Developing an electrochemical method for the measurement of organophosphate hydrolase

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Introduction

Organophosphate (OP) compounds are highly toxic compounds, often found as pesticides and nerve agents, that act as cholinesterase (ChE) inhibitors (Wang & Sun, 2021). OP poisoning occurs when these chemicals bind to acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), causing a build-up of acetylcholine in the blood. This leads to symptoms such as vomiting, miosis, seizures, and eventually death if left untreated.

Organophosphate hydrolase (OPH) is an enzyme that acts as a bioscavenger, catalytically hydrolyzing OP compounds before they irreversibly bind to ChE (Wang & Sun, 2021). Sensors such as the ChemDx quantitatively measure AChE activity, indicating whether there has been exposure to an OP compound through electrochemical activity (Kasten, 2018). The ChemDx provides an electrochemical platform onto which an assay for the therapeutic monitoring of OPH can be built. The purpose of this project was to develop an electrochemical method for the measurement of OPH in relevant biological matrices.

Materials and Methods

Cyclic voltammetry (CV) was performed with three sweeps at scan rate 100 mV/s using the MultiEmStat LR (Graph 1). Test strips were prepared by pipetting 25 µL of paraoxon diluted in phosphate-buffered saline (PBS) and 25 µL of PBS onto a DRP-110 electrode. Paraoxon was selected because its oxidation peak did not overlap with the oxidation peak for its hydrolysis product 4-nitrophenol (4-NP) at a 2 µM concentration. The breakdown of paraoxon is shown in Figure 1.

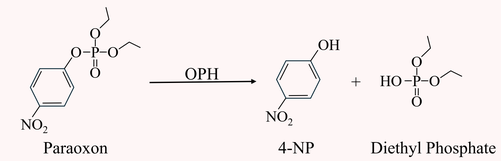
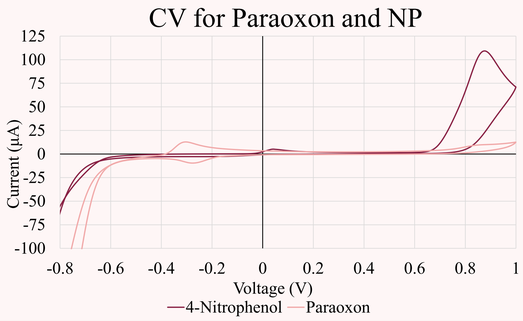


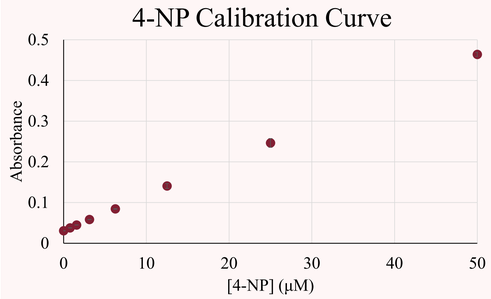
Figure 1 (left): Breakdown mechanism of paraoxon into its metabolites, 4-NP and diethyl phosphate.



Graph 1 (left): CV scans of paraoxon and 4-NP. The oxidation peak for paraoxon occurs at around −0.30 V while the peak for 4-NP occurs at around 0.85 V. Chronoamperometry sweeps were conducted at 0.85 V to scan for 4-NP.

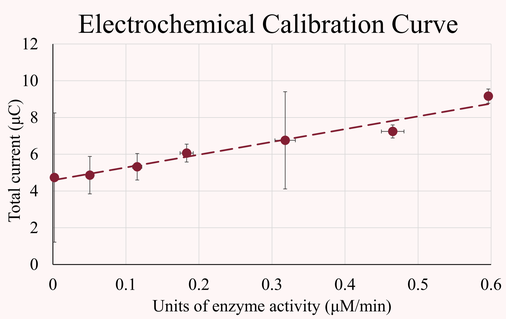
Materials and Methods (continued)

A spectrophotometric assay was conducted on known concentrations of 4-NP to create a calibration curve (Graph 2) as a reference method for the electrochemical sensor. The plate was prepared by pipetting 100 µL of each concentration of 4-NP and 100 µL of PBS in each well. A 70% serial dilution for eight concentrations of OPH was prepared with a starting concentration of 1 µM. Absorbance was measured for each concentration at a wavelength of 405 nm using the SpectraMax 384 Plus. Beer's law was used to calculate the amount of 4-NP produced, which was then used to calculate enzyme activity.



