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ACCELERATED PUBLICATION

Molecular Modeling of Human Lanosterol 14α-Demethylase Complexes with Substrates and Their Derivatives

D. V. Mukha[1], S. I. Feranchuk[2], A. A. Gilep[1], and S. A. Usanov[1]*

[1]*Institute of Bioorganic Chemistry, National Academy of Sciences of Belarus, ul. Kuprevicha 5/2, 220141 Minsk, Belarus; E-mail: usanov@iboch.bas-net.by*
[2]*United Institute of Informatics Problems, National Academy of Sciences of Belarus, ul. Surganova 6, 220012 Minsk, Belarus*

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Abstract—Lanosterol 14α-demethylase (CYP51A1) is a key enzyme in sterol biosynthesis. In humans, this enzyme is involved in the cholesterol biosynthesis pathway. The majority of antifungal drugs are aimed at the inhibition of CYP51 in fungi. To elucidate the molecular mechanisms of highly specific protein–ligand recognition, we have developed a full-atomic model of human CYP51A1 and performed docking of natural substrates and their derivatives to the active site of the enzyme. The parameters of the binding enthalpy of substrates, intermediates, and final products of the reaction of 14α-demethylation were estimated using the MMPB(GB)SA algorithm. Dynamic properties and conformational changes of the protein globule upon binding of the ligand near the active site have been investigated by the molecular dynamics method. Our studies reveal that hydroxylated intermediate reaction products have a greater affinity than the initial substrates, which facilitates the multistage reaction without accumulation of intermediate products. The contribution to the free energy of steroid ligand binding of 30 amino acids forming the substrate-binding region of CYP51A1, as well as the influence of their substitutions to alanine on the stability of the protein molecule, has been clarified using alanine scanning modeling. We demonstrate that the most serious weakening of the binding is observed in the case of substitutions Y137A, F145A, V149A, I383A, and R388A. The results of molecular modeling are in agreement with the data obtained by analysis of primary sequences of representatives of the CYP51 family.

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Key words: CYP51A1, lanosterol 14α-demethylase, molecular modeling, docking, molecular dynamics, mutagenesis, alanine scanning

Steroid hormone biosynthesis plays an essential role in growth and development of most eukaryotes as well as of some prokaryotes. Human lanosterol 14α-demethylase (CYP51A1) belongs to the superfamily of heme proteins called cytochrome P450. To subfamily CYP51 also belong enzymes participating in the biosynthesis of steroids (Fig. 1a). CYP51 is a single subfamily of cytochrome P450 whose representatives are found in all kingdoms of living organisms. The structure of CYP51 demonstrates a high degree of conservatism [1]. More than 174 protein sequences belonging to CYP51 subfamily can now be found in the NCBI RefSeq database. Lanosterol 14α-demethylase in humans is involved in cholesterol biosynthesis (Fig. 1a). It has also been shown that the product of the reaction of lanosterol 14α-demethylation (FF-MAS) regulates gamete maturation, thus affecting the functioning of the reproductive system [2].

The important role of CYP51 in biosynthesis of steroids makes it suitable target in the development of drugs, species selectively inhibiting its activity [3]. Sterol 14α-demethylase is vitally important enzyme for fungi (Fig. 1a), since it participates in biosynthesis of the component of cell membrane—ergosterol [3].

CYP51 is of a great importance as a target against the protozoa causing some infection diseases. Inhibitors of the new generation now being developed as well as the recently marketed drug posaconazole are able to suppress the development of *Trypanosoma cruzi in vivo* (the agent of Chagas disease) by inhibiting CYP51 activity of the parasite [4]. The possibility of applying cytochrome P450 inhibitors against *Trypanosoma brucei* (the cause of sleeping sickness) and some species of the *Leishmania* [5] is promising.

Abbreviations: CYP, cytochrome P450; MMPB(GB)SA, molecular dynamics/the model of Poisson–Boltzmann (or Generalized Born model)/surface area.

* To whom correspondence should be addressed.

