Could *o*-Aminoquinones of Estrogen Metabolites Serve as Platforms for Redox Cycling?

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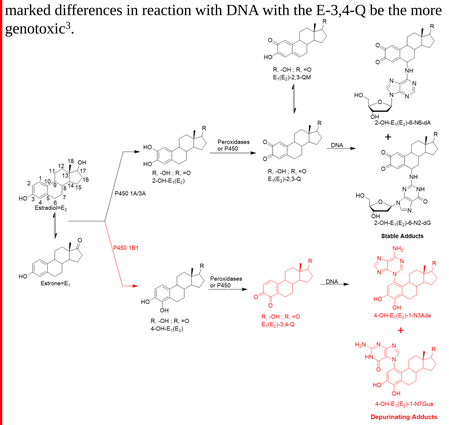
Introduction

Oxidative metabolism of estrogen can produce catechols and further oxidation lead to *o*-quinones, reactive electrophiles capable of binding to DNA. It is also thought that redox cycling between catechols and *o*-quinones can produce genotxic ROS. Of the two isomeric catechol estrogens, 2-OHE and 4-OHE, 4-OHE has been show a procarcinogen in animal models[1] while 2-OHE is not even they both possess similar oxidation potentials[2]. The corresponding *o*-quinones however show marked differences in reaction with DNA with the E-3,4-Q be the more genotoxic[3].



Castagnooli Jr. et.al, identified an *o*-aminoquinone structure produced by the regiospecific attack of nitrogen nucleophiles on an electrophilic quinone derived from poison oak and poison ivy catechols[4]. They resulting adducts displayed an intense red color with an absorbance around 480 nm. Yusuf J. Abul-Hajj and Dipti Khasnis explored the reaction of estrogen quinones with propylamine in aprotic organic solvents and isolated mainly condensation products[5]. We have explored the reaction of nitrogen nucleophiles with estrogen *o*-quinones in the presence of a proton source know to promote conjugate addition. Specifically, the reaction product of estrogen-3,4-quinone with γ-aminobutryic acid (GABA) has been characterized and its potential as redox platform is presented.

Materials and Methods

Synthesis of 1-GABA-E-3,4-Q

One equivalent of 4-OHE_1 was combined with five equivalents of MnO_2 in acetonitrile. This mixture was stirred at 0° C for 30 minutes to for the E-3,4-Q. Five equivalents of 4-amintobutyic acid (GABA) dissolved in .2 M acetate buffer pH 4.5-5 is added to the newly formed E-3,4-Q. This solution was allowed to react at room temperature for 30-40 minutes to form the 1-amino-E-3,4-Q. Reaction can be monitored visually by the formation of the dark red product. The solution was then filtered to remove the MnO_2 and the remaining solution rotary evaporated to remove the solvent mixture. This crude product can be stared in the dry state at -78° C.

Purification of 1-GABA-E-3,4-Q

The dried crude product was dissolved in a minimal volume of methanol for injection in the HPLC. The 1-GABA-E-3,4-Q is purified on a gradient that begins at 70% water and 30% methanol and reaches 100% after 20 minutes. The HPLC was monitored at two wavelengths 280 nm and 480 nm. Estrogen metabolites absorb well in the 280 nm range and the dark red product absorbs well at both the 280 nm and 480 nm range. The product peak eluted at 12 minutes approximately at 85% methanol 10% water. The product peak was collected for all the injections and the solvents were removed via rotary evaporation to yield the pure product. This can be stored under vacuum or at -78° C.

NMR of 1-GABA-E-3,4-Q

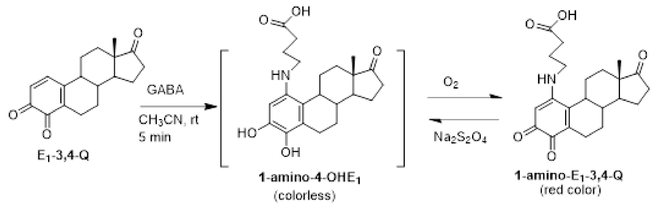
Approximately 8 mg of the 1-GABA-E-3,4-Q was dissolved in 99.9% pure deuterated methanol. A dried glass NMR tube and pipet were used to try to minimize water in the sample. Such a low mass of product was used because of stacking that occurs at higher concentrations. This broadens the peaks of the NMR.

Cyclic Voltammetry of 1-GABA-E-3.4-Q

Cyclic voltammetry was performed in a 125 mL glass electrochemical cell equipped with 5 ports and a stir bar. The cell was equipped with a glassy carbon working electrode, a platinum counter electrode and Ag/AgCl reference electrode (all reported potentials relative to this reference electrode). The electrodes were connected to a Uniscan potentiostat interfaced to a PC using UniChem software. Measurements were made in dry, degassed acetonitrile (50 mL) containing 0.1M n-Bu_4NPF_6 as an electrolyte. Analyte concentrations were typically 2.5 mM.

