

ELECTROCHEMICAL CLEAVAGE AND OXIDATION OF PEPTIDES BY ROXY EC SYSTEM COMBINED WITH ESI-MS

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ABSTRACT

Today, chemical and enzymatic cleavage of peptides and proteins followed by mass spectrometry has become one of the most widely used analytical techniques in characterization of these species. It was also shown that an electrochemical oxidation of peptides containing tyrosine (Tyr) or tryptophan (Trp) can lead to specific cleavage next to these amino acids^[1]. Coupling of electrochemical oxidation to mass spectrometry with electrospray ionization (EC/ESI-MS) may become an alternative to routinely used methodologies. In fact, this technique can help to distinguish phosphorylated Tyr residues from unphosphorylated ones^[2]. The electrochemical oxidation of wide range of peptides was researched by applying a potential ramp from 0 to 2 or 3 V respectively during passage of the sample through an electrochemical cell. To study more about the process, two kinds of working electrodes have been used: Glassy Carbon (GC) and Magic Diamond (MD). All experiments were performed in acidic conditions, 1% formic acid (v/v), to get optimal cleavage yield. Nonetheless, a significant percentage of peptides containing Tyr or Trp followed the hydroxylation process (MH⁺ +16 m/z ions present in the mass spectra). MS/MS spectra of cleavage products in all experiments were analyzed to reveal the real structure. The results confirm the oxidation mechanism presented in earlier published papers^[3]. Coupling an electrochemistry system (EC) *on-line* with electrospray mass spectrometry (ESI-MS) allows analysis of the oxidation products of peptides, especially those containing Tyr or Trp. Fragmentation of peptides occurred by hydrolysis at the C-terminal side of Tyr gives an idea that the EC/ESI-MS system can be used for selective peptide digestion without usage of expensive enzymes.

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INTRODUCTION

An electrochemical conversion of many target compounds with a mass spectrometry (MS) detection has become utilization for a wide range of applications. It can be a model for some metabolites development, signal enhancement in MS and finally, electrochemical cleavage of proteins and peptides, that is shown below. **The ROXY system** for *on-line* electrochemistry (EC-MS) was used to research cleavage and oxidation of peptides containing tyrosine or tryptophan.



Figure 1. Working electrodes. (From left: GC, MD, Pt, Au)



Figure 2. Electrochemical cell (Left) and EC-MS system (Right).

METHODS AND MATERIALS

The **ROXY EC System** (Antec, Zoeterwoude, The Netherlands) for single compound screening includes the ROXY potentiostat equipped with a ReactorCell (**Figure 2**), infusion pump and all necessary LC connections, was connected *on-line* with mass spectrometer Bruker Esquire 3000 (Leipzig, Germany). The ROXY EC System was controlled by Antec Dialogue software and all data were processed with Bruker Daltonics DataAnalysis programme.

A 20 μM peptides solutions in 10% acetonitrile in 1% formic acid were pumped at a constant flow rate of 2 μL/min through the ReactorCell using an infusion pump. The outlet of the reactor cell was connected directly to the ESI-MS source. The scan mode was used to register the MS Voltammogram. Working electrode potential was ramped from 0 – 3 V with the scan rate of 5 mV/s in the half cycle.

Two electrodes were tested: Glassy Carbon and Magic Diamond (**Figure 1**).

RESULTS

In **Figure 3** the MS Voltammograms are shown for GGYR peptide. The data for MS Voltammograms were recorded using a scan mode. Simply, the potential ramp was performed in range between 0 and 3 V and scanned with 5 mV/s rate in the half cycle on MD working electrode.

For the potential higher than 1.8 V cleavage of peptide can be received on the C-terminal site of tyrosine. Fragment with reduced mass by 2 m/z was also present in the mass spectrum. The extracted ion chromatogram for the m/z of GGYR peptide (m/z of 452) and its fragment GGY (m/z of 296) are shown in **Figure 3** as MS Voltammograms. Furthermore with potential higher than 1.8 V hydroxylation of peptide was also observed.

An experiment under the same conditions was performed for other peptides containing tyrosine (peptides with sequence: GLYR, LGYR and LFYR), but no cleavage was observed.

