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Cyclic Voltammetric Studies of Nitroimidazoles in DMSO in the presence of Cysteine and other Weak Acids. Implications for the Biological Reactivity of Nitroimidazoles

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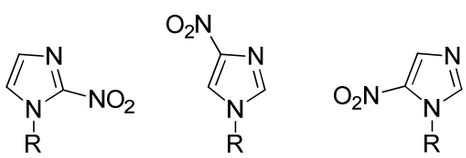
A number of nitroimidazoles (NI's) are used as drugs to treat a variety of infections by anaerobic microorganisms. In all cases the NI's are believed to require in vivo reduction in order to be activated. However, the actual active forms of the drugs as well as the mechanisms of action are not known for certain. One widely accepted theory is that the active form is a 1 e^- reduction product, either the radical anion or the protonated radical, which causes cell death by inflicting extensive damage to the organism's DNA. Other research suggests that it may be the 2 e^- product, the nitroso, that is the active species. Nitrosobenzenes are known to react with thiols like glutathione and cysteine in vitro and in vivo. Thus an alternative hypothesis is that cell death is caused by deactivation of key proteins and/or the disturbance of cellular redox balance due to adduct formation between the NI and cysteine residues.



2-NI 4-NI 5-NI

Nitrosoimidazoles are generally very difficult to isolate due to their high reactivity, making it hard to study their reactions with thiols by conventional physical organic chemistry methods. In principle, voltammetry could provide an alternative means to study these reactions, since it can be used to both generate the nitroso and observe product formation. However, the interpretation of the voltammetry is not straightforward because, although the reduction of the nitro can be made to stop at the radical anion stage, it cannot be stopped at the nitroso stage. Once the radical anion is protonated the reduction continues to the hydroxylamine. Nonetheless, in previous studies using nitrobenzene as a model we found indirect evidence for the reaction between the nitroso intermediate and cysteine (boc- and methyl ester-protected). Most notably, the reduction to the hydroxylamine proceeds much faster in the presence of cysteine than with other acids of similar strength in DMSO. Studies with nitrosobenzene and thiols in aqueous solution show that thiols initially react with the nitroso to form a semi-mercaptal which can then be thiolytically cleaved to give the hydroxylamine among other products, eq 1-2. Voltammetry of mixtures of nitrosobenzene show that this reaction also occurs in DMSO and that the semi-mercaptal intermediate can be oxidatively detected. An oxidation peak at the same potential is produced upon reduction of nitrobenzene in the presence of cysteine, and scan rate studies show that the species responsible for this peak is formed prior to the hydroxylamine, consistent with what would be expected for the semi-mercaptal. Thus a reasonable explanation for the increased rate of reduction of nitrobenzene in the presence of cysteine is rapid removal of the 2 e^- intermediates due to the formation of the semi-mercaptal.

$$ArNO + RSH \rightarrow ArN(OH)(SR) \quad (1)$$
$$ArN(OH)(SR) + RSH \rightarrow ArNH(OH) + RSSR \quad (2)$$

In this paper the results of similar voltammetric studies with simple 2-, 4- and 5-NI's will be described. Initial work shows that the voltammetry of the NI's in DMSO generally parallels that observed with nitrobenzene, although there are some differences between the NI's. In particular, the >1 e^- reduction products of the 5-NI's appear to be less stable than those of the 2 and 4-NI's. In all cases, as with nitrobenzene, the reduction to the hydroxylamine appears to proceed faster with cysteine than other acids of similar strength. However, unlike nitrobenzene, the hydroxylamine products cannot be directly detected in the voltammograms. This is likely because they also react rapidly with cysteine leading to thioether adducts between the imidazoles and cysteine. This is significant because there is mass-spectral evidence that these types of products are formed with cell proteins upon treatment with NI drugs. Altogether, these initial results suggest that cyclic voltammetry in DMSO will be able to provide useful information about the reactivity of reduced NI's with cysteine and other thiols.