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Surface-modified electrodes in the mimicry of oxidative drug metabolism

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ABSTRACT

This review discusses different approaches that have been taken to mimic oxidative drug metabolism as executed by members of the Cytochrome P450 (CYP450) family of enzymes in humans. Non-modified electrodes can be used to produce some of the oxidative drug metabolites observed *in vivo* but their scope is rather limited. Modifying electrodes with simple cofactors in analogy to those observed in CYP450 but without the protein scaffold extends these possibilities and notably allows driving reactions following a CYP450-like mechanism. The review ends with approaches to immobilize CYP450s or analogs thereof on electrodes to mimic the *in vivo* drug metabolism fully. We discuss future perspectives with respect to the advantages and the disadvantages of each level of complexity and possible ways forward.

Contents

1. Introduction

It is a long journey before a new chemical entity enters the market as a pharmaceutical product. An important part of preclinical and clinical studies relates to the study of drug metabolism. A new drug may be converted into pharmacologically active, inactive or even toxic metabolites *in vivo*. The regulatory authorities therefore require studies summarized under the term ADME (absorption, distribution, metabolism and excretion) to assure the safety and the efficacy of newly developed pharmaceuticals [1,2]. Preclinical ADME studies are performed in experimental animals ranging from mice to monkeys prior to use in humans, due to safety considerations. In order to investigate drug metabolism at an early stage of new drug development, it is thus important to predict potential metabolites, to characterize them analytically and to synthesize them to evaluate their toxicity.

In vitro methods for drug metabolism research usually use extracts of rat or human liver microsomes that contain enzymes of the CYP450 family. The discovery of CYP450 enzymes traces back to the early 1960s when a pigmented protein that binds carbon monoxide and exhibits an absorption maximum at 450 nm was discovered in liver microsomes of pigs and rats [3,4]. It was subsequently shown that CYP450s contain an iron protoporphyrin IX (heme) as cofactor. This heme-containing protein was later identified as a *b*-type cytochrome and therefore called Cytochrome P450 [5].

The CYP450 family comprises many isoforms, such as CYP1A2, 2A6, 2B6, 2D6 and 2E1, with different substrate specificities [6], despite the fact that sequence similarity is as high as 80% [7]. CYP450 catalysis requires a constant supply of NADPH as the electron source

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Table 1
Overview of different modified electrodes

Species	Electrodes	Immobilization strategy	Techniques	Substrate catalysis	Ref.
PMMT	Platinum/carbon	Adsorption	CV	Cyclo-octene and stilbene	[40]
Co(*p*-OH)TPP	Vitreous carbon	Electropolymerize	CV	2-mercaptoethanol	[41]
cobalt corrin-polyion	Carbon	Electropolymerize	CV	No	[42]
$FePcCl_{16}$	Graphite	Adsorption	CV, CA	2-mercaptoethanol	[43]
CYP2C9	Gold	Thiophenol SAM	CV, HPLC	Tolbutamide	[49]
CYP2C9	Gold	Organic acid SAM	CV, HPLC	Warfarin	[50]
CYP2E1	Gold	Nanoparticles	EIS, TEM, CV	Rifampicin	[51]
CYP3A4	Gold	Organic acid SAM	QCM, LC-MS	Verapamil, quinidine	[52]
CYP2E1	Gold	Cysteamine SAM	CV	*P*-nitrophenol	[53]
CYP3A4	Gold	Thiolate SAM	CV, HPLC	Testosterone	[54]
CYP2C9/CYP2D6	Gold	SAM	CV, HPLC	Bufuralol and warfarin	[56]
CYP3A4	Glassy carbon	Nanoparticles	CV, HPLC	Nifedipine	[57]
CYP2B4	Screen-printed carbon	Amine SAM	CV, CA	Cocaine	[59]
Fungal monooxygenase	Glassy carbon	Mwcnt-nf-pei	AFM, CV	*N*-hexadecane	[60]
CYP6A1	EPG	Ddab film	CV, MS	Aldrin or heptachlor	[61]
CYP1A2	Carbon cloth	Sulfonate film	CV, GC	Styrene	[62]
CYP3A4	ITO	Electrostatic	CV	Testosterone	[63]
CYP2B4	Glassy carbon	adsorption	CV	Aminopyrine, benzphetamine	[65]

and CYP450 reductase to deliver electrons to the active site, both of which are costly and require continual addition upon extended incubations [8]. Additionally, it can be very difficult to detect drug metabolites or to isolate and to study reactive intermediates from incubations with microsomes, due to the complexity of the biological sample. Reactive intermediates may also react further, for example, with proteins, and escape detection. Thus, new approaches capable of generating oxidative drug metabolites and allowing their analysis are needed to gain better understanding of oxidative drug metabolism (Phase-I metabolism) and of synthesizing drug metabolites at a scale that allows toxicological studies in experimental animals (see review on "Electrosynthesis methods and approaches for the preparative production of metabolites from parent drugs" in this Special Issue).

