Toxicity Screening Biosensors using In-situ Metabolite Generation in Ultrathin DNA-Enzyme Films

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Rapid detection of DNA damage could serve as a basis for in-vitro genotoxicity screening for new organic compounds, millions of which are made each year, at an early stage in their commercial development. Ultrathin films 20-40 nm thick containing myoglobin or cytochrome P450cam and DNA grown layer-by-layer on electrodes were activated by hydrogen peroxide, and the enzyme in the film generated the metabolite styrene oxide from styrene. This styrene oxide reacted with double stranded (ds)-DNA in the same film, mimicking metabolism and DNA damage in the human liver. DNA damage in the film was detected by square wave voltammetry (SWV) by using catalytic oxidation with tris-(2,2'-bipyridine)Ru(II) and by monitoring the binding of tris-(2,2'-bipyridine)Co(III). Damaged DNA reacts more rapidly than intact ds-DNA with electrochemically generated tris-(2,2'-bipyridine)Ru(III), giving SWV peaks at $\sim$1 V vs. SCE that grow larger with reaction time. tris-(2,2'-bipyridine)Co(III) binds more strongly to intact ds-DNA, and its SWV peaks at 0.04 V decreased as DNA was damaged. Little change in SWV signals was found for incubations of DNA/enzyme films with unreactive organic controls or hydrogen peroxide. Capillary electrophoresis and HPLC-MS/MS suggested that styrene oxide adducts of DNA bases formed under similar reaction conditions in solution as those used for the DNA-enzyme electrodes. The catalytic SWV method was more sensitive, providing multiple measurements over a 5 min. metabolite generation time.