Expanding Role of Electrochemistry/Mass Spectrometry in Life Sciences

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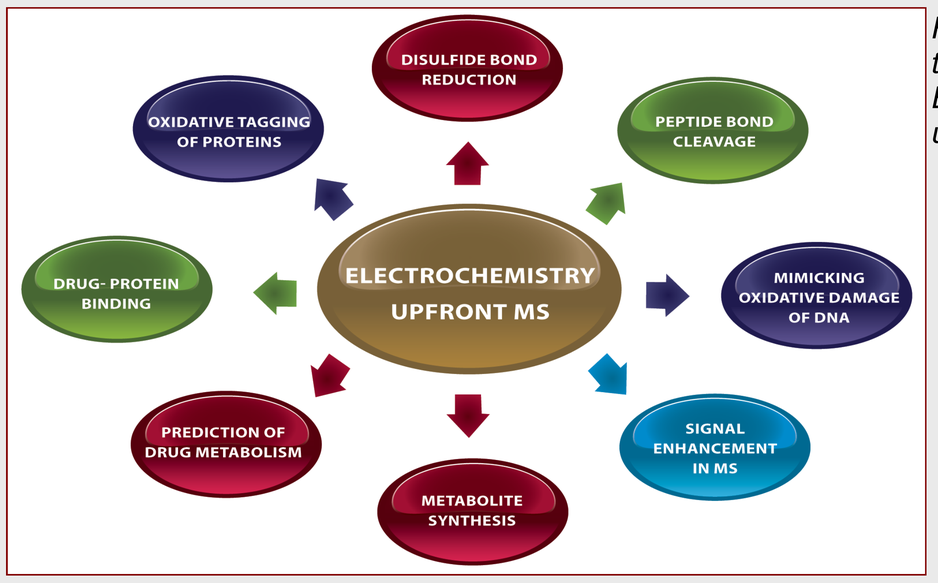


Figure 1: Application areas of Electrochemistry upfront MS.

Introduction

Electrochemistry (EC) in combination with mass spectrometry creates a powerful platform to simulate various oxidation and reduction processes in life sciences. Electrochemistry is a complementary technique to traditional in vivo or in vitro metabolism studies, and delivers the oxidative metabolic fingerprint of a (drug) molecule in a very short time. Furthermore, protein/peptide cleavage, disulfide bonds reduction and covalent drug-protein binding can be performed in the electrochemical cell. Mass spectrometry delivers selective and sensitive detection and allows for unambiguous identification of all products generated in the electrochemical cell.

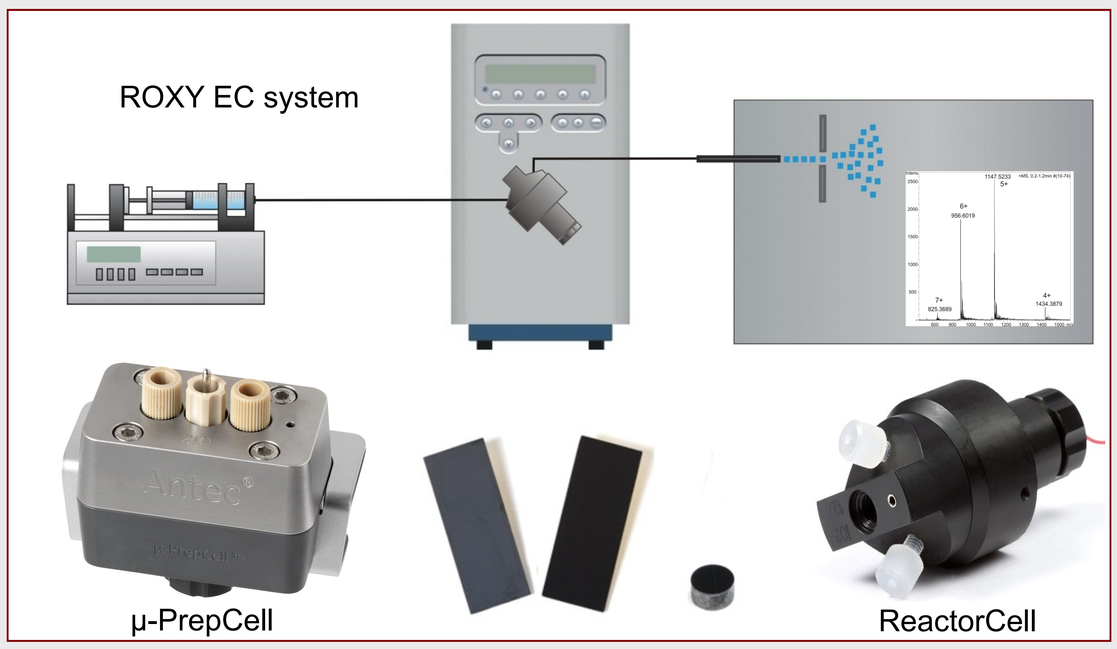
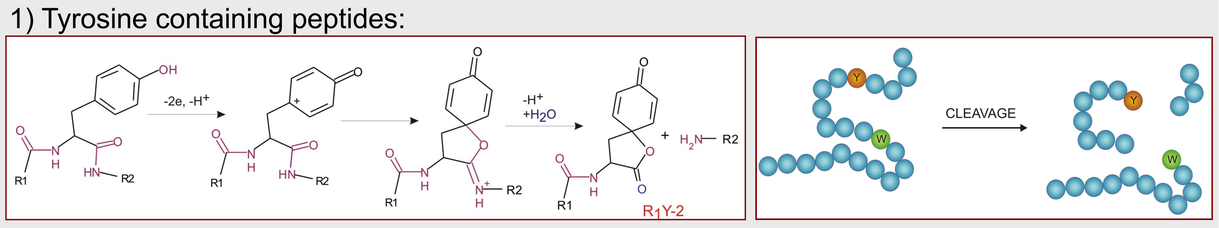


