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Electrochemistry of Cytochromes P450: Analysis of Current–Voltage Characteristics of Electrodes with Immobilized Cytochromes P450 for the Screening of Substrates and Inhibitors

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Abstract—In the current study, an approach to elucidating the substrate specificity of cytochromes P450 based on the analysis of current–voltage characteristics of voltammograms and amperograms is proposed. Data on the electrochemical behavior of bioelectrodes with immobilized cytochromes P450 2B4, 1A2, 3A4, 11A1 (P450scc), and 51b1 (*Mycobacterium tuberculosis* sterol 14α-demethylase or CYP51 MT) in the presence of typical substrates and inhibitors for these hemoprotein forms are reported. Immobilization of the enzymes was accomplished by using graphite screen-printed electrodes modified with gold nanoparticles and with the synthetic membrane-like compound didodecyldimethylammonium bromide. The method of electro-analysis can be applied to the search of potential substrates and inhibitors of cytochromes P450 and to creation of multichannel electrochemical plates (chips, panels) with immobilized cytochromes P450.

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Cytochromes P450 discovered in the 1950s [1] are the most active participants in the metabolism of xenobiotics such as therapeutic drugs, food supplements, and environmental chemicals and pollutants; they are also involved in the metabolism of endogenous compounds: saturated and unsaturated fatty acids, eicosanoids, steroids, sterols, vitamin D, retinoids, and bile acids. P450-catalyzed metabolism of foreign compounds leads to formation of toxic metabolites, influencing genetic processes and carcinogenesis [2]; such multifunctionality of cytochromes P450 determines theirs great clinical significance. In recent years, a variety of approaches to investigating cytochrome P450 activity as well as to conducting a high-throughput screening of potential substrates and inhibitors for cytochrome P450 superfamily members have been proposed. These approaches are based on the use of recombinant enzymes and on the analysis of immunological, spectral, fluorescent, chromatographic (HPLC), and mass-spectrometric (LC/MS) parameters of analytical systems [3-7].

Most promising in this regard are electrochemical systems based on recombinant forms of cytochromes P450. Application of these systems enables standardization of the format of analysis and investigation of only the hemoproteins, without their redox partners and without additional label compounds, via direct electron transfer between protein and electrode [8-11]. Besides, bioelectrochemical approaches make it possible to miniaturize a sensor and, in perspective, to obtain the prototype of a "lab-on-a-chip" device [12-15]. Electrochemical methods based on the analysis of current–voltage characteristics of systems are quite fast-acting. Use of nanoparticles (metal nanoparticles, nanostructured polymers, carbon nanostructures) allows the investigator to significantly enhance the sensitivity of electrochemical methods [16, 17].

The aim of the current study was to develop methods of analysis of current–voltage characteristics of electro-

Abbreviations: Bz, benzphetamine; CV, cyclic voltammetry; DDAB, didodecyldimethylammonium bromide; DPV, differential pulse voltammetry; E_{pa} and E_{pc}, potentials of anode reduction and cathode oxidation peaks, respectively; IPA, intermittent pulse amperometry; SWV, square wave voltammetry; Ts, testosterone.

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chemical cytochromes P450-containing systems with a view to revealing substrates and inhibitors of various hemoprotein classes.

MATERIALS AND METHODS

Equipment. Electrochemical measurements were carried out using an AUTOLAB potentiostat (Eco Chemie, The Netherlands) with GPES software. All the measurements were taken at room temperature. Electrochemical studies of cytochromes P450 2B4, 1A2, and 3A4 were done in 0.1 M potassium-phosphate buffer containing 0.05 M NaCl (pH 7.4), those of cytochrome P450 51b1 were done in 0.1 M potassium-phosphate buffer containing 0.04% Triton X-100, 0.05 M NaCl (pH 7.4), and those of cytochrome P450 11A1 were done in 0.1 M potassium-phosphate buffer containing 1% Triton X-100, 0.05 M NaCl (pH 7.4). Screen-printed graphite electrodes were used as working electrodes and screen-printed Ag/AgCl as reference electrodes (Elkom, Russia). The diameter of the working electrode was 2 mm. The potentials are all referred to the Ag/AgCl reference electrode.

Cyclic voltammograms (CV) were registered at the scan rate 10 to 100 mV/sec. Parameters used in square wave voltammetry (SWV, reduction, aerobic conditions) were as follows: initial potential, 100 mV; final potential, –600 mV; step potential, 5 mV; amplitude, 20 mV; frequency, 10 to 100 Hz. Parameters used in differential pulse voltammetry (DPV) were as follows: pulse amplitude, 25 mV; initial potential, 100 mV; final potential, –600 mV; step potential, 1 mV; pulse duration, 50 msec.

Current measurements of intermittent pulse amperometry (IPA) were carried out by scanning with the aid of an AndCare 9600 96-well electrochemical reader (96-well Sensor Plate Monitor) and of 96-well carbon sensor plate (Alderon Biosciences, USA) in aerobic conditions at the potential $E = -300$ mV. The volume of electrolyte was 100 µl.

Spectral studies were conducted using a Carry 100 Scan UV-VIS spectrophotometer; fluorescent spectra were measured using a Scan Array Express spectrofluorimeter (Perkin Elmer, USA).

