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Electrochemically Driven Drug Metabolism by Membranes Containing Human Cytochrome P450

Yasuhiro Mie,* Masaaki Suzuki, and Yasuo Komatsu

Research Institute of Genome-based Biofactory, National Institute of Advanced Industrial Science and Technology (AIST), 2-17-2-1 Tsukisamu-higashi, Sapporo 062-8517, Japan

Received December 1, 2008; E-mail: yasuhiro.mie@aist.go.jp.

Human cytochrome P450s (CYPs) play the most crucial role in drug metabolism.[1] Single nucleotide polymorphisms of CYPs are partially responsible for differences in drug effects among individuals.[2] Also, drug metabolism via the CYPs can cause drug−drug/drug−food interactions that result in toxicities and so on.[2,3] Drug metabolism reaction measurements using CYPs are very important to identify the aforementioned issues and especially in drug development.

The active center of CYP consists of an iron protoheme that is activated via electron transfer reactions. NADPH and NADPH regenerating enzyme systems are generally used to supply electrons to CYP, and the activity of CYP is determined in the presence of these components via HPLC or photometric analyses.[4] However, assay components are expensive and analyses are time-consuming. Replacing the conventional electron supply system with an electrode is a promising approach to develop inexpensive and rapid assays.[5] Some reports have dealt with direct electron transfer reactions from an electrode to human isolated CYP samples.[5]

On the other hand, membranes (microsomes) containing human CYP and CYP-reductase (CPR) are frequently used in conventional assays for drug research[6] due to their easy preparation and low cost. Fairly recently, Sultana et al. observed direct electron transfer reactions to CPR in microsomes containing both CYP and CPR. They immobilized microsomes on a polycation-coated electrode and observed the electrocatalytic oxidation of styrene by CYP through electron transfers from the electrode.[8] However, catalytic reactions were slow and not detected by voltammetry.

Since CYP, CPR, and microsome components (lipid) have hydrophobic regions, we expected that a hydrophobic electrode surface might facilitate the immobilization of microsomes containing CYP and CPR (CYP/CPR-microsomes) and drive efficient drug metabolism reactions. Herein, we demonstrate electrons supplied from an electrode to CYP/CPR-microsomes on an electrode coated with hydrophobic thin films and observe electrochemically driven drug metabolisms by voltammetry.

We adopted benzenethiolate (BT), ethanethiolate (ET), naphthalenethiolate (NT), and 1-dodecanethiolate (DT) as hydrophobic coating materials. Aminoethanethiolate (AET) was used as a hydrophilic cation coating material for comparisons. Immobilizations of microsomes containing human CYP3A4 and CPR from *E. coli* on monolayer films of thiolates were examined.

Contact angle experiments revealed that the surface hydrophobicity increased in the order AET/Au < ET/Au, BT/Au, NT/Au < DT/Au (Table 1). For all surfaces, the immobilization of microsomes was confirmed by QCM and IR-RAS (Figure S1) measurements. Amounts of microsomes immobilized on ET, BT, NT, and DT surfaces were similar to each other and less than half of those on AET (Table 1). Based on these results, microsomes might adsorb with lipid bilayer forms on cationic AET surfaces[8] and with monolayer forms on others; however, further work is needed for definite confirmation.

Table 1. Thiolate Coating Effects on Immobilization and Electrochemical Responses of Microsomes Containing Human CYP and Reductase

coated thiolates	contact angle[a] (deg)	microsomes[b] (ng cm^{-2})	voltammetric peak[c]
aminoethanethiolate	42	1056	not observed
ethanethiolate	76	451	poor
benzenethiolate	77	465	well-defined
naphthalenethiolate	81	526	well-defined
dodecanethiolate	108	458	not observed

[a] Measured before microsome modification. [b] Amount of microsome immobilized on the surface as measured by QCM. [c] Voltammetric response of CYP obtained at conditions outlined in Figure 1.

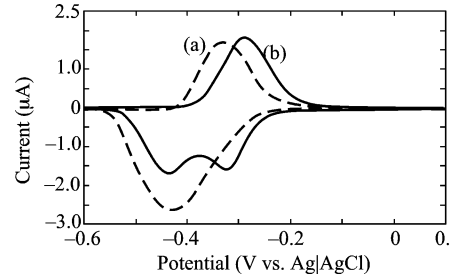

Figure 1. Background subtracted cyclic voltammograms of microsomes containing CYP3A4 and CPR on gold electrodes modified with naphthalenethiolate monolayers at 20 V s^{-1} in 0.1 M phosphate buffer (pH 7.4) at 25 °C in the absence (a: dashed line) and presence (b: solid line) of CO.

