Inosoxy Catalyzed Aliphatic Nitro-Compound Electroreduction

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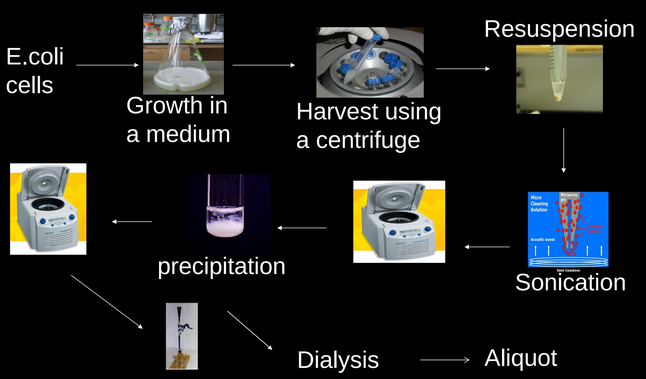
Background:

- Nitroalkanes are widely being used for many purposes like industrial solvents, fuel in racing cars or rockets, chemical intermediates and also in explosives like TNT and Nitroglycerine.
- These compounds when released into the environment and inhaled by human beings cause headaches, nausea, convulsions, neuropathy and can **irreversibly damage the DNA double helix** acting as carcinogens.
- The activation of these xenobiotic nitro compounds by **inosoxy enzyme (a p450 like enzyme)** can be a potent explanation for the nitro compound related health issues.

Purpose:

- The purpose of the study is to show that the oxygenase domain of inducible nitric oxide synthase (iNOSoxy) acts as an efficient electrocatalyst for nitroalkane reductions on the surfactant films of pyrolytic graphite electrodes.
- The electrochemical process used was Cyclic Voltammetry and by immobilizing the enzyme on the electrode using a surfactant, the activation of different nitroalkanes (nitromethane, nitroethane and nitropropane) by the inosoxy enzyme was studied.

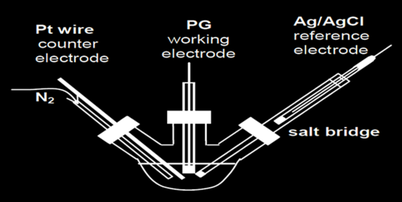
Protein purification:



- Inosoxy protein was purified and the concentration of the enzyme was determined by using p450 assay.
- The specific activity was determined by the Greiss assay.

Electroanalytical method:

- Electrochemical analysis was performed using 3 electrode cyclic voltammetry.
- Enzyme was embedded in cast surfactant thin films on pyrolytic graphite electrode to achieve direct electron transfer.



Electrode Modification Procedure:

- inosoxy is immobilized in DDAB surfactant film on a basal-plane pyrolytic graphite (PG) disc electrode (inosoxy/DDAB/PG).
- Before film casting, the PG electrode is polished using 400-grit sandpaper followed by 1µm, 0.3µm, 0.05µm alumina slurries.
- The polished electrode is then rinsed and sonicated in distilled water. inosoxy/DDAB surfactant film is prepared by casting 10µl of 10mM DDAB in H^2O followed by inosoxy solution.
- The modified PG electrode is then allowed to dry in air for more than 12 hours. DDAB emulsion is prepared by dissolving DDAB powder in deionized water.
- The emulsion is then sonicated for at least 2 hours or until the solution becomes clear.

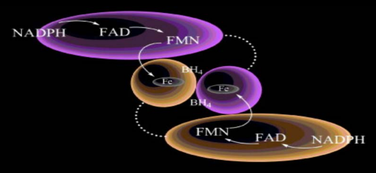
Procedure for the Cyclic Voltammetry Experiment:

- The electrochemical experiments were conducted using a three-electrode system.
- Ag/AgCl (1M KCl) was used as reference electrode and all potentials were reported versus this reference.
- A platinum wire was employed as the auxiliary electrode. Modified PG electrode was used as working electrode.
- The potentiostat was assembled and 15 ml of acetate buffer 0.1M was used as the buffer.
- The Cyclic voltammetry cell was purged with nitrogen gas for 15 to remove oxygen and other impurities.
- Care was taken that the air in the cell stays inert. The voltammogram with inosoxy\DDAB\PG was taken first.
- 0.3 mm nitromethane was added to the 15ml buffer and stirred and purged with nitrogen for 5 minutes and a measurement was taken.
- The same process was repeated for 0.4mm, 0.6,0.8,1.0,1.2,1.4,1.6 mm and 1.8 mm.
- Different scan rates, 0.02, 0.04, 0.08,0.1, 0.12, 0.15, 0.18, 0.2 volts\second were used to study the effects of different scan rates at different concentrations.

Results:

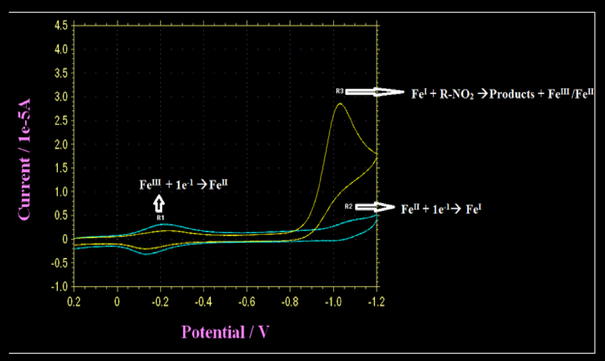
The electrochemical behavior of inosoxy in surfactant film:

- In the presence of Nitroalkanes a **new peak R3**, with significantly higher current appears at potentials relative to the R2(-1.05v)
- This new peak is accompanied by the loss of **reoxidation return of inosoxy**.