Reagents. The following reagents were used: didodecyldimethylammonium bromide (DDAB), $HAuCl_4 \cdot 3H_2O$, sodium borohydride, lanosterol, ketoconazole, metyrapone, testosterone (Ts), benzphetamine (Bz), cholesterol (Chl), 7-pentoxyresorufin, 7-ethoxyresorufin, and Triton X-100 (Sigma-Aldrich, USA).

In electrochemical experiments we used freshly prepared 1 mM lanosterol in isopropanol, 10 mM cholesterol in ethanol, 14 mM cholesterol-calibrator (Pointe-Scientific Inc, USA), 50 mM benzphetamine solution in 0.1 M potassium-phosphate buffer, 0.05 M NaCl, 20 mM metyrapone solution in 0.1 M potassium-phosphate buffer, 0.05 M NaCl, 3.6 mM ketoconazole in acetone–dimethylformamide mixture (1 : 1), and 1 mM 7-pentoxyresorufin or 7-ethoxyresorufin in ethanol.

Synthesis of DDAB-stabilized solution of gold nanoparticles was carried out as described in [18]. Colloidal gold solution stabilized by DDAB in chloroform was spectrally characterized: $\lambda_{max} = 520$ nm [19, 20]. The concentration of gold nanoparticles in 0.1 M DDAB in chloroform was calculated in accordance with the stoichiometry of the reaction (5 mM).

Cytochrome P450 2B4 (CYP2B4) (17-18 nmol/mg, $A_{418}/A_{278} = 1.5$) was isolated from the microsomal fraction of rabbit liver induced by phenobarbital and purified as described in [21]. Concentration of P450 2B4 was determined by formation of a complex of the cytochrome P450 reduced form with carbon monoxide using the extinction coefficient $\varepsilon_{450} = 91$ $mM^{-1} \cdot cm^{-1}$ [22]. P450 1A2 (CYP1A2) (10-12 nmol/mg, $A_{393}/A_{278} = 0.9$) was isolated from the microsomes of 3-methylcholanthrene-induced rabbits [23]. P450 1A2 concentration was determined by formation of a complex of the cytochrome reduced form with carbon oxide using the extinction coefficient $\varepsilon_{450} = 104$ $mM^{-1} \cdot cm^{-1}$ [24].

Employed in this study were 100 µM cytochrome P450 2B4, 96 µM cytochrome P450 1A2, and 20 µM cytochrome P450 51b1 [25] (the latter was a gift of Prof. V. M. Govorun from the Institute of Physico-Chemical Medicine, Moscow, Russia).

Recombinant P450 3A4 (59 µM) and recombinant P450scc (CYP11A1, 1700 µM), both containing His_6-tag, were kindly presented by Prof. S. A. Usanov (Institute of Bioorganic Chemistry, Minsk, Belarus).

Preparation of electrodes. Onto the surface of the working graphite electrode was applied 2 µl of 5 mM colloidal gold solution in 0.1 M DDAB in chloroform; after evaporation of the chloroform (10 min), 2 µl of the hemoprotein was loaded onto the electrode surface. Electrodes were allowed to stay for 12 h at 4°C in a humid chamber to prevent their total drying.

For experiments in anaerobic conditions, argon was passed through the buffer solution of electrolyte for 30 min.

Product analysis (formaldehyde formation) after electrolysis was run for 30 min at 37°C by adding to 0.5 ml electrolyte and 0.5 ml Nash reagent (containing 4 M ammonium acetate, 0.1 M glacial acetic acid, and 0.04 M acetyl-acetone). Optical density was measured at 421 nm. Formaldehyde concentration was calculated using the extinction coefficient $\varepsilon_{412} = 4$ $mM^{-1} \cdot cm^{-1}$ [26, 27].

RESULTS AND DISCUSSION

Gold nanoparticles stabilized by the synthetic membrane-like surfactant DDAB provide effective electron transport between the graphite electrode and the

cytochrome P450 heme. Synthetic lipid membrane with colloidal gold, DDAB/Au, contains a sufficient amount of water to maintain hemoprotein structure and, also, secures fixation of enzymes on graphite printed electrodes. Earlier it was shown [18, 28, 29] that the DDAB/Au/P450 2B4, DDAB/Au/P450 1A2, and DDAB/Au/P450 51b1 electrodes have stable electrochemical parameters, which permits such chemically modified electrodes to be used as sensors in studies of substrates and/or inhibitors of these cytochrome P450 forms.

Modification of surfaces of screen-printed graphite electrodes with 0.1 M DDAB/Au in chloroform with subsequent inclusion of cytochromes P450 3A4 or P450 11A1 (P450scc) into the membrane-like matrix also leads to direct electron transfer from electrode to heme. DDAB/Au/P450 electrodes become electroactive when picomolar amounts of enzyme are loaded onto the electrode.

Since test systems based on cytochromes P450 are in great demand, various methods for high-throughput screening of substrates and inhibitors are being actively developed [30, 31]. The majority of proposed methods are based on measuring the activity of human liver microsomes or the activity of recombinant cytochromes P450, expressed together with reductase (baculosomes), towards studied substrates by direct techniques (LC/MS, HPLC) or measuring the activity of cytochromes P450 using a fluorescent probe. The analysis of cassette incubation of probe substrates ("the cocktail" approach or n-in-one technique) is also employed [32]. Spectral optical methods can only provide information about the type of binding, although their efficiency is high enough.

