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Mechanism of Intermolecular Interactions of Microsomal Cytochrome P450s CYP17 and CYP21 Involved in Steroid Hormone Biosynthesis

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Abstract—Protein–protein interactions play a significant role in regulation of functional activity of cytochrome P450s. The aim of the present study was to elucidate the molecular interactions between steroidogenic enzymes CYP17 and CYP21 localized in endoplasmic reticulum membranes of adrenal cortex and involved in biosynthesis of corticosteroid hormones. In the present work, we demonstrate for the first time the direct interaction at molecular level between highly purified human recombinant cytochrome P450s in a mixed reconstituted system. The dependence of the interaction between CYP17 and CYP21 on concentration of the redox-partner – NADPH-cytochrome P450 reductase – is demonstrated, and it is shown that electrostatic interactions play a crucial role in the interaction between CYP17 and CYP21.

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Key words: cytochrome P450, CYP17, CYP21, protein–protein interaction, steroid hormone biosynthesis

Steroid hormones are important components of the human hormonal system. Most reactions of steroid hormone biosynthesis are catalyzed by cytochrome P450 (P450, CYP). Cytochrome CYP17 and CYP21 are located in the endoplasmic reticulum membranes and catalyze a number of sequential hydroxylation reactions of the steroid hormone biosynthesis pathway in mammals (Fig. 1). Abnormalities in the functions of these enzymes result in serious changes in hormone metabolism and cause severe disorders in humans [1, 2]. Cytochrome CYP17 (17α-hydroxylase/17,20-lyase) is a key enzyme of steroid hormone biosynthesis. CYP17 is unique due to its ability to catalyze two independent reactions in the same active center, the 17α-hydroxylase and 17,20-lyase reactions. Furthermore, the ratio of these activities is physiologically important and may direct steroid hormone biosynthesis to the production of either corticoid or sex hormones [3]. CYP21 (steroid 21-hydroxylase) catalyses reactions of progesterone (P4) and 17α-hydroxyprogesterone (17OHP4) conversion to 11-deoxycorticosterone (DOC) and 11-deoxycortisol (DCS), respectively, providing an important step in aldosterone and cortisol biosynthesis [4].

There are a number of factors affecting productivity of steroid hormone biosynthesis: expression level of enzymes, distribution of steroid hormone biosynthesis enzymes in organs and tissues, availability of redox partners of enzymes, and presence of protein- and non-protein effectors of these systems. Change in the level of catalytic activity is one of the methods of fine regulation of steroid hormone biosynthesis. Thus, CYP17 is one of the few cytochrome P450s involved in steroid hormone biosynthesis that are phosphorylated [5]. It is hypothesized that this posttranslational modification may influence catalysis [6].

Electron transfer from NADPH-cytochrome P450 reductase (CPR) to cytochrome P450 is an important mechanism of regulation of steroid hormone biosynthesis [7]. Formation of a functional complex between cytochrome P450 and CPR is a prerequisite for productive electron transfer [8]. The availability of electrons supplied by CPR is a rate-limiting factor for most of the reactions catalyzed by microsomal cytochrome P450. The amount of cytochromes P450 in the liver and steroidogenic tissues significantly exceeds the amount of CPR (ratio approximately 20 : 1) [9]. CPR concentration in adrenal microsomes is 4 times higher than in testicular microsomes [10]. This favors production of C19-steroids,

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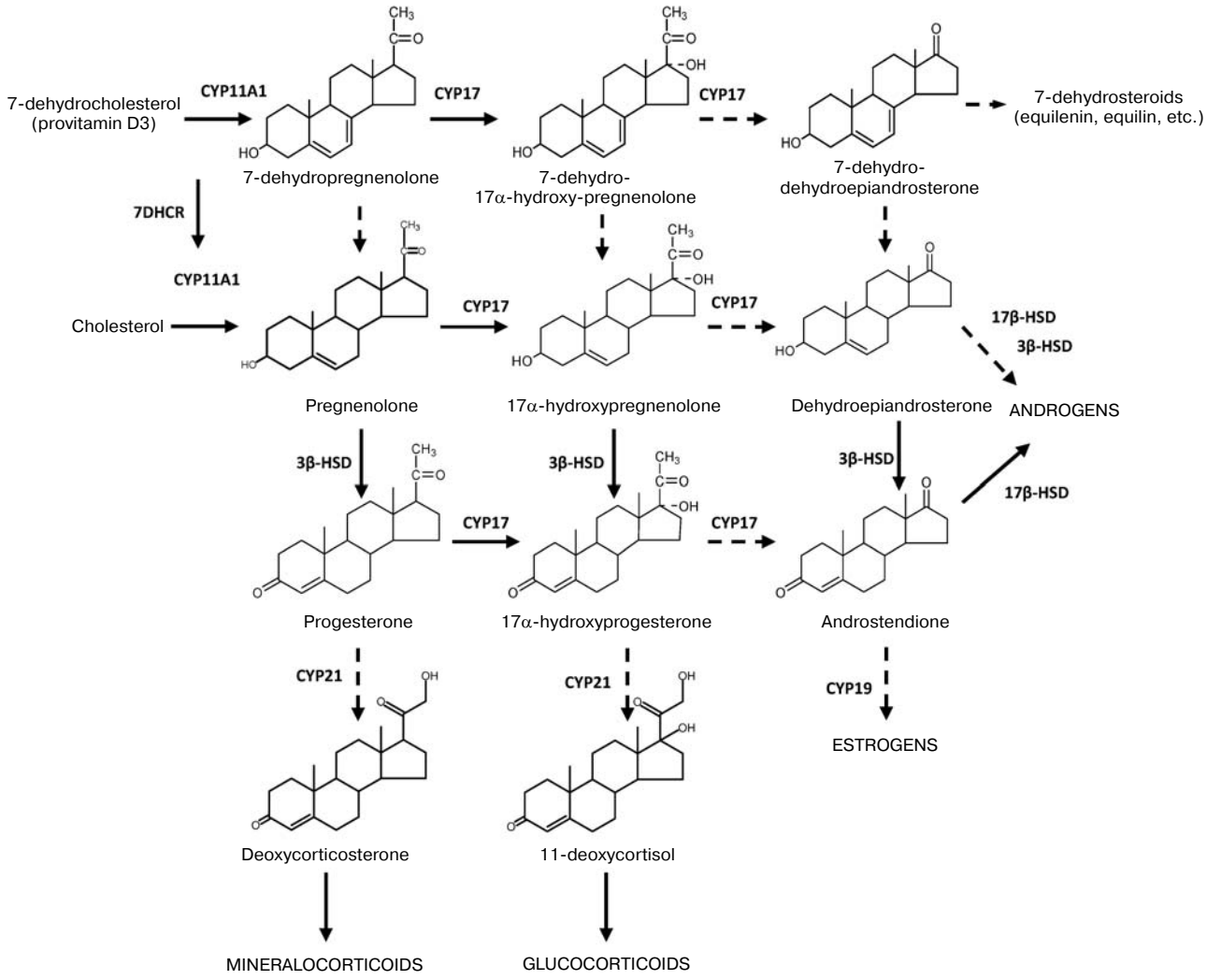

