Evaluation of Liver Microsomes for Genotoxicity Sensors—Voltammetry of the Microsomes

by Sadagopan Krishnan and James F. Rusling

Toxicity of chemicals used by humans is a major concern, and is responsible for ~30% of drug development failures.[1] Identifying toxicity at very early stages of development promises to lower discovery and development costs, which currently run to $1 billion per drug. Toxicity often involves metabolism. Liver microsomes are used extensively by the pharmaceutical industry as a convenient source of metabolic enzymes in assessing metabolism and inhibition. In particular, the cytochrome P450s (cyt P450) are the major "Phase I" enzymes in liver microsomes that metabolize lipophilic drugs and pollutant molecules.[2-4]

Genotoxicity occurs when a chemical or its metabolite damages DNA in a living organism. We are developing electrochemiluminescence-based genotoxicity screening arrays for drugs and other chemicals to apply at very early stages of development as a toxicity screening method. As test compounds, we are using known genotoxic agents like nitrosamines, benzo[a]pyrene, and styrene. We recently reported several studies using pure cyt P450 enzymes in genotoxicity screening sensors and arrays in which DNA damage for enzyme-generated metabolites is monitored.[5-9] However, pure human cyt P450s are not commercially available, and their isolation and purification are labor intensive. This creates a serious bottleneck in the development of arrays featuring a collection of metabolic enzymes.

In the current study, we are evaluating rat liver microsomes as bioactivation components in the genotoxicity sensors. For this purpose, we plan to combine the microsomes with DNA and suitable polyions in thin films that can metabolize substrates and facilitate resulting detection of DNA damage as the genotoxicity endpoint. Rat liver microsomes are cheap, commercially available sources of cyt P450s and other metabolic enzymes. With this perspective, we first investigated the voltammetry of prototype thin films from rat liver microsomes and polyions of opposite charge on pyrolytic graphite electrodes. We found that the voltammetric properties were mainly associated with oxidoreductases in these microsomal films,[10] which presumably transfer electrons to cyt P450s in metabolic reactions. Figure 1 shows cyclic voltammograms (CV) of (PDDA/microsomes)$_3$ films on rough pyrolytic graphite (PG) electrodes with increasing scan rates (where PDDA = poly (diallyldimethylammonium chloride), a polycation). Non-ideal quasi-reversible thin film voltammetry was observed for these films and we used CV to measure an apparent surface electron transfer rate constant of 30 s-1.

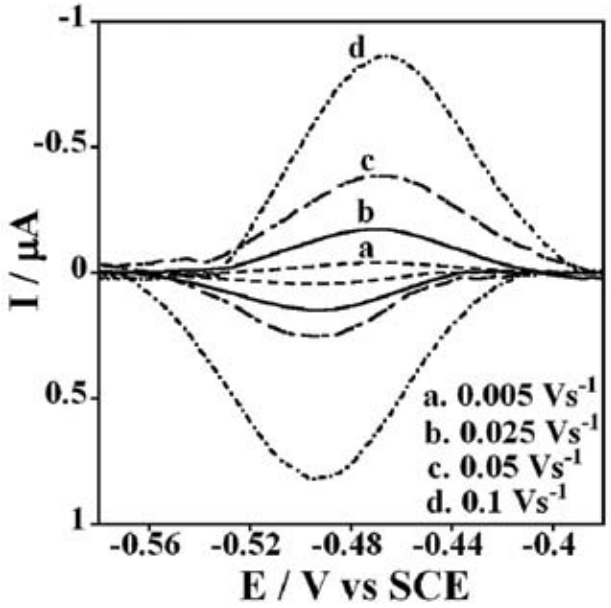

Fig. 1. *Background subtracted cyclic voltammograms of (PDDA/microsomes)^3 films on rough PG electrodes in phosphate buffer (50 mM, pH 7.0) + 0.1 M KCl purged with nitrogen with increasing scan rates. (Adapted with permission from Ref. 10. Copyright 2007, Elsevier).*

Figures 2a and 2b show CVs of PDDA/microsomes films in oxygen and with increasing concentrations of H_2O_2 respectively. Only small increases in catalytic reduction current were observed under these conditions. All the observed