Electrochemical approaches, owing to their high sensitivity, are much more perspective for the analysis of enzyme–substrate and enzyme–inhibitor interactions. The attempts to develop a method of potentiometric analysis of enzyme–substrate interactions show little promise because of the lack of direct correlation between the shifts of the reduction potential in the presence and absence of substrate [33-35]. Some substrates do not shift the reduction potential of cytochrome P450, as in the case with cytochrome P450cam and camphor [36] as well as with cytochrome P450 2B4 and benzphetamine or aminopyrine [28, 35]. In the case of cytochrome P450scc (P450 11A1), cholesterol induced anodic shift of 80-100 mV [37, 38]. Effect of substrates on the reduction potential of cytochrome P450 2C9 was studied in [33]. Diclofenac induced no shift in the oxidation–reduction potential of hemoproteins, while positive shifts induced by torsemide, tolbutamide, and S-warfarin were found to be insignificant, i.e. 4 to 22 mV. Verapamil, midazolam, quinidine, and progesterone did not induce a shift of the redox potential of cytochrome P450 3A4 [39]. Besides, such an approach is ineffective in the search for cytochrome P450 inhibitors.

To develop the algorithm for the search of potential substrates and inhibitors of cytochromes P450, we tested a variety of electrochemical methods. Electrodes with immobilized cytochromes P450 2B4, 1A2, 3A4, 11A1 (P450scc), and 51b1 (CYP51 MT) were studied in the presence of substrates and inhibitors of these protein forms.

Figure 1 presents the amperometric curves at the potential –400 mV of the following systems: DDAB/Au/P450 2B4, DDAB/Au/P450 2B4 + benz-

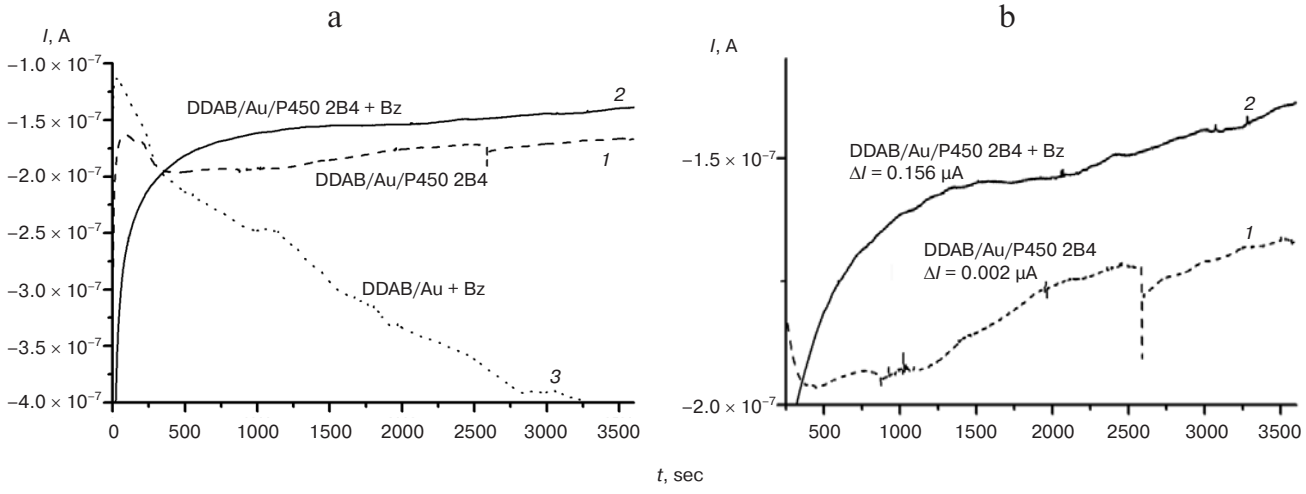

Fig. 1. a) Amperometric response of a screen-printed DDAB/Au/P450 2B4 electrode (*1*), of DDAB/Au/P450 2B4 electrode in the presence of 250 μM benzphetamine (*2*), and of DDAB/Au electrode in the presence of 250 μM benzphetamine (*3*) when a constant potential of $E = -400$ mV was applied. b) Amperometric response of a screen-printed DDAB/Au/P450 2B4 electrode (*1*) and of DDAB/Au/P450 2B4 electrode in the presence of 250 μM benzphetamine (*2*). The applied potential $E = -400$ mV.

Table 1. Electrochemical parameters of DDAB/Au/P450 electrodes as determined from cyclic voltammetry (CV) and from voltammetric analysis (SWV, DPV)

Cytochrome P450	$(E_{pa} + E_{pc})/2 = E_{1/2}$ (mV) vs. Ag/AgCl
2B4	−323 (CV) −327 (DPV)
1A2	−370 (DPV) −382 (SWV)
3A4	−337 (SWV)
51b1 (CYP51)	−273 (CV)
11A1 (P450scc)	−360 (SWV) E_{red} (E_{pc})

Note: Values presented in this table were obtained by averaging results of 3-5 experiments.