Figure 2: Upper panel: Instrumental set-up of ROXY™ EC system. Bottom panel: μ-PrepCell™ (left) and ReactorCell™ (right). Comparison of working electrodes (μ-PrepCell™ vs. ReactorCell™).

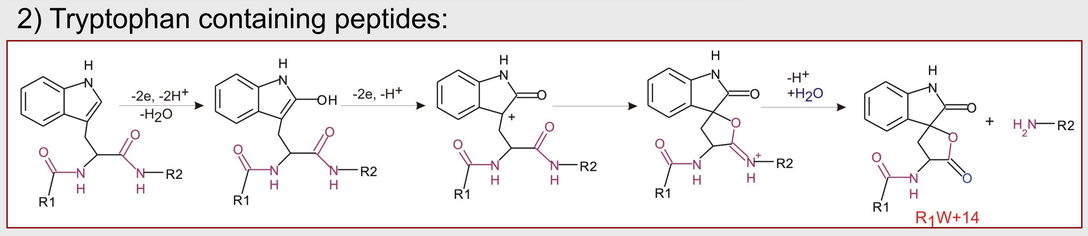
Electrochemical Digestion of the Peptides

Proposed cleavage mechanism of Tyr–containing Peptides (1) and Trp–containing Peptides (2) [Ref 1]:

1) Tyrosine containing peptides:



2) Tryptophan containing peptides:



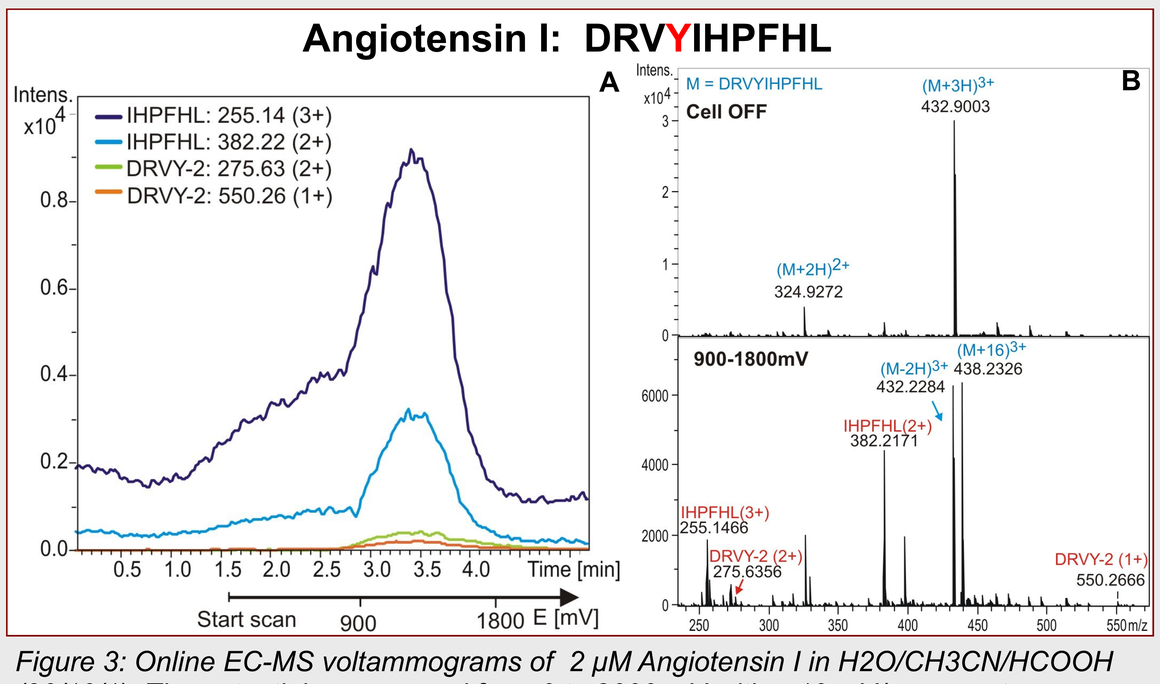
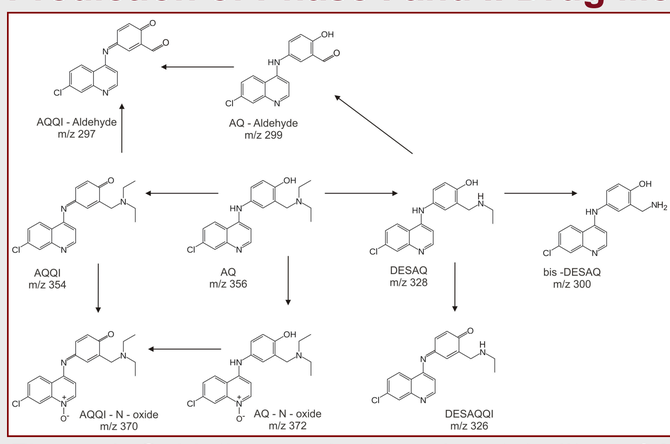


Figure 3: Online EC-MS voltammograms of 2 μM Angiotensin I in H2O/CH3CN/HCOOH (90/10/1). The potential was ramped from 0 to 2000 mV with a 10 mV/s scan rate.
***(A):** EIC of cleavage products: IHPFHL (2+) = 382.22 and IHPFHL (3+) = 255.14; DRVY-2 (2+) = 275.63 and DRVY-2 (1+)= 550.26; **(B):** Mass spectrum of Angiotensin I with Cell OFF (no oxidation) and mass spectrum of oxidized Angiotensin I (accumulated area of 900-1800mV and corrected for background signals). Cleavage fragments are indicated in red.*

Prediction of Phase I and II Drug Metabolism



Metabolite	Electrochemical oxidation	Microsomal Incubation
AQ	+	+
AQQI	+	+ only with GSH
DESAQ	+	+
DESAQQI	+	+ only with GSH
bis - DESAQ	-	+
AQ - Aldehyde	+	+
AQQI - Aldehyde	+	-
AQ-N-oxide	-	+
AQQI -N- oxide	+	-

Figure 4: Oxidation products and metabolites of Amodiaquine. Table: comparison of products formed by electrochemical oxidation an d microsomal incubation [3].

