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Role of Positively Charged Residues Lys267, Lys270, and Arg411 of Cytochrome P450scc (Cyp11A1) in Interaction with Adrenodoxin

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Abstract—Cytochrome P450scc and adrenodoxin are redox proteins of the electron transfer chain of the inner mitochondrial membrane steroid hydroxylases. In the present work site-directed mutagenesis of the charged residues of cytochrome P450scc and adrenodoxin, which might be involved in interaction, was used to study the nature of electrostatic contacts between the hemeprotein and the ferredoxin. The target residues for mutagenesis were selected based on the theoretical model of cytochrome P450scc–adrenodoxin complex and previously reported chemical modification studies of cytochrome P450scc. In the present work, to clarify the molecular mechanism of hemeprotein interaction with ferredoxin, we constructed cytochrome P450scc Lys267, Lys270, and Arg411 mutants and Glu47 mutant of adrenodoxin and analyzed their possible role in electrostatic interaction and the role of these residues in the functional activity of the proteins. Charge neutralization at positions Lys267 or Lys270 of cytochrome P450scc causes no significant effect on the physicochemical and functional properties of cytochrome P450scc. However, cytochrome P450scc mutant Arg411Gln was found to exhibit decreased binding affinity to adrenodoxin and lower activity in the cholesterol side chain cleavage reaction. Studies of the functional properties of Glu47Gln and Glu47Arg adrenodoxin mutants indicate that a negatively charged residue in the loop covering the Fe_2S_2 cluster, being important for maintenance of the correct architecture of these structural elements of ferredoxin, is not directly involved in electrostatic interaction with cytochrome P450scc. Moreover, our results indicate the presence of at least two different binding (contact) sites on the proximal surface of cytochrome P450scc with different electrostatic input to interaction with adrenodoxin. In the binary complex, the positively charged sites of the proximal surface of cytochrome P450scc well correspond to the two negatively charged sites of adrenodoxin: the "interaction" domain site and the "core" domain site.

Key words: cytochrome P450scc, site-directed mutagenesis, heterologous expression, protein–protein interactions

Cytochrome P450scc is the terminal monooxygenase of the inner mitochondrial membrane electron transfer chain that catalyzes the conversion of cholesterol to pregnenolone. Adrenodoxin reductase and adrenodoxin are redox proteins that are directly involved in electron transfer from NADPH to cytochrome P450scc to activate molecular oxygen. Adrenodoxin transfers electrons, which it receives in turn from adrenodoxin reductase, to cytochrome P450scc. During electron transfer, the components of the electron transfer chain form specific complexes in which electrons are transferred from one redox center to another. Electrostatic interactions are predominant contacts in redox partner complex formation [1]. The intrinsic molecular mechanism of the interaction of adrenodoxin with adrenodoxin reductase and cytochrome P450scc, however, is still not clear. Site-directed mutagenesis of negatively charged residues of adrenodoxin and positively charged residues of adrenodoxin reductase or cytochrome P450scc confirms the importance of ionic contacts for the interactions [2-6].

Recently, based on site-directed mutagenesis of the positively charged cytochrome P450scc surface residues and molecular modeling of cytochrome P450scc using cytochrome P450BM3 as a template, we built a theoretical model of the complex of cytochrome P450scc and adrenodoxin [7]. According to this model and a model that was previously built based on the structure of only cytochrome P450cam [8], residue Arg411 of the proximal surface of cytochrome P450scc is spatially located very close to the "nucleus" of interaction with the redox part-

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ner. Taking into consideration the conservative nature of this amino acid residue among cytochromes P450 from different species, the distance to the proximal ligand of heme, Cys422 (10 amino acid residues), and the multipoint character of the interaction, we suggested that this residue might be important for formation and/or stabilization of the complex between adrenodoxin and cytochrome P450scc. In addition, using bifunctional reagents to covalently cross-link the complex between adrenodoxin and cytochrome P450scc and subsequent structural analysis [9] of the complex indicate that the polypeptide fragment Leu401-Trp417 of the cytochrome P450scc molecule appears to be involved in the interaction with adrenodoxin. Moreover, two (Lys403 and Lys405) of four positively charged amino acids of this fragment of cytochrome P450scc directly participate in electrostatic interaction with adrenodoxin, as confirmed by site-directed mutagenesis experiments [5].

