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Orientation of Cytochrome P450scc in Langmuir−Blodgett Monolayers

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Monolayers of cytochrome P450scc and its complex with adrenodoxin were formed by Langmuir techniques and covalently immobilized on the solid substrates. The orientation of hemeprotein molecules was studied using polyclonal antibodies specific to the intact cytochrome P450scc molecule and its tryptic fragments F1 and F2, representing N- and C-terminal parts of the hemeprotein molecule. Specific interactions of the Langmuir films of cytochrome P450scc with adrenodoxin were investigated, and the position of the ferredoxin binding site at the hemeprotein molecule was identified. It was shown that the molecular orientation of the P450scc−AD (adrenodoxin) complex at the water−air interface is dependent on the surface density of the monolayer. The P450scc molecules do not denature upon spreading on the water surface. The formed monolayer can be transferred from the air−water interface to the surface modified with siloxane polymer and covalently immobilized without damage to the structure. A model considering the mode of orientation of cytochrome P450scc molecules on the air−water interface in dependence on the surface pressure is discussed.

1. Introduction

A large number of potential applications for organized protein monolayers has recently motivated considerable research activity in this field.[1,2] Construction of specific interaction-directed, self-assembled protein films has been performed at the air−water interface. In-plane order can be greatly enhanced by exploiting the combination of planar orientation and mobility which this interface provides. This was demonstrated by the spontaneous formation of two-dimensional crystalline antibody domains upon binding to the antigen functionalized lipid monolayer.[3,4] Self-assembled monolayers have been constructed on the surface of gold from biotin-functionalized thiols and other components such as streptavidin, biotinylated Fab fragments, and secondary antibodies.[5,6]

The ability to orient the protein molecules into defined organized layers is practically attractive for biochemical applications. Generally, the protein molecules are asymmetric structurally and functionally. The hydrophobic, hydrophilic, and charged sites are separated on the external surface of the globule; thus protein molecules spread on water surface are likely to be subject to reorientation as a response to external factors. Dubrovsky et al., studying the dependence of surface density versus the deposition pressure, proposed a model of molecular packing of IgG in Langmuir monolayers.[7,8] According to this studies, the orientation of the molecules in the film depends on the surface pressure. The model has been recently proved by measuring the change of the film thickness at the water−air interface with the increase of surface pressure by means of ellipsometry.[9] Salesse et al. have shown that the orientation of rhodopsin molecules on the water surface also depends drastically on surface pressure.[10] However, the possibility to organize the molecules on the water surface is not sufficient for manufacturing of the film with desired molecular disposition. The film should be deposited on some solid support and therefore another problem arises as to whether or not the molecular orientation remains unchanged upon deposition.

Mitochondrial cytochrome P450scc (CYP11A1) is the mixed function monooxygenase which catalyses the initial step of steroid hormones biosynthesis−cholesterol side chain cleavage reaction to form the pregnenolone. The electrons used for monooxygenase reaction are received from NADPH through electron transfer chain consisting of two proteins: the FAD-containing adrenodoxin reductase and [2Fe-2S] ferredoxin called adrenodoxin (AD). Cytochrome P450scc forms stable complex with AD, and the interaction of the P450scc and AD has been reported to be mainly electrostatic.[11] P450scc films can be deposited on solid supports by Langmuir−Schaefer techniques using either hydrophobic or hydrophilic adsorption.[12] Moreover, the cytochrome is most likely not to denature on the water surface because after being transferred from the interface,

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it still exhibits specific electron transfer activity.[13] However, up to now the orientation of the hemeprotein molecules in LB films is unknown.

