Exploring Galactose Oxidase as an Electrochemical Probe for Complex Sugars

Galactose Oxidase (GalOx) is a single polypeptide, Type 2 mononuclear copper oxidase, with a molecular weight of 68.5KDa. The protein active site consists of a Cu(II)center and a tyrosyl radical. The Cu(II) is bound by two histidine (His 581, His 496) and two tyrosine (Tyr 272 and Tyr 495). The geometry of the catalytic active center is a distorted square pyramidal. The fifth ligand is occupied by H_2O or acetate, both monodentate ligands that are easily substituted. GalOx primary function is the oxidation of alcohols to aldehydes. Its preferred substrate is D-(+)-galactose (Gal), but it also oxidizes complex sugars where Gal is present and organic alcohols. Several electrochemical studies have been published looking at the direct or mediated electron transfer between the enzyme and the electrode surface. However, a full characterization of the redox process at the electrode surface has not been done, neither the full potential of this enzyme for the development of biosensors, or as a redox catalyst has been exploited. The goal of this project is to assess whether GalOx could serve as an electrochemical probe for the detection of complex sugars. We proposed to study the electrochemical oxidation of galactose, lactose, galactosamine, raffinose and stachyose by GalOx. Because these sugars contain Gal, the hypothesis is that an electrochemical biosensor for Gal could be used to quantify them based on the relative content of other sugars to Gal. An electrochemical biosensors array would be necessary to identify a given sugar and its composition. Our long-term goal is to develop an electrochemical biosensors array for the detection of multiple sugars and explore its application for the detection of endotoxins in gram-negative bacterialike *Salmonella enterica*, *Escherichia coli*, and *Pseudomonas aeruginosa*.