Electrochemistry in the mimicry of oxidative drug metabolism
Nouri-Nigjeh, Eslam

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2011

Citation for published version (APA):
Chapter 5
Electrocatalytic Activation of Hydrogen Peroxide on a Platinum Electrode in the Imitation of Oxidative Drug Metabolism by Cytochrome P450s

Electrochemistry in combination with mass spectrometry has shown promise as a versatile technique not only in the analytical assessment of oxidative drug metabolism, but also for small-scale synthesis of drug metabolites. However, electrochemistry is generally limited to reactions initiated by direct electron transfer. In the case of substituted-aromatic compounds, oxidation proceeds through a Wheland-type intermediate where resonance stabilization of the positive charge determines the regioselectivity of the anodic substitution reaction, and hence limits the extent of generating drug metabolites in comparison with in vivo oxygen insertion reactions. In this study, we show that the electrocatalytic oxidation of hydrogen peroxide on a platinum electrode generates reactive oxygen species, presumably surface-bound platinum-oxo species, which are capable of oxygen insertion reactions in analogy to oxo-ferryl radical cations in the active site of Cytochrome P450. Electrochemical oxidation of lidocaine at constant potential in the presence of hydrogen peroxide produces both 3- and 4-hydroxylidocaine, suggesting reaction via an arene rather than a Wheland-type intermediate. No benzyl hydroxylation was observed, excluding the presence of freely diffusing radicals. The results of the present study extend the possibilities of electrochemical imitation of oxidative drug metabolism to oxygen insertion reactions.

* Submitted as: Nouri-Nigjeh, E.; Bischoff, R.; Bruins, A.P.; Permentier, H.P. Electrocatalytic activation of hydrogen peroxide on a platinum electrode in the mimicry of oxidative drug metabolism by Cytochrome P450s, Chemical Research in Toxicology, Submitted.
Electrocatalytic Activation of Hydrogen Peroxide on a Platinum Electrode in the Imitation of Oxidative Drug Metabolism by Cytochrome P450s

5.1 INTRODUCTION

Drug metabolism and toxicological studies are shifting toward the earlier stages of drug discovery and development. This makes it indispensable to develop fast and accurate analytical techniques capable of detecting and characterizing drug metabolites. Electrochemistry in combination with mass spectrometry has shown promise as a versatile technique not only in the analytical assessment of oxidative drug metabolism, but also for small-scale synthesis of drug metabolites [1-4]. However, electrochemistry is mainly restricted to reactions initiated by single electron transfer (SET), such as N-dealkylation, or hydroxylation of substituted aromatic ring systems [5, 6]. Further research is thus required to extend the range of electrochemical techniques to cover the full range of in vivo drug metabolism by imitating reactions initiated by hydrogen atom transfer (HAT) or oxygen insertion.

Lidocaine, a local anesthetic drug with a variety of in vivo metabolites, has been used as a test compound in the evaluation of new electrochemical techniques [7, 8]. The in vivo oxidative drug metabolism of lidocaine by Cytochrome P450s (CYP) encompasses N-dealkylation, N-oxidation, and aromatic and benzylic hydroxylation reactions [9-11]. An early study showed that electrochemistry can imitate the N-dealkylation reaction by application of a constant positive potential of less than 1.5 V vs. Pd/H₂ in an on-line electrochemical cell coupled to a mass spectrometer [12], where the reaction proceeds through initial electron transfer from the tertiary amine moiety to the electrode to generate an imine intermediate followed by hydrolysis and cleavage after intramolecular rearrangement [13]. N-oxidation of lidocaine was achieved through electrochemical reduction of molecular oxygen and generation of hydrogen peroxide in a two-compartment electrochemical cell under air atmosphere [7]. This reaction proceeds most likely through a peroxide intermediate [14].

Whereas the only oxidation product at potentials below 1.5 V is N-dealkylation [10], we have recently shown that oxidation at more positive potentials (optimum at 3.0 V) using 99/1 (v/v) acetonitrile/water and tetrabutylammonium perchlorate (TBAP) as supporting electrolyte results in 4-hydroxylation [8]. Four-hydroxylation is probably initiated by electron transfer from the aromatic ring moiety to generate a Wheland-type intermediate, that leads to the hydroxylation product through deprotonation and an anodic substitution reaction (Scheme 1A) [15, 16]. The presence of an amide substituent on the aromatic ring decreases the oxidation potential compared with an unsubstituted benzene ring and directs the anodic substitution reaction toward the 4-position by resonance-stabilization. Since the anodic substitution reaction proceeds through the Wheland-type intermediate, the regioselectivity of the reaction excludes the formation of the 3-hydroxylidocaine. In contrast, in vivo aromatic hydroxylation is believed to follow a concerted oxygen insertion mechanism by oxo-ferryl radical cations (Compound I) in CYP and...
Electrochemistry in the Mimicry of Oxidative Drug Metabolism