The P450scc molecule consists of two structural domains linked by a short loop in the middle part of the polypeptide chain. Limited proteolysis of the P450scc with trypsin may result in the formation (in equimolar ratio) of two large polypeptide fragments: F1 (Ile1−Arg250) and F2 (Asn257−Ala481).[14] Polyclonal antibodies specific to whole P450scc molecule (anti-P450scc) and corresponding tryptic fragments F1 (anti-F1) and F2 (anti-F2) have been used to study the topology of the hemeprotein in the inner mitochondrial membrane.[15,16]

Here we present the study of the dependence of orientation of cytochrome molecules in the film on the deposition pressure. In fact, if we expose the cytochrome P450scc film to the solutions of domain-specific antibodies, the binding of anti-F1 and anti-F2 antibodies will depend on how cytochrome molecules are oriented in the film. If F1 fragment is exposed on the surface, the binding reaction will occur with anti-F1 and be weak with anti-F2, and vice versa. To check the complexability of hemeprotein in densely packed monolayer, we used the AD-binding reaction; hence the relative location of AD-binding site was found.

2. Experimental Section

Abbreviations. Abbreviations used are as follows: P450scc, cytochrome P450scc; AD, adrenodoxin; BSA, bovine serum albumin; TBS, Tris buffered saline, 30 mM Tris(hydroxymethyl)-aminomethane, 150 mM NaCl, 0.05% NaN_3, pH 7.4; Blocking Buffer, 1% BSA, 0.05% Tween-20 in TBS; Binding Buffer, 0.3% BSA in TBS; Wash Buffer TBST, 0.05% Tween-20 in TBS; GOPTS, (3-glycidoxypropyl)trimethoxysilane; FITC, fluorescein isothiocyanate.

2.1. Materials. P450scc and AD were affinity-purified using specific adsorbents from bovine adrenocortical mitochondria.[17,18] Affinity-purified polyclonal rabbit antibodies specific to AD, P450scc, and F1, F2 domains are available from previous studies.[15,19] Blocker BSA and Protein A/G were purchased from Pierce. GOPTS (reagent grade), sodium hydrogen carbonate and sodium chloride (A.C.S. reagent grade), sodium azide, FITC, and Tween-20 (reagent grade), DMF (HPLC grade), and tris(hydroxymethyl)aminomethane (ultrapure grade) were purchased from Aldrich. Sephadex G-25M was purchased from Pharmacia Biotechnology. All reagents were used as received. Water was Milli-Q purified with resistance of 18.2 MΩ cm.

2.2. Modification of the Supports. Fused quartz disks with polished surfaces (5 mm diameter and 2.5 mm thickness) were carefully cleaned by soaking in fresh chromic acid, rinsed in water, dried under nitrogen flux, and silanized with GOPTS under vacuum in an apparatus has been described previously,[7] according to the method developed by Malmqvist.[20] The surface density of the deposited poly(organosiloxane) polymer film was controlled by the microgravimetric method[8] and its average value for GOPTS was found to be 4−8 nmol/cm^2. Prepared in this way, the surface remains hydrophilic and has exposed active epoxy groups which react readily with ϵ-amino groups of lysine. This method of surface preparation has been successfully used for the immobilization of Langmuir−Blodgett films of antibodies,[8] Protein A,[22] and glutation *S*-transferase.[21]

2.3. Deposition of the Films. P450scc monolayers were formed in a Langmuir Trough (MM-MDT Inc., Russia), which has been equipped with home-made Teflon trough with dimensions of 55 × 200 × 7 mm and volume of 95 mL. The trough was treated with methylene chloride before the monolayer formation and then dried with nitrogen. Protein monolayer formation as well as transfer to the GOPTS-activated quartz substrates was performed from the surface of 50 mM carbonate buffer containing 150 mM NaCl, pH 7.4. Relatively high ionic strength is necessary to minimize electrostatic interactions between charged groups of the protein and quartz substrate as well as to exclude nonspecific interactions between protein molecules. P450scc solution (200 μL of 80 μM P450scc) in TBS was spread on the subphase by Hamilton rheodyne syringe. The typical estimation for the concentration of the protein in a subphase is 0.16 nmol/mL. After 5 min of exposition, monolayers were compressed at the rate of 2 mm/s. Transfer of P450scc films from the subphase surface onto the activated supports was performed by "touching" the silanized support in parallel mode to the subphase surface (analogous to the Langmuir−Schaefer method). The supports were lifted after the touching with vertical speed of 1 cm/s. After film deposition, the samples were dried under nitrogen flux, incubated for 10 h at 4 °C, washed with TBST and water, and dried under nitrogen flux. The same procedure was used for AD−P450scc complex. This complex was formed by means of dialysis of hemeprotein and ferredoxin mixture (1:2 molar ratio) in 20 mM Tris buffer, pH 7.4.