Table 2. Electrochemical Michaelis constants K_m calculated from the results of amperometric titration of DDAB/Au/P450 electrodes

Electrode	K_m	
DDAB/Au/P450 2B4 + benzphetamine	13 μM	50 μM [26]
DDAB/Au/P45011A1 + cholesterol	830 μM (on titration with 14 mM standard cholesterol solution) 17 μM (on titration with 10 mM cholesterol in ethanol)	K_D 4 μM [40]
DDAB/Au/P45051b1 + lanosterol	30 μM	1 μM [41]

phetamine (Bz), DDAB/Au + Bz. The applied potential for amperometry corresponds to the redox potential of the studied hemoprotein (Table 1). As follows from Fig. 1a, the time dependences of the current in the case of electrocatalytic reaction and in the case of reaction not catalyzed by the enzyme differ significantly. The difference in currents for the reaction of catalytic reduction of oxygen (curve *1*) and for benzphetamine N-demethylation reaction with oxygen reduction (curve *2*) (Fig. 1b) corresponds to $\Delta I(O_2 + Bz) - \Delta I(O_2) = 154$ nA. The electrochemical catalytic constant k_{cat} calculated by formation of the benzphetamine N-demethylation product, formaldehyde, was found to be 12 min^{-1}.

Amperometric titration at controlled potential makes it possible to calculate the apparent electrochemical Michaelis constant for cytochrome P450 substrates. To calculate the apparent electrochemical Michaelis constant, the Lineweaver–Burk method of double inverse coordinates ($1/I$; $1/[S]$) was applied, where I is a difference between background and steady-state current value at the corresponding substrate concentration S. Table 2 presents the results of amperometric titration for cytochrome P450 2B4 and benzphetamine, for cytochrome P450scc and cholesterol, and for P450 51b1 and lanosterol.

Figure 2 presents the results of amperometric titration of DDAB/Au/P450 11A1 (P450scc) electrode by standard cholesterol solution.

The electroanalytical characteristics were studied based on current–voltage electrode responses that were registered using cyclic voltammetry and voltammetric analysis, i.e. by SWV and DPV, respectively. Figure 3 presents maximal peak currents of SW voltammograms, corrected for the baseline, for enzyme electrodes in the presence of substrates and/or inhibitors: maximal amplitude of the current before (*1*) and after the addition of the substrate benzphetamine (*2*); after the addition of camphor (*3*), which is not a substrate for P450 2B4; after the addition of the inhibitor metyrapone (*4*), and, lastly, after the addition of the substrate benzphetamine (*5*). The amplitude of catalytic current of SW voltammogram was increased upon benzphetamine addition up to 160% (*2*); in the presence of metyrapone there occurs a 20% decrease in the current amplitude compared to the basic value (*4*). The mechanism of inhibitory action of metyrapone is based on its binding to heme iron and on lipophilic binding of metyrapone to the hydrophobic regions of the protein [42]. The decrease in peak current is possibly explained by the competition of metyrapone

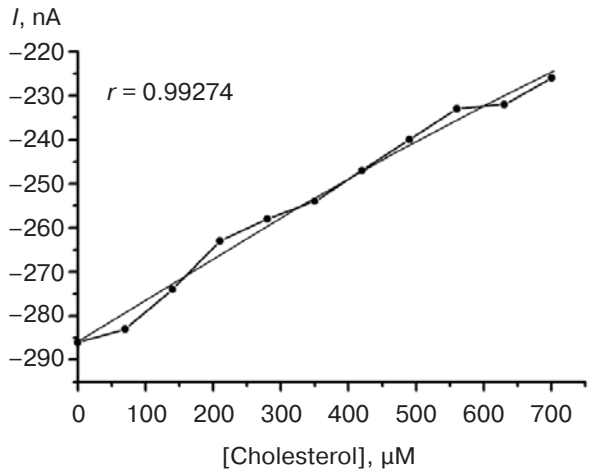

Fig. 2. Amperometric response of a screen-printed DDAB/Au/P450 11A1 electrode to increasing concentration of cholesterol (5-μl aliquots of 14 mM cholesterol) at $E = -500$ mV (vs. Ag/AgCl). Electrolyte volume is 1 ml of 100 mM potassium phosphate buffer plus 50 mM NaCl containing 1% Triton X-100, pH 7.4.