Fig. 1. Scheme of steroid hormone biosynthesis pathway in Chordata.

sex hormone precursors, because increase in CPR concentration stimulates mainly the CYP17 17,20-lyase reaction, but not the 17α-hydroxylation reaction.

Furthermore, cytochrome b_5 plays an important role in the intracellular regulation of biosynthesis of androgens at the level of CYP17. It is shown that cytochrome b_5 mainly stimulates 17,20-lyase activity of CYP17 (5-10-fold), but has no effect on the 17α-hydroxylation reaction [11]. It is hypothesized that cytochrome b_5 is an allosteric effector that can facilitate complex formation between cytochrome P450 and NADPH-cytochrome P450 reductase. As a result of structural rearrangements, cytochrome P450 turns to be more accessible for interaction with CPR [12]. The cytochrome b_5 isoform – outer mitochondrial membrane cytochrome b_5 (OMb_5) – was also found in cells. OMb_5 may also influence androgen biosynthesis [13], but the physiological role of OMb_5 is not clearly understood. It is necessary to emphasize the specificity of interaction between cytochrome CYP17 and cytochrome b_5, because regulatory protein IZA (adrenal inner zone antigen), that contains heme as a prosthetic group and has a structural features similar to cytochrome b_5, did not stimulate the catalytic activity of CYP17 [14].

3β-Hydroxysteroid dehydrogenase type II (HSD3B2) influences mineralocorticoid (aldosterone) and glucocorticoid (cortisol) biosynthesis by competing with CYP17 for metabolism of pregnenolone (P5) and 17α-hydroxypregnenolone (17OHP5) [15]. High HSD3B2 expression level combined with low CYP17 catalytic activity turns biosynthesis to aldosterone production. In contrast, low level of HSD3B2 expression combined with high level of catalytic activity of CYP17 favors the production of androgens.

Despite the great progress in investigation of enzymatic systems of steroid biosynthesis, the role of protein–protein interactions between cytochromes P450 involved in steroid hormone biosynthesis is still not sufficiently studied.

The aim of the present study was to elucidate the molecular interactions between cytochromes CYP17 and

CYP21 involved in steroid hormone biosynthesis. In the present work we demonstrate for the first time the mutual effect of CYP17- and CYP21-monooxygenase systems involved in human steroid hormone biosynthesis.

MATERIALS AND METHODS

Materials. The following chemicals were used in the work: P4, 17OHP4, P5, sodium cholate, Triton X-100, NADPH, phenylmethylsulfonyl fluoride (PMSF), δ-aminolevulinic acid (ALA), SDS, Coomassie brilliant blue R-250, Hepes, Tris-buffer, arabinose, ampicillin, kanamycin, cholesterol oxidase (from *Cellulomonas* species) (Sigma, USA); Ni-NTA agarose (Qiagen, USA); Emulgen 913 (Kao Atlas, Japan); agarose, isopropyl-β-D-thiogalactopyranoside (IPTG), and dithiothreitol (DTT) (Gibco BRL, USA); Bacto-Tryptone, Bacto-Peptone, and Bacto-Yeast extract (Difco Laboratories, USA); Bio-Gel HTP (Bio-Rad, USA); methylene chloride (Ekos-1, Russia). Restriction enzymes and other enzymes for DNA modification were from New England Biolabs (USA) and Promega (USA).