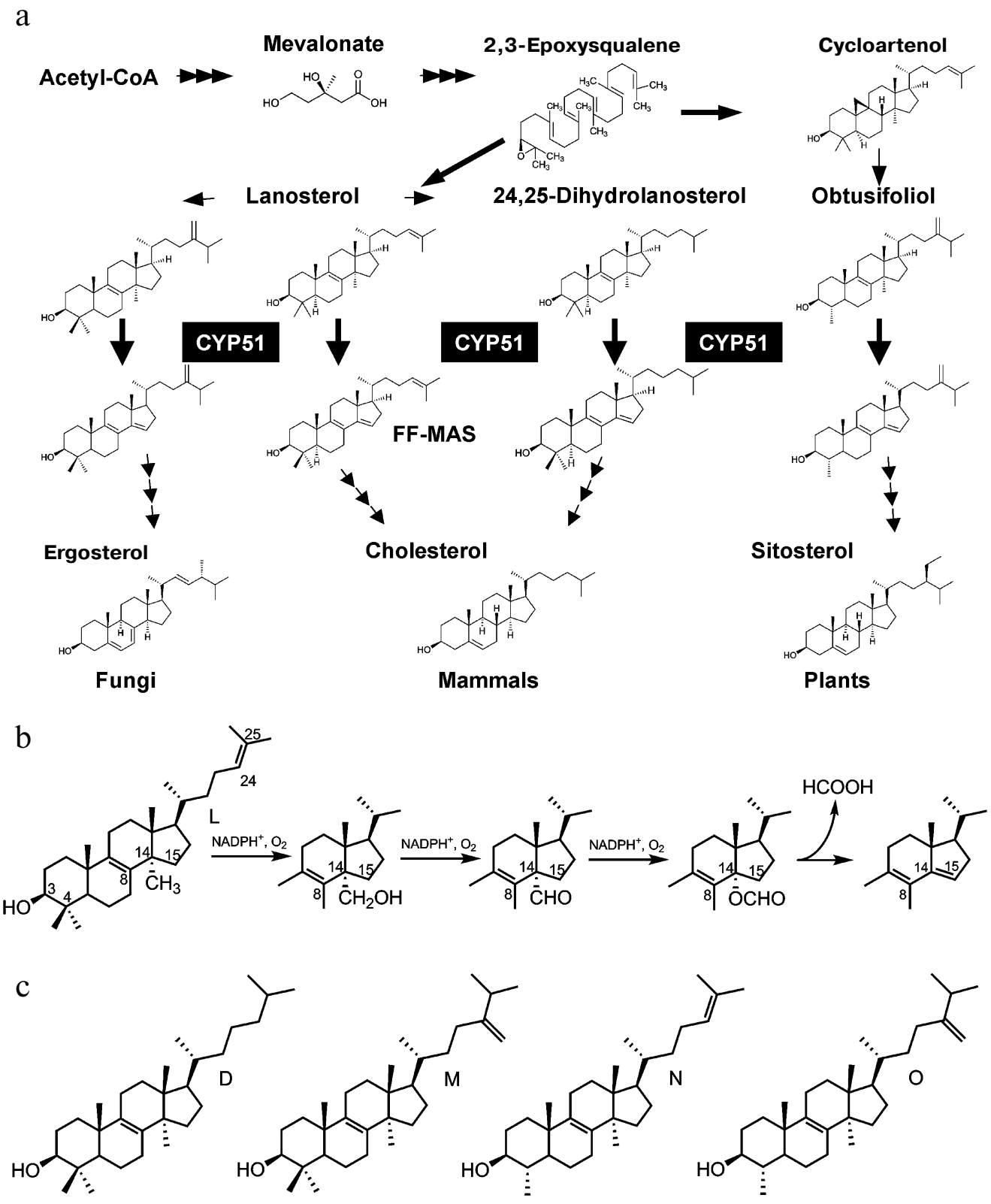

Fig. 1. a) Role of CYP51 in biosynthesis of sterols in fungi, mammals, and plants; b) mechanism of reaction of sterol 14α-demethylation catalyzed by CYP51; c) natural substrates of the enzyme: L, lanosterol; D, 24,25-dihydrolanosterol; M, 24-methylene dihydrolanosterol (eburicol); N, norlanosterol; O, obtusifoliol.

CYP51 is considered a promising target for suppression of *Mycobacterium tuberculosis* infection [6]. However, a very important goal is increasing the selectivity of inhibitors to avoid simultaneous effect of a drug on CYP51 of both parasite and host.

Most currently used inhibitors of CYP51 suppress the activity of the human enzyme. Knowledge of structural peculiarities of human CYP51 is of a principle importance for the development of new drugs directed against infectious microorganisms that might demonstrate strict species specificity, eliminating the interaction with the human ortholog. Computer modeling is now a powerful approach for studying the protein targets of pharmaceutical compounds [7]. Docking of inhibiting compounds to the protein active site of CYP51 is used to explain their antifungal activity [8]. Computer modeling of CYP51 was successfully used to explain the effect of the substitution of some amino acid residues in the enzyme from *Mycosphaerella fijiensis* [9] and to characterize the active site of the CYP51 from *Cryptococcus neoformans* [10]. Theoretical models of CYP51 built by homology modeling were successfully used for screening of azole inhibitors interacting with CYP51 from *Candida albicans* [11-13], *Aspergillus fumigatus* [12], and *Penicillium digitatum* [14].

CYP51 catalyzes the reaction of methyl group cleavage at position C_{14} of lanosterol or its analogs that results in double bond formation at position 14 of the steroid molecule (Fig. 1b). In humans lanosterol and 24,25-dihydrolanosterol serve as substrates for CYP51. Experiments *in vitro* have shown that human lanosterol 14α-demethylase catalyzes reaction with participation of 24-methylene dihydrolanosterol, norlanosterol, and obtusifoliol [15]. The reaction of lanosterol 14α-demethylation is multi-step process that consists of three sequential oxidation reactions (Fig. 1b). It is thought that initially substrate is converted to the 30-hydroxy- and then to the 30-aldehyde derivative. As a result of the third oxidation reaction, the 14-formyloxy-derivative is formed, which spontaneously decomposes to form the final demethylation reaction product and formic acid [16]. Overall in the course of the reaction three molecules of O_2 and three molecules of NADPH are consumed.

The aim of the present studies was to elucidate structure–function peculiarities of CYP51A1 that determine the interaction of the enzyme with substrates and their derivatives in the region of the enzyme active site. For this purpose, we docked substrates and their derivatives formed during the sterol 14α-demethylation reaction to the active site of CYP51A1 using physical force field. The structures of the complexes were optimized using molecular dynamics calculations. The data of molecular dynamic optimization were used to calculate enthalpy of interaction of enzyme with ligand in the active site using the Poisson–Boltzmann method to take into account the energy of electrostatic interactions in the medium with alternating dielectric permeability. The role of amino acid residues in interaction with ligands of steroid nature in the active site was determined by alanine scanning modeling.

Thus, in the present work we built the full-atomic model of CYP51A1 that describes the interaction of human sterol 14α-demethylase with substrates and their derivatives and predicts the character of conformational changes taking place in the protein during complex formation with ligand. Using molecular dynamics, we calculated the energy parameters for complexes of CYP51A1 with steroids that cannot be obtained based on the static model. Since the interaction of CYP51A1 with substrate in the substrate-binding region is extremely important for formation of tight complexes of cytochrome P450 and inhibitors [3], the approach used in the present work can be used to estimate the affinity of tested compounds to the enzyme target.