2.4. Microgravimetric and Ellipsometric Measurements. Standard resonators 2B, 10 MHz, with aluminum-plated polished electrodes were used for microgravimetric measurements. For calibration of the resonators the frequency shift induced by the deposition of bilayers of cadmium arachidate was measured. The sensitivity of the resonator was found to be 2.95 ng Hz^{-1} cm^{-2}. This method was described in ref 22. Ellipsometric measurements were performed using a PCSA null ellipsometer LEPh-2 (Special Bureau for Scientific Devices of the Siberian branch of the Russian Academy of Sciences, Novosibirsk) using a He−Ne laser (wavelength, 632.8 nm). For deposited films an angle of incidence of 70° and two-zone technique were used. The data were treated according to a model of two isotropic layers. The upper layer represents the protein layer and the lower one accounts for superficial imperfections of the support. The accuracy of the device is 0.02° with respect to ellipsometric parameters ψ and Δ. The technique is described in detail elsewhere.[9] The evaluation of film thickness was made assuming 1.5 for refractive index of monolayer. This value was obtained for multilayer thick films.

2.5. Fluorescence Measurements. Antigen−antibody and hemeprotein−AD specific interactions were monitored by a fluorescence technique using Protein A/G−FITC and AD−FITC conjugates, respectively. Fluorescence was measured by a Zeis Axioplan microscope (Zeiss Co., Germany) equipped with a mercury lamp, a set consisting of BP 450−490, FT 510, and BP 515−565 filters, and a 40× objective. Images were acquired by CCD camera CH260 (Photometrix Co., Germany) at −35 °C for exposure time of 2800 ms and area of acquisition of 87 × 87 μm^2. Five images at different places for every sample were acquired, and the integrated fluorescence intensity over all pixels was averaged over acquisitions. The integrated intensity dispersion of acquisitions of one sample was less than 4% and the dispersion over different samples deposited under equal conditions was less than 3%, which shows good reproducibility of the monolayer formation and deposition. The images were uniform, revealing normally no visible inhomogeneites of the film.

2.6. Labeling of Protein A/G and AD with FITC. Conjugates of FITC with Protein A/G or AD were prepared in accordance with method described in ref 24. Gel filtration on a Sephadex G-25M column (1.5/20 cm) in 30 mM Tris buffer, pH

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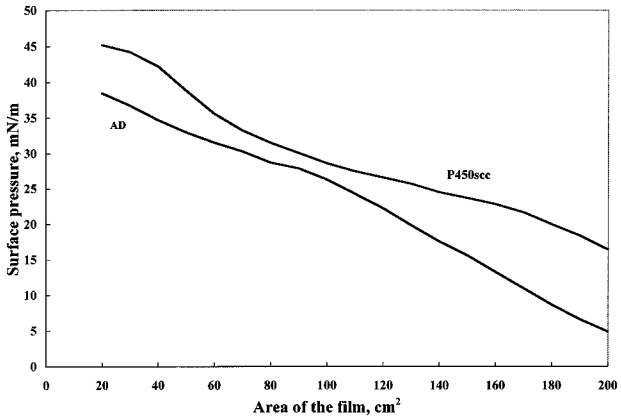

Figure 1. Compression isotherms of cytochrome P450scc (P450scc) and adrenodoxin (AD) at 23 °C on the aqueous subphase: 50 mM carbonate buffer containing 150 mM NaCl, pH 7.4.