Expression of human CYP17 and CYP21. *Escherichia coli* JM109 competent cells were transformed with corresponding plasmids containing human CYP17 or CYP21 and molecular chaperones GroES-GroEL [16, 17]. The transformed cells were screened on Petri dishes with LB-agar containing ampicillin (100 μg/ml) and kanamycin (40 μg/ml). A 0.5-liter portion of TB-medium containing 100 mM potassium-phosphate buffer, pH 7.4, rare salt solution, ampicillin (100 μg/ml), and kanamycin (40 μg/ml) was inoculated with 3 ml of overnight culture, and the mixture was incubated in thermostatic orbital shaker at 37°C and 180 rpm. After reaching absorbance A_{600} ~ 0.4, protein expression was stimulated by adding IPTG (0.5 mM), also adding ALA (0.65 mM), arabinose (4 mg/ml), ampicillin (100 μg/ml), and kanamycin (40 μg/ml). After 48 h of incubation in the thermostatic orbital shaker at 26°C and 140 rpm, the cells were cooled for 1 h and collected by centrifugation at 3000 rpm for 10 min. The pellet was suspended in buffer A (50 mM Tris-HCl buffer, pH 7.4, 20% glycerol, 0.3 M NaCl) containing 0.5 mM PMSF and 50 μM P4 (1 volume of cells/4 volumes of buffer). The cells were frozen at temperature –73°C.

Isolation and purification of recombinant proteins. Cells were thawed and treated using Emulsiflex C5 (Avestin, Canada). Recombinant cytochrome P450 was solubilized from membranes by adding drops of Emulgen 913 to final concentration 1% (3 mg detergent per mg protein) under continuous mixing for 1 h. The suspension was centrifuged 1 h at 22,000 rpm to remove non-solubilized membrane structures. The supernatant was applied to a Ni-NTA-agarose column equilibrated with buffer A. The column was washed with 2-3 volumes of buffer A containing 0.2% Emulgen 913 and then with 10 volumes of buffer A containing 0.2% Emulgen 913 and 100 mM glycine (buffer B). The proteins were eluted from the column with buffer B containing 50 mM histidine and 0.2% Emulgen 913 (0.5% for CYP21). Fractions containing CYP17 or CYP21 were pooled and applied to a column with hydroxyapatite equilibrated with 10 mM potassium phosphate buffer, pH 7.4. The column was washed with 10 volumes of 50 mM potassium phosphate buffer, pH 7.4, containing 20% glycerol, 50 μM progesterone, 0.2% Emulgen 913 (0.5% for CYP21), and 0.1 mM DTT. The proteins were eluted from the column with 0.3 M potassium-phosphate buffer, pH 7.4, containing 20% glycerol, 50 μM progesterone, 0.2% Emulgen 913 (0.5% for CYP21), and 0.1 mM DTT. The highly purified heme proteins were stored at temperature –73°C.

Highly purified recombinant rat NADPH-cytochrome P450 reductase was purified as previously described [18].

Analytical methods. Cytochrome P450 concentration was determined spectrophotometrically using coefficient $\varepsilon_{450\text{-}490} = 91\ mM^{-1}\cdot cm^{-1}$ for absorbance of the complex of the reduced heme protein with CO [19]. CPR concentration was assigned from the absorbance spectrum using molar extinction coefficient $\varepsilon_{456} = 21.4\ mM^{-1}\cdot cm^{-1}$ [20]. Protein homogeneity was determined by SDS-PAGE.

Determination of CYP17 17α-hydroxylase activity. The 17α-hydroxylase activity of recombinant CYP17 was determined in a reconstituted system containing CYP17 and CPR and in mixed system containing CYP17, CYP21 (or CYP2C19), and CPR at 37°C in 50 mM potassium-phosphate buffer, pH 7.2. Aliquots of the concentrated proteins (0.5 μM P450 and 1 μM CPR, if not mentioned otherwise) were mixed and incubated at room temperature for 5 min. P5 dissolved in ethanol was added to the reaction mixture to final concentration 50 μM and incubated 10 min at 37°C. The reaction was started by adding NADPH to final concentration 0.25 mM. Aliquots (0.5 ml) were taken from the incubation mixture at definite time intervals. The reaction was stopped by boiling. Cholesterol oxidase was added to the mixture and incubated for an additional 30 min at 37°C. The reaction was stopped by adding 5 ml of methylene chloride. The mixture was vigorously shaken, and the layers were separated by centrifugation at 3000 rpm for 10 min. The aqueous layer was carefully removed, and the organic layer was dried under a flow of argon. Steroids were solved in 100 μl of methanol and analyzed by HPLC (Hewlett Packard HP 1090 series II/L, equipped with a μ-Bondapak C18-column (3.9 × 300 mm) and a diode array detector).

Determination of CYP21 catalytic activity. CYP21 activity was determined in a reconstituted system containing CYP21 and CPR and in a mixed system containing CYP21, CYP17, and CPR at 37°C in 50 mM potassium phosphate buffer, pH 7.2. Activity was measured as described for CYP17 in the system containing 0.05 μM

cytochrome P450 and 0.1 μM CPR (if not mentioned otherwise). 17OHP4 was added to final concentration 50 μM.

Determination of CYP17 and CYP21 catalytic activity at physiological ratios of the proteins. CYP17 activity was determined in a reconstituted system containing CYP17 and CPR (25 : 1) based on data obtained about CYP17, CYP21, and CPR expression levels in adrenal cortex [21]. P5 was added to final concentration 50 μM. CYP21 activity was determined in a reconstituted system containing CYP21 and CPR (5 : 1), and 17OHP4 was added to final concentration 50 μM. Interaction between CYP17 and CYP21 was estimated in a mixed reconstituted system containing CYP17, CYP21, and CPR (25 : 5 : 1).

All experiments were performed at least twice, and the averaged data are presented.

Modeling of tertiary structure of CYP17. The tertiary structure of CYP17 was modeled using the crystal structure of CYP1A2 as a template as described previously [22].