formation of an arene intermediate and does not involve the Wheland-type intermediate (Scheme 1B) [17]. Therefore, for electrochemistry to imitate in vivo oxidative drug metabolism more closely, the generation of reactive oxygen species (ROS) that mimic the generated ROS during catalytic activation of molecular oxygen by CYP is required.

Electrochemistry-mediated aromatic hydroxylation

Platinum electrodes are widely used for the oxidation of organic compounds as they are stable even at high positive potentials. They are also used for electrocatalytic oxidation of hydrogen peroxide to generate molecular oxygen [18, 19]. This reaction supposedly begins by binding of H₂O₂ to electrochemically generated Pt(II) sites on the electrode surface, and it is overall a two-electron and two-proton transfer process, i.e. H₂O₂ → 2H⁺ + O₂ + 2e⁻, depending strongly on the pH, electrode surface condition as well as the O₂ and H₂O₂ concentrations [18-20].
Electrocatalytic Activation of Hydrogen Peroxide on a Platinum Electrode in the Imitation of Oxidative Drug Metabolism by Cytochrome P450s

However, the generation of ROS during the electrocatalytic oxidation of H$_2$O$_2$ on the Pt electrodes has not been studied yet. Here we report that electrocatalytic oxidation of H$_2$O$_2$ on a Pt electrode leads to aromatic hydroxylation of lidocaine in the 3-position thus extending the electrochemistry-based imitation of P450-mediated, oxidative metabolism of lidocaine.

5.2 EXPERIMENTAL PROCEDURES

Reagents. Tetrabutylammonium perchlorate (TBAP, 86893), hydrogen peroxide (30 vol % in water (31642)), H$_2$O with 97 atom % $^{18}$O (H$_2^{18}$O, 329878), and lidocaine (L7757) were purchased from Sigma-Aldrich. Throughout this article, H$_2$O$_2$ denotes 30% H$_2$O$_2$ in water. Water was purified by a Maxima Ultrapure water system (ELGA, High Wycombe, Bucks, UK). Ultra-pure HPLC grade acetonitrile (ACN) was purchased from Merck. 3-Hydroxylidocaine (CAS No. 34604-55-2) was purchased from Toronto Research Chemicals Inc.

Electrode preparation. The surface of the working electrode was polished with a lapping sheet (Micromesh grade 3200) prior to each experiment. After mechanical polishing the surface was washed with ethanol and air-dried.

Electrochemical experiments. Electrochemical oxidations were performed with a home-made potentiostat controlled by a MacLab system (ADInstruments, Castle Hill, NSW, Australia) and EChem v.1.52 software (eDAQ, Denistone East, NSW, Australia). A two-compartment electrochemical cell was constructed by using a porous Vycor tip with Teflon heat shrink (MF-2064, Bioanalytical Systems (BASI), West Lafayette, IN, USA) to separate compartments of the working and auxiliary electrodes. The working electrodes were platinum (MF-2013, BASI) or glassy carbon (MF-2012, BASI) disks, respectively, and the auxiliary electrode was a platinum wire (MW-4130, BASI). Potentials were measured against a silver wire pseudo-reference electrode (MF-2017, BASI), which was placed in the working electrode compartment. All experiments were performed at ambient temperature, while the auxiliary electrode and working electrode compartments were constantly purged with argon and synthetic air, respectively. Solutions containing 10 mM lidocaine and 0.1 M TBAP dissolved in ACN/H$_2$O$_2$ 99/1 (v/v) were subjected to constant potential oxidation for 30 min prior to LC-MS analysis. Samples were collected from the working electrode compartment and diluted 100 times in water containing 10 $\mu$M acetaminophen as internal standard for LC-MS signal normalization, immediately after the batch oxidations and stored at room temperature until LC-MS analysis.