MATERIALS AND METHODS

Computer modeling of the interaction of the enzyme with low molecular weight ligands includes several steps: molecular dynamics of the ligands, enzyme structure minimization, docking of ligands into the active site of CYP51A1, molecular dynamics of the complexes, and estimation of the energy of complex formation.

The tertiary model of CYP51A1 was built based on the data found in the RCSB Protein Data Bank (3LD6). As ligands we used lanosterol, 24,25-dihydrolanosterol, 24-methylene-dihydrolanosterol (eburicol), norlanosterol, obtusifoliol, and their hydroxy-, aldehyde-, and formyloxy-derivatives, as well as the products of the reactions of their 14α-demethylation, in all 25 compounds (Fig. 1). To create the models of ligands, we used the force field GAFF (General Amber force field). Algorithm AM1-BCC [17, 18] was used to reproduce electrostatic potential of the ligand molecule. Force field FF03 was used for parameterization of the protein part of the heme protein. The prosthetic group of CYP51A1 was parameterized according to the data of Oda and coauthors [19] for the high-spin state of the heme ($S = {}^5/_2$).

Docking was performed using the UCSF Dock v.6.3 program. The algorithm of flexible docking demonstrated insufficient efficiency since it resulted in partial overlapping of ligand atoms during the search for energetically beneficial complex structure. In this connection we used an algorithm of rigid docking for multiple ligand conformations. Ligand conformations were obtained by clusterization of structures from the trajectory of molecular dynamic with duration 1 ns. Docking was carried out in two steps. In the first step molecules were placed into the active site of protein using potential taking into account van der Waals and electrostatic interactions (grid score). The conformations obtained were sorted using scoring function GB/SA [20].

From the structures obtained as a result of docking of complexes, ligand conformations having minimal potential energy and satisfying criteria of functional positioning were chosen. For the latter, we choose the ultimate distance of 6 Å from heme iron to the center of oxidation in the substrate (methyl radical at C_{14} or functional group replacing it).

Molecular dynamics of the complexes of cytochrome CYP51A1 with substrates and their analogs was carried out in Amber 10 [21]. Molecular complexes were subjected to relaxation during 1 ns (without counting time spent for heating the system to 300 K). The integration step did not exceed 2 fs. Molecular dynamics was performed with application of implicit solvent GB (Generalized Born) with cut-off distance for electrostatic interactions of 15 Å and using the Langevin thermostat. During the following 500 ps of molecular dynamics after equilibration, snapshots of the system were recorded for calculation of enthalpy (ΔH) using the MMPB(GB)SA method [22]. The data for calculation of thermodynamic parameters of receptor, ligand, and complex were extracted from a single molecular-dynamic trajectory.

Alanine scanning was modeled using the Amber 10 program. For alanine scanning we used amino acid residues whose atoms in molecular dynamic experiments were able to form contacts with the steroid substrate molecule. The following amino acid residues were included in this list (hereinafter amino acid numbering is according record NP_000777 of the NCBI RefSeq database): V136, Y137, L140, T141, F145, V149, Y151, F158, Q161, K162, L165, F240, H242, W245, L291, M310, L314, L316, H320, T312, I383, M384, I385, M386, M387, R388, I456, M493, I494, H495. All together 30 amino acid residues were chosen. Due to dynamic plasticity of the protein molecule, the list includes amino acid residues, which do not have direct access to the substrate-binding cavity according to the crystal structure but sporadically become close to the substrate during molecular dynamics.

Protein sequences of cytochrome P450 belonging to the CYP51 family were aligned using the MUSCLE algorithm [23, 24]. For alignment we used amino acid sequences from the RefSeq database having at least 36% identity with the sequence of human CYP51A1 (NP_000777) and degree of overlapping more than 66%. A total of 174 amino acid sequences corresponding to the chosen criteria were found in the database. The minimum rating BLASTP 2.2.24 under comparison with the amino acid sequence of human CYP51A1 was 170 points. All sequences used for alignment are identical based on the position of a cysteine residue forming a coordination bond with the heme iron. On the basis of the alignment, we built a phylogenetic tree in ClustalX v.2.11.

Calculations necessary for docking and molecular dynamics were performed using the SKIF cluster supercomputer at the United Institute of Informatics Problems of the National Academy of Sciences of Belarus.

RESULTS

Docking of the substrates and their derivatives to the active site of CYP51A1. The resolution of the tertiary structure of substrate-free human CYP51A1 and in a complex with azoles did not reveal the nature of substrate-binding site of the heme protein, while attempts to crystallize CYP51A1 in a complex with substrates have been unsuccessful. Therefore, we used the approach consisting of modeling of the complex of CYP51A1 with substrates and its analogs. Docking resulted in monotype distribution close to the heme of all 25 molecules studied (Fig. 2; see color insert). The substrate-binding site of CYP51A1 is formed by α-helices B′, C, I, loops B′-C, F-G, as well as polypeptide chain fragments after α-helix K and between elements of β5 (nomenclature of the elements of secondary structure is shown in accordance with the work of Poulos et al. [25]). The distance between heme iron and carbon atom C_{30} of the steroid molecule is varied from 4.5 to 6 Å. The hydroxyl group at the carbon atom in the third position of the steroid molecule is faced in the direction of the substrate access channel to the active site. The cavity formed by α-helices B′ and C comprises the carbon atoms of the aliphatic side chain of the steroid molecule. Comparison of the crystal structures of CYP51A1 without ligand (3JUV), in complex with ketoconazole (3LD6), and in complex with econazole (3JUS) indicates that the enzyme undergoes significant conformational changes during complex formation with the ligand. The presence of the cavity comprising the side chain atoms of the steroid molecule is characteristic for the structures of CYP51 in a complex with azole-containing inhibitors. In substrate-free structure, due to mobility of B′-C loop, this cavity is reduced and is no longer connected with the heme pocket. The methyl group at carbon atom 14 is located near the heme iron (distance less than 6 Å). Substrate in the active site is surrounded mostly by hydrophobic amino acid residues. Residue L140, located in α-helix B′, forms a contact with the β-methyl substituent at carbon atom 4. The hydroxyl group at carbon 3 is able to form hydrogen bonds with the oxygen atom of isoleucine 385 and nitrogen of methionine 386.