Microsomes on BT and NT surfaces showed well-defined peak currents in voltammograms (Figure 1a). On the other hand, microsomes on AET and DT surfaces showed no voltammetric peaks at the present conditions, and those on the ET surface showed subtle peak currents (less than 10% of that on NT). ET did not work as an efficient promoter for direct electrochemistry in spite of similar surface hydrophobicity to BT and NT surfaces, indicating that hydrophobic layers of aromatic compounds on an electrode surface are effective for direct electron transfers. This could be reasonable considering that monolayer films of unsaturated oligophenylene thiolates show better electronic conductance than those of saturated alkanethiolate.[9]

Figure 1a shows the cyclic voltammogram of CYP/CPR-microsomes on an NT-coated electrode. Clear reduction and oxidation peaks around −0.40 V under an Ar atmosphere were observed. This potential is in good agreement with the reported value (−0.41 V) of the formal potential (midpoint potential) determined by a titration method for CYP3A4 bound to lipids.[10] In the presence of small amounts of oxygen, the oxidation current disappeared at a scan rate lower than 0.2 V s^{-1} while a catalytic reduction current was observed (like Figure S2). This behavior is

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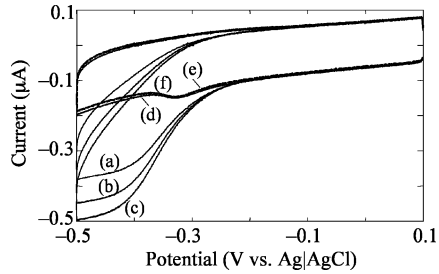

Figure 2. Effect of substrate and inhibitor on cyclic voltammograms of microsomes containing CYP3A4 and CYP-reductase on gold electrodes coated with naphthalenethiolate monolayers at 0.1 V s^{-1} in 0.1 M phosphate buffer (pH 7.4) at 25 °C. (a) Without substrate and inhibitor; (b) 30 μM testosterone; (c) 130 μM testosterone; (d) 500 μM ketoconazol; (e) 500 μM ketoconazol + 30 μM testosterone; (f) 500 μM ketoconazol + 130 μM testosterone.

characteristic of the electrochemical response of some heme proteins immobilized on an electrode and corresponds to the reduction of dissolved oxygen.[5]

To further confirm the electrode reaction involves electron transfer of heme proteins, voltammetry was carried out in the presence of CO. Figure 1b shows the voltammogram obtained in a buffer solution bubbled with CO. The oxidation peak appeared at a more positive potential by 50 mV than that without CO, indicating the peak current from heme proteins.[11] On the other hand, the reductive current separated into two peaks. One appeared at more positive potentials and the other remained. To understand more precisely the obtained peaks of CYP/CPR-microsomes, voltammetric measurements of CYP-microsomes (without CPR) and CPR-microsomes (without CYP) were carried out (Figure S3). Both showed a couple of redox peaks with a formal potential of −0.41 V for CPR-microsomes and −0.38 V for CYP-microsomes, and the former showed no potential shift while the latter showed a positive shift by adding CO. This indicates that the peak splitting and the potential shift in voltammograms of CYP/CPR-microsomes in the presence of CO (Figure 1b) would be due to the positive shift by the component of CYP-microsomes. The ratio of electrical charges of cathodic currents at −0.32 and −0.44 V in Figure 1b was 1:1.4. This was roughly consistent with the ratio (1:1.7) of CYP/CPR in microsomes. Since there were no obvious peaks in the voltammogram of the modified electrode with control microsomes (without CYP and CPR), redox peaks of CYP-microsomes and CPR-microsomes could be derived from electron transfers of CYP and CPR enzymes, respectively. Figure 1 also indicates that no reoxidation current of CPR was observed in the voltammogram of CYP/CPR-microsomes while it appeared in the voltammogram of CPR-microsomes, although reasons for this are currently unclear.

As electron transfers from the electrode to CYP/CPR-microsomes were observed, we expected electrochemically driven drug metabolism reactions. Figure 2 shows voltammograms in the absence and presence of testosterone, a substrate of CYP3A4 (experimental details are in the Supporting Information). Although in the absence of testosterone the catalytic current was observed (Figure 2a) due to a reduction of oxygen as described above, the currents further increased with the concentration of testosterone (Figure 2b−c). The addition of ketoconazole, a CYP3A4 inhibitor that coordinates to the heme iron,[12] caused little catalytic current (Figure 2d−f). These results show that the electrode drove the catalytic reaction of CYP3A4 in microsomes. The apparent K_m value estimated from the relationship between catalytic currents and concentrations of testosterone with an Eadie−Hofstee plot (Figure S4) was ca. 63 ± 15 μM, which is consistent with the reported value 70.8 μM as determined by conventional methods.[13] To confirm drug metabolism reactions, electrolyses and HPLC analyses were done. The main metabolite, 6β-hydroxytestosterone (6β-HOTST), was produced (Figure S5), and this is consistent with the standard solution phase reaction. Although one unidentified significant peak was also found at a shorter retention time than 6β-HOTST, this product might be derived from the oxidation of other parts of the steroid backbone. The rate of 6β-HOTST formation was calculated to be 42 pmol/min/pmol CYP. The value was comparable with that (89 pmol/min/pmol CYP) evaluated by a conventional method. Importantly, the electrode modified with CYP/CPR-microsomes provided a larger amount (270 pmol) of 6β-HOTST than that (140 pmol) of the electrode modified with CYP-microsomes, even though the amount of CYP on each electrode surface was estimated to be equal from the noncatalytic voltammogram. The enhanced metabolite formation by the existence of CPR suggests that the electrons transferred from the electrode to CPR and then to a CYP-like pathway utilized in nature and the electron path is more efficient than the direct electron supply to the CYP.

In summary, electrochemically driven drug metabolism reactions by CYP/CPR-microsomes can be facilitated using simple hydrophobic electrode surfaces. This work may aid the development of electrochemical assays of CYP for drug research and bioreactor/biosensor applications utilizing these enzymes.

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Supporting Information Available: Descriptions of experimental methods and additional figures. This material is available free of charge via the Internet at http://pubs.acs.org.

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