A Novel Enzyme Chip Using Localized Electrochemistry

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Background

Electrochemical Enzymatic Redox
- Redox (Reduction/Oxidation) gain or loss of electrons in a chemical reaction
- Oxidation of glucose is catalyzed by glucose oxidase (GOx)
  \[ \beta-D-glucose + O_2 \rightarrow \text{gluconic acid} + H_2O \]
- K4 ions are deposited on or within the PAH layer and optical signal decreases and stabilizes

Current Enzyme Biosensors
- Use electrochemical redox to determine concentration of substrate in solution
- Are limited to detecting a single substrate because of this electrochemical method

Differential Interferometry
- Technique has been used to measure the redox of [Fe(CN)6]3- and [Ru(NH3)6]3+ ions in a localized area of an electrode

Materials & Methods

Enzyme Chip (Figure 1)
- An electrode on a silicon wafer
- Poly(methyl methacrylate) (PMMA) spincoated on half of the chip
- Chip is spincoated with poly(allylamine hydrochloride) (PAH)
- PAH is saturated with Potassium Hexacyanoferrate (K4) ions via electrodeposition
- Device is functionalized with n-succinimidyl iodoacetate (SIA) crosslinker and GOx

Differential Interferometer (Figure 2)
- Measures the difference between two parallel laser beams
- One beam strikes the PMMA reference while the other strikes the functionalized electrode
- Electrochemical cell operated by traditional 3 electrode setup

Solutions in Chamber
- Electrodeposition of K4 done in 50 mM K4 and 25 mM Phospate buffer
- Tests run in a 25 mM Phosphate buffer solution containing 37.5 mM NaCl and glucose concentrations varying from 5 to 100 mM

Results

K4 Saturation
- Positively charged PAH attracts negative K4 ions
- Increased ion concentration causes increased optical signal
- K4 ions are deposited on or within the PAH layer and optical signal decreases and stabilizes

Electrodeposition
- Graphs below show that K4 redox is present even when there is no K4 in solution.
- Redox from deposited K4 is too weak to cause a change in optical signal
- Non-linear current shows that redox is present
- Washing does not remove K4

Electrotransfer
- The red (deposited K4) and blue (H2O2) CV curves show that K4 and H2O2 redox occurs in a very similar voltage range
- K4 can assist in electron transfer due to its proximity to the electrode

Conclusions

Optical Method
- The redox reaction of glucose, catalyzed by glucose oxidase, is detectable by the differential interferometry method
- The optical and electrochemical redox responses correspond with one another

K4 is a Redox Catalyst
- The K4 ions assist in redox of glucose by mediating the transfer of electrons between the electrode and the enzyme

Concentration Dependence
- The optical signal is dependent on the concentration of glucose in solution

Future Work

Other Substrate – Enzyme Systems
- Expand the method to other systems such as lysine-lysine oxidase and lactate-lactate oxidase

Multi-Enzyme Biosensor
- Test for multiple substrates using a single electrode via optical signal

Applications
- Comprehensive monitoring and diagnosis of disease in biofluids (e.g. serum, urine, saliva)

References