Since existing docking algorithms do not take into account the movement of atoms of the enzyme molecule, we used molecular dynamics in which complexes were moved in the phase space in the direction to the energy minimum.

Quality of models. Quality control of the created models was performed at each step of the modeling process. We tested the possibility of overlapping of atoms and stereoisomerization of amino acid residues. The models obtained after molecular dynamics were further investigated for the correspondence of the parameters of molecular structure to the values known from experiments on X-ray crystal structure resolution. The reaching of relaxation during the molecular dynamics was deter-

mined based on achieving a plateau of parameters of root mean square deviation of C_α-atoms of amino acids ($RMSDC_\alpha$) and potential energy of the system. To estimate the adequacy of the models used, we compared the minimized structures taken at the end of each calculated molecular dynamic trajectories and the structures of human CYP51A1 resolved by X-ray analysis [3] by PROCHECK program [26, 27]. The structures were further analyzed based on distribution of amino acids on the Ramachandran map (Table 1) and the values of G-factor (Table 2). No steric overlapping of atoms or stereoisomerization of amino acids was found in the models. The model is characterized by slightly lower G-factor value as compared to structures taken from PDB. This appear to be connected with the fact that structures resolved using crystallography are characteristic equilibrium, averaged values of bond lengths, values of flat and dihedral angles, while the models represent a "snap-shot" of a dynamic system. The distribution of φ- and ψ-angles of amino acids in models differs insignificantly from that in structures resolved by crystallographic analysis. On average, the models are not inferior in quality to the structures obtained experimentally.

Enthalpy of complex formation with ligands. The affinity of enzyme to substrate, together with the reaction rate, is one of the parameters characterizing its catalytic properties. The criterion of affinity is the Gibbs free energy including two components: enthalpy and term that depends on enthalpy. Accounting for the latter in the frame of the molecular-mechanic model is difficult since in that approximation the model is initially devoid of many parameters that determine normal modes. It is thought that the difference in the entropy changes during interaction with similar ligands is rather small and might not be taken into account. We estimated entropy for interaction of CYP51A1 with steroid ligands that serve as the substrates for human lanosterol 14α-demethylase, their derivatives formed during the demethylation reaction, and the reaction products. According to the obtained data, the ability of ligand to interact with sub-

Table 1. Distribution of amino acids among regions of the Ramachandran plot

Structure	Ramachandran map			
	main region, %	additional region, %	generously allowed region, %	disallowed region, %
3JUS, chain A	90.2	9.5	0.0	0.3
3JUS, chain B	89.5	10.3	0.0	0.3
3JUV	89.5	9.7	0.5	0.3
3LD6, chain A	92.0	7.7	0.0	0.3
3LD6, chain B	92.3	7.5	0.0	0.3
Worst values for models	88.4	11.3	1.3	0.5

Table 2. Values of G-factor

Structure	G-factor		
	dihedral angles	covalent bonds	total value
3JUS, chain A	–0.16	0.52	0.10
3JUS, chain B	–0.25	0.53	0.06
3JUV	–0.32	0.50	0.01
3LD6, chain A	–0.12	0.53	0.14
3LD6, chain B	–0.19	0.55	0.10
Worst values for models	–0.24	0.15	–0.07