Abstract

The metabolism of estrogen can lead to the formation of two isomeric *o*-quinones, estrogen-2,3-quinone (E-2,3-Q) and estrogen-3,4-Q (E-3,4-Q). The more reactive E-3,4-Q is genotoxic and can damage DNA by forming apurinic sites; whereas, E-2,3-Q does not form apurinic sites. Estrogen quinones may also be involved in redox cycling to produce reactive oxygen species (ROS), which is another genotoxic pathway. What is not yet clear, is why E-3,4-Q would undergo redox cycling while the non-carcinogenic E-2,3-Q would not. Nitrogen nucleophiles react with the E-3,4-Q at the 1-position. With DNA bases as the nitrogen (sp^2) nucleophile, the adducts formed are catechols. We have investigated the reaction of saturated nitrogen (sp^3) nucleophiles with both E-3,4-Q and E-2,3-Q. E-3,4-Q reacts quickly with 4-aminobutyric acid (GABA) at the 1-position to form a red, *o*-aminoquinone product whereas E-2,3-Q does not react with GABA to form an *o*-aminoquinone. The reaction proceeds through an electron rich catechol intermediate which is oxidized by the original E-3,4-Q to produce equal amounts 1-amino-E-3,4-Q and the catechol of E-3,4-Q, 4-hydroxyestrogen (4-OHE). If the reaction is done in the presence of an oxidant (MnO_2), 4-OHE is not produced, only the 1-amino-E-3,4-Q is observed. The 1-amino-E-3,4-Q can be reduced to a catechol with sodium dithionite to produce the 1-amino-4-OHE catechol. This catechol is reoxidized to the *o*-aminoquinone when exposed to air indicating a possible redox cycling platform. Cyclic voltammogram analysis of an *o*-aminoquinone, versus an Ag/AgCl reference electrode, displays reversible behavior with first and second reduction potentials of -0.965 V and -1.440 V, respectively. These values are close to 0.5 V, lower than E-3,4-Q reduction potentials. Since the oxidation potentials of 2-OHE and 4-OHE are almost identical, perhaps ROS are produced from nitrogen adducts of E-3,4-Q in the form of *o*-aminoquinones.

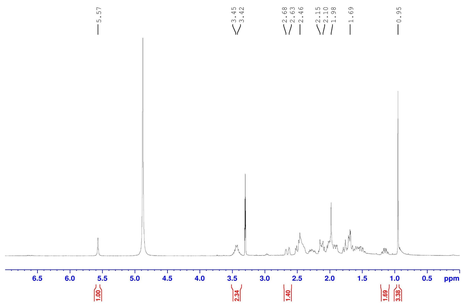


Conclusion

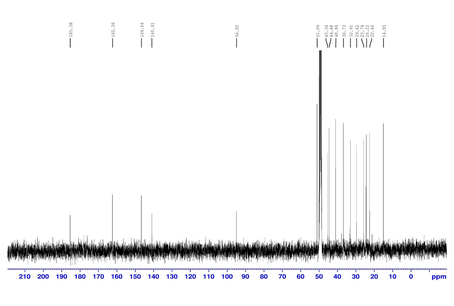
We were successful in synthesizing the 1-GABA-E_1-3,4-Q by reacting estrone-3,4-quinone with γ-aminobutyric acid (GABA) in an acetonitrile/acetate buffer solution. The bright red colored compound was characterized by 1-D and 2-D NMR analysis and high resolution mass spectrometry. We demonstrated that the *o*-aminoquinone compound could reversibly convert between *o*-quinone and catechol by reduction with sodium dithionite in acidic medium followed by air oxidation back to *o*-quinone at higher pH. Visually, this cycling can be observed by the change in color of the compound and was monitored by UV/VIS spectrometry. Cyclic voltammetry displayed a quasi-reversible profile with a large negative first reduction potential. This reduction potential was made more positive in the presence of HCl consistent with the dithionite reduction.

Future work involve reaction of N-acetyllysine and n-butylamine nucelophiles with the estrogen quinone. In addition, the ability of these compounds to produce ROS *in vitro* will be examined to see if *o*-aminoquinone estrogen adducts can serve as platforms for redox cycling.

