CAPSULES notes and applications from BASi

359

September 2000

# Detection of Toxins by Measurement of Enzyme Inhibition

## Purpose

There are a number of toxins (e.g., cyanide and azide) that can act through inhibition of enzyme activity. Therefore, the concentrations of such species can be measured using biosensors based on the activity of the appropriate enzyme.

## Reference

Reagentless Mediated Laccase Electrode for the Detection of Enzyme Modulators, F. Trudeau, F. Daigle, and D. Leech, Anal. Chem. 69 (1997) 882-886.

## Method

The biosensor used in this study was based on the enzyme laccase, which is an oxygenase that uses copper centers to catalyze oxidation reactions coupled to the 4 electron reduction of oxygen to water. Therefore, the substrate for the biosensor is oxygen. After the oxygen reduction, the active site of the enzyme must be regenerated by a 4 electron reduction of the oxidized copper centers. Ideally, this should be achieved by direct electron transfer from the electrode to the active site, but in practice this is not possible, since the active site is buried within the protein. Therefore, electron transfer has to be achieved using mediators, which are small, redox-active molecules that shuttle the electrons from the electrode surface to the active site (***F1***). In this study, two osmium bipyridine complexes were used. In the biosensor, these were covalently bound, together with the enzyme, to a polymer immobilized on the electrode surface. The advantage of such "wired" enzyme biosensors is that the leaching that occurs with mobile mediators cannot occur.

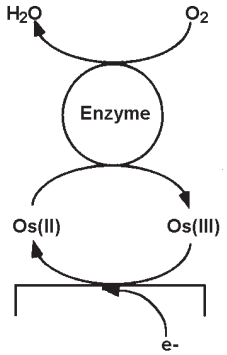


*Figure 1. Scheme for the catalytic reduction of oxygen using laccase.*

The first step in this study was the investigation of the electron transfer reaction in solution between the enzyme and the monomeric osmium mediator complexes ($[Os(bipy)_2Cl_2]^+$ (Osbpy) and $[Os(bipy)_2(MeIm)Cl]^+$ (OsMeIm) where MeIm = methyl imidazole). The behavior of the species immobilized on the surface of a glassy carbon electrode within the redox polymer was then characterized.

## Results

The redox potential of the mediator must be more negative than the redox potential of the laccase enzyme (~+570 mV vs. Ag/AgCl) and more positive of the redox potential of oxygen (~-200 mV). The redox potentials of the monomeric osmium complexes used as the mediator in the solution studies were -20 mV for Osbpy and +130 mV for OsMeIm.

The cyclic voltammograms for Osbpy in the absence and presence of laccase are shown in ***F2a*** and ***F2b***, respectively. The increase in current in the presence of the enzyme is characteristic of a catalytic reaction, as is the change from an asymmetric peak to a sigmoidal response. In this case, the catalytic reaction is the enzymatic reduction of oxygen (the involvement of oxygen is shown by the decrease in the magnitude of the catalytic current when oxygen is removed from the solution). A value of $(2.2 \pm 0.6) \times 10^6\ M^{-1}s^{-1}$ for the rate of the electron transfer reaction between the mediator and the enzyme was calculated from the cyclic voltammograms; that is, the rate of electron transfer is fast.

The cyclic voltammograms of the immobilized redox polymer/enzyme in the absence (**a**) and in the presence of sodium azide (**b-e**) (which acts as an

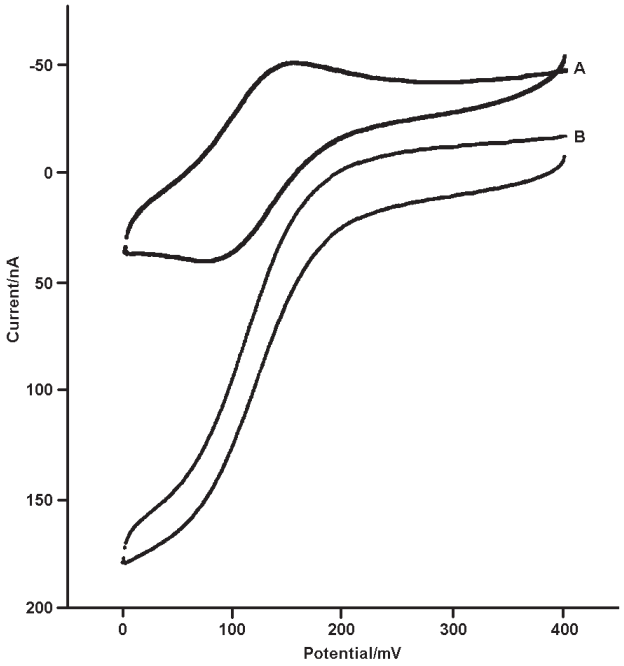


*Figure 2. Cyclic voltammograms of Osbpy (0.1 mM) in the absence (a) and in the presence (b) of laccase (0.12 µM) in 0.05 M acetate buffer (pH 4.7). Scan rate = 2 mV $s^{-1}$, all potentials measured with reference to Ag/AgCl. Adapted with permission from primary reference. Copyright American Society 1997.*

inhibitor) are shown in ***F3***. The sigmoidal shape that is characteristic of a catalytic reaction can be seen in the absence of the inhibitor. However, as the concentration of the inhibitor is increased, the catalytic current decreases, and the shape of the voltammogram tend towards a symmetric peak shape that is characteristic of a species adsorbed on the electrode surface. However, it was found that the reaction with azide was irreversible; that is, the activity of the enzyme could not be restored by rinsing the electrode. Partial activity could be restored when using cyanide as the inhibitor, but this required washing for 1-2 hours. This irreversibility limits the usefulness of this system as an inhibition biosensor.

Biosensors based on inhibition have also been developed based on tyrosinase (for cyanide (1) and organophosphorus pesticides (2)).

## References

*1. M.H. Smit and G.A. Rechnitz, Anal. Chem. 65 (1993) 380-385.*
*2. W.R. Everett and G.A. Rechnitz, Anal. Chem. 70 (1998) 807-810.*

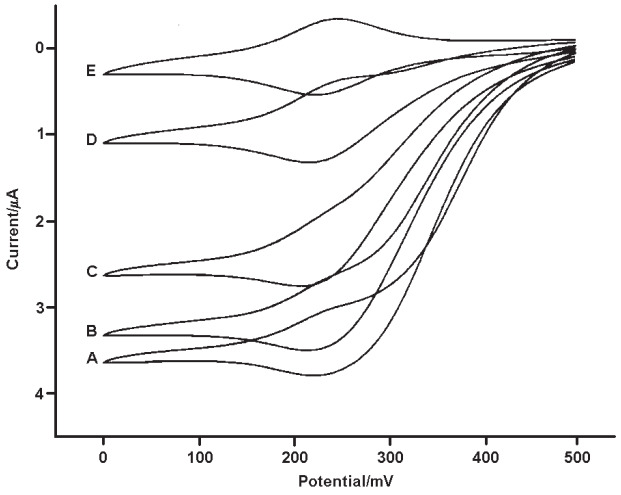


*Figure 3. Cyclic voltammograms of polymer-modified electrode (laccase and osmium complex immobilized in polymer) in absence (a) and in presence of sodium azide (2.5 µM (b), 7.5 µM (c), 32.5 µM (d), and 5 mM (e)) in 0.05 M acetate buffer (pH 4.7). Scan rate = 2 mV $s^{-1}$, all potentials measured with reference to Ag/AgCl. Adapted with permission from primary reference. Copyright American Society 1997.*