7.4, was used for separation of FITC−Protein A/G or FITC−AD conjugates from free label. P450scc concentration was determined from CO-difference spectra (dithionite-reduced preparation) using an extinction coefficient of 91 mM^{-1} cm^{-1} for difference in the absorbance between 450 and 490 nm.[23] AD concentration was calculated using an extinction coefficient of 9.8 mM^{-1} cm^{-1} at 414 nm.[17] The labeling degree of Protein A/G was calculated using a molar extinction coefficient for FITC $\epsilon_{493} = 62$ mM^{-1} cm^{-1} in 30 mM Tris buffer, pH 7.4. For AD labeling the ferredoxin concentration was determined using an extinction coefficient of $\epsilon_{600} = 2.1$ mM^{-1} cm^{-1} (there is no FITC absorbance in this range). AD absorption at 493 nm was then determined using an extinction coefficient of 6.1 mM^{-1} cm^{-1}. To calculate the FITC net absorption, the value of ferredoxin absorption was subtracted from the absorption of AD−FITC conjugate. The molar ratio of FITC to Protein A/G was found to be 3:1, and that for FITC−AD conjugate was 1.4:1.

2.7. Immunological Binding of Domain-Specific Antibodies to P450scc and AD−P450scc Films. After P450scc or AD-P450scc film deposition, the supports were incubated in Blocking Buffer for 1 h at 37 °C with gentle stirring for blocking the excess of binding sites. The immunological binding with anti-P450scc, anti-F1, anti-F2, or anti-AD IgG was performed from the Binding Buffer at antibody concentration of 8 μg/mL with gentle stirring at 37 °C for 1 h. Continuous washing with TBST was performed before coupling with Protein A/G-FITC conjugate. Specific binding with conjugate (0.5−1.0 μg/mL) was performed from TBST.[24] Washing twice with TBST and water and drying in nitrogen flux were performed before the fluorescence measurement.

2.8. Specific Binding of AD−FITC Conjugate to P450scc Monolayer. After hemeprotein film deposition, the quartz disks were incubated with 20 μM FITC−AD in 20 mM sodium phosphate buffer, pH 7.4 for 30 min. Washing twice with 50 mM phosphate buffer and water and drying in nitrogen flux were carried out before the fluorescence measurement.

3. Results and Discussion

3.1. Immobilization and Characterization of Langmuir Films of Cytochrome P450scc and P450scc−AD Complex. Monolayers of the cytochrome P450scc were prepared as described in the Experimental Section. Analysis of the $\pi-A$ isotherms presented on Figure 1 can lead to the conclusion that both cytochrome P450scc and AD form rather dense and stable films on the water−air interface. These monolayers can be easily transferred to the activated surface of a quartz support using the Langmuir−Schaefer technique and immobilized. In contrast to P450scc, AD is rather hydrophilic and is adsorbed weakly to the activated support.

Microgravimetric techniques were used to control the immobilization of a protein monolayer on a siloxane polymer (GOPTS) surface. The deposition of a compressed monolayer of P450scc−AD complex (at 35−40 mN/m) on the surface of the resonator resulted in a frequency shift of 146−151 Hz. This corresponds to a mass loading on the electrode surface of $\Delta m = 431-448$ ng/cm^2, and the surface density of the protein monolayer was calculated to be 6−6.5 pmol/cm^2. The area per molecule of the complex in the immobilized monolayer was calculated to be 25−28 nm^2.

As yet there are no crystallographic data on the structure of P450scc−AD complex. To estimate the dimensions of the complex, we used the equation proposed by Wherland and Gray[25]

$$R = 0.717M^{1/3}$$

where R is the radius of the protein complex and M is its molecular weight. Assuming that P450scc−AD complex is spherical and its molecular weight is the sum of molecular weights of P450scc and AD (56 400 and 12 500, respectively), the diameter of the hemeprotein complex was estimated to be 5.5−6.0 nm, and its cross section is about 27−30 nm^2. The latter value is in agreement with that calculated from microgravimetric measurements.