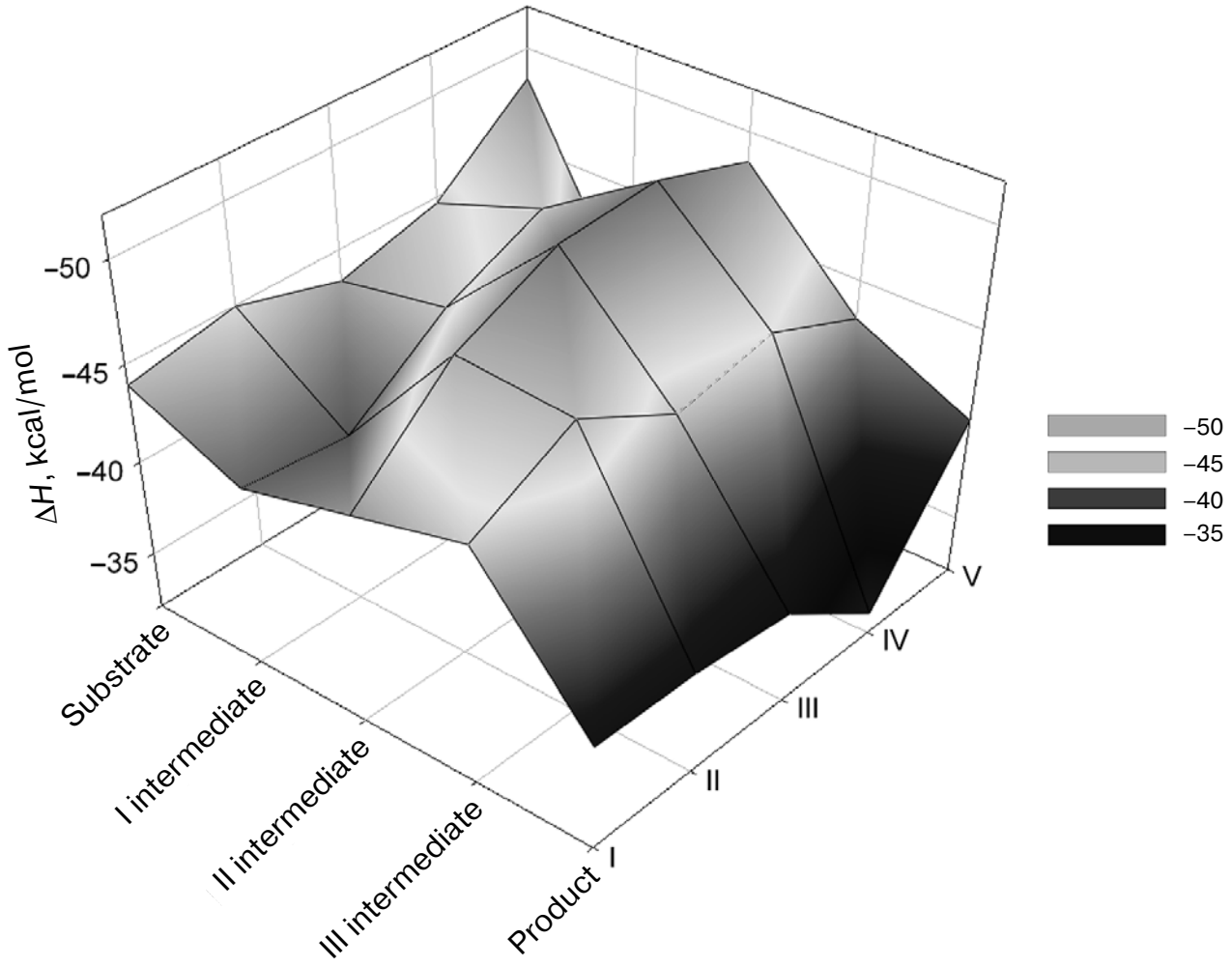

Fig. 3. Changes in strength of ligand binding in the active site of CYP51A1 during the reaction of sterol 14α-demethylation. I, O; II, L; III, D; IV, M; V, N. The order of substrates was chosen arbitrarily.

strate-binding site of lanosterol 14α-demethylase changes during the reaction (Fig. 3). The highest absolute values of the enthalpy of interaction is found for aldehyde derivatives of the substrates.

The main contribution to the energy of complex formation between enzyme and substrates and their derivatives are indirect disperse interactions and effects of desolvation. Hydrophobicity of the initial substrates (parameter logP) and reaction products do not differ dramatically. Incidentally, the created molecular-mechanic model is able to predict a sharp decrease in the affinity of enzyme to products of reaction of sterol 14α-demethylation.

Alanine scanning. To determine the contribution of amino acids to the interaction with ligands of steroid nature, we modeled the effect of substitution of these amino acids for alanine. Based on results on the alanine scanning modeling, we obtained data on changes in enthalpy of interaction of the enzyme ($\Delta\Delta H$) with the ligands (Fig. 4; see color insert). The most evident decrease in binding is observed in the case of substitutions Y137A, F145A, V149A, I383A, and R388A. Substitutions L140A, L165A, F240A, L314A, and I494A result in moderate impairment of binding affinity.

For substitutions T141A, Q161A, H242A, H320A, T321A, R388A we observed destabilization of the protein globule, which is reflected in an increase in potential energy of the molecule. The index shown in Fig. 5 reflects changes in the potential energy landscape due to which a mutant form will possess different conformational potential energy characteristic from the native enzyme.

For many polar and charged amino acid residues $\Delta\Delta H$ is below zero. This indicates that substitution of this amino acid for nonpolar alanine favors contacts with hydrophobic ligands in the binding region. However, side chains of amino acids T141, Y151, Q161, H320, T321, and H495 participate in formation of hydrogen bonds stabilizing the structure of the protein globule. The replacement these amino acid residues decreases the overall stability of the