Graph 2 (left): Calibration curve for the spectrophotometric sensor correlating production of 4-NP to absorbance measured. R^2 = .9955. Since the R^2 is greater than .99, the curve is reliable. The equation generated by the line is $y = 0.0087x + 0.0311$. Errors bars represent ±2 standard error.

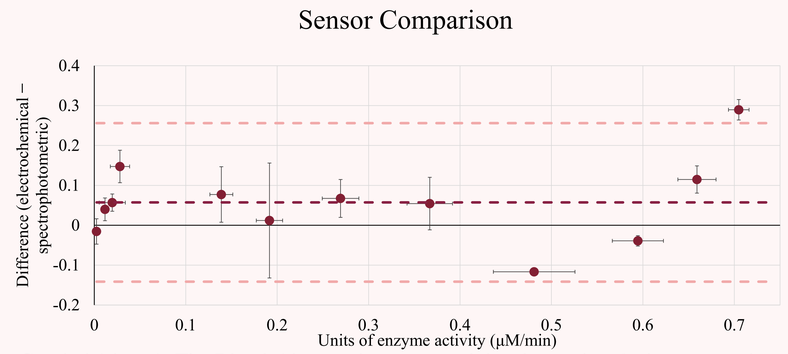
Each concentration was electrochemically evaluated with an applied potential of 0.85 V using chronoamperometry. Total current over 30 seconds was calculated by taking area under the curve. A calibration curve for the electrochemical sensor was created by correlating units of enzyme activity from spectrophotometry and total current over 30 seconds (Graph 3). All measurements were collected in triplicate.



Graph 3 (left): Calibration curve for the electrochemical sensor correlating units of enzyme activity calculated from the assay and total current over 30 seconds. R^2 = .9599. Since the R^2 is greater than .95, the calibration curve is reliable. The equation generated by the line is $y = 6.95x + 4.5868$. Errors bars represent ±2 standard error..

Twelve unknown concentrations of OPH were prepared. Spectrophotometry and chronoamperometry scans were run using the same procedure, and units of enzyme activity were calculated for each method. The difference between the two methods were calculated to determine the accuracy of the comparator (Graph 4).

Results



Graph 4 (above): The Bland-Altman plot compared the electrochemical sensor to the spectrophotometric sensor and showed good agreement between the two sensors. The mean difference (electrochemical − spectrophotometric) was 0.057 µM/min (red line) with upper limit of 0.256 µM/min and lower limit of −0.141 µM/min (pink lines). Since the upper and lower limit range includes 0, the sensor does not consistently overestimate or underestimate OPH activity. Errors bars represent ±2 standard error.

Conclusion

The hydrolysis of paraoxon by OPH can be detected by CA scans. This serves as proof of concept that developing a sensor for the measurement of OPH is a viable route for bioscavenger monitoring. This will allow OPH to be administered as a prophylactic and measured in subjects who could be exposed to possible nerve agents and agricultural workers constantly exposed to OP containing pesticides. Rapid decrease of OPH activity could indicate OP exposure, triggering further confirmatory testing. Overall, the electrochemical sensor had good agreement with the spectrophotometric assay. An OPH concentration lower than 10 nM could not be detected by the sensor. Higher concentrations also showed greater disagreement. In future studies, a pesticide that is stable long-term is necessary to ensure stability while maintaining accuracy.

References

Kasten, S. A. (2018). *Point-of-care in-vitro diagnostic device for the amperometric detection of cholinesterase activity in whole blood for indication of exposure to cholinesterase inhibiting substances* (US. Patent No. 10913967). U.S Patent and Trademark Office. https://patents.google.com/patent/US10913967B2/en

Wang, L., & Sun, Y. (2021). Engineering organophosphate hydrolase for enhanced biocatalytic performance: A review. *Biochemical Engineering Journal*, *168*. https://doi.org/10.1016/j.bej.2021.107945