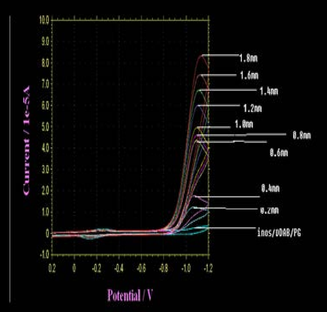
"Crystal structure of inosoxy molecule"

The cyclic voltammogram dramatically changes after introducing nitromethane. There are 2 pairs of redox couples assigned to Fe^{III}/Fe^{II} (formal potential at -0.24V vs. Ag/AgCl) and Fe^{II}/Fe^{I} (formal potential at -1.05V vs. Ag/AgCl), respectively. Upon addition of nitromethane, a new peak, R^c, appears at -1.05V vs. Ag/AgCl in parallel with the disappearance of the Fe^{II}/Fe^{I}. **The new Rc peak appearing at potentials more positive than Fe^{II}/Fe^{I} redox couple and the disappearance of the later are both indicative of an electro catalytic process mediated by inosoxy.**



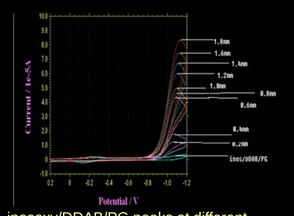
Catalytic Efficiency as a Function of Concentration:

- The catalytic efficiency is expressed in terms of the ratio of the catalytic peak current in presence of the substrate(R3) and the current of hemin in the absence of the substrate.

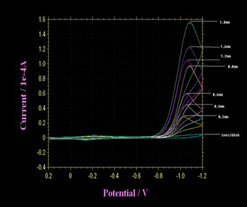


inosoxy/DDAB/PG peaks at different concentrations of nitromethane in 0.1 M acetate buffer at 0.1 V/S scan rate.

The catalytic current increases with increasing concentrations of the Nitroalkanes.



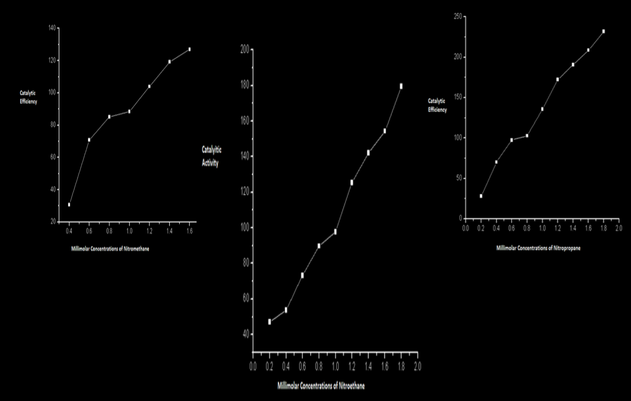
inosoxy/DDAB/PG peaks at different concentrations of **nitroethan**e in 0.1 M acetate buffer at 0.1 V/S scan rate.



inosoxy/DDAB/PG peaks at different concentrations of **nitropropane** in 0.1 M acetate buffer at 0.1 V/S scan rate.

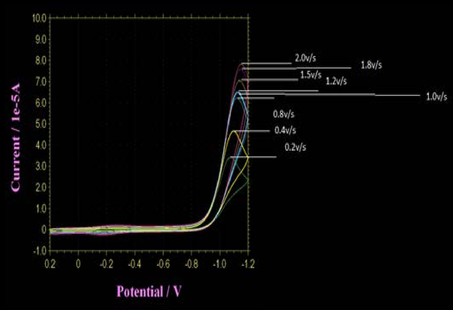
Origin Graphs:

The Catalytic Efficiency of inosoxy increases as the concentration of the nitroalkane increases as per the data analyzed by ORIGIN*

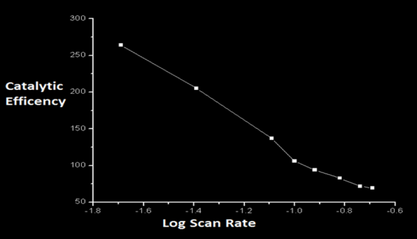


Catalytic Efficiency as a function of scan rate:

- Increasing **scan rate shortens the timescale** within which the catalytic reaction is monitored. As expected the catalytic efficiency, defined by the ratio I^{cat} / I^{0} is larger at small scan rate and gradually drops as the scan rate increase.
- This behavior is typical of electrocatalytic process and can be used to extract kinetic information.



1.3 mm nitromethane in 0.1M acetate buffer at different scan rates.



1.3Millimolar nitromethane at different Scan rates

Conclusions:

- The Concentration of the Nitroalkanes used and the Scan rate effect the Catalytic efficiency of the enzyme inosoxy
- The activation of xenobiotic nitro compounds by inosoxy enzyme (a p450 like enzyme) can be a potent explanation for the nitro compound related health issues.

• Acknowledgements:

We want to thank all the members of **Dr.Bayachou's lab** for the support to accomplish this Study.