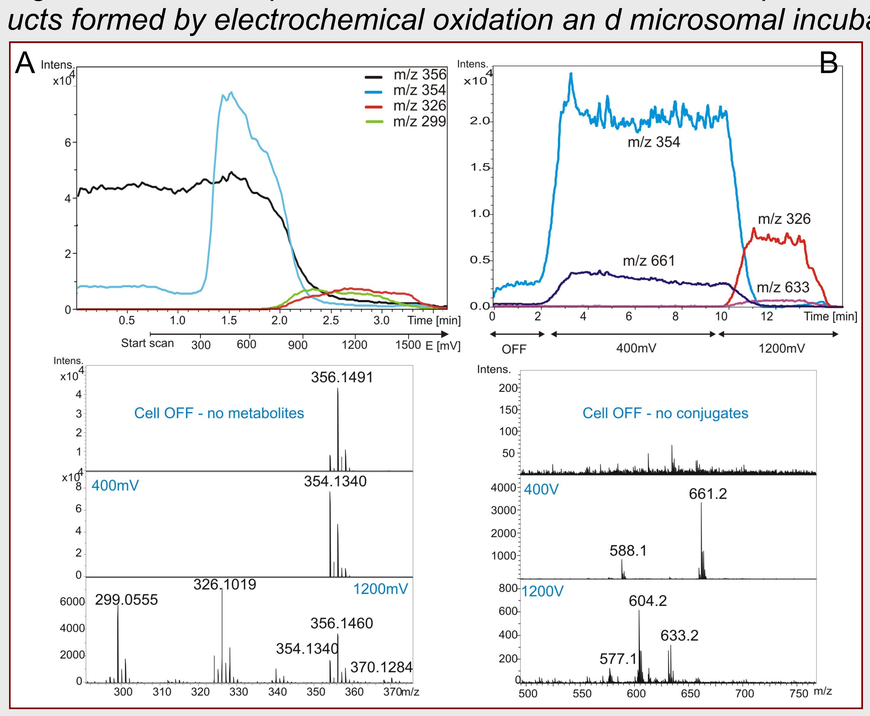


*Figure 5 **(A):** 2-D MS Voltammogram of Amodiaquine (Scan mode) and mass spectra of phase I metabolites of Amodiaquine. **(B):**. Example of EICs of Metabolite 1 (m/z 354) and its conjugate (m/z 661) and Metabolite 2 (m/z 326) and its conjugate (m/z 633). Conjugation was performed in DC mode. The mass spectra of the conjugation products formed with different potential. The spectrum with cell OFF confirms that the conjugates are formed ONLY if potential is applied.*

Drug – Protein adduct Formation

Amodiaquine (AQ) was oxidized in the ReactorCell by applying continuous scan 500-1000 mV with 50 mV/s rate. AQ underwent oxidation by dehydrogenation to quinine imine (AQQI). Figure 6 shows the adduct formation of electrogenerated AQQI with β-Lactalbumin. The shift in the m/z values after the reaction with the reactive drug metabolites clearly indicates the occurrence of covalent drug-protein adduct formation.

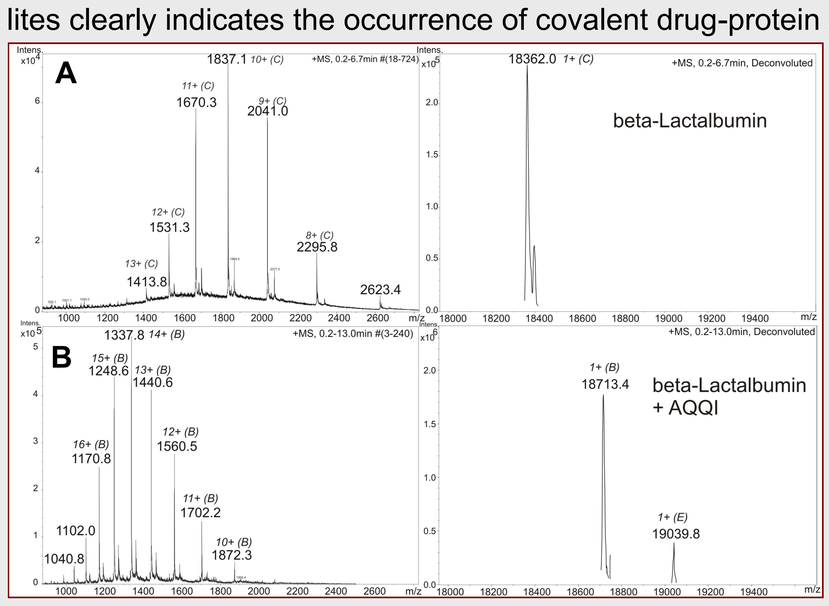


Figure 6: Mass spectra and deconvolution results of unmodified β-Lactalbumin (A) and after reaction with AQQI (B). Courtesy of Prof. Dr. Jerzy Silberring (AGH University of Science and Technology, Kraków, Poland).

Conclusions

Using the ROXY™ EC system on-line with MS results in fast generation of metabolites (seconds vs. days or weeks using in-vitro and/or in-vivo methods), access to phase II reactions as well as reactive metabolites. Amiodaquine was successfully used as model drug to mimic the oxidative metabolic pathway in the human liver by on-line EC/MS.
Phase I and II metabolites, which were already known from the literature as detoxification products in vivo, were generated in the EC reactor cell and on-line identified by MS using either amodiaquine alone or in the presence of glutathione.
Electrochemistry up front MS can be applied for fast and selective protein and peptide cleavage (as a promising new approach to enzymatic digestion).
Overall, EC/MS represents a powerful analytical technique to study Nature's redox reactions.

Acknowledgements

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References

[1] Roeser J. et al., *Anal. Chem*. 82 (18) (2010) 7556
[2] Lohmann W. et al., *LC-GC Europe.* January (2010) 1
[3] Faber H et al., *Angew. Chem. Int. Ed. Engl.,* 50 (2011) A52