RESULTS

Influence of CYP17 on catalytic activity of CYP21 in a reconstituted system. To elucidate the influence of the interaction between CYP17 and CYP21 on the catalytic activity, the proteins were studied in a mixed reconstituted system containing CYP17, CYP21, and CPR in different proportions (Table 1). To prevent competition for substrate between CYP17 and CYP21, we used 17OHP4 as substrate for CYP21 in this experimental series, because human CYP17 is highly specific to Δ^5-type steroids (17OHP4 is not a substrate for the 17,20-lyase reaction). It is shown that CYP21 catalytic activity with 17OHP4 as substrate decreases after addition of CYP17 in the reconstituted system containing CYP21 and CPR. The inhibitory effect of CYP17 addition depends on [cytochrome P450]/[reductase] ratio. At limiting reductase amount ([cytochrome P450]/[reductase] = 1 : 0.5), 42% inhibition of CYP21 catalytic activity occurs. At the ratio of concentrations 1 : 1, CYP21 activity decreases by 23%, and increase in CPR concentration (ratio 1 : 2) causes practically complete recovery of CYP21 catalytic activity (8% decrease in activity). The inhibitory effect of CYP17 addition could be explained by competition of CYP17 and CYP21 for interaction with CPR, i.e. for supply of electrons from the flavoprotein.

Influence of CYP21 on catalytic activity of CYP17 in the reconstituted system. The addition of CYP21 in the reconstituted system containing CYP17 and CPR caused more evident decrease in CYP17 catalytic activity (Table 1). To prevent competition for substrate between CYP17 and CYP21, we used P5 as a substrate for CYP17 in this experimental series since human CYP21 uses predominantly Δ^4-steroids as substrates. Analogously, inhibitory effect of CYP21 addition on CYP17 activity depends on the ratio [cytochrome P450]/[NADPH-cytochrome P450 reductase]. At limiting CPR amount ([cytochrome P450]/[NADPH-cytochrome P450 reductase] = 1 : 0.5), 53% inhibition of CYP17 catalytic activity occurs. At the ratio 1 : 1, CYP17 activity decreases by 40%. In contrast to the system described above, increase in concentration ratio to 1 : 2 does not lead to recovery in CYP17 catalytic activity (35% decrease in activity at ratio 1 : 2). In this case decrease in CYP17 catalytic activity after CYP21 addition could not be explained only by competition for interaction with CPR. The results suggest the formation of an enzymatic complex where CYP17 has lower affinity to CPR compared with the reconstituted system containing only one cytochrome P450, or CYP21 may inhibit CYP17 activity directly.

Table 1. CYP17 and CYP21 catalytic activity in simple and mixed reconstituted systems

Protein	P450/CPR ratio		
	1/0.5	1/1	1/2
CYP17	0.38 ± 0.01	1.22 ± 0.07	1.33 ± 0.07
CYP17 + CYP21	0.18 ± 0.03	0.73 ± 0.06	0.87 ± 0.05
% of reaction compared with system containing only CYP17 and CPR	47	60	65
CYP21	31.27 ± 3.16	49.70 ± 3.90	53.57 ± 0.87
CYP21 + CYP17	18.00 ± 1.70	38.10 ± 3.12	49.02 ± 0.71
% of reaction compared with system containing only CYP21 and CPR	58	77	92

Note: CYP17 catalytic activity (min^{-1}) was measured by formation of 17OHP5 from P5; CYP21 catalytic activity (min^{-1}) was measured by formation of DCS from17P4 as described in "Materials and Methods".

