structures of the investigated trapping agents glutathione (GSH) and ß-lactoglobulin A (ß-LGA). Both molecules contain one free thiol group that can act as nucleophile.

Method

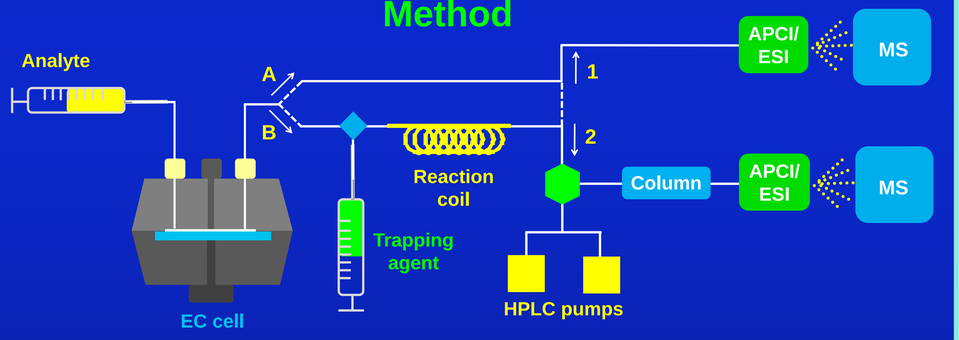


Figure 3: Instrumental on-line setup for the EC-(LC)-MS analysis of pyrene. A) Identification and characterization of oxidation products. B) Studies on reactivity of oxidation products towards SH groups.

- The pyrene containing solution was pumped through an electrochemical thin-layer cell (EC cell) while applying either a potential ramp (recording of mass voltammograms) or a constant potential.
- A) Oxidation products were identified via direct APCI-MS detection (A1) and further characterized by means of HPLC-APCI-MS (A2)
- B) Adduct formation of products was studied by incubation with a trapping agent and subsequent ESI-MS analysis: GSH adducts (B1) and ß-LGA adducts (B2)

Summary and Conclusion

- EC/MS is a promising tool for simulating metabolic processes of PAHs.
- Quinones were found to be the major oxidation products of pyrene. Distinction between two different isomers was achieved by means of HPLC separation.
- Adduct formation of pyrene quinones with glutathione and ß-lactoglobulin A was observed. This may play a role in the toxicity of PAHs.

Results and Discussion

A: Identification and characterization of oxidation products

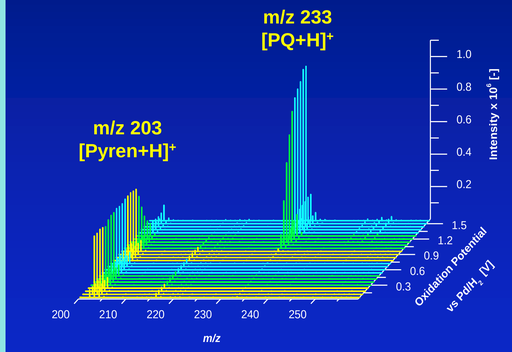


Figure 4: Mass voltammogram of pyrene. The intensitiy of the parent compound (m/z 203) decreases, while new signals arise. The major oxidation product with m/z 233 can be attributed to the formation of pyrene quinones (PQ).

- Quinones are the major oxidation products of pyrene (Figure 4)
- Two different quinone isomers are formed as can be seen in Figure 6
- Possible structures are shown in Figure 5

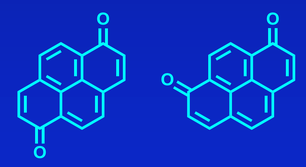


Figure 5: Proposed structures of pyrene quinones as known from in vivo studies.[1]

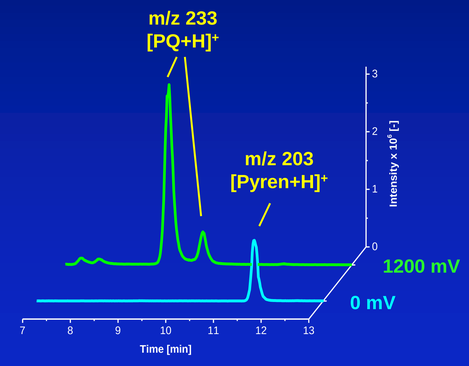


Figure 6: HPLC-APCI-MS chromatograms of pyrene before and after oxidation at 1200 mV. The appearance of two different peaks for pyrene quinone (m/z 233) indicates the formation of two isomers.