Ellipsometry was used to measure the thickness of the immobilized monolayers. At 35−40 mN/m the thickness of the film of P450scc−AD complex was found to be 4.6−5.0 nm, which is comparable to the diameter of the complex calculated. It can be concluded that the protein complex formed a densely-packed monolayer on the surface of the siloxane polymer.

We also examined the molecular orientation of the cytochrome P450scc and its complex with adrenodoxin in LB films immobilized at different surface pressures. These molecular assemblies are probed using polyclonal antibodies toward various parts of the cytochrome molecule and adrenodoxin. Purified recombinant Protein A/G labeled with FITC was used as a secondary antibody in the immunochemical procedure. Gene fusion of the Fc-binding domains of Protein A and Protein G has led to the production of functionally chimerical protein with extended binding specificity to the IgG molecule.[24] The scheme of the immunocomplex formed is presented in Figure 5. The immobilized P450scc film was incubated in the Binding Buffer containing IgG, and binding with fragment-specific antibodies occurred. Then, after carefully rinsing the film with Wash buffer and drying it, the film was incubated with Binding Buffer containing Protein A/G-FITC conjugate. Control experiments have been performed for all types of specific binding to estimate the nonspecific adsorption of proteins to the film surface: rabbit anti-mouse polyclonal antibodies have been used for the immunological binding instead of antibodies specific to P450scc. The background fluorescence corresponds to nonspecific adsorption of antibodies and Protein A/G is indicated for each measurement. We assumed that in all experiments antigen−antibody and IgG (Fc)−Protein A/G reactions proceed equally for each type of antibody. In addition, we propose that modification of three from a total 43 lysines[26] of protein globule by FITC does not influence drastically the binding properties of Protein A/G toward Fc fragment of antibody molecule.

3.2. Specific Binding of Anti-F1 and Anti-F2 Antibodies to the Monolayer of P450scc. The binding of these antibodies to the film of cytochrome P450scc with respect to the surface pressure of the monolayer is presented in Figure 2. The binding level is proportional

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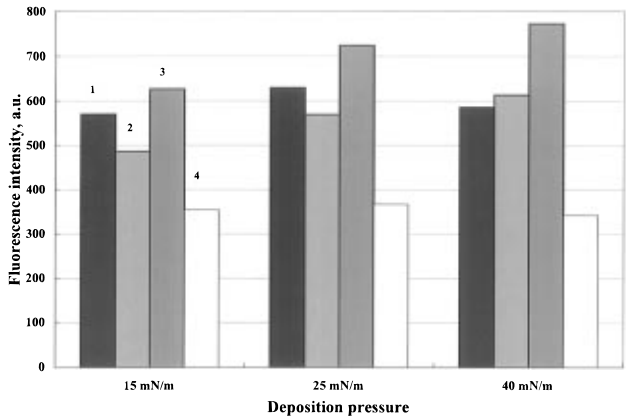

Figure 2. Comparison of binding of anti-F1 (1), anti-F2 (2), and anti-P450scc (3) antibodies to the Langmuir films of cytochrome P450scc immobilized at the different surface pressures. The baseline (4) indicates the level of nonspecific adsorption of proteins to the film surface. The binding performed is described in the Experimental Section.

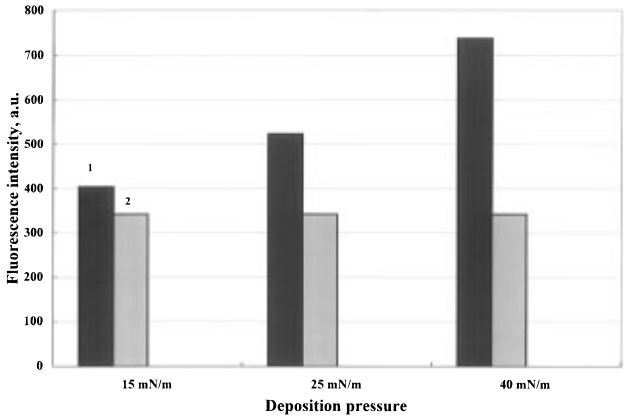

Figure 3. Specific binding of the FITC-labeled adrenodoxin to the Langmuir films of cytochrome P450scc immobilized at the different surface pressures (1). The baseline (2) indicates the level of nonspecific adsorption of proteins to the film surface. The binding performed is described in the Experimental Section.

to the fluorescence measured. For the anti-F2 and anti-P450scc antibodies, the amount of IgG bound to the film is increased with increasing surface pressure; however for the anti-F1 it is initially increased within the range of 15−25 mN/m and then decreased to 40 mN/m.