Molecular modeling of the cytochrome P450scc–adrenodoxin complex indicates that there is a salt bridge between Glu47 of adrenodoxin and Lys267 of cytochrome P450scc. Chemical modification of cytochrome P450scc lysine residues [10] indicates the importance of Lys267 and Lys270 for interaction with adrenodoxin. On the other hand, residue Glu47 of adrenodoxin is located in the loop covering the Fe_2-S_2 cluster. The contribution of each residue of this loop to the function of adrenodoxin was estimated by sequential deletion analysis of each residue in the loop [11]. The evidence for importance of individual residue using deletion is an effective method for understanding the nature of the interaction between the proteins. However, deletion of amino acids in a significant structural site and possible interaction region do not allow discussing its role unambiguously.

To clarify the role of the above-mentioned amino acid residues in interaction and/or in orienting the best geometry of the two proteins for effective electron transfer, we are presently using the site-directed mutagenesis of these residues. Adrenodoxin mutants Glu47Gln and Glu47Arg and cytochrome P450scc mutants Lys67Gln, Lys270Gln, and Arg411Gln were constructed, expressed in *E. coli*, purified to apparent homogeneity, and characterized compared to the wild type proteins.

The results indicate that Arg411 of cytochrome P450scc directly participates in electrostatic interaction with adrenodoxin, while residue Glu47 of adrenodoxin localized in the loop near the Fe_2-S_2 cluster does not determine electron transfer properties of the ferredoxin.

MATERIALS AND METHODS

Site-directed mutagenesis. Site-directed mutagenesis of cytochrome P450scc and adrenodoxin was done using the Quick Change (Stratagene, USA) kit of chemicals with the following mutagenic primers:

cytochrome P450scc

Lys267Gln
5′-CCTCTACTGCCTCCTGCAGAGTGAGAAGAT-GCTC-3′ (sense)
GAGCATCTTCTCACTCTGCAGGAGGCAGTA-GAGG (antisense)

Lys270Gln
GCCTCCTGAAAAGTGAGCAGATGCTCTTGGAG-GATG
CATCCTCCAAGAGCATCTGCTCACTTTTCAGGA-GGC

Arg411Gln
CAAAGACCTCATCCACTTCCAGAACCTGGGC-TTTGGC
GCCAAAGCCCAGGTTCTGGAAGTGGATGAGGT-CTTTG

adrenodoxin

Glu47Gln
GGTTTTGGTGCATGTCAGGGAACCTTGGCTTG
CAAGCCAAGGTTCCCTGACATGCACCAAAACC

Glu47Arg
GGTTTTGGTGCATGTCGGGGAACCTTGGCTTG
CAAGCCAAGGTTCCCCGACATGCACCAAAACC.

The presence of desired substitution was confirmed by automatic sequencing on an A377 DNA sequencer (Applied Biosystems, USA).

Expression and purification of proteins. Recombinant proteins of the cytochrome P450-dependent monooxygenase system from bovine adrenocortical mitochondria (cytochrome P450scc, adrenodoxin, and adrenodoxin reductase) were expressed in *E. coli*. The plasmid (pTrc99A) containing cDNA encoding the mature form of cytochrome P450scc was kindly presented by Prof. M. R. Waterman (Vanderbilt University, USA). The plasmids containing cDNA encoding mature adrenodoxin (pBA1159) and adrenodoxin reductase (pBAR1607) were kindly presented by Prof. A. Sagara (Kochi Medical School, Japan).

Adrenodoxin reductase, adrenodoxin, and their mutants were purified according to published procedures [5, 12].

Isolation and purification of cytochrome P450scc (wild type and mutant forms of cytochrome P450scc) were carried out according to a scheme developed for purification of substrate-bound high-spin form of the hemeprotein [13].