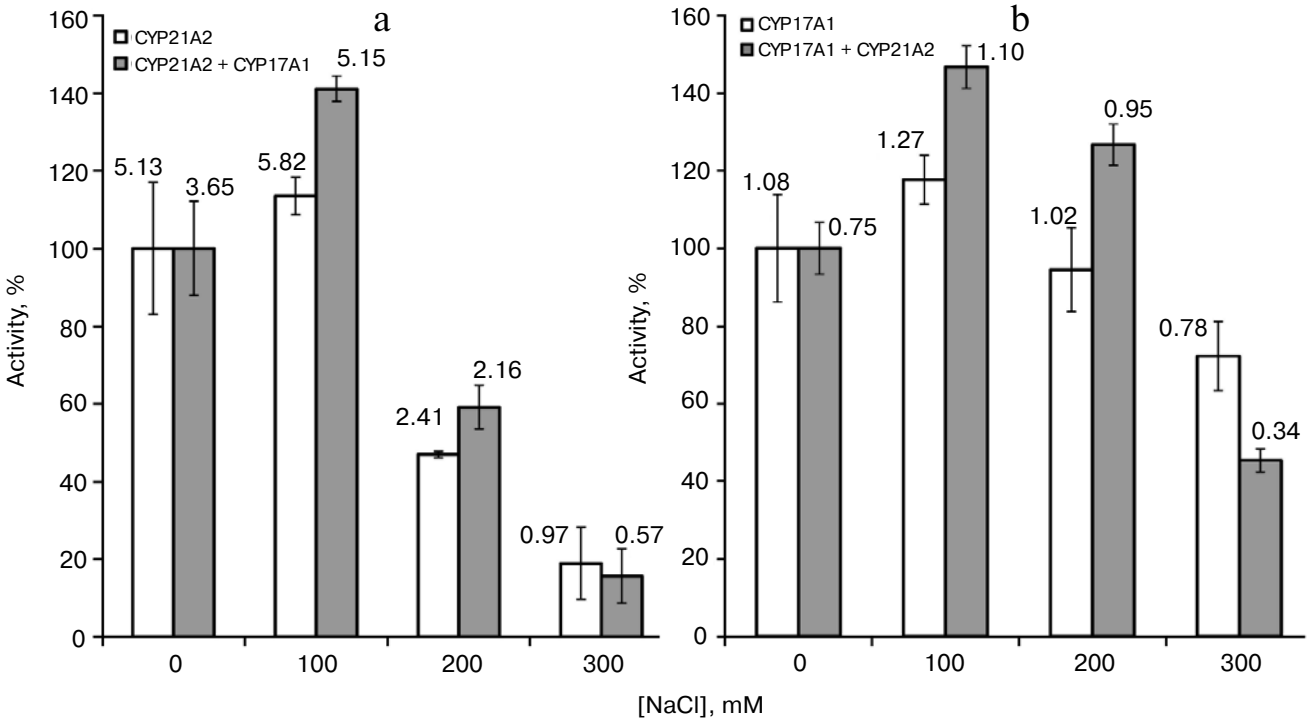

Fig. 2. Effect of ionic strength on formation of DCS from 17OHP4 catalyzed by CYP21 (a) and 17OHP5 from P5 catalyzed by CYP17 (b) in simple and mixed reconstituted systems.

Influence of ionic strength on CYP17 and CYP21 activity. To clarify the mechanism of interaction between cytochromes P450, the effect of ionic strength on catalytic activity was studied. Catalytic activity of CYP17 and CYP21 was measured in a mixed reconstituted system containing CYP17 and CYP21 at different NaCl concentrations (Fig. 2). CYP17 activity in the binary system increases with an increase in ionic strength reaching a maximum at 100 mM; further increase in NaCl concentration leads to decrease in CYP17 activity. Analogously, CYP17 has maximal activity at 100 mM NaCl in the mixed system, and further increase in NaCl concentration leads to decrease in CYP17 activity. The inhibitory effect of CYP21 addition is decreased with increase in ionic strength from 0 to 200 mM. The inhibitory effect increases at 300 mM NaCl.

Similarly, it was shown that CYP21 activity in the binary system increases with increase in ionic strength with maximal effect at 100 mM, while further increase in NaCl concentration leads to decrease in CYP21 activity. CYP21 has maximal activity at 100 mM NaCl in the mixed system, and further increase in NaCl concentration leads to decrease in CYP21 activity. The inhibitory effect of CYP17 addition is decreased with increase in ionic strength from 0 to 200 mM. The inhibitory effect increases at 300 mM NaCl.

The data indicate that electrostatic interactions play a significant role in the interaction between CYP17, CYP21, and NADPH-cytochrome P450 reductase.

It is important to note that neither presence of additional enzyme nor alteration of ionic strength influence stereoselectivity of the steroid hydroxylation (Fig. 3).

Specificity of interaction between CYP17 and CYP21. To confirm the specificity of interaction between CYP17 and CYP21, the interaction between CYP17 and microsomal cytochrome CYP2C19 was studied. CYP2C19 was chosen because of significant homology between CYP17, CYP21 and the CYP2 family; the proteins have similar folding and use CPR as the electron donor. However, CYP2C19 and CYP17 have different expression localization. CYP2C19 is mainly expressed in liver and metabolizes xenobiotics [23]. CYP2C19 effectively metabolizes tricyclic antidepressants (amitriptyline, clomipramine, and imipramine), anticonvulsants and anti-epileptics (diazepam, primidone, phenytoin, phenobarbital, and nordazepam), proton pump inhibitors (omeprazole, pantorazole, lansoprazole, rabeprazole, and esomeprozole), antimalarial drug proguanil, nonsteroidal antiinflammatory drugs (diclofenac and indomethacin) as well as warfarin, clopidogrel, nelfinavir, and voriconazole [24]. It is reasonable to suppose that interactions between cytochrome CYP17 and CYP2C19 are not specific, due to the different localization and substrate specificity of these enzymes. Indeed we found that addition of CYP2C19 to the reconstituted system containing CYP17 and CPR in different proportions does not significantly change CYP17 catalytic activity, as was observed after CYP21 addition (Table 2).