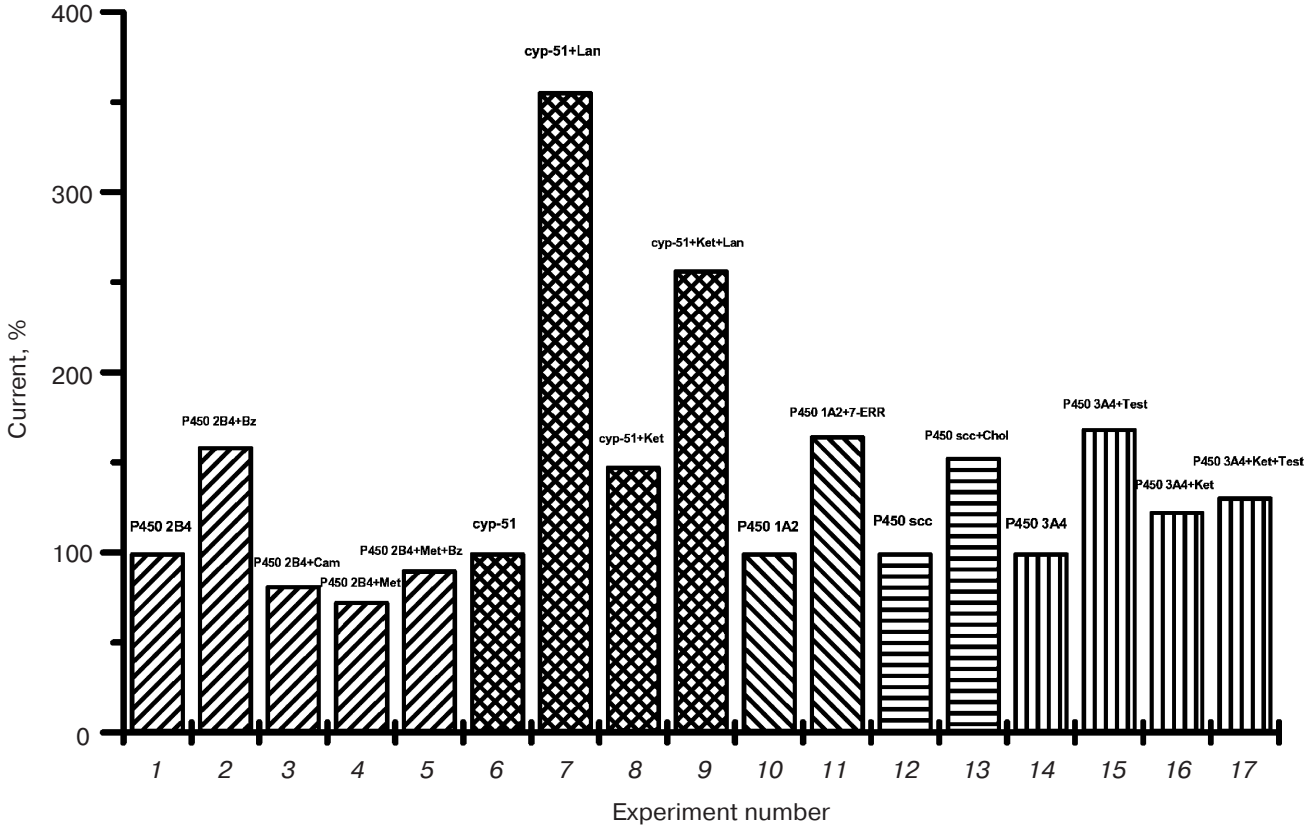

Fig. 3. Peak intensity of reductive SWV of screen-printed electrodes in aerobic buffer (with baseline correction): *1*) DDAB/Au/P450 2B4; *2*) DDAB/Au/P450 2B4 + 250 μM benzphetamine (Bz); *3*) DDAB/Au/P450 2B4 + 25 μM camphor; *4*) DDAB/Au/P450 2B4 + 1 mM metyrapone; *5*) DDAB/Au/P450 2B4 + 1 mM metyrapone + 250 μM benzphetamine (Bz); *6*) DDAB/Au/P450 51b1; *7*) DDAB/Au/P450 51b1 + 10 μM lanosterol; *8*) DDAB/Au/P450 51b1 + 36 μM ketoconazole; *9*) DDAB/Au/P450 51b1 + 36 μM ketoconazole + 10 μM lanosterol; *10*) DDAB/Au/P450 1A2; *11*) DDAB/Au/P450 1A2 + 5 μM 7-ethoxyresorufin; *12*) DDAB/Au/P450 11A1; *13*) DDAB/Au/P450 11A1 + 200 μM cholesterol; *14*) DDAB/Au/P450 3A4; *15*) DDAB/Au/P450 3A4 + 200 μM testosterone; *16*) DDAB/Au/P450 3A4 + 36 μM ketoconazole; *17*) DDAB/Au/P450 3A4 + 36 μM ketoconazole + 200 μM testosterone.

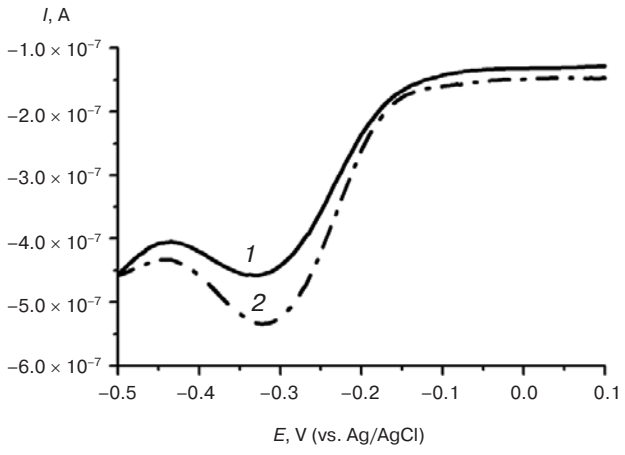

Fig. 4. Reductive DPV of screen-printed DDAB/Au/P450 3A4 electrode before (*1*) and after the addition of 200 μM testosterone (Ts) (*2*). Electrolyte volume is 1 ml 100 mM potassium phosphate buffer plus 50 mM NaCl, pH 7.4.

with oxygen for the binding to heme iron and for substrate binding. Camphor does not increase the catalytic current (*3*) because it is not metabolized by cytochrome P450 2B4 [43].

Similar results were obtained in our earlier studies of electrocatalysis of sterol-14α-demethylase (CYP51 MT, P450 51b1) in the presence of the substrate lanosterol and the inhibitor ketoconazole (Fig. 3, experiments *6-9*) [29].

