Electrochemistry On-Line with Electrospray Mass Spectrometry: Insights into Oxidative Metabolism of Tamoxifen

Haiteng Deng and Gary J. Van Berkel

Chemical and Analytical Sciences Division, Oak Ridge National Laboratory
Oak Ridge, TN 37831-6365

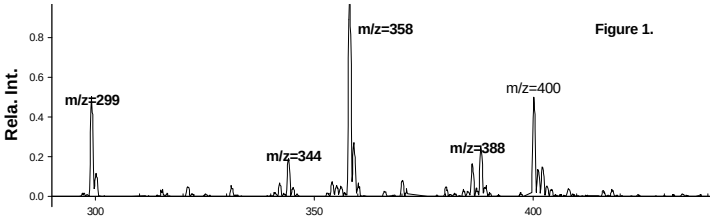
Introduction. A technique coupling an electrochemical flow cell on-line with electrospray mass spectrometry (EC/ES-MS) was established and used to investigate biological redox reactions. Using the oxidation of dopamine as the model system, we have characterized this EC/ESMS system. Studies of the oxidation of tamoxifen show that this technique in combination with MS/MS and on-line HPLC separations is a powerful tool in studying the redox chemistry of biologically important molecules.

Experimental. The thin layer flow cell (BAS, total cell volume ~ 1.1 µL) used a 16 µM spacing gasket to separate the glassy carbon working electrode from the Pt counter electrode. The cell was controlled by an PAR Model 173 potentiostat, and the potential and current readouts from potentiostat were directed to the mass spectrometer computer system by a dual channel PE Nelson Model 970A A/D interface. All EC/ES-MS on-line experiments were performed on an PE SCIEX API165 single quadrupole and API365 triple quadrupole mass spectrometer

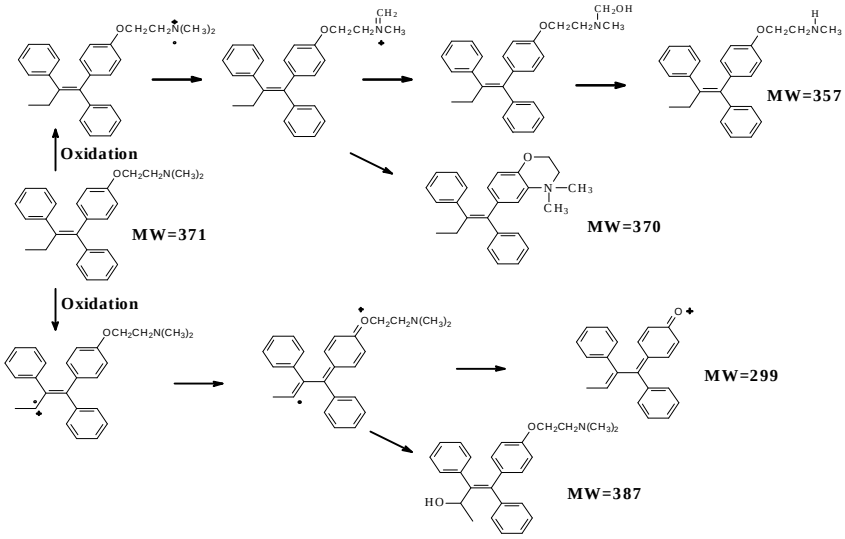
Results and Discussion. Dopamine is an important neurotransmitter and its electrochemical oxidation has been well studied. The major oxidation product of dopamine is dopamine quinone confirmed by CID. Using this reaction as the model system, we found that the electrolysis efficiency decreases with the solvent flow rates. At the typical flow rate for electrospray (30 µl/min), the electrolysis efficiency reaches 75% and the response time, i.e., the time for an electrochemically generated product to travel from the cell to the mass spectrometer is 5.2 second. Nucleophilic reactions of dopamine quinone with nucleotides, cysteine, glutathione, aniline, and PhSH were also investigated that demonstrates this experimental set-up is a powerful means to study biologically relevant chemical followup reactions.