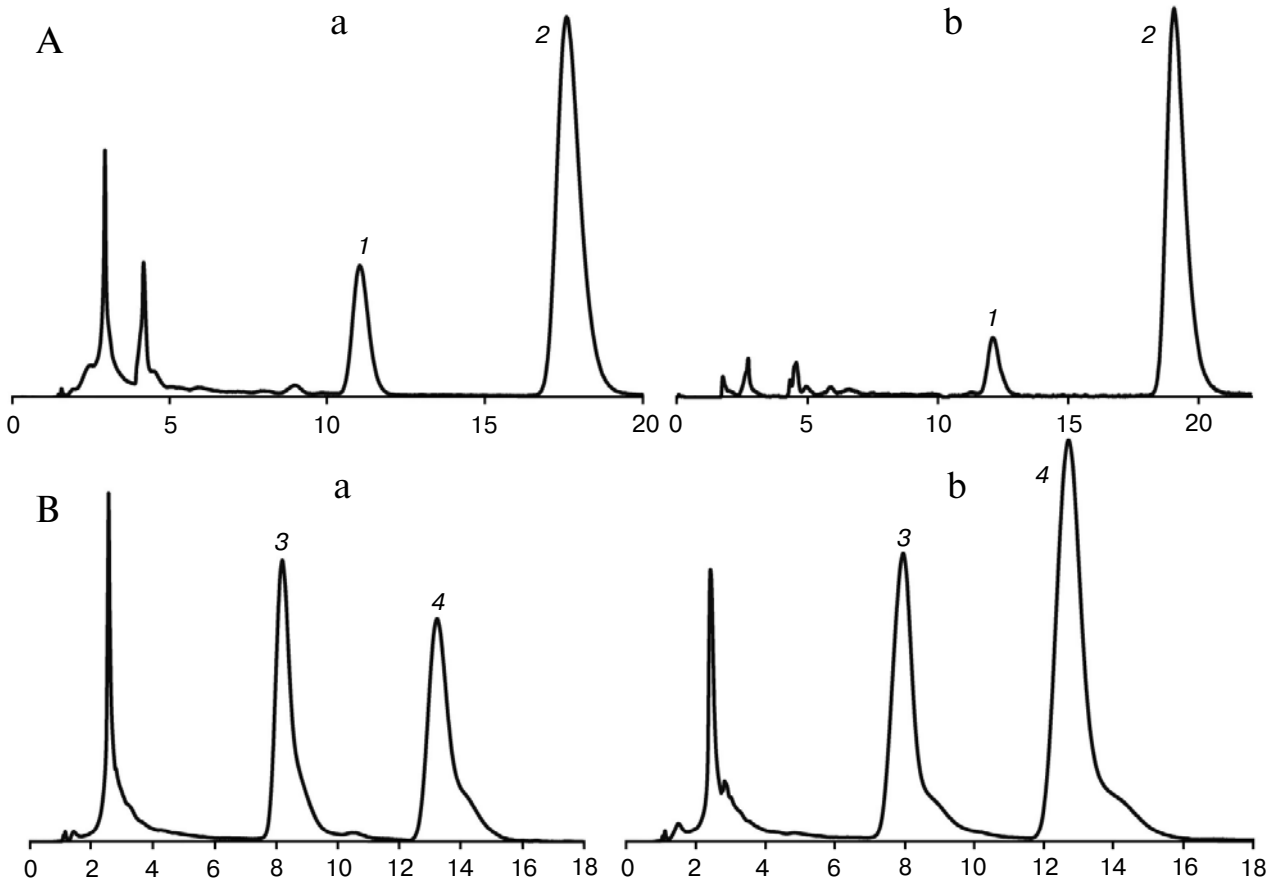

Fig. 3. HPLC profile of products formed during metabolism of: A) P5 by cytochrome CYP17 (a) or by mixture of cytochrome CYP17 and CYP21 (b); B) 17OHP4 by cytochrome CYP21 (a) or by mixture of cytochrome CYP21 and CYP17 (b). *1*) 17OHP5, m/z 333; *2*) P5, m/z 317; *3*) DCS, m/z 347; *4*) 17OHP4, m/z 331.

The data confirm the high specificity of interaction between CYP17 and CYP21. We did not study interaction between CYP2C19 and CYP21 because inhibitory effect of CYP17 on CYP21 is nonspecific and could be explained by competition for interaction with the redox partner.

Interaction between CYP17 and CYP21 at naturally occurring protein ratio. Using data concerning CYP17, CYP21, and CPR expression level in adrenal cortex [21], the interaction between CYP17 and CYP21 at physiological protein ratio was examined. The interaction was estimated in a reconstituted system containing CYP17, CYP21, CPR at the protein ratio of 25 : 5 : 1. It demonstrated that addition of CYP17 to the reconstituted system containing CYP21 decreases CYP21 activity more than 4-fold. However, addition of CYP21 to a reconstituted system containing CYP17 leads to 55% decrease in

Table 2. Interaction of CYP17 and CYP2C19

Protein	P450/CPR ratio		
	1/0.5	1/1	1/2
CYP17	0.47 ± 0.01	0.67 ± 0.04	0.98 ± 0.04
CYP17 + CYP2C19	0.55 ± 0.02	0.70 ± 0.06	0.93 ± 0.05
% of reaction occurs compared with system containing only CYP17 and CPR	117	104	95

Note: CYP17 catalytic activity (min^{-1}) was measured by formation of 17OHP5 from P5, as described in "Materials and Methods".

CYP17 catalytic activity. This means that *in vitro* examination of catalytic activity of the enzymes involved in steroid biosynthesis in binary reconstituted systems at the equimolar ratio not always reflects the real processes taking place in the cell. It is necessary to carry out additional studies of P450–P450 interactions of the components of the steroid biosynthesis system.

DISCUSSION

Cytochromes P450 represent a large superfamily of monooxygenases involved in metabolism of a variety of endogenous (steroids, bile acids, fatty acids, prostaglandins, leukotrienes, and biogenic amines) and exogenous (xenobiotics) substances. NADPH-cytochrome P450 reductase serves as a universal redox partner for microsomal cytochrome P450. Considerable quantitative predominance of microsomal cytochrome P450 compared to CPR is important feature of cytochrome P450-dependent systems. It is supposed that aggregation of cytochrome P450, i.e. various availability to redox partner, could minimize the effect of NADPH-cytochrome P450 reductase insufficiency on catalytic activity of cytochrome P450. Formation during catalysis of multienzyme complexes suggested the presence of multiple protein–protein interactions via multiple binding sites. Indeed, it is demonstrated for a number of microsomal cytochromes P450 involved in drug metabolism that catalytic activity of one cytochrome P450 isoform can be affected by the presence of another cytochrome P450 isoform in a reconstituted system as a result of heteromeric complex formation. Existence of protein–protein interactions between different cytochrome P450 isoforms was shown for the pairs of human cytochrome P450 CYP2C9–CYP2C19 [27] and CYP2A6–CYP2E1 [28], rabbit CYP1A2–CYP2B4 [26] and CYP1A2–CYP2E1 [29], human CYP3A4–CYP1A2 [30], and human CYP2C9–CYP2D6 [31]. There are a number of factors that cause the changes in catalytic activity of cytochrome P450 in the presence of other heme protein isoforms. Competition for the interaction with CPR plays a significant role, but it is not the only mechanism. Ionic interactions, conformational changes of the cytochrome P450 structure, changes in substrate-binding site and CPR-binding site, competition for substrate, and formation of heteromeric complexes exhibiting activity that differs from the monomers are considered as potential mechanisms of protein–protein interactions between various cytochrome P450 isoforms [25].