Cytochrome P450 3A4 is the most functionally significant among cytochromes P450: it metabolizes 225 substrates of which 191 are therapeutic drugs; among the 97 known inhibitors of this enzyme, 87 are therapeutic drugs. Cytochrome P450 3A4 is involved in the metabolism of 34% of therapeutic drugs which are now in use [43]. Analysis of interaction of cytochrome P450 3A4 with testosterone by DPV shows that upon enzyme–substrate interactions the catalytic current can be registered (Fig. 3, experiments *14-17*, and Fig. 4). The ratio of maximal amplitudes of reductive currents in the presence of testosterone (Ts) and without substrate can be expressed

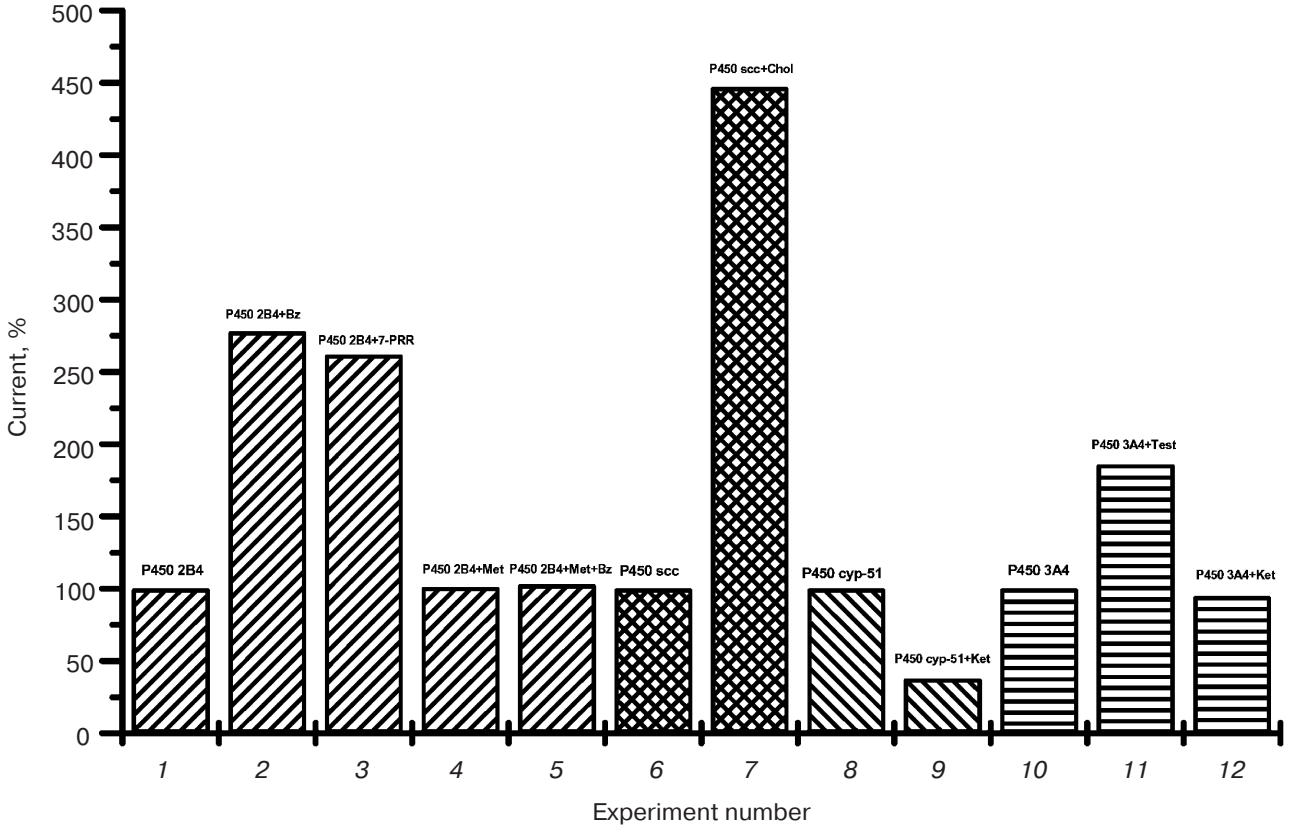

Fig. 5. Peak intensity of intermittent pulse amperometry of screen-printed electrodes in aerobic buffer ($E = -300$ mV): *1*) DDAB/Au/P450 2B4; *2*) DDAB/Au/P450 2B4 + 500 μM benzphetamine (Bz); *3*) DDAB/Au/P450 2B4 + 10 μM 7-pentoxyresorufin; *4*) DDAB/Au/P450 2B4 + 200 μM metyrapone; *5*) DDAB/Au/P450 2B4 + 200 μM metyrapone + 500 μM benzphetamine (Bz); *6*) DDAB/Au/P450scc; *7*) DDAB/Au/P450scc + 100 μM cholesterol; *8*) DDAB/Au/P450 51b1; *9*) DDAB/Au/P450 51b1 + 72 μM ketoconazole; *10*) DDAB/Au/P450 3A4; *11*) DDAB/Au/P450 3A4 + 200 μM testosterone; *12*) DDAB/Au/P450 3A4 + 36 μM ketoconazole.

as $I_{DPV}(O_2 + Ts)/I_{DPV}(O_2) = 1.7$; the inhibitor ketoconazole (36 mM) lowers this ratio to 1.3.