Analytical methods. Analysis of protein content of recombinant bacterial cells after expression and monitoring of the purity of final preparations was done by SDS-

PAGE in 12% gel using a Mini Protean II system (Bio-Rad, USA). Immunochemical identification of recombinant proteins was done by immunoblotting analysis [14]. The concentrations of cytochrome P450scc, adrenodoxin reductase, and adrenodoxin were determined using molar extinction coefficients 91 $mM^{-1}\cdot cm^{-1}$ at 450 nm, 11 $mM^{-1}\cdot cm^{-1}$ at 450 nm, and 10 $mM^{-1}\cdot cm^{-1}$ at 414 nm, respectively [15, 16].

Spectral characterization of mutant forms of cytochrome P450scc and adrenodoxin. Spectrophotometric measurements were done using a Shimadzu UV-3000 (Shimadzu, Japan) spectrophotometer. The concentration of cytochrome P450scc and its denatured form, cytochrome P420, were determined from carbon monoxide difference spectra of the dithionite-reduced hemeprotein using molar extinction coefficients 91 $mM^{-1}\cdot cm^{-1}$ at 450 nm and 114 $mM^{-1}\cdot cm^{-1}$ at 420 nm according to the equation given in [17]. The recording of reduced carbon monoxide difference spectra was also used to assess the expression level of cytochrome P450scc in *E. coli* as well as to determine cytochrome P450scc concentration at the intermediate steps of its isolation and purification. The content of high spin form of cytochrome P450scc in the purified cytochrome P450scc was calculated from absolute absorbance spectra using the ratio $\Delta A_{390\text{-}470}/\Delta A_{416\text{-}470}$ [18] equal to 0.4 and 2.2 for low- and high-spin forms of cytochrome P450scc, respectively. The ratio of peak intensities tyrosine/tryptophan $\Delta A_{287\text{-}228.3}/\Delta A_{295\text{-}290.5}$ in the second derivatives of the ultraviolet spectra of cytochrome P450scc was determined according to [19].

Circular dichroism spectra were recorded using a JASCO J-720 spectropolarimeter (Jasco, Japan). Measurements were carried out in 10 mM phosphate buffer, pH 7.4, at room temperature. The concentration of adrenodoxin for circular dichroism measurements in the visible region of the spectra was 40 μM (0.5 cm cell), while in the far ultraviolet region the concentration was 20 μM (0.1 cm cell). The content of the elements of secondary structure in cytochrome P450scc was calculated from the ultraviolet circular dichroism spectra using the Dichropro 2.5 program.

Enzyme assay. Cholesterol side chain cleavage activity of cytochrome P450scc and its mutant forms was analyzed according to [20] using HPLC on a Zorbax-Sil column (4.6 × 250 mm) in the system hexane–isopropanol (3 : 1) by the amount of progesterone formed from the reaction product (pregnenolone) in the presence of cholesterol oxidase. Deoxycorticosterone was used as an internal standard.

Cytochrome *c* reduction was assayed in 20 mM Hepes buffer, pH 7.2, containing 0.1% Tween-20, 0.1 mM EDTA, 0.1 mM DTT, 50 mM NaCl, 20 μM cholesterol, 0.05 μM adrenodoxin reductase, 1 μM adrenodoxin, and 25 μM horse heart cytochrome *c*. The reaction was started by addition of 100 μM NADPH. The reduction of cytochrome *c* was monitored at 550 nm, and the activity was calculated using molar extinction coefficient 20 $mM^{-1}\cdot cm^{-1}$.

Registration of spin changes in cytochrome P450scc during interaction with adrenodoxin. The spectral changes reflecting changes of spin state of cytochrome P450scc induced by adrenodoxin binding were recorded as previously described [21]. The apparent dissociation constants for cytochrome P450–adrenodoxin complex were determined in 20 mM Hepes, pH 7.2, containing 0.1% Tween-20, 0.1 mM EDTA, 0.1 mM DTT, 50 mM NaCl, and 20 μM cholesterol [22]. The concentration of cytochrome P450scc in the sample was 1 μM. The change in the optical density $\Delta A_{100} - \Delta\varepsilon_{390\text{-}420\ nm} = 110\ mM^{-1}\cdot cm^{-1}$ was accepted as a 100% change in the spin state [17]. Recording of the reduced carbon monoxide difference spectrum proved the absence of denatured cytochrome P450scc in the sample.