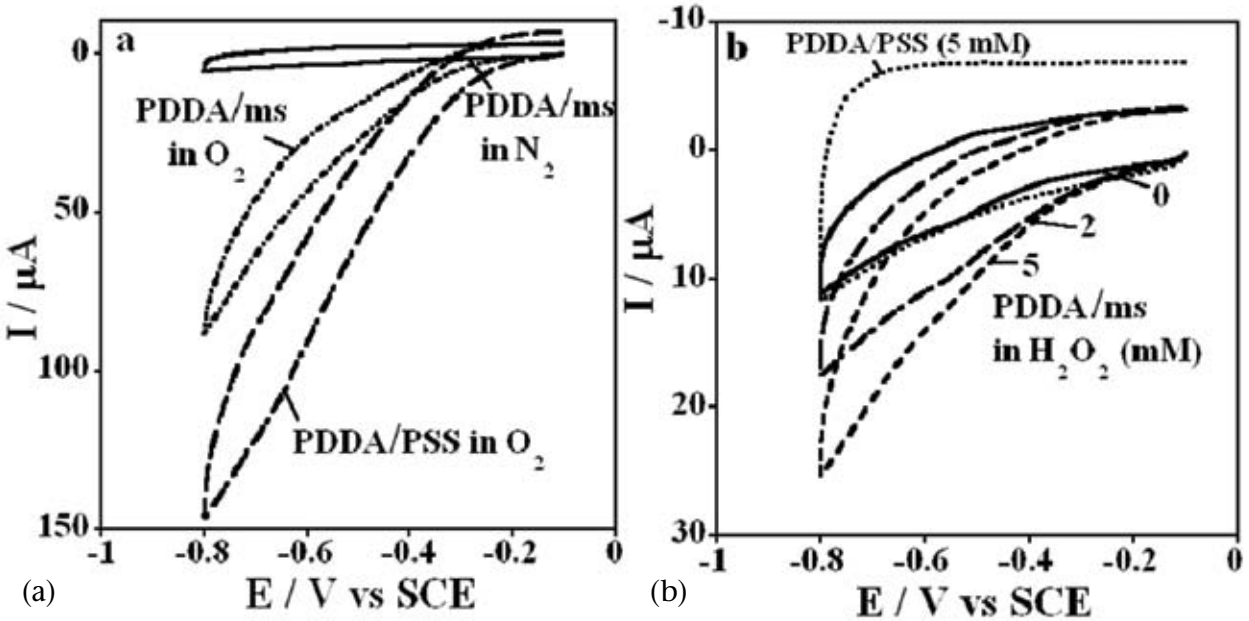

Fig. 2. *Cyclic voltammograms at 0.1 V s-1 for films on rough PG electrodes in phosphate buffer (50 mM, pH 7.4) + 0.1 M KCl showing the influence of (a) purging with oxygen; (b) H2O2 on the CVs of PDDA/microsomes films. Where PSS = polystyrene sulfonate, a polyanion used in this study. (Adapted with permission from Ref.10. Copyright 2007, Elsevier).*

results suggested that the CVs can be attributed mainly to oxidoreductases in the microsomes rather than cyt P450s. It is possible that the peaks are due to cyt P450 reductases.Error! Bookmark not defined.

The fact that oxidoreductases in these films readily accept electrons gave us the confidence that microsomes can be used to replace pure enzymes for bioactivation of organic compounds and drugs in genotoxicity sensors and arrays. Testing of this hypothesis is currently underway. Prototype genotoxicity sensor arrays made using layer-by-layer assembly of DNA, liver microsomes, and electrochemiluminescent (ECL) metallopolymer have shown promising preliminary results.

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About the Authors

Sadagopan Krishnan is a graduate student in the Department of Chemistry at the University of Connecticut, Storrs, U.S. He is pursuing his PhD under the supervision of Prof. James Rusling. He may be reached at sadagopan.krishnan@uconn.edu.

James F. Rusling is a professor of chemistry and pharmacology at the University of Connecticut, Storrs, U.S. He received his BS in chemistry from Drexel University in 1969 and PhD in chemistry from Clarkson College of Technology in 1979. He is a Fellow of The Electrochemical Society and past chair of the ECS Organic and Biological Electrochemistry Division. He may be reached at james.rusling@uconn.edu.