The intensity of fluorescence is a function of antigen surface density. However, the avidity of antibodies to antigen-containing films may depend on combinations of different factors: antigen surface concentration, accessibility of antigenic determinants to antibodies, and the orientation of P450scc molecules. It is impossible to determine the influence of these factors individually on the binding, when the fluorescence intensity is rising with respect to the surface pressure. However, the decreasing fluorescence intensity shows that the accessibility of antigenic determinants exposed to F1 domain becomes lower in a closely packed monolayer as compared to that of the other part of the molecule. Taking into account that the accessibility of antigenic determinants on the surface of protein globules is dependent on their orientation in the film, and assuming that P450scc molecules do not denature on the interface, the data obtained indicates that at 40 mN/m the F2 fragment of the P450scc molecule is exposed to the water phase more than the F1 fragment.

3.3. Specific Binding of Adrenodoxin to the Monolayer of P450scc. The data on the chemical modification and the site-directed mutagenesis suggest that the AD−P450scc complex is stabilized by electrostatic interactions between carboxylate groups of AD and lysine ϵ-amino groups of P450scc.[27−29] The dissociation constant of the complex was found to range from 10 to 100 nM.[30] In order to determine the position of the binding site of adrenodoxin on the surface of the P450scc molecule within a densely packed monolayer, we measured the specific binding of the AD−FITC conjugate with the immobilized films. It was found that the chemical modification of AD by FITC increases the dissociation constant of the complex but does not drastically influence the ability of the hemeprotein to react with ferredoxin in solution.[33] The relationship between the fluorescence of the FITC−AD conjugate bound to the P450scc monolayer and the surface pressure is presented on Figure 3. An increase in the deposition pressure causes an increase in the intensity of the fluorescence. It seems most likely that the AD binding site of the P450scc molecule is exposed to the water phase either at low (15 mN/m) or at high (40 mN/m) surface pressures. Moreover, the orientation of the molecules in a densely packed monolayer would allow favorable electrostatic interactions between P450scc and AD surfaces. Other intermolecular interactions between polar residues and close van der Waals contacts between hydrophobic residues of P450scc molecules can stabilize these molecular assemblies, since the film preserves the AD-binding ability after being transferred to the support.

We can compare our results with the experimental data on the membrane organization of mitochondrial cytochrome P450scc that has been recently published. On the basis of studies of the limited proteolysis of the inner mitochondrial membrane and immunochemical assay of P450scc, Usanov et al.[16,31] demonstrated that hemeprotein molecules can penetrate into the membrane. This supports the conclusion that P450scc molecules contain pronounced hydrophobic regions on their external surface. Previous experiments have also revealed that sites responsible for interactions of P450scc with AD molecules are exposed to the matrix (hydrophilic) side of the inner mitochondrial membrane: the specific activity of cytochrome P450scc (conversion of cholesterol to pregnenolone), that is incorporated into the membrane, was found to be inhibited by binding with antibodies toward the P450scc molecule and its fragments F1 and F2.[19] It is likely that the inhibition of the activity is caused by specific interactions between the antibodies and cytochrome molecules, which prevent the formation of the cytochrome−adrenodoxin complex. On the other hand, anti-F2 antibodies showed higher binding capacity to the matrix side of the inner mitochondrial membrane as compared to the anti-F1.[16] This agrees well with our data.