RESULTS

As follows from the theoretical model of the electron transfer complex of cytochrome P450scc and adrenodoxin [7], Lys267 of the heme protein may be involved in electrostatic interaction with Glu47 of ferredoxin. To check this hypothesis, two series of mutants were constructed and expressed in *E. coli*: mutants of cytochrome P450scc and mutants of adrenodoxin.

Site-directed mutagenesis of Glu47 of adrenodoxin. Mutants of adrenodoxin have been constructed, expressed

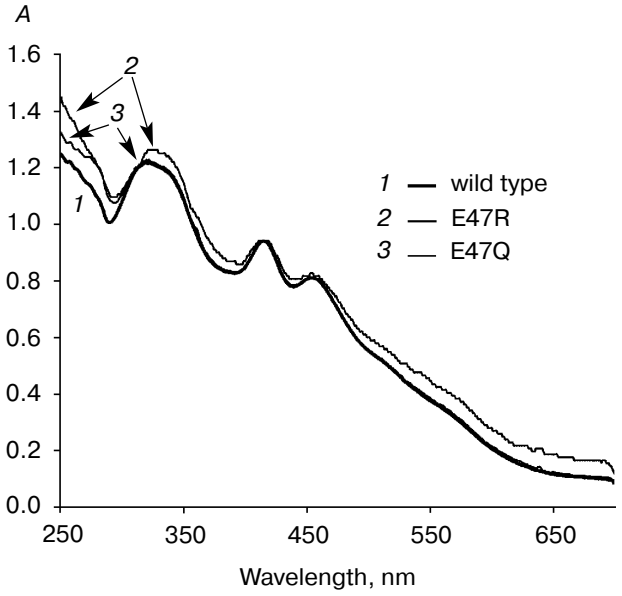

Fig. 1. Absolute absorbance spectra of oxidized adrenodoxin and its mutants Glu47Gln and Glu47Arg. Spectra were recorded in 10 mM sodium phosphate buffer, pH 7.4.

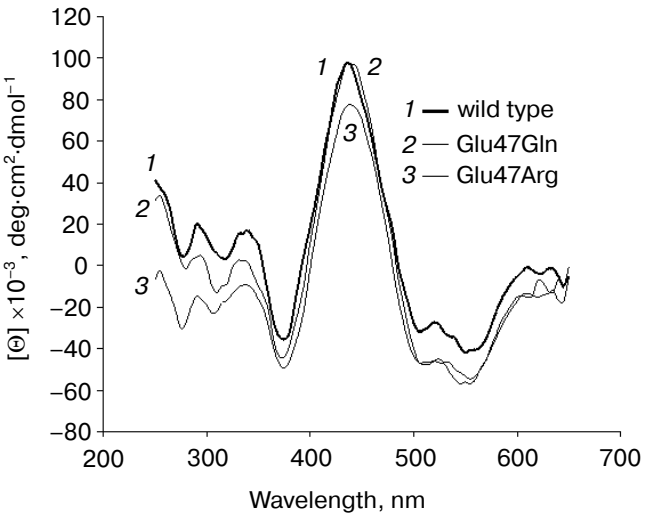

Fig. 2. Circular dichroism spectra of oxidized adrenodoxin and its mutant forms Glu47Gln and Glu47Arg in the ultraviolet region. The samples contained 40 μM adrenodoxin in 10 mM sodium phosphate buffer, pH 7.4.

as holoproteins in *E. coli*, and purified to homogeneity (spectrophotometric index A_{414}/A_{280} = 0.78-0.86). The recombinant proteins show correct assembly of the Fe_2-S_2 cluster confirmed by the absorbance (Fig. 1) and CD spectra (Fig. 2), which were indistinguishable from those for the wild type proteins. No dramatic changes were observed in the far-ultraviolet region of the CD spectrum, but there was a slight effect of mutation Glu47Arg in the visible region at 440 nm.

Functional properties of the mutants of adrenodoxin are presented in Table 1. As follows from the table, dissociation constants (K_d) of the complex with cytochrome P450scc for the mutants Glu47Gln and Glu47Arg exhibit values of the same range as wild type adrenodoxin. To determine the effect of adrenodoxin mutations on its interaction with adrenodoxin reductase, turnover of cytochrome *c* reduction was determined using Glu47Gln and Glu47Arg mutants compared to the wild type ferredoxin. The values of rate constants for reduction of cytochrome *c* are quite similar for the wild type and the mutants. Hence, the mutation of a negatively charged amino acid in adrenodoxin near the Fe_2-S_2 cluster (between two cysteine residues, Cys46 and Cys52) does not affect the protein–protein interaction with the redox partner.