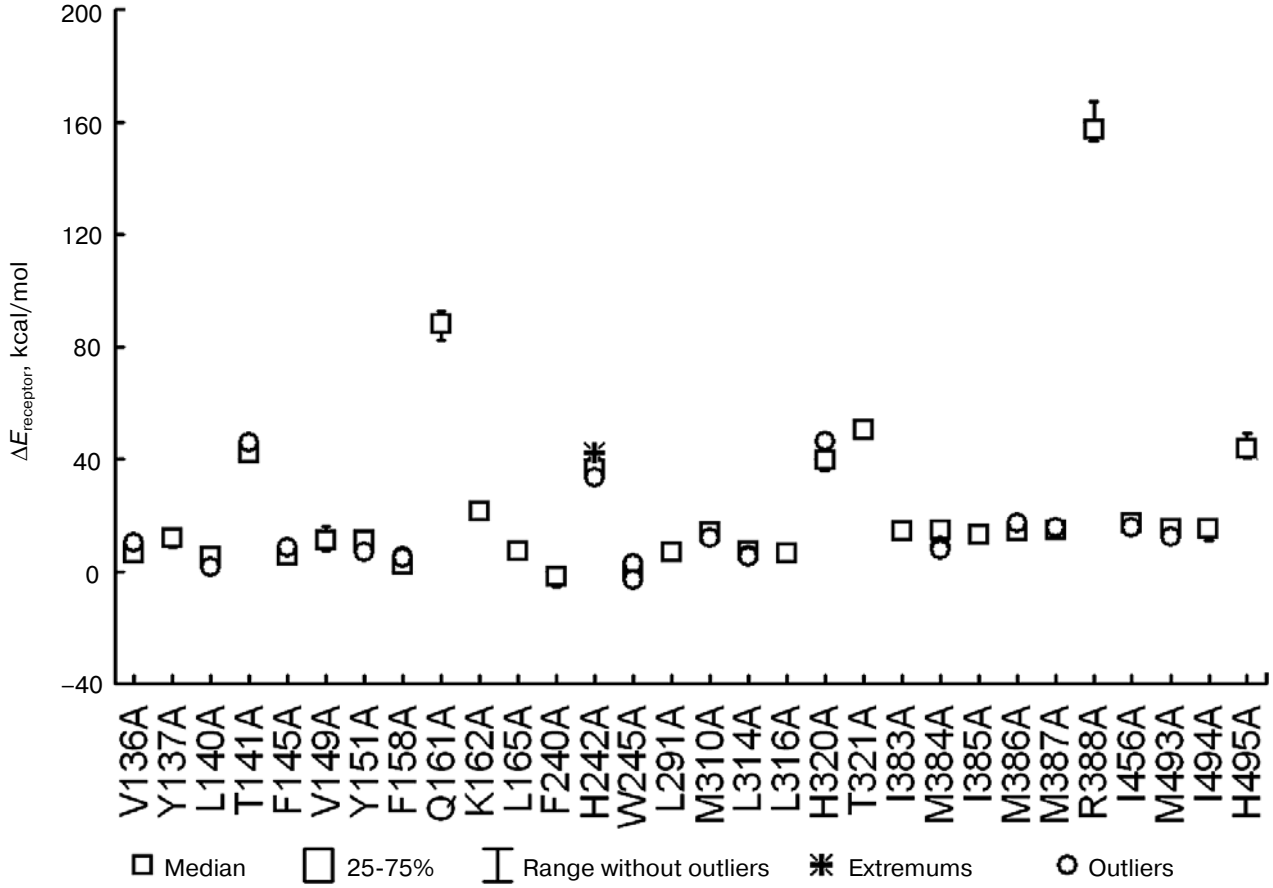

Fig. 5. Changes in potential energy of CYP51A1 due to substitutions of amino acids for alanine.

protein globule (Fig. 5). Residue H320 forms a salt bridge with residue D237 creating an interaction of α-helices I and F. The side chain of residue H240 in the presence of substrate is exposed at the protein surface and therefore its substitution to alanine increases the potential energy of the molecule. It is interesting that substitutions H320A and H495A increase the affinity to the final reaction products more than to substrates and intermediate reaction products. Consequently, these substitutions may be negatively reflected on the kinetics of the demethylation reaction.

The effect of each substitution of an amino acid for alanine does not exceed 4 kcal/mol. Besides the effect on the binding enthalpy in the native conformation, substitutions might result in displacement of the global minimum of potential energy of the protein molecule in the phase space, but to study this phenomenon one need additional resource-consuming investigation.

DISCUSSION

As follows from the data presented in this work, the orientation of steroid molecules in the substrate-binding site of human CYP51A1 coincides in detail with the arrangement of estradiol in the active site of CYP51 from *Mycobacterium tuberculosis* [28]. However, in the active site of human CYP51A1 the substrates are located much closer to the heme and α-helix I. As follows from the model (Fig. 2), the space between ring D of the steroid molecule and the heme in the active site of human CYP51A1 is enough to host the formyloxy-group attached to C_{14}. Close to ring D of estradiol in the structure 1X8V a charged side chain of arginine is located, while in human CYP51A1 this region forms a hydrophobic pocket for interaction with the side chain of a steroid molecule. Conservative residue Y137 (human CYP51A1) is located similarly in the molecule of CYP51 from *M. tuberculosis*. The side chain of residue H320 in human CYP51A1 is declined more than for 120° compared to residue H259 in CYP51 from *M. tuberculosis*. Thus, the imidazole group of residue H259 in CYP51 of *M. tuberculosis* brings it together with the substrate molecule, while in human CYP51 it is vice versa, this group is removed from the substrate-binding site. It is suggested that this structural feature determines much lower affinity of the human enzyme to fluconazole [3]. Amino acid residue

L140 of human CYP51A1 forms a contact with the 4β-methyl group of a steroid substrate (if it is present).

Based on the molecular dynamics data, the substrate-binding region in the F-G loop and amino acid residues forming the access channel of the substrate to the active site of human CYP51A1 is most flexible. Some amino acid residues of α-helix H are characterized by some degree of mobility. These data are in agreement with the values of coefficients of Debye–Waller factor obtained by X-ray analysis. Conformational changes touching α-helixes B′ and C probably occur only in the process of complex formation with substrate or inhibitor in the active site, and after this process the enzyme structure is stabilized. As a result of complex formation with ligand in the substrate-binding site of human CYP51A1, the number of coils in α-helices F and G is increased.

Human CYP51A1 demonstrates a similar degree of affinity to all tested substrates including those found in the human body as well as those found in other living organisms, as is confirmed by *in vitro* experiments [1]. The values of enthalpy of binding determined using the models were slightly higher than might be expected from the experimental data. This appears to be connected with the hydrophobicity of the tested compounds (logP octanol–water > 5), for which existence in surrounding water molecules is energetically unfavorable. To solvate hydrophobic compounds is in practice very difficult since most of steroid compounds used for docking are almost insoluble in water (Fig. 1c). This feature of ligands results in systematically overestimated enthalpy of binding using the MMPB(GB)SA method, in which one of the terms in the formula of enthalpy determination is the energy of ligand solvation.