LC-MS/(MS) analysis. LC-MS experiments on 100-times diluted samples were carried out on an LC-Packings Ultimate HPLC system (LC-Packings, Amsterdam, the Netherlands) coupled to an API 365 triple quadrupole mass spectrometer (MDS Sciex, Concord, ON, Canada) upgraded to EP10+ (Ionics, Bolton, ON,
Canada) with electrospray ionization in the positive mode using the TurboIonSpray source. The MS parameters were as follows: ion spray voltage 4800 V, OR 40 V, and RNG 120 V, with scans between m/z 100-600 (step size 1.0 amu, dwell time 1 ms). MS/MS parameters were as follows: ion spray voltage 5000 V, OR 40 V, RNG 170 V, and collision energy 20 eV (step size 1.0amu, dwell time 1 ms). Heated turbo gas (nitrogen) with a flow rate of 1.0 L/min was used at 450 °C for LC-MS/MS analysis.

A C₁₈ reversed-phase column (GraceSmart RP 18 5μm, 2.1×150 mm; Grace Davison, Lokeren, Belgium) was used at a flow rate of 200 μL/min. Solvent A: H₂O/ACN 95/5 (v/v) with 0.1 % formic acid; Solvent B: ACN/H₂O 95/5 (v/v) with 0.1 % formic acid. Five and 50 μL of a diluted oxidation product mixture were subjected to LC-MS and MS/MS analysis, respectively, using a linear gradient of 5-50 % B in 20 min. Peak heights were normalized with respect to the peak height of acetaminophen as internal reference compound.

5.3 RESULTS AND DISCUSSION

Electrochemical oxidation of lidocaine at +3.0 V from a solution of ACN/H₂O 99/1 (v/v) has been reported to result in 4-hydroxylation. The N-oxide was formed in a chemical reaction with electrochemically generated hydrogen peroxide.(8) In the present study, oxidation at +3.0 V in a solution of ACN/H₂O 99/1 (v/v), yielded two oxidation products with m/z 251 and one with m/z 267 (Figure 1-a). LC-MS/MS analysis of the two m/z 251 products (Figures 1-b and 1-c) suggests that both are aromatic hydroxylation products. Benzylic hydroxylation was ruled out since it is expected to give water loss upon fragmentation by collision induced dissociation (CID) [7]. Peak II co-eluted with 3-hydroxylidocaine which lead us to the conclusion that peak I corresponds to 4-hydroxylidocaine.
Figure 1. (a) LC-MS analysis of lidocaine oxidation products from a solution of acetonitrile/hydrogen peroxide 99:1 (v/v) with 0.1 M tetrabutylammonium perchlorate at +3.0 V on a platinum electrode. Extracted ion chromatograms are shown of the MH⁺ ions at m/z 267, and m/z 251 before (solid line), and after (dashed line) addition of 0.25 μM 3-hydroxylidocaine as a standard. (b and c) Collision induced dissociation fragmentation spectra of peaks I and II (c) measured with a collision energy of 20 eV.
MS/MS analysis of the m/z 267 oxidation product, showed a fragment at m/z 249, corresponding to loss of a water molecule, in addition to the m/z 86 fragment, from the tertiary amine part of lidocaine. In order to elucidate the structure of this oxidation product, we synthesized 3,4-dihydroxylidocaine by pulsed electrochemical oxidation of 3-hydroxylidocaine as described before [8]. 3,4-dihydroxylidocaine co-eluted with the m/z 267 oxidation product and had the same CID fragment at m/z 249 (Figure 2-a). For further confirmation, 3,4-dihydroxylidocaine was synthesized from 3-hydroxylidocaine in the presence of H_2^{18}O resulting in an oxidation product with m/z 269. CID produced two fragments at m/z 249 and 251, which are attributed to the loss of H_2^{18}O or H_2^{16}O, respectively (Figure 2-b). Taken together this confirms the identity of the m/z 267 oxidation product as 3,4-dihydroxylidocaine. As expected, production of 3,4-dihydroxylidocaine from 3-hydroxylidocaine was easier than hydroxylation of lidocaine due to the presence of two electron-donating substituents. Further oxidation to 3,4-benzoquinone lidocaine was not observed under our conditions.

The yield for the 3- and 4-hydroxylation products, and the 3,4-dihydroxylation products of lidocaine at constant potentials ranging from +1 to +5 V on a platinum electrode are shown in Figures 3-a and 3-b, respectively. All hydroxylation products showed a maximum yield at +3.0 V.