Site-directed mutagenesis of cytochrome P450scc. The wild type cytochrome P450scc and the Lys267Gln, Lys270Gln, and Arg411Gln mutants were expressed in *E. coli* with similar yield, indicating the absence of dramatic changes in the protein folding after the single amino acid replacements. All of the cytochrome P450scc mutants were purified according to the previously described procedure [13] using Ad-Sepharose (immobilized recombinant adrenodoxin) and found to have similar spectral properties to wild type cytochrome P450scc. It is evident from this fact that replacements Lys267Gln, Lys270Gln, and Arg411Gln do not dramatically affect adrenodoxin binding and the cytochrome P450scc mutants still retain the ability to interact with the immobilized redox partner. All mutants of cytochrome P450scc have been purified by this procedure to apparent homogeneity, as confirmed by SDS-PAGE. The spectral properties of the P450scc mutants are summarized in Table 2. As follows from the table, cytochrome P450scc and the mutants are purified as high-spin hemeproteins and do not contain the denatured form of enzyme, cytochrome P420.

Table 1. Functional properties of adrenodoxin mutants

Adrenodoxin	Cytochrome *c* activity, min^{-1}	Activity in cholesterol side chain cleavage reaction, %	Dissociation constant (K_d) of complex with P450scc, μM
Wild type	213	100	0.269
E47Q	210	130	0.362
E47R	210	120	0.220

Table 2. Spectral properties of mutant forms of cytochrome P450scc

P450scc	$i_{393/280}$*	Content of high-spin form, %	Second derivatives of the UV-spectra: (287-228.3)/(295-290.5)	C_{CO}/C_{abs}, %**
Wild type	0.85	77	1.1	92
R411Q	0.63	81	1.23	84
K267Q	0.76	70	1.19	61
K270Q	0.66	70	1.24	84

* Homogeneity index for P450scc preparations which reflects the presence of impurities and apoenzyme.
** Ratio of P450scc concentrations determined from the CO-difference and absolute absorbance spectra.

The absence of significant differences in the second derivative of the ultraviolet spectra, which are very sensitive to any changes in protein conformation [19] and reflect the average polarity of the microenvironment of the tyrosine residues present in cytochrome P450scc, supports our conclusion on the absence of dramatic changes in the conformation of the mutant forms of cytochrome P450scc after replacements Lys267Gln, Lys270Gln, and Arg411Gln. To test directly whether binding of adrenodoxin to cytochrome P450scc is affected by the above-mentioned amino acid substitution in P450scc, we carried out spectrophotometric titration of cytochrome P450scc with adrenodoxin.

Figure 3 shows the titration curve of cytochrome P450scc–adrenodoxin interaction. The dissociation constant (K_d) for the complex between Arg411Gln cytochrome P450scc mutant and adrenodoxin was found to differ about 4-fold as compared to the complex between wild type cytochrome P450scc and adrenodoxin. Similar spectral changes were observed for the cytochrome P450scc Lys405Gln mutant [5]. Based on this observation, we suggest that there are at least two distinct positively charged "clusters" on the proximal face of cytochrome P450scc. One interaction site is formed by amino acid residues Arg425, Arg426, Lys338, and Lys342, while the other neighboring surface interaction site is composed by amino acids Lys403, Lys405, and Arg411 and is responsible for the specific geometry between the two interacting proteins required for efficient electron transfer. The dissociation constants for the complexes between adrenodoxin and cytochrome P450scc Lys267Gln and Lys270Gln mutants reveal only slight changes in affinity compared to the wild type hemeprotein.