3.4. Orientation of AD−P450scc Complex in the Monolayer. In order to determine the comparative orientation of AD and P450scc molecules at the interface, monolayers of AD−P450scc complex were formed at different surface pressures and immobilized on an acti-

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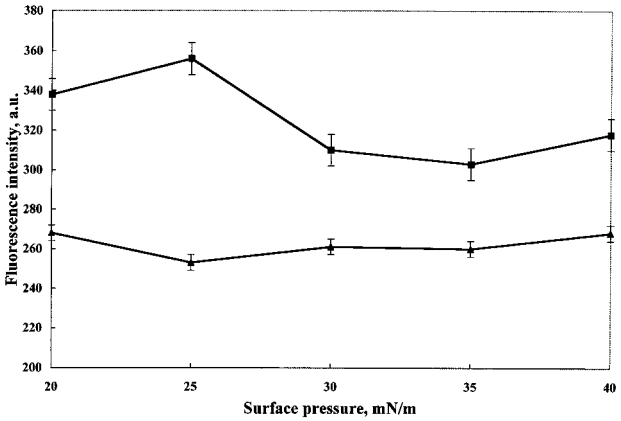

Figure 4. Specific binding of anti-AD antibodies to the Langmuir films of a complex of cytochrome P450scc−adrenodoxin immobilized on activated quartz surfaces at the different surface pressures (squares). The baseline (triangles) indicates the level of nonspecific adsorption of proteins to the film surface. The binding performed is described in the Experimental Section.

vated surface. Modeling of the complex has suggested that there are two lysine pools which can effectively react with a GOPTS activated support: one pool belongs to the AD molecule, which contains 5 lysine residues, and the other one belongs to the P450scc molecule, containing 30 lysine residues.

In fact, the orientation of the immobilized complex on the support depends on the comparative orientation of the AD and P450scc at the interface. It should be noted that immobilized films were incubated with 50 mM TBS containing 1 M NaCl and 0.3% of sodium cholate in order to separate the covalently immobilized AD−P450scc complex and noncovalently attached AD molecules. It is well-known that under such conditions the AD and cytochrome molecules are separated and the complex cannot form.[32] Such treated AD−P450scc monolayers were then incubated with anti-AD antibodies to determine the complex orientation. If both the AD and the P450scc parts of the complex are bound to the activated surface, the structure of the immobilized complex does not change after washing with salt/detergent solution, and AD molecules exposed to the surface can be detected using anti-AD antibodies. Otherwise, if the complex is oriented on the interface in such a way that its AD part is exposed to the subphase, it is safe to assume that after the immobilization only the P450scc part of the complex is bound to the surface and the AD part is washed off by the salt/detergent solution. The specific binding of anti-AD antibodies with the surface should be insignificant in this case. The first situation probably occurs at low surface pressures while the second one can be expected at high surface pressures.

The binding of anti-AD antibodies to the AD-P450scc films with respect to the surface pressure of the monolayer is presented on Figure 4. At the surface pressure ranging from 20 to 25 mN/m the specific binding is increased, but then it decreases within the 25−30 mN/m interval and becomes practically constant up to 40 mN/m. We believe that the decrease in binding of the anti-AD antibodies to the film at surface pressures of 25−40 mN/m reflects the interaction of the P450scc−AD complex with the GOPTS-activated support mainly through the P450 moiety, and the AD is washing with 1 M NaCl and 0.3% sodium cholate.

Let us summarize the explanations that have been put forward for the results obtained. At low surface pressures (∼15 mN/m) either AD or P450scc parts of the complex are exposed to the air−water interface and the molecules are randomly oriented (Figure 5). The surface density of