Electrochemistry has been used as a versatile technique for the mimicry of oxidative drug metabolism [9–24], since it allows generating drug metabolites and reactive intermediates under controlled conditions, avoiding the complexity of the *in vitro* or *in vivo* systems [25]. Direct electrochemistry on metal or carbon electrodes proved to be a powerful tool for the synthesis of a wide range of metabolites due to N-dealkylation, N-oxidation, S-oxidation or P-oxidation reactions [26]. However, a number of reactions that are catalyzed by CYP450s could not be reproduced on non-modified electrodes, including aliphatic hydroxylation. Moreover, electrochemistry on metal-based or carbon-based electrodes produces these metabolites through mechanisms that do not resemble CYP450-mediated reaction pathways, making them unsuitable to mimic and to study such reactions in greater mechanistic detail.

In order to mimic the natural enzyme system more closely, approaches to immobilize CYP450s on different electrodes have been promoted [27–29], including different bare electrodes, clay-modified electrodes, phospholipid-modified electrodes and electrodes modified with multilayer films, in which the electrode serves as the supplier of electrons to drive the CYP450 catalytic cycle and thus to expand the range of reactions leading to oxidative drug metabolites. Immobilizing redox-active metalloporphyrins or CYP450 enzymes on electrodes has been shown to facilitate their use as catalysts in electrochemical cells, due to a fast, direct, reversible electron transfer between the redox center and the electrode surface [30]. Different strategies to immobilize CYP450 were reported [31], for example, adsorption to bare electrodes or thin films, layer-by-layer adsorption, encapsulation in polymers of gels, and covalent attachment to a self-assembled monolayer (SAM). However, the active redox site in CYP450s is deeply embedded in the interior of the enzyme, which insulates it and impedes electron transfer from the electrode, resulting in a diminished biocatalytic activity of directly immobilized enzymes on bare electrodes [32]. Regioselective immobilization of CYP450s on SAM-modified electrodes was shown to be beneficial in this respect (Table 1).

In this review, we summarize the applications of metalloporphyrin and CYP450 modified electrodes in the field of drug metabolism research, and introduce the reader to different strategies for the preparation of such modified electrodes.

2. Metalloporphyrin-modified electrodes

Due to the importance and the versatility of the porphyrin macrocycle and its metalated complexes in nature, considerable efforts have been devoted to understanding, mimicking and expanding the role of metalloporphyrins [33]. The past two decades witnessed considerable progress in biomimetic applications of synthetic metalloporphyrins in, among others, olefin epoxidation [34,35], alkane hydroxylation [36] and the electrocatalytic reduction of O_2 [37,38]. As iron is located in the active center of CYP450s, iron complexes are the most widely studied metalloporphyrins, although other metal ions, such as manganese, ruthenium and cobalt, have also been investigated. One of the shortcomings of immobilized metalloporphyrins in view of mimicking drug metabolism is their poor regioselectivity, for example, with respect to aromatic hydroxylation reactions.

Mimicking the catalytic reaction mechanism of CYP450s with immobilized metalloporphyrins is a challenge. Reduction of the central metal ion in the presence of O_2 to produce an oxo-iron intermediate or H_2O_2 has been reported [39]. For example, a manganese porphyrin (Fig. 1a) polymer film deposited on a platinum or carbon electrode surface by electropolymerization of a pyrrole-monosubstituted manganese tetraphenylporphyrin (PMMT) was designed [40], where the pyrrole was covalently linked to the phenyl groups. However, steric hindrance limited the efficiency of the electrochemical polymerization process of the metalloporphyrin monomer. Cauquis et al. reported that connecting the polymerizable group to the macrocycle through a flexible chain improved the electropolymerization efficiency. Platinum or carbon electrodes coated with electropolymerized pyrrole-substituted manganese tetraphenylporphyrin films were used for the catalytic epoxidation of cyclo-octene and stilbene with molecular oxygen.

An electropolymerized cobalt porphyrin-modified vitreous carbon electrode was reported by Bedioui et al. to catalyze the oxidation of 2-mercaptoethanol [41]. Cobalt (*p*-tetrakishydroxyphenyl) porphyrin (Co(*p*-OH)TPP, Fig. 1b) was immobilized by repeated potential scans between –1.4 and 1.4 V [*versus* saturated calomel electrode