Analysis of SW voltammograms for cytochrome P450scc, cleaving the side chain of cholesterol with formation of pregnenolone, enables registering the catalytic current caused by cholesterol. The ratio of amplitudes of maximal currents of SW voltammograms for the corresponding reductive processes (reduction of oxygen and reduction of cholesterol (Chl)) corrected for the baseline can be expressed as $I_{SWV}(O_2 + Chl)/I_{SWV}(O_2) = 1.5$.

Cytochrome P450 2B4 catalyzes the O-dealkylation of 7-pentoxyresorufin with formation of the fluorescent metabolite resorufin. To test the electrocatalytic activity of DDAB/Au/P450 2B4 electrode, the substrate 7-pentoxyresorufin was loaded (together with the enzyme) onto this electrode. Electrolysis was conducted in the vertical and the horizontal mode with subsequent measurement of fluorescence on a Perkin-Elmer Scan Array Express fluorescence spectrophotometer (λ_{ex} = 530 nm, λ_{em} = 590 nm). For electrode calibration, electrodes with the known resorufin concentration were prepared (extinction coefficient ε_{571} = 69,700 $M^{-1}\cdot cm^{-1}$) [44]. As a result of electrocatalytic reaction, the formation of the fluorescent metabolite resorufin was registered. Resorufin formation, as determined from the calibration curve, corresponds to 4 nmol per electrode in the planar regime, and to 10 nmol per electrode in the vertical mode. Taking into account that about 10% of loaded cytochrome P450 is electroactive, the electrocatalytic activity of cytochrome P450 2B4 is 0.12-0.5 pmol resorufin/pmol enzyme per min, which is comparable with O-dealkylation activity of the microsomal P450-monooxygenase system [45].

To study a number of enzymes and/or substrates, multichannel electrochemical readers were used (Alderon Biosciences; http://www/alderonbiosciences.com). The intensity of currents of intermittent pulse amperometry (IPA) upon scanning using a plane-table AndCare 9600 electrochemical potentiostat and a (12 × 8) sensor array on a 96-well plate in aerobic conditions is shown in Fig. 5. It is to be noted that in studies conducted under the classical scheme (using the square wave voltammetric technique or multichannel electrochemical reader), similar current values were obtained. Registered were: increase in catalytic current in the presence of substrates

of appropriate cytochrome P450 forms; inhibition of current upon addition of inhibitor; inhibition of catalytic current in the case of initially added inhibitor with subsequent addition of substrate (Figs. 3 and 5).

Thus, based on the results of amperometry, cyclic voltammetry, voltammetric analysis (DPV and SWV), and intermittent pulse amperometry, it is possible to conduct a search for and to study the kinetic parameters of potential substrates and inhibitors of cytochrome P450. The proposed electrochemical approach is a sort of a bio-bar code of cytochrome P450 for determination of substrate/inhibitor P450 competence (Figs. 3 and 5).