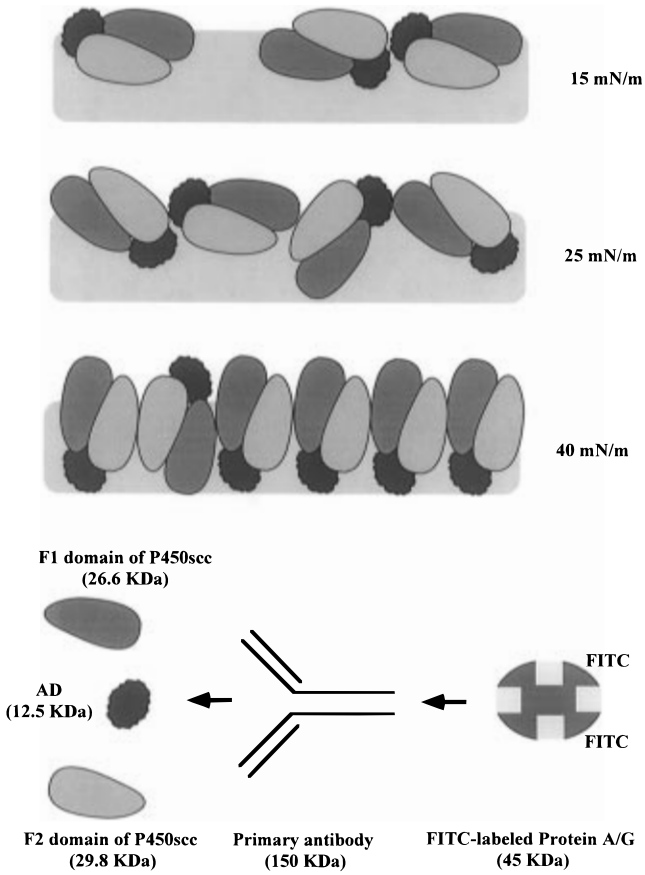

Figure 5. Model of molecular orientation of P450scc−AD monolayer at the water−air interface at the different surface pressures, and the schematic view of the immunochemical binding of specific antibodies and FITC−Protein A/G conjugate to the monolayer.

the protein is not high and the film thickness is irregular. The second structure ($\pi = 25$ mN/m) represents the film with maximum concentration of ferredoxin at the interface; the film is rather dense. Upon further pressure increase the AD−P450scc complexes begin to change their mutual orientation. The distance between the surface of the support and the AD molecules exceeds the length of the spacer group of the GOPTS-activated surface (1.4 nm), and therefore the AD molecules being transferred to the support are not able to react with epoxy groups. At a pressure of 40 mN/m the complexes achieve the position where only P450scc molecules can interact with the activated support and AD molecules are turned to the water phase (Figure 5).

The decrease in the binding of anti-AD antibodies with compressed films of P450scc−AD complex could also be explained by changing the conformation mobility of hemeprotein molecules, which is required for the interaction with AD. This may lead to a decrease in the affinity of P450scc to ferredoxin. We note, however, that even in a densely packed monolayer hemeprotein molecules react with the FITC-modified AD (Figure 3), and so this assumptions can be excluded.

Our results demonstrate that the molecular orientation of the P450scc−AD complex at the water−air interface is dependent on the surface density of the monolayer. During the compression of the monolayer, the orientation of the molecules alter. The accessibility of F2 fragments of cytochrome molecules in a monolayer, obtained at highest surface pressure, to correspondent antibodies is greater than accessibility of F1 fragments, and the AD part of the P450scc−AD complex is mainly exposed to the subphase. Covalent attachment of the oriented protein monolayers

to the solid surfaces activated with organosilane polymer is rather complex when compared to the adsorption of protein molecules from solution. An important advantage of this method is that the reaction of ϵ-amino groups of lysines located on the surface of the protein globule with the activated surface, have made it possible to maintain the molecular orientation of the immobilized monolayer.

Conclusions

1. The P450scc molecules do not denature upon spreading on the water surface and being transferred to the solid support. The monolayer consists of the cytochrome P450scc molecules exhibiting a well-defined surface pressure relationship, reflected in an alternation of the molecular orientation at the surface.

2. Our results demonstrate that monolayers of cytochrome P450scc and P450scc−AD can be transferred from the air−water interface, even in very expanded states, to the solid substrate and covalently immobilized without damage to the structure. On the basis of the results obtained, we proposed a model of orientation of P450scc molecules in the monolayer.

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