To study the effect of the electrode material, constant potential oxidations were repeated on a glassy carbon electrode in the presence of H_2O_2. Only very small amounts of hydroxylation and dihydroxylation products were detected on this type of electrode. Moreover, aromatic hydroxylation products were completely absent at potentials above +2.0 V (see Figures 3-c and 3-d). The surface properties of the platinum electrode are therefore critical in the electrocatalytic oxidation of H_2O_2 to allow aromatic hydroxylation of lidocaine.
Electrocatalytic Activation of Hydrogen Peroxide on a Platinum Electrode in the Imitation of Oxidative Drug Metabolism by Cytochrome P450s

Figure 2. Collision induced fragmentation spectra of 3,4-dihydroxylidocaine. Synthesized by square-wave pulses from lidocaine in the presence of H₂¹⁶O (a) or from 3-hydroxylidocaine in the presence of H₂¹⁸O (b). (R represents the rest of lidocaine molecule)

If hydroxylation of lidocaine were initiated by HAT, we should have observed extensive benzylic hydroxylation, as shown previously for the Fenton reaction [7]. Since no benzylic hydroxylation was observed in the present study, a hydroxylation reaction based on a HAT mechanism by freely diffusing radicals, e.g. hydroxyl radicals, is unlikely. Accordingly, generation of the 3-hydroxylation product on a Pt
Electrochemistry in the Mimicry of Oxidative Drug Metabolism

electrode in the presence of \( \text{H}_2\text{O}_2 \) appears to follow an oxidation mechanism that is neither initiated by direct electron transfer nor by a HAT mechanism due to freely diffusing radicals. We hypothesize that electrocatalytic oxidation of \( \text{H}_2\text{O}_2 \) on a Pt electrode might involve surface-bound platinum-oxo species capable of promoting oxygen insertion in a mechanism reminiscent of the reaction of oxo-ferryl radical cations during the aromatic hydroxylation of substrates by CYP. Oxygen insertion by the putative platinum-oxo species might proceed through an arene oxide intermediate that results in both 3- and 4- hydroxylation products after tautomerization, as shown in Scheme 2.

Figure 3. Relative product ion distribution for the 3- and 4-hydroxylation products of lidocaine and the 3,4-dihydroxylation product versus the applied potential from a solution of 10 mM lidocaine in acetonitrile/hydrogen peroxide 99/1 (v/v) with 0.1 M tetrabutylammonium perchlorate on a platinum electrode (a, b), and a glassy carbon electrode (c, d).
Electrocatalytic Activation of Hydrogen Peroxide on a Platinum Electrode in the Imitation of Oxidative Drug Metabolism by Cytochrome P450s

The role of the putative platinum-oxo species that were generated during electrocatalytic oxidation of $\text{H}_2\text{O}_2$ in the hydroxylation of lidocaine were further studied by adding 1 vol % pyridine. Pyridine may react either with reactive intermediates of lidocaine, notably the arene intermediate, or with reactive platinum-oxo species directly, as illustrated in Scheme 2. In fact, oxidation of lidocaine in the presence of pyridine only led to 4-hydroxylidocaine and a pyridine-oxide product at $m/z$ 96. This indicates that reaction between pyridine and the putative platinum-oxo species competes with the formation of 3-hydroxylidocaine but not with that of 4-hydroxylidocaine. This is consistent with the mechanism in Scheme 1A where 4-hydroxylation occurs through direct oxidation. Therefore, pyridine addition supports the generation of the putative platinum-oxo species and their selective reaction toward the more abundant substrate, i.e. pyridine, during the electrocatalytic oxidation of $\text{H}_2\text{O}_2$. Further study of the Pt electrode surface and reactive intermediates should provide more insight into nature of the putative platinum-oxo species and the reaction mechanism.

Scheme 2. Oxygen transfer mechanism by surface-bound platinum-oxo species based on a concerted oxygen insertion mechanism, proceeding via an arene oxide intermediate.
5.4 CONCLUSIONS

Lidocaine is metabolized in vivo to aromatic hydroxylation products at the 3- and 4-position as well as to the benzylic hydroxylation product, through the action of P450 enzymes. Direct electron transfer oxidation by electrochemistry leads only to 4-hydroxylidocaine, whereas freely diffusing hydroxyl radicals from the Fenton reaction generate all three products. Electrocatalytic oxidation of hydrogen peroxide on a platinum electrode in the presence of lidocaine produces both 3- and 4-hydroxyldiocaine without concomitant benzylic hydroxylation. We suggest that this selectivity is due to generation of reactive surface-bound platinum-oxo species that are capable of oxygen insertion in analogy to oxo-ferryl radical cations, which are the main reactive species in the catalytic cycle of P450. The results of this study thus extend the application of electrochemistry in the imitation of oxidative drug metabolism by P450 in that it mimics oxygen insertion reactions.
5.5 REFERENCES


Electrochemistry in the Mimicry of Oxidative Drug Metabolism