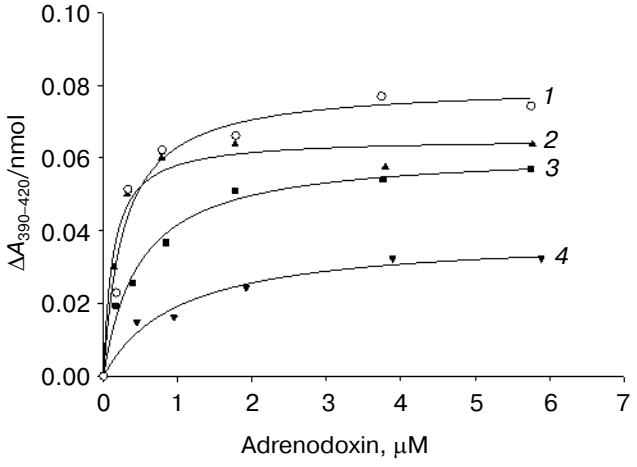

Fig. 3. Titration curves reflecting the interaction of wild type cytochrome P450scc (*1*) and its mutant forms Lys267Gln (*2*), Lys270Gln (*3*), and Agr411Gln (*4*) with adrenodoxin. Titration experiments were carried out in 20 mM Hepes, pH 7.2, containing 0.1% Tween-20, 0.1 mM EDTA, 0.1 mM DTT, 50 mM NaCl, and 20 μM cholesterol. The cytochrome P450scc concentration was 1 μM.

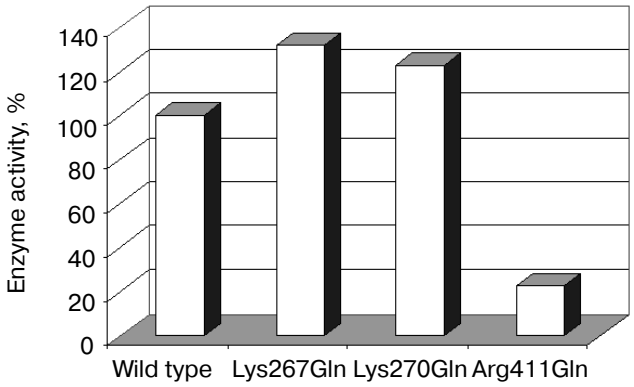

Fig. 4. Activity of wild type cytochrome P450scc and its mutant forms Lys267Gln, Lys270Gln, and Agr411Gln in the cholesterol side chain reaction in a reconstituted system. The reaction was carried out in 25 mM Hepes, pH 7.2, containing 0.1% Tween-20, 0.1 mM EDTA, 0.1 mM DTT, 100 μM cholesterol, 1 μM adrenodoxin reductase, 2 μM adrenodoxin, 1 μM cytochrome P450scc, and an NADPH-regenerating system.

Thus, based on replacements in cytochrome P450scc we investigated in the present work and taking into account the theoretical model of electron transfer complex as well earlier data on chemical modification of amino acid residue Lys267 and Lys270 of cytochrome P450scc, we conclude that these residues apparently do not directly participate in electrostatic interaction with adrenodoxin.

The activity of wild type cytochrome P450scc and its mutants in cholesterol side chain cleavage reaction, estimated as pregnenolone formation, are presented in Fig. 4. As follows from the figure, substitution of Arg411 for Gln in cytochrome P450scc results in a decrease in the activity, which represents only 25% of the activity of the wild type hemeprotein, which is in good agreement with the titration data. Thus, the change in catalytic activity of the cytochrome P450scc Arg411 mutant arises from the reduction of its affinity to the redox partner.

DISCUSSION

The cytochrome P450 superfamily comprises a huge number of proteins with diverse functions and activities. However, the three dimensional structure has been solved for only a few representatives of this superfamily. To get information about the structure–function relationships in cytochromes P450 with unknown 3D structure, it is necessary to use alternative methods: molecular modeling based on the known structure of homologous protein as template, chemical modification, site-directed mutagenesis, etc. In the present study mutants Lys267Gln,