Based on the results of the modeling performed in the present work, we conclude that aldehyde derivatives of steroids possess higher affinity to the active site than the initial substrates, but the latter bind more tightly than the final reaction products (Fig. 5). This facilitates the flow of the multi-step reaction without accumulation of intermediate reaction products. The cleavage of the methyl group at carbon atom 14 and formation of a double bond sharply decreases affinity of enzyme to ligand, which results in liberation of the reaction product from the active site.

For comparison of the data obtained by molecular modeling of human CYP51A1 with the data of phylogenetic analysis, we aligned amino acid sequences of some representatives of the family of steroid 14α-demethylases (Fig. 6; see color insert). The majority of conservative residues undoubtedly perform important structural roles supporting the architecture of the CYP51 molecule. Conservation during evolution of amino acid residues forming a hydrophobic cavity required for interaction with substrate does not look strict, mostly due to interchangeability of hydrophobic amino acids. Nevertheless, comparison of the effects of substitution of amino acids for alanine (based on $\Delta\Delta H$) estimated using molecular modeling with the degree of conservativeness of the amino acids in proteins of the CYP51 family indicates that substitutions with maximal values of $\Delta\Delta H$ affect the most conservative residues (Fig. 7). These residues include Y137, F145, V149, I383, and R388. The degree of conservatism of these residues is higher than for other residues involved in formation of the substrate-binding site, which is in agreement with their contribution to interaction with the natural substrates of CYP51A1 determined by modeling of alanine scanning.

Residue F145 interacts with the side chain and carbon atom 18 of the steroid substrate located in the active site of CYP51A1. The substitution of this amino acid by leucine in the heme protein from *C. albicans* combined with other substitutions, in particular K143R (K162R in the sequence of human enzyme) affords resistance to pathogen with respect to fluconazole [29]. Substitution of residue Y118 in CYP51 from *C. albicans* (Y137 in human CYP51A1) sharply decreases affinity to azole inhibitors [30]. This residue is highly conservative among representatives of the family of sterol 14α-demethylases. There is experimental evidence indicating that residues Y137 and F145 are necessary for catalytic activity of human CYP51A1 [31]. Substitutions of residues Y76F and F83Y corresponding to amino acids in CYP51 from *M. tuberculosis* result in loss of ability to bind substrate [31].

Residue R388 is absolutely conserved among the members of the CYP51 family. It interacts with the propionate group of ring A of the heme. As shown using modeling of alanine replacements in the present paper, residue R388 is also important for interaction with ligands since the side chain of this amino acid strongly affects electrostatic potential in the binding region. Substitution of this residue results in both impairment of substrate binding and significant destabilization of the protein globule. Residues Y151, F158, and K162 are not strictly conservative and belong to a polypeptide chain fragment whose conformation does not coincide in CYP51 from *Mycobacterium* spp. and eukaryotes. Residues Y151 and K162 are replaced in many CYP51 from different organisms by the hydrophobic amino acid phenylalanine.

Residue I383 is extremely conserved; there are only few substitutions for the structurally similar amino acid leucine. For sterol 14α-demethylase from *Mycosphaerella graminicola*, it was shown that substitution of residue I381 (corresponding to I383 of human CYP51A1) to valine inactivates the enzyme [32], which is in agreement with our data on the structural importance of residue I383 for human CYP51A1 obtained by alanine scanning. The degree of evolutionary conservatism is demonstrated by two proline residues preceding I383 in the polypeptide chain that assure conformational interruption of α-helix K and convergence of residue 383 with the active site pocket. Residue L291 positioned more distant from the