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REFERENCES

1. Omura, T., and Sato, R. (1962) *J. Biol. Chem.*, **237**, 1375-1376.
2. Nebert, D. W., and Russell, D. W. (2002) *The Lancet*, **360**, 1155-1162.
3. Kumar, R. A., and Clark, D. S. (2006) *Curr. Opin. Chem. Biol.*, **10**, 162-168.
4. Chen, H., and Morgan, J. A. (2206) *Enzyme Microb. Technol.*, **38**, 760-764.
5. Lee, M.-Y., Park, C. B., Dordick, J. S., and Clark, D. S. (2005) *Proc. Natl. Acad. Sci. USA*, **102**, 983-987.
6. Sakai-Kato, K., Kato, M., Homma, H., Toyo'oka, T., and Utsunomiya-Tate, N. (2005) *Anal. Chem.*, **77**, 7080-7083.
7. Turpeinen, M., Korhonen, L. E., Tolonen, A., Uusitalo, J., Juvonen, R., Raunio, H., and Felkonen, O. (2006) *Eur. J. Pharm Sci.*, **29**, 130-138.
8. Estabrook, R. W., Faulkner, K. M., Shet, M. S., and Fisher, C. W. (1996) *Meth. Enzymol.*, **272**(B), 44-51.
9. Bistolas, N., Wollenberger, U., Jung, C., and Scheller, F. W. (2005) *Biosens. Bioelectron.*, **20**, 2408-2423.
10. Shumyantseva, V. V., Bulko, T. V., and Archakov, A. I. (2005) *J. Inorg. Biochem.*, **99**, 1051-1063.
11. Udit, A. K., and Gray, H. B. (2005) *Biochem. Biophys. Res. Commun*., **338**, 470-476.
12. Bakker, E., and Telting-Diaz, M. (2002) *Anal. Chem.*, **74**, 2781-2800.
13. Mehrvar, M., and Abdi, M. (2004) *Anal. Sci.*, **20**, 1113-1126.
14. Wang, J. (2005) *Analyst*, **130**, 421-426.
15. Murphy, L. (2006) *Curr. Opin. Chem. Biol.*, **10**, 177-184.
16. Pumera, M., Sanchez, S., Ichnose, I., and Tang, J. (2007) *Sensors and Actuators*, **123**, 1195-1205.
17. Tansil, N., and Gao, Z. (2006) *Nanotoday*, **1**, 28-37.
18. Shumyantseva, V. V., Bulko, T. V., Rudakov, Yu. O., Kuznetsova, G. P., Samenkova, N. F., Lisitsa, A. V., Karuzina, I. I., and Archakov, A. I. (2007**)** *J. Inorg. Biochem.*, **101**, 859-865.
19. Berthell, D., Brust, M., Schiffrin, D. J., and Kiely, C. (1996) *J. Electroanal. Chem.*, **409**, 137-143.
20. Han, X., Cheng, W., Zhang, Z., Dong, S., and Wang, E. (2002) *Biochim. Biophys. Acta*, **1556**, 273-277.
21. Karuzina, I. I., Zgoda, V. G., Kuznetsova, G. P., Samenkova, N. F., and Archakov, A. I. (1999) *Free Rad. Biol. Med.*, **26**, 620-632.
22. Omura, T., and Sato, R. (1964) *J. Biol. Chem.*, **239**, 2379-2385.
23. Imai, Y., Hashimoto, C., Satake, H., Girardin, A., and Sato, R. (1980) *J. Biochem.*, **88**, 489-503.
24. Hashimoto, C., and Imai, Y. (1976) *Biochem. Biophys. Res. Commun.*, **68**, 821-827.
25. Shavkunov, A. S., Lazarev, V. N., Zgoda, V. G., Govorun, V. M., Levi, P., Yanssen, P., and Archakov, A. I. (2003) *Biomed. Khim.*, **49**, 145-152.
26. Nash, T. (1953) *Biochem. J.*, **55**, 416-421.
27. Kanaeva, I. P., Dedinskii, I. R., Scotselyas, E. D., Krainev, A. G., Guleva, I. V., Sevryukova, I. F., Koen, Ya. M., Kuznetsova, G. P., Bachmanova, G. I., and Archakov, A. I. (1992) *Arch. Biochem. Biophys.*, **298**, 395-412.
28. Shumyantseva, V. V., Bulko, T. V., Rudakov, Yu. O., Kuznetsova, G. P., Samenkova, N. F., Lisitsa, A. V., Karuzina, I. I., and Archakov, A. I. (2007) *Biochemistry (Moscow)*, *Suppl. Ser. B: Biomed. Chem.*, **1**, 327-333.
29. Shumyantseva, V. V., Bulko, T. V., Kuznetsova, G. P., Lisitsa, A. V., Ponomarenko, E. A., Karuzina, I. I., and Archakov, A. I. (2007) *Biochemistry (Moscow)*, **72**, 658-663.
30. Smith, D., Sadagopan, N., Zientek, M., Reddy, A., and Cohen, L. (2007) *J. Chromatogr. B*, **850**, 455-463.
31. Persson, K. P., Ekehed, S., Otter, C., Lutz, M., McPheat, J., Masimirembwa, C. M., and Andersson, T. B. (2006) *Pharm. Res.*, **23**, 56-69.
32. Tupeinen, M., Jouko, U., Jorma, J., and Olavi, P. (2005) *Eur. J. Pharm. Res.*, **24**, 123-132.
33. Johnson, D. L., Lewis, B. C., Elliot, D. J., Miners, J. O., and Martin, L. L. (2005) *Biochem. Pharmacol.*, **69**, 1533-1541.
34. Shukla, A., Gillam, E. M., Mitchll, D. J., and Bernhardt, P. V. (2005) *Electrochem. Commun.*, **7**, 437-442.
35. Shumyantseva, V. V., Ivanov, Yu. D., Bistolas, N., Scheller, F. W., Archakov, A. I., and Wollenberger, U. (2004) *Anal. Chem*., **76**, 6046-6052.
36. Lei, C., Wollenberger, U., Jung, C., and Scheller, F. W. (2000) *Biochem. Biophys. Res. Commun.*, **268**, 740-744.
37. Shumyantseva, V. V., Deluca, J., Bulko, T. V., Carrara, S., Nicolini, C., Usanov, S. A., and Archakov, A. I. (2004) *Biosens. Bioelectron*., **19**, 971-976.
38. Shumyantseva, V. V., Bavastrello, V., Carrara, S., Riley, D. J., Bulko, T. V., Skryabin, K. G., Archakov, A. I., and Nicolini, C. (2005) *Biosens. Bioelectron.*, **21**, 217-222.
39. Joseph, S., Rusling, J. F., Lvov, Y. M., Friedberg, T., and Fuhr, U. (2003) *Biochem. Pharmacol.*, **65**, 1817-1826.
40. Lepesheva, G. I., Strushkevich, N. V., and Usanov, S. A. (1999) *Biochim. Biophys. Acta*, **1434**, 31-43.
41. Lepesheva, G. I., and Waterman, M. R. (2004) *Mol. Cell. Endocrinol.*, **215**, 165-170.
42. Ortiz de Montellano, P. R. (ed.) (1995) *Cytochrome P450*, 2nd Edn., Plenum Press, New York, pp. 307-317.
43. Lewis, D. F. V. (2001) *Guide to Cytochrome P450. Structure and Function* (Taylor and Francis, eds.) London-New York, pp. 90-93.
44. Kitson, T. M. (1996) *Bioorg. Chem.*, **24**, 331-339.
45. Prough, R. A., Burke, M. D., and Mayger, R. T. (1978) *Meth. Enzymol.*, **52**, 372-377.