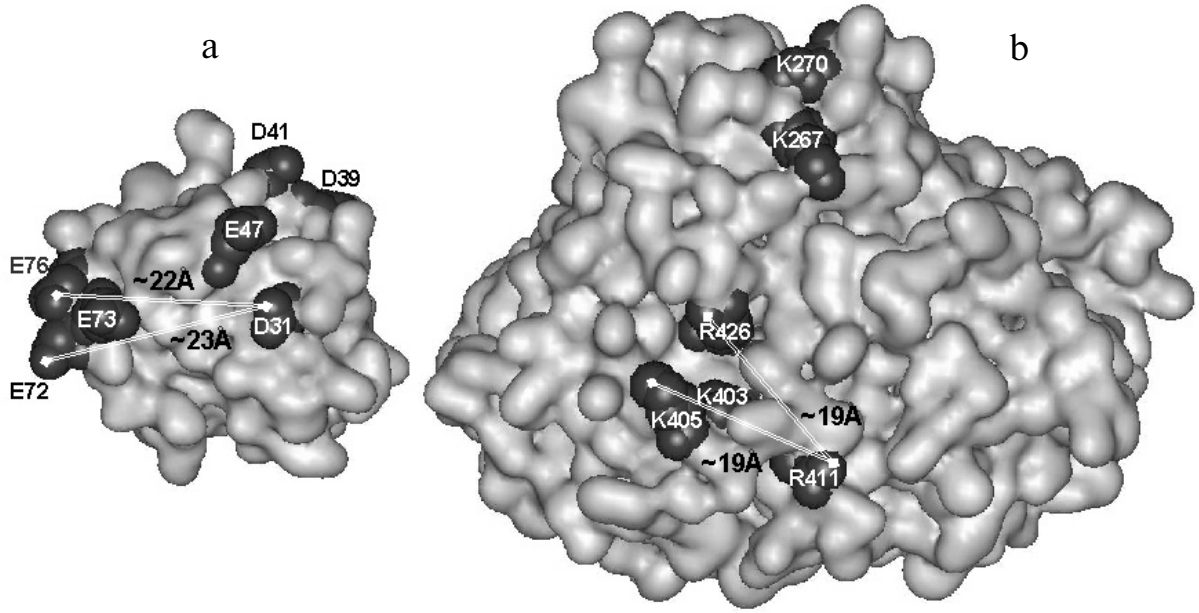

Fig. 5. Sites of interaction on the surface of adrenodoxin (a) and cytochrome P450scc (b). Shown are amino acid residues involved in interaction and the distance between these residues. Three-dimensional coordinates: adrenodoxin, crystal structure (PDB: 1AYF); cytochrome P450scc, theoretical model [29].

Lys270Gln, and Arg411Gln of cytochrome P450scc and Glu47Gln and Glu47Arg of adrenodoxin were synthesized on the basis of theoretical model of the complex between cytochrome P450scc and adrenodoxin [7] and the role of residues Lys270 and Arg411 of P450scc in the interaction with adrenodoxin was investigated.

Amino acid residues Lys267 and Lys270 of cytochrome P450scc are located in H α-helix and H-I loop [7] of cytochrome P450scc, respectively, and have been previously mapped by chemical modification as residues involved in the interaction with adrenodoxin [10]. Residue Arg411 of cytochrome P450scc is located between amino acid residues of random coil, where Lys405 and Lys403 were identified as sites for interaction with adrenodoxin, and conservative heme-binding peptide, which contains Arg425 and Arg426, is also involved in electrostatic interactions with negatively charged adrenodoxin residues. The distribution of mutated residues on the surface of P450scc is presented in Fig. 5.

The data obtained in the present work indicate that only replacement of the arginine residue at position 411 (Arg411) has indeed affected the functional properties of cytochrome P450scc. Of particular interest is the similarity of effects caused by replacement of Lys405Gln and Arg411Gln in cytochrome P450scc on the character of electrostatic interaction with adrenodoxin. This similarity led us to suggest that there are different types and strength of electrostatic contacts, which occur on the proximal surface of cytochrome P450scc. In the first step, adrenodoxin, being reduced by adrenodoxin reductase, interacts selectively with positively charged residues of the "cluster" of cytochrome P450scc at the proximal side of the hemeprotein and then the complex is fixed by "second order contacts". Indeed, complex formation and its biological implication (efficient electron transfer) require spatial proximity (optimal distance for electron tunneling) and certain geometry (conformational "tuning", angle of approach, size and shape of interface) [23] of redox centers. As follows from the model of the complex of cytochrome P450scc with adrenodoxin, it can be seen that some amino acid residues near the iron–sulfur cluster are close to the heme-binding region, especially to the loop covering the Fe_2-S_2 cluster from one side, and surface residues around the heme may participate in complex fixation. According to the theoretical model built for the complex of cytochrome P450scc and adrenodoxin, residue Glu47 of adrenodoxin is located close to the iron–sulfur cluster and Lys267 residue of cytochrome P450scc. Nevertheless, neutralization of the ionic charge of this adrenodoxin amino acid residue or even changing the charge of this residue to the opposite charge does not result in dramatic changes in complex formation and do not confirm their participation in electrostatic interaction with cytochrome P450scc. Spectral characterization and functional analysis of adrenodoxin mutants Glu47Gln and Glu47Arg reveal no differences compared to the wild type protein. However, deletion of residue Glu47 in the loop surrounding the Fe_2-S_2 cluster shows dramatic

