A Step Towards a Discriminative Neurotoxin Biosensor: Guarding of Acetylcholinesterase from Organophosphate Compounds

Yuanyuan Zhang, Jeffrey Kirsch, Mary Arugula, and Aleksandr Simonian
Auburn University Materials Research and Education Center
Department of Materials Engineering, Auburn University, AL 36849

Previously, we reported the possibility for protection of acetylcholinesterase from organophosphate neurotoxins by guarding the enzyme with organophosphate hydrolase. This was achieved through layer-by-layer assembly of enzyme armored carbon nanotubes and placing the OPH layer directly over the AChE layer. However, this approach did not allow for the sensitivity of AChE to be preserved. Here, the sensors were constructed with a "protection pad", such that an OPH terminal layer-by-layer assembly was placed underneath the inlet of the flow cell, and an AChE terminal "detection pad" was placed directly over the electrode. To detect each concentration, the flow rate was reduced to require the 50 μL "bullet" of analyte to pass over each pad for a total of 15 minutes. Acetylthiocholine was used to test the activity of AChE before and after exposure to each concentration of neurotoxin. The result shows that OPH can destroy paraoxon (PX), the model organophosphate, before it can irreversibly inhibit AChE, while methyl carbamate, a non-OP, is unaffected by the OPH and inhibits the AChE. A control sensor was built without any OPH to show the nominal inhibition of AChE from paraoxon and methyl carbamate. The real limit of detection of the neurotoxin sensor is shown to be in the 10-100 pM range, and protection from OP neurotoxins can be achieved up to 100 μM. Other enzymes could be employed in a neurotoxin sensor to enhance the selectivity of the sensor and provide discriminate detection among OP neurotoxins and from other cholinesterase inhibitors.