Figure 4 shows the mass spectra corresponding to ReactorCell OFF (control measurement) and with 2.0 V applied during the SAWGSWS peptide passage. This shows that for peptides containing more than one aromatic amino acid multiple hydroxylation can be obtained during following experiment.

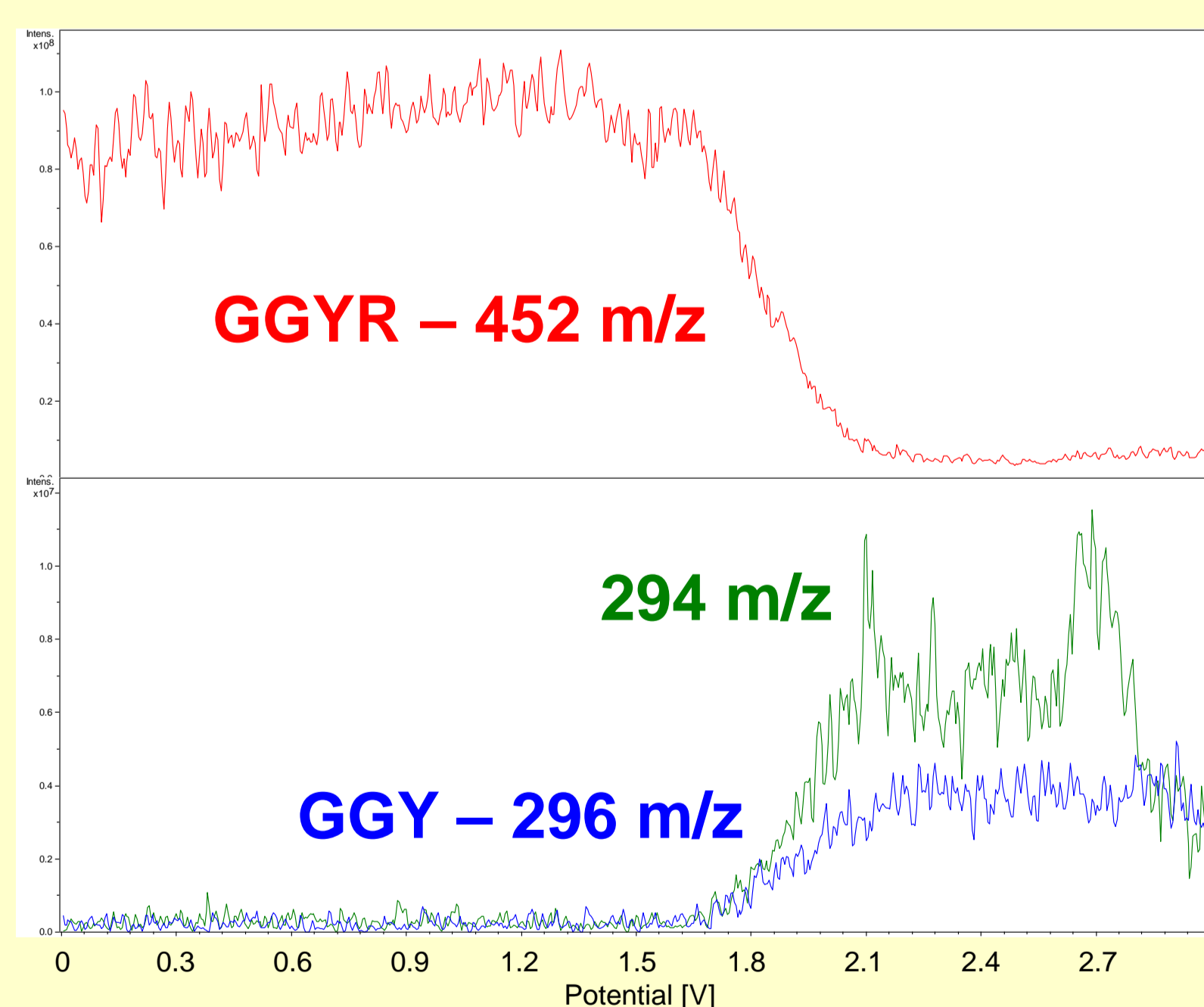


Figure 3. Oxidation of GGYR peptide on MD electrode (Top) and oxidation products (Bottom).