In the present study we attempted to elucidate the molecular interactions between cytochromes CYP17 and CYP21 located in the endoplasmic reticulum of adrenal cortex and involved in corticoid hormone biosynthesis. This work is important due to the absence of reliable data about molecular interactions between microsomal cytochromes P450 involved in steroid hormone biosynthesis in endoplasmic reticulum membranes. Moreover, study of protein–protein interactions between microsomal cytochromes P450 involved in steroid hormone biosynthesis gives the possibility to carry out complex assessment of drug influence on enzymatic systems, including the enzymatic systems involved in steroid hormone biosynthesis.

We have shown for the first time that addition of CYP17 to the reconstituted system causes a 23% decrease in CYP21 catalytic activity, while CYP21 addition to reconstituted system leads to 40% decrease in CYP17 catalytic activity at equimolar ratio of proteins in the reaction mixture. If CPR concentration was reduced to the ratio 1 : 0.5 (against cytochrome P450), the presence of CYP17 caused 42% decrease in CYP21 activity, while the presence of CYP21 caused 54% decrease in CYP17 activity. Almost no inhibitory effect of CYP17 on CYP21 catalytic activity was observed when CPR concentration was raised to the ratio 1 : 2, but such effect was not noted for CYP17 (35% decrease in activity).

Thus, specific interaction at the molecular level and interference between CYP17 and CYP21 was demonstrated for the first time. Dependence of the protein effect on catalytic activity on concentration of redox partner – NADPH-cytochrome P450 reductase – was also shown.

The effect of solution ionic strength on proteins interaction was analyzed. Increase in ionic strength decreased the inhibitory effect. This fact indicates a significant role of electrostatic forces in the interaction between the proteins. The interaction was also studied at the physiologic ratio of the proteins.

Molecular modeling of CYP17 three dimensional structure and analysis of the recently obtained CYP21 crystal structure [32] makes it possible to elucidate structural elements involved in the interaction between these proteins. It can be hypothesized, based on analysis of the distribution of amino acid residues on the surface of the proteins, that positively charged amino acids of α-helices B and C of CYP17 and negatively charged amino acids of α-helices F, G, I and DE loop of CYP21 are involved in interaction, as well as negatively charged amino acids of the BC-loop and β3-sheet of CYP17 and positively charged amino acids of α-helices F and G of CYP21 (Fig. 4; see color insert). It should be mentioned that CYP17 and CPR interaction region [33-35], located at the proximal surface of CYP17, and the region of interaction between CYP17 and CYP21 are partly overlapping (in the region of α-helix C). The CYP21 and CYP17 interaction region, located on the distal surface of CYP21, does not overlap with the redox partner interaction region [32, 36]. This can explain significant decrease in CYP17 catalytic activity in the presence of CYP21. The crystal structure of CYP17 is not available, so the models used are not precise enough and there is no possibility to study the energy of the complex and to model docking. It is necessary to get more data based on crystal structures and results of site-directed mutagenesis of amino acids involved in the interaction.

Protein–protein interactions between CYP17 and CYP21 are an additional mechanism of fine regulation of corticoid hormone biosynthesis. CYP17 is a unique enzyme due to its ability to catalyze two different types of reactions using the same active site. CYP17 may direct steroid hormone biosynthesis pathways either to the production of mineralocorticoids, glucocorticoids, or sex hormones. One of the possible assumptions explaining functional importance of the dependence of catalytic activity of CYP17 on the presence of CYP21 is that CYP21 may regulate production of corticoid hormones and could impede sex hormone biosynthesis when coexpressed with CYP17 in adrenal cortex. Adrenal cortex is morpho-functionally divided for three layers: zona glomerulosa (produces mineralocorticoids), zona fasciculata (produces glucocorticoids), and zona reticularis (produces sex hormones). There is a minimal concentration of CYP17 in zona glomerulosa. Inhibition of CYP17 activity in the presence of CYP21 may redirect the hormone biosynthesis pathway to mineralocorticoid production. Co-expression of CYP17 and CYP21 is observed in zona fasciculata. The 17α-hydroxylase activity dominates in the absence of cytochrome b_5, and sex hormones are produced in negligible amounts. Inhibition of CYP17 activity in the presence of CYP21 favors the maximal predomination of glucocorticoid synthesis. CYP21 is not expressed in zona reticularis, so 17,20-lyase activity of CYP17 dominates. CYP17 17,20-lyase activity is stimulated in the presence of cytochrome b_5. In addition, the 17,20-lyase activity can be regulated by the available CPR concentration [37].

In summary, a balanced system regulating adrenal cortex hormones biosynthesis is formed. Taking into account the importance of these enzymes in steroid hormone biosynthesis, further studies on the effect of protein- and non-protein effectors, including specific CYP17 inhibitors used as therapeutics, on the given enzyme complexes are needed.