The antiestrogen tamoxifen (TMX) is a widely used drug for breast cancer prevention and treatment. The metabolites detected in women treated with this drug are N-desmethyl TMX (357 Da), N-Didesmethyl TMX (343 Da), 4-OH TMX (387 Da), TMX N-oxide (387 Da), α-OH TMX (387 Da), TMX epoxide (385 Da). Some metabolites such as 4-OH TMX and/or α-OH TMX are a hepatocarcinogen in rats**.** Previous studies suggested that the oxidation product of TMX is a phenanthrene derivative (369 Da) as that from the UV radiation of TMX [1]. In the present work, TMX and related compounds, 4-OH TMX, α-OH TMX, clomiphene, toremifene, and diethylstilbestrol were studied. Cyclic Voltammograms show that oxidation of TMX and related compounds is an irreversible process. Compounds with 4-OH group such as 4-OH TMX and diethylstilbestrol have a lower oxidation potential. EC/ESMS studies show that major oxidation product for TMX, α-OH TMX, toremifene, and clomiphene is the demethylation product. But oxidation of 4-OH TMX and diethylstilbestrol is by dehydrogenation. Oxidation of clomiphene has no hydroxylation products which indicates that the ethyl group play an important role for hydroxylation of TMX. In general, the electrochemical oxidation products of TMX in H_2O/CH_3OH

(1/1 v/v) have same m/z ratios (observed as $(M+H)^+$) as those metabolic activation products of TMX, e.g., 344, 358, and 388 as shown in Fig.1.



CID mass spectrum of the oxidation product of TMX at *m/z* 358 shows that it comes from demethylation of TMX. In comparison with CID spectrum of the phenanthrene derivative, formed by photoactivation of TMX, we found that the electrochemical oxidation product of TMX at *m/z* 370 is a mixture of the structural isomers. CID mass spectrum of the oxidation product of TMX at *m/z* 388 shows the major fragment at *m/z* 72. However, CID mass spectra of α-OH TMX and 4-OH TMX display a similar dissociation pattern. In order to determine the structures of the oxidation products of TMX at *m/z* 388, bulk electrolysis of TMX (100 μM in water with 50mM NH_4Ac and 7.5% HAc) was carried out at 1.2 V vs Ag/AgCl for two hours. The electrolysis products were separated using a PGC column. By comparison with the retention time of α-OH TMX and 4-OH TMX in same mobile phase, we have found that the major oxidation product of TMX at *m/z* 388 appears to be α-OH TMX. By monitoring the major oxidation products of TMX with the scanning of the electrode potential, we found that demethylation occurs at the lower potential than hydroxylation. The ratios of the demethylation and hydroxylation products are significantly different in alcoholic and aqueous solution which indicates there are two oxidation intermediates. Because the major products from electrochemical oxidation and from metabolic activation of tamoxifen are the same, these two oxidation processes may produce the same reactive intermediates. Based on results from this work and the previous work on TMX and amides [2-3], the oxidation mechanisms of TMX are proposed as shown below.



[1] Wang, J., et al., Talanta **1997**, 45, 273. [2] Kochi, J. K., et al., J. Am. Chem. Soc. **1998**, 120, 6931. [3] Hanzlik, R. P., et al., J. Org. Chem. **1989**, 54, 2446.

H. D. acknowledges support through an appointment to the ORNL Postdoctoral Research Associates Program administered jointly by the Oak Ridge Institute for Science and Education and ORNL. This work was supported by National Cancer Institute under Interagency agreements DOE 0485-F053-A1 and NCI Y1-CB-0016-01 and by the Division of Chemical Sciences, Office of Basic Energy Sciences, United States Department of Energy (via a Cooperative Research and Development Agreement with Perkin-Elmer SCIEX Instruments (CRADA No. ORNL96-0458)), under contract DE-AC05-96OR22464 with ORNL. ORNL is managed by Lockheed Martin Energy Research Corp. The α-OH TMX was provided by Dr. McClelland of the University of Toronto.