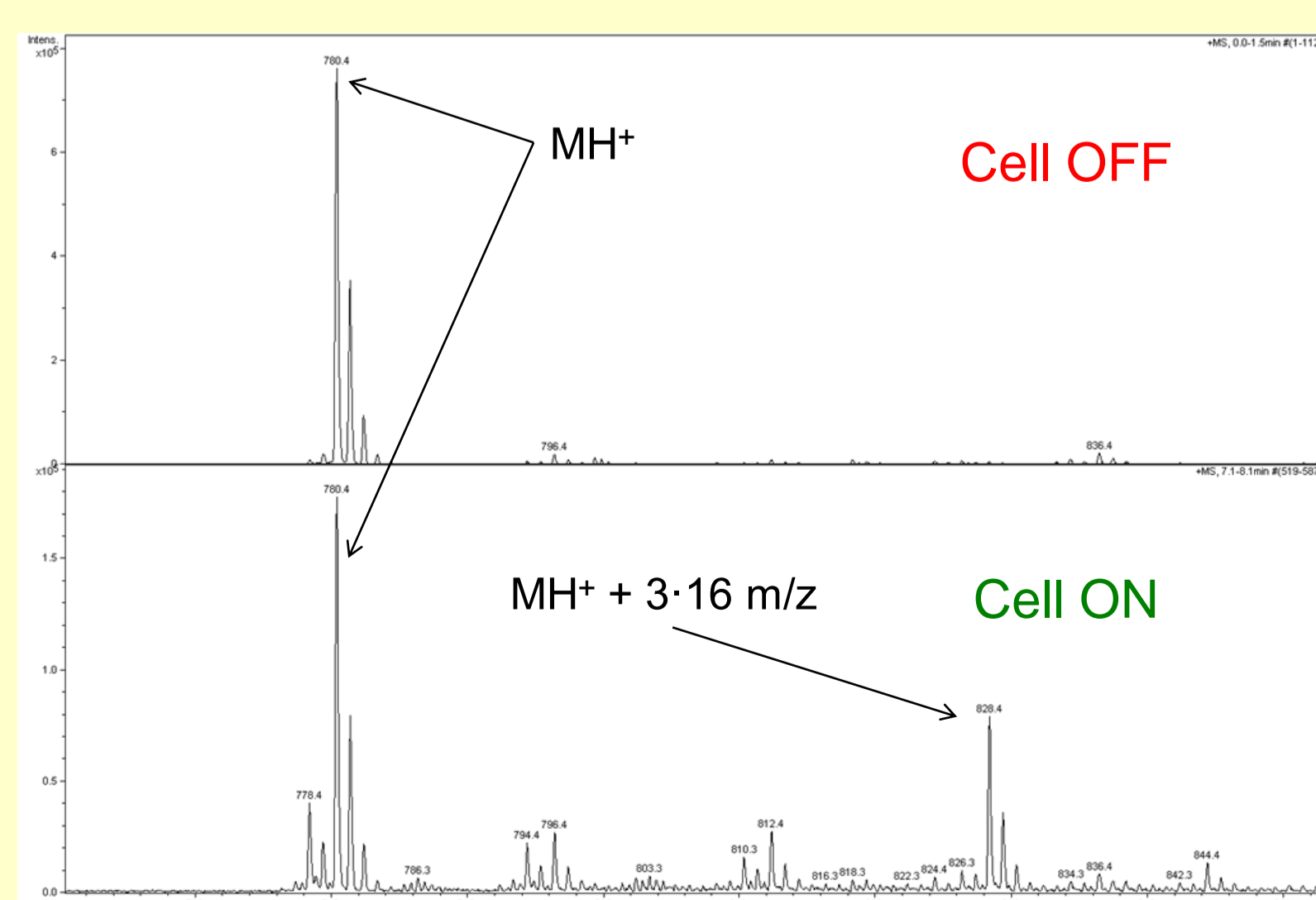


Figure 4. Oxidation of SAWGSWS peptide on MD electrode, cell off (Top), cell on (Bottom).

DISCUSSION

When amino acids like **tyrosine** or **tryptophan** are present in peptides or proteins molecules, the oxidized intermediates can follow intramolecular reactions that lead to the cleavage process. The experiment shows that this can result in specific cleavage of the C-terminal peptide bond to tyrosine, but not for tryptophan as it was published earlier^[3].