reduction in electron transfer properties of the protein [11]. These results appear to highlight that the extent of perturbation of the protein structure caused by deletion from its ideal geometry in the wild type protein is correlated to the impairment of its catalytic activity.

The loop covering the Fe_2-S_2 cluster of adrenodoxin appears to have been selected by evolution to compensate a structural site in vertebrate-type ferredoxins. The replacement of the whole loop by a similar segment of plant ferredoxins containing only four amino acids or its whole deletion would not reduce in a dramatic way the electron transfer function of the system as sequential deletion of a single residue of the loop (as shown for restrictocin-non-glycosylated single chain basic protein of 149 amino acid residues [24]). It is very likely that structural space due to the loss of a single residue is substituted or compensated for by neighboring residues, i.e., the spatial distance between prosthetic groups of the redox partners changed because of different volume of side radicals of amino acids and this, in turn, may lead to changes in the electron pathways and as a result reflect on the activity of the ferredoxin.

Since the sites of interaction of adrenodoxin with adrenodoxin reductase and cytochrome P450 are overlapping [25], and cross-linked adrenodoxin–adrenodoxin reductase complex indicates two sites of the redox partners for complex formation [26], adrenodoxin, as well as plant ferredoxins [27] and putidaredoxin [28], can have a binding patch on the surface localized in the "structural" domain but not in the loop covering the Fe-S cluster. The alignment of ferredoxins from different sources allows predicting potential binding sites Asp31, Asp39, and Asp41 in adrenodoxin: residues Asp39 and Asp41 have been suggested earlier as sites for interaction with redox partners [26, 29] and residue Asp31 is mentioned in [30].

Taking into consideration that (i) residues Asp34 and Asp38 of putidaredoxin participate in interaction with cytochrome P450cam [31] (41st and 45th amino acid residues of adrenodoxin, respectively), (ii) adrenodoxin is not able to substitute for putidaredoxin in the reaction of camphor hydroxylation [32], (iii) terpredoxin exhibits only 2% of the activity of putidaredoxin in the reconstituted camphor monooxygenase system [33], and (iv) 3D structures of adrenodoxin and terpredoxin superimpose with smaller differences than 3D structures of adrenodoxin and putidaredoxin would suggest that adrenodoxin and terpredoxin should have common residues participating in interaction with cytochromes P450 which are lacking in putidaredoxin. Such a residue might be Asp31 located in the C-helix of the core domain of adrenodoxin. In this case, sites for interaction with adrenodoxin reductase and cytochrome P450scc will be localized on the opposite sides of adrenodoxin.

Thus, the results of site-directed replacement of Arg411 of cytochrome P450scc confirm multipoint character of the interaction of the hemeprotein with ferredoxin. At the same time, proposed architecture of a theoretical complex [7] implicating the involvement of an ion pair between Lys267 of cytochrome P450scc and Glu47 of adrenodoxin in complex stabilization has not been confirmed by experimental data using site-directed mutagenesis. Nevertheless, our results are in agreement with the conclusion concerning multipoint character of interaction P450scc with adrenodoxin. Arg411 of P450scc participates in electrostatic interaction with adrenodoxin, but this residue is not paired exactly with a complementary charged residue of adrenodoxin. The possibility remains of the existence of two interaction sites on the surface of the redox partner in the mitochondrial monooxygenase system.

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