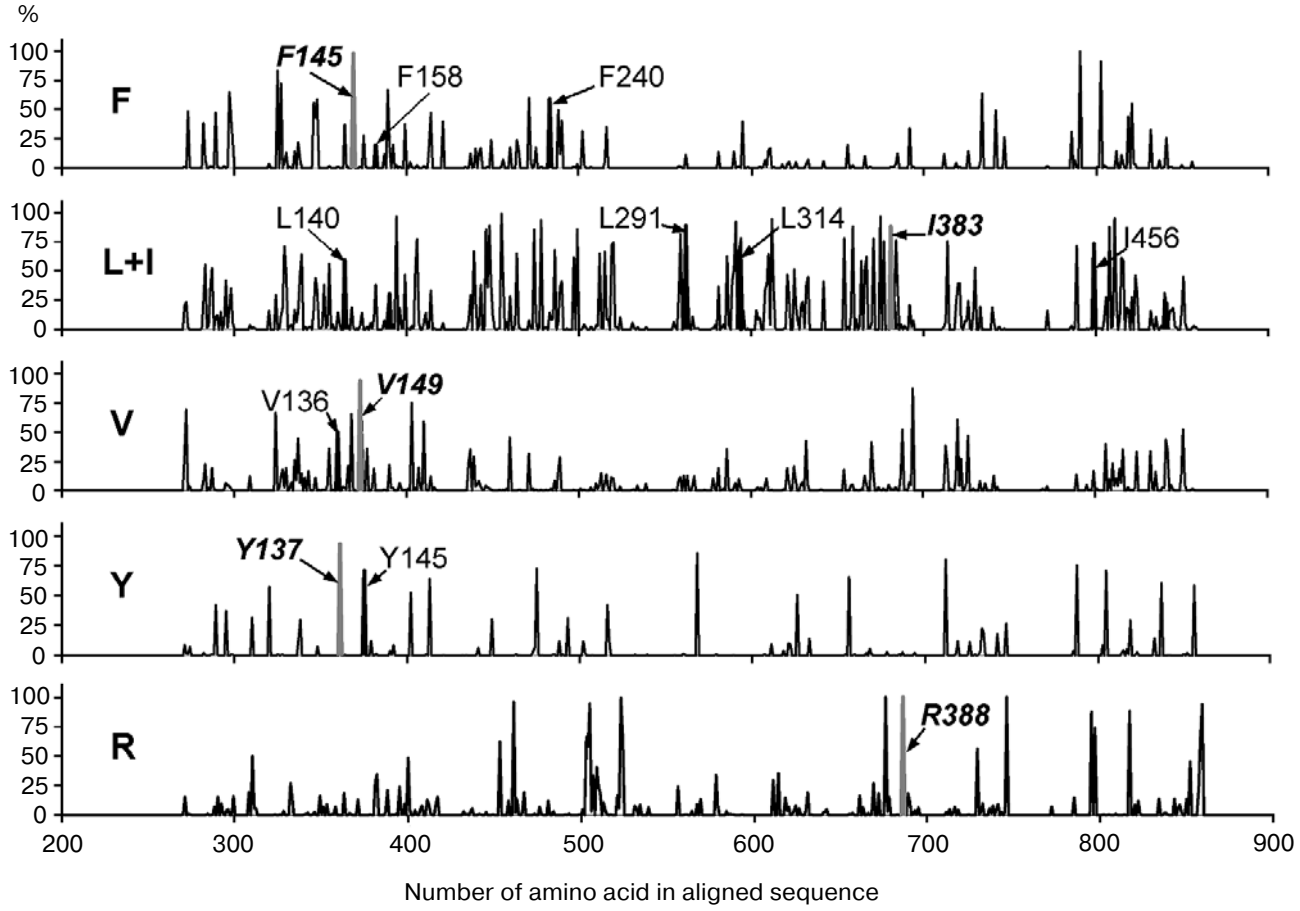

Fig. 7. Diagram of conservative amino acids in aligned protein sequences of different CYP51 molecules. Columns included in the diagram do not contain more than 5% of gaps. Leucine and isoleucine were taken as equivalent. Amino acids participating in formation of contacts with substrate in the active site of CYP51 are marked. Amino acids which substitution by alanine results in the most significant changes of the affinity to substrate are shown in italic.

active site demonstrates high degree of conservatism (Fig. 7) in accordance with its function—supporting mutual location of α-helices H, I, and C. This residue forms hydrophobic contacts with residues L165 (α-helix C), L302, M310, and L311 (α-helix I).

Residue L140 is characteristic for CYP51 of Chordata and fungi, while in sterol 14α-demethylases of *Trypanosoma*, plants, and the majority of bacteria this position is taken by phenylalanine [15], the side chain of which has much larger volume. The position of residue L140 in the active site of human CYP51A1 relative to steroid substrates confirms the above suggestion on the role of this residue in selectivity of CYP51 with respect to the substituent at carbon atom 4 of the steroid skeleton that determines substrate specificity of orthologs of CYP51 from different kingdoms of living organisms [15].

Substitutions of various amino acids to alanine are not always critically reflected in the overall structure of CYP51. For example, substitution C442A in CYP51 from *M. tuberculosis* (PDB nomenclature 1U13) does not change the elements of secondary structure of β5. The $RMSD_{C\alpha}$ between corresponding elements of the mutant heme protein and the wild type enzyme is only 0.137 Å [28].

The substitution Y136F in CYP51 from *Histoplasma capsulatum* decreases the sensitivity of the microorganism to voriconazole and fluconazole [33]. The similar substitution Y132F in CYP51 from *C. albicans* is associated with an increase in the resistance of the pathogen to fluconazole [29]. This position corresponds to residue Y141 in human CP51A1, which according to the model data directly interacts with ligands in the active site. The nonessential character of this substitution for vitality of the microorganism may be explained by the fact that phenylalanine in that position is found among different representatives of living organisms.

There are some data that indicate that substitution P230L (loop F-G) in CYP51 from *C. albicans* is associated with increased resistance of this microorganism to high molecular weight azole inhibitors such as posaconazole and itraconazole [12]. This may be connected with the geometry of these molecules, due to which during com-

plex formation the inhibitor occupies some part of the substrate access channel in the active site. The substitution of the corresponding residue H242 in human CYP51A1 to alanine weakly affects the affinity of the enzyme to steroid molecules (Fig. 4) since they are completely accommodated in the cavity close to the heme. Due to this, a similar mutation in CYP51 of *C. albicans* does not affect the wild type activity of sterol 14α-demethylase and increases vitality of microorganisms in the presence of the inhibitor.

The nature of the amino acid at position 385 (isoleucine) is variable. In fungi and some other eukaryotes this amino acid is replaced by threonine, serine, or glutamine. The M493/I494 residue pair is moderately conserved: among various organisms these residues are sometimes replaced by hydrophobic amino acid residues such as leucine, valine, or phenylalanine.

Results of the computer modeling are in a good agreement with experimental data and are confirmed by analysis of the alignment of the amino acid sequences of the representatives of the CYP51 family. The detailed description of the architecture of the active site of human CYP51A1 is of great significance for rational strategy of the development of effective new drugs not having side effects.

Thus, in the present work we developed a full-atomic model describing the interaction of human sterol 14α-demethylase with substrates and their derivatives. The model predicts the character of conformational changes taking place in the protein globule during complex formation with a ligand. Using molecular dynamics we determined thermodynamic parameters of the molecule that cannot be obtained based on the static model. The approach used in this paper allows estimation of the effect of particular amino acid residues on the thermodynamic parameters of interaction of human CYP51A1 with its substrates and their derivatives. The model of human CYP51A1 is useful for estimation of the affinity of inhibitors to the enzyme. An application of calculation methods for determination of the affinity of small organic molecules to isoforms of CYP molecules will allow early determination of possible side effects of drugs on the human organism as a result of inhibition of the activity of cytochrome P450-dependent monooxygenase systems.

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