B: Reactivity of pyrene quinones towards thiol groups

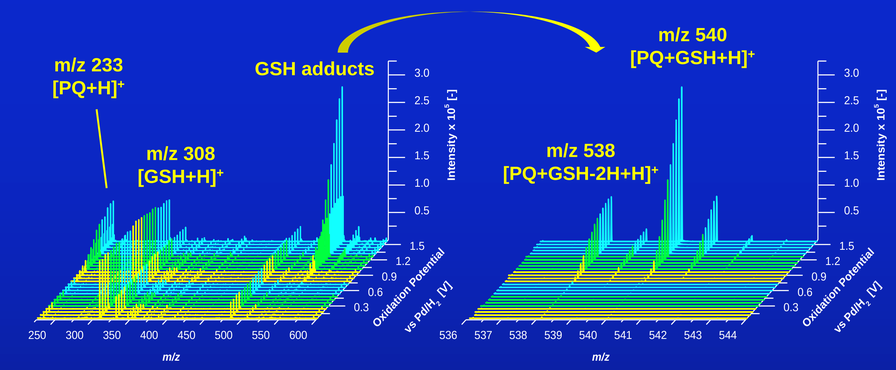


Figure 7: Mass voltammogram of pyrene after addition of glutathione (GSH). Besides the signal for pyrene quinone (PQ, m/z 233), additional signals with m/z 538 and m/z 540 arise. Based on exact masses and fragmentation pattern, these signals were identified as glutathione adducts of PQ.

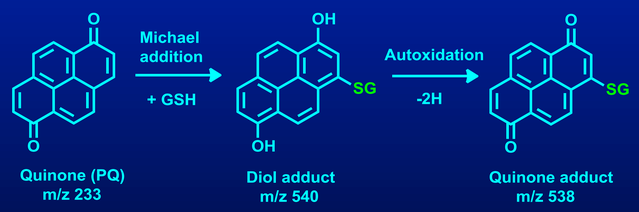


Figure 8: Proposed structures of GSH adducts of pyrene quinone and their formation pathway.

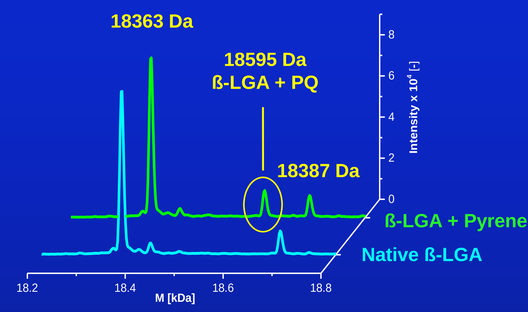


Figure 9: Deconvoluted mass spectra of native β-LGA and β-LGA after incubation with pyrene oxidized at 1200 mV. The additional signal with a mass difference of 232 Da compared to the native protein indicates the formation of an adduct of pyrene quinone with ß-LGA.

- Pyrene quinones show high reactivity towards SH groups
- Formation of adducts was observed with glutathione as well as with ß-lactoglobulin (Figures 7 and 9)
- Initial glutathione diol adducts undergo further oxidation to rebuild the quinoid structure (Figure 8) [2]

Literature

[1] Harper, K., Br J Cancer 1957, 11, 499-507.

[2] Murty, V. S., Penning, T. M., Chem Biol Interact 1992, 84, 169-188.

Acknowledgement

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