Bioelectrochemistry of Drug Metabolising Human Flavin-Containing Monooxygenase

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The mammalian flavin-containing monooxygenase (FMO) is a widely distributed enzyme that catalyses the NADPH-dependent oxygenation of a wide variety of drugs and xenobiotics to polar metabolites that are readily excreted [1-2]. They constitute the second most important human monooxygenase system after cytochromes P450. To date, five different genes encoding for functional forms of human FMO proteins have been identified. One of these expressed genes, FMO3, is the predominant FMO present in adult human liver and exhibits genetic polymorphisms. Mutant alleles in this enzyme contribute to the disease known as trimethylaminuria where the patients have reduced capacity for *N*-oxidation of the dietary-derived aliphatic amine trimethylamine [3].

The gene encoding the human FMO3 [4] has been cloned in an expression vector in our laboratory and the resulting soluble recombinant protein expressed in *E.coli* and purified. The presence of a tightly bound FAD cofactor makes these proteins amenable to electrochemical measurements.

Here we report the first direct electrochemical characterisation of human FMO3 using both non-oriented entrapped protein on glassy carbon electrode (GC) and covalently linked protein on gold electrode. The recombinant protein was initially immobilised on GC electrode using the cationic surfactant DDAB (didodecyldimethylammonium bromide) to help the interaction with electrode. Direct electrochemistry was carried out under anaerobic conditions at room temperature, using cyclic voltammetry. The voltammograms showed a single redox couple with a midpoint potential of – 0.42 V (vs. Ag/AgCl). The peak current was linear with scan rate up to 150 mV/s^{-1}, indicating that the protein was adsorbed on the electrode surface.

Cyclic voltammetry was also carried out under anaerobic conditions with protein immobilised on gold electrode after functionalisation with sulfhydryl-reactive cross-linkers. Finally, the ability of the immobilised enzyme to catalyse the turn over of specific substrates was followed electrochemically. The findings constitute the first step towards the creation of electrochemical sensors for HTS of drug metabolism [5].

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