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REFERENCES

1. Forest, M. G. (2004) *Hum. Reprod. Update*, **10**, 469-485.
2. Yanase, T. (1995) *J. Steroid Biochem. Mol. Biol.*, **53**, 153-157.
3. Hall, P. F. (1991) *J. Steroid Biochem. Mol. Biol.*, **40**, 527-532.
4. Kominami, S., Ochi, H., Kobayashi, Y., and Takemori, S. (1980) *J. Biol. Chem.*, **255**, 3386-3394.
5. Dufau, M. L., Minegishi, T., Buczko, E., Kitamura, M., Delgado, C., and Namiki, M. (1989) *Ann. N. Y. Acad. Sci.*, **564**, 57-76.
6. Zhang, L. H., Rodriguez, H., Ohno, S., and Miller, W. L. (1995) *Proc. Natl. Acad. Sci. USA*, **92**, 10619-10623.
7. Miller, W. L. (2005) *Endocrinology*, **146**, 2544-2550.
8. Miwa, G. T., West, S. B., Huang, M. T., and Lu, A. Y. (1979) *J. Biol. Chem.*, **254**, 5695-5700.
9. Estabrook, R. W., Franklin, M. R., Cohen, B., Shigamatzu, A., and Hildebrandt, A. G. (1971) *Metabolism*, **20**, 187-199.
10. Yanagibashi, K., and Hall, P. F. (1986) *J. Biol. Chem.*, **261**, 8429-8433.
11. Onoda, M., and Hall, P. F. (1982) *Biochem. Biophys. Res. Commun.*, **108**, 454-460.
12. Hlavica, P., Schulze, J., and Lewis, D. F. (2003) *J. Inorg. Biochem.*, **96**, 279-297.
13. Ogishima, T., Kinoshita, J. Y., Mitani, F., Suematsu, M., and Ito, A. (2003) *J. Biol. Chem.*, **278**, 21204-21211.
14. Min, L., Strushkevich, N. V., Harnastai, I. N., Iwamoto, H., Gilep, A. A., Takemori, H., Usanov, S. A., Nonaka, Y., Hori, H., Vinson, G. P., and Okamoto, M. (2005) *FEBS J.*, **272**, 5832-5843.
15. Conley, A. J., and Bird, I. M. (1997) *Biol. Reprod.*, **56**, 789-799.
16. Nishihara, K., Kanemori, M., Kitagawa, M., Yanagi, H., and Yura, T. (1998) *Appl. Environ. Microbiol.*, **64**, 1694-1699.
17. Harnastai, I. N., Gilep, A. A., and Usanov, S. A. (2006) *Protein Expr. Purif.*, **46**, 47-55.
18. Gilep, A. A., Guryev, O. L., Usanov, S. A., and Estabrook, R. W. (2001) *Biochem. Biophys. Res. Commun.*, **284**, 937-941.
19. Omura, T., and Sato, R. (1964) *J. Biol. Chem.*, **239**, 2370-2378.
20. Porter, T. D., Wilson, T. E., and Kasper, C. B. (1987) *Arch. Biochem. Biophys.*, **254**, 353-367.
21. Rehman, K. S., Carr, B. R., and Rainey, W. E. (2003) *J. Soc. Gynecol. Investig.*, **10**, 372-380.
22. Pechurskaya, T. A., Lukashevich, O. P., Gilep, A. A., and Usanov, S. A. (2008) *Biochemistry (Moscow)*, **73**, 806-811.
23. Shimada, T., Misono, K. S., and Guengerich, F. P. (1986) *J. Biol. Chem.*, **261**, 909-921.
24. Gardiner, S. J., and Begg, E. J. (2006) *Pharmacol. Rev.*, **58**, 521-590.
25. Backes, W. L., and Kelley, R. W. (2003) *Pharmacol. Ther.*, **98**, 221-233.
26. Backes, W. L., Batie, C. J., and Cawley, G. F. (1998) *Biochemistry*, **37**, 12852-12859.
27. Hazai, E., and Kupfer, D. (2005) *Drug Metab. Dispos.*, **33**, 157-164.
28. Tan, Y., Patten, C. J., Smith, T., and Yang, C. S. (1997) *Arch. Biochem. Biophys.*, **342**, 82-91.
29. Kelley, R. W., Cheng, D., and Backes, W. L. (2006) *Biochemistry*, **45**, 15807-15816.
30. Yamazaki, H., Gillam, E. M., Dong, M. S., Johnson, W. W., Guengerich, F. P., and Shimada, T. (1997) *Arch. Biochem. Biophys.*, **342**, 329-337.
31. Subramanian, M., Low, M., Locuson, C. W., and Tracy, T. S. (2009) *Drug Metab. Dispos.*, **37**, 1682-1689.
32. Zhao, B., Lei, L., Kagawa, N., Sundaramoorthy, M., Banerjee, S., Nagy, L. D., Guengerich, F. P., and Waterman, M. R. (2012) *J. Biol. Chem.*, **287**, 10613-10622.
33. Nikfarjam, L., Izumi, S., Yamazak, T., and Kominam, S. (2006) *Biochim. Biophys. Acta*, **1764**, 1126-1131.
34. Auchus, R. J., and Miller, W. L. (1999) *Mol. Endocrinol.*, **13**, 1169-1182.
35. Im, S. C., and Waskell, L. (2011) *Arch. Biochem. Biophys.*, **507**, 144-153.
36. Robins, T., Carlsson, J., Sunnerhagen, M., Wedell A., and Persson, B. (2006) *Mol. Endocrinol.*, **20**, 2946-2964.
37. Miller, W. L., Auchus, R. J., and Geller, D. H. (1997) *Steroids*, **62**, 133-142.