Reversible voltammetry of microsomes genetically-enriched with human cyt P450s in films of polyions - J. Rusling, N. Sultana (University of Connecticut), and J. Schenkman (University of Connecticut Health Center)

Cytochrome P450 enzymes in the human liver metabolize lipophilic pollutants and drugs to products that may be react with DNA in a major genotoxicity pathway. Different human cyt P450 enzymes may act preferentially on certain molecules, or produce different metabolite distributions from the same molecule. Thus, it is important to screen the toxicity of metabolites that are formed by the different isoforms of cyt P450s. We have been developing toxicity screening sensors containing enzymes and DNA that product the metabolites and measure their relative reactivity with DNA by detecting DNA damage. The use of different cyt P450 isoforms in these sensors is limited by labor intensive isolation and purification of the enzymes from genetically altered bacteria. In this paper we explore the possibility of using commercially-available microsomes that have been genetically engineered to containing significant amounts of a single human cyt P450. We have prepared films of these microsomes layered with polycations on rough pyrolytic graphite electrodes, and measured direct the voltammetry of human cyt P450 1A2 and 3A4 in these films. The Fe^{III}/Fe^{II} redox couple of the iron heme enzymes in the microsomes gave chemically reversible voltammetry at potentials similar to those of the pure cyt P450 enzymes. Cyclic and square wave voltammograms of these thin microsome-polyion films had the characteristics of non-ideal quasireversible thin-film voltammetry. The microsome-polycation films showed peroxidase activity as measured electrochemically after addition of 2 μM hydrogen peroxide. The use of cyt P450-enriched microsomes in genotoxicity screening sensors will be discussed.