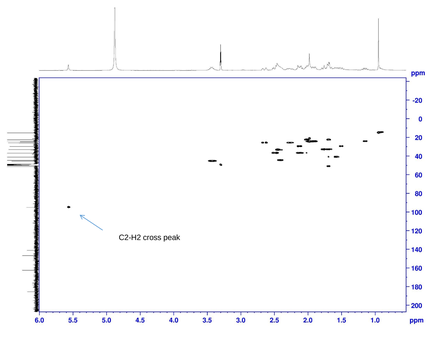
Results



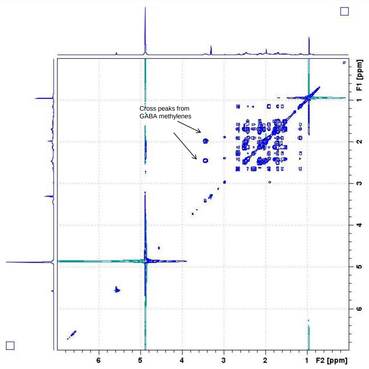
Proton NMR of the 1-GABA-E-3,4-Q



Carbon NMR of the 1-GABA-E-3,4-Q



HSQC of 1-GABA-E-3,4-Q



TOCSY of 1-GABA-E-3,4-Q

Figure 1: NMR of 1-GABA-E-3,4-Q

Above are the NMR form the 1-GABA-E-3,4-Q. The HSQC is a 1-bond spectrum. The carbon spectrum is not yet perfect. The concentration of product is low so the carbon peaks are very small, and some cannot be seen. If the concentration is increased then stacking occurs. We are currently working on obtaining a better carbon spectrum. This will also improve the HSQC. Some important peaks to help prove the structure of the 1-GABA-E-3,4-Q are the methylene next to the nitrogen of GABA and the single hydrogen on the A ring. The methylene next to the nitrogen has a carbon shift of 45.34 and proton shift of 3.45. The single hydrogen on the A ring has a shift of 5.57 and is a singlet .The carbon that the hydrogen is attached to has a shift of 94.93

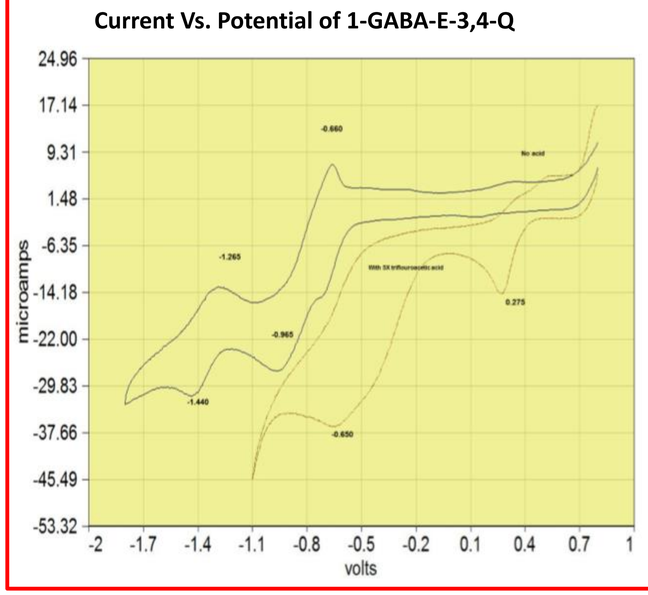


Figure 2: Cyclic Voltammogram of 1-GABA-E-3,4-Q

Without acid, the redox cycle is quasi reversible. The first and second reduction potentials are -0.965 and -1.440, respectively. Once the acid is added, the reduction potential becomes more positive making that quinone easier to reduce. In the presence of 1M HCl, the first and second reduction potentials are 0.275 and -0.650, respectively. This change in reduction potentials supports the need to acidify the quinone in **Figure 3** before reduction with sodium dithionite.

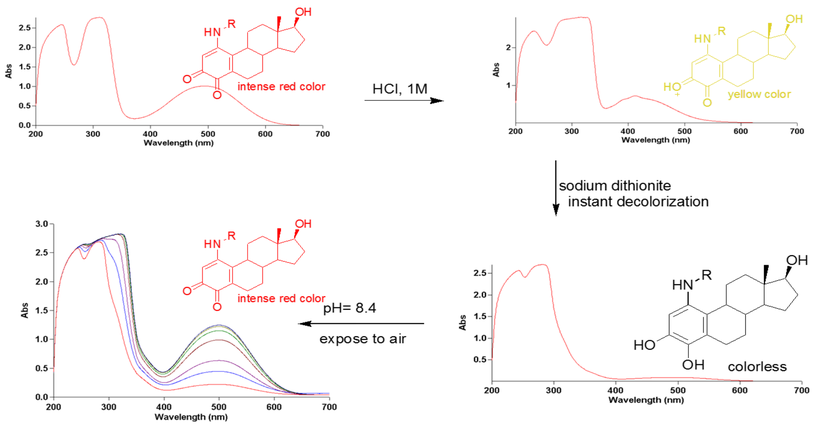


Figure 3: Redox Cycling of 1-GABA-E-3,4-Q

Above are UV Vis spectrums of the compounds. The first spectrum shows the quinone with its intense red color with the absorbance peak at 500 nm. Acid is then added to the quinone to make it easier to reduce. This acidification causes a color change to a yellow solution shifting the absorbance peak to around 410 nm. The amino quinone is then reduced to the amino catechol by sodium dithionate. Once this reduction is complete the solution is completely colorless indicated by the absence of a prominent peak at 500 nm or 410 nm. This indicates that only the amino quinones are highly colored. The fully reduced quinone at pH 8.4 is exposed to the air where is slowly oxidizes back to the quinone and regains its intense red color as evidences by the increase in absorbance at 500 nm as time goes on.

References

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