Presence of 294 m/z ion in **Figure 3** additionally prove proposed oxidation mechanism for tyrosine presented in **Figure 5**.

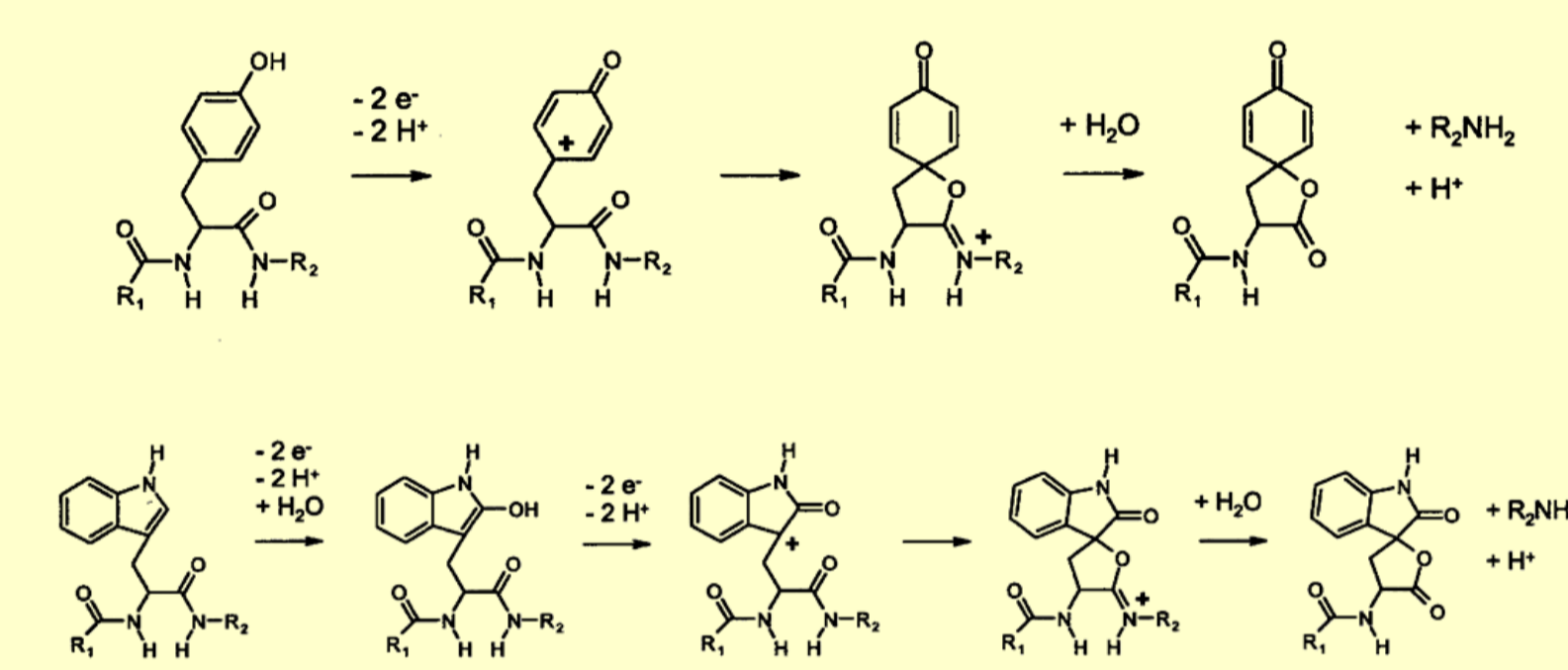


Figure 5. Proposed reaction mechanism for electrochemical oxidation and cleavage at tyrosine (Top) and tryptophan (Bottom). R1 and R2 are the parts of the protein N-terminal and C-terminal, respectively, to tyrosine and tryptophan.

CONCLUSIONS

The electrochemical cleavage of peptides is possible to achieve and specific for peptides containing tyrosine residues. This indicates the possibility to develop electrochemistry as an instrumental alternative to enzymatic or chemical protein digestion. The merits are the short time of measurement and easy coupling of the electrochemical flow cell to MS.

On the other side, protein oxidation reactions that do not produce protein cleavage, e.g. hydroxylation process, still complicate the resulting oxidation. Furthermore, protein sequence highly influences the oxidation reaction.

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