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Molecular modelling of 17α-hydroxylase-17,20-lyase

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New methods in treatment of hormone-dependent diseases like prostate or breast cancer have become a major subject in medical and pharmaceutical research. Because of the direct correlation of cancer growth and hormone concentration inhibition of hormone biosynthesis reveals a promising strategy in cancer therapy. The key enzyme of androgen biosynthesis is the cytochrome P450 system 17α-hydroxylase-17,20-lyase. To gain deeper insights into the structure and function of this enzyme, whose crystal structure is still unknown we present in this paper a theoretical 3D-model of the human 17α-hydroxylase-17,20-lyase. The model was built by homology modelling using the crystal structure of the P450 CYPeryF as a template. After energy minimisation followed by molecular dynamics simulation the refined model exhibits reasonable protein geometry and a good protein folding quality. For evaluation of protein stability the structure was subjected to molecular dynamics in a waterbox under almost physiological conditions using the GROMACS program. The protein structure and folding remains stable even after 300 ps of free molecular dynamics simulation. The calculation of interaction fields employing the program GRID was used to characterise the active site of the protein. Subsequent docking studies with the natural substrate pregnenolone and further molecular dynamics of the protein-substrate-complexes enabled us to propose a putative binding-site for the physiological substrates.

1. Introduction

The cytochrome P450 superfamily comprises a large number of hemoproteins. About 500 individual members of this have been sequenced until now. More than 60 different enzymatic reactions are known to be catalysed by P450s, most of them are involved in the metabolism of a huge variety of endogenous and exogenous substrates. Several other P450 families are involved in specific biosynthesis of steroid hormones [1]. The general molecular mechanism for the majority of cytochrome P450s is activation and cleavage of molecular dioxygen. During the catalytic cycle a single oxygen is inserted into the substrate while the other oxygen atom leaves the reaction forming a water molecule. The reaction requires two reduction equivalents (i.e. two electrons and two protons) delivered by a specific electron transport system. These different electron transport systems are used for classification of cytochrome P450s.

Class I cyctochrome P450s utilise NADH and a primitive iron-sulphur redoxin or FAD-containing reductase. In contrast class II P450s use a FAD- and FMN-dependent reductase to transfer electrons from NADPH. Most bacterial and mitochondrial cytochromes belong to class I, whereas the microsomal enzymes represent class II P450s [2, 3].

The 17α-hydroxylase-17,20-lyase is a microsomal cytochrome P450 system anchored to the membrane of the endoplasmatic reticulum. The enzyme specifically converts progesterone and pregnenolone to androgens via 17α-hydroxylation (needed also for glucocorticoid production) followed by the cleavage of the side-chain [4, 5]. Because of its key role in biosynthesis of androgens, inhibition of the enzyme results in a total blockade of androgen production. Thus the enzyme has become an interesting target in the treatment of prostate cancer [6]. Development of potent enzyme inhibitors requires a more detailed understanding of enzyme structure and function. The 3D-structure of the enzyme is unknown however and the membrane anchor still prevents the protein from crystallisation. Therefore we found it to be of interest to medicinal chemists to construct a theoretical 3D-model of the protein by homology modelling.

2. Investigations, results and discussion

2.1. Homology and secondary structure

The evolution of the cytochrome P450 superfamily led to an early differentiation and separation of many subfamilies yielding low homologies of only < 40% to each other.

Until now five cytochrome P450 proteins have been crystallised [7–11] and are publicly available. The last one, the eucaryotic CYPnor, has not been taken into consideration for model construction, because this enzyme is a NO reductase and does not belong to the group of monooxygenases.

Despite of the low sequence homology the overall structure and 3D-folding of the four bacterial P450 proteins is highly conserved. In general the topology of the cytochrome P450 system contains four β-sheets and approximately 13 α-helices. The conserved common 'core' is located in the C-terminal region and includes all segments necessary for P450-type functions like heme-binding, oxygen- and redox-partner-binding sites. Whereas the more variable N-terminal segment is associated with the substrate-binding [12, 13].

2.2. The sequence alignment

Because of the low sequence homology alignment procedures based on the sequence only must fail. Therefore the sequence of CYP17 was aligned to a structure alignment based on a 3D-superposition of four cytochrome P450 crystal structures (CYPeryF, CYPterp, CYPBM3, CYPcam [7–10]). The alignment was available from the FSSP-server (EMBL) [14]. The FSSP alignment including the model sequence was compared and manually refined to the information proposed by Lewis et al. in a multialignment of many CYP P450 sequences [2]. The characterisation of the model sequence and the determination of secondary structures was done with the help of secondary structure prediction programs and in comparison with known highly conserved sequential motifs in the crystal structures [14].

In this context the accuracy of several different structure prediction programs was evaluated (Predator, SSPAL,

GOR etc) by predicting the secondary structural elements in the crystallised cytochrome P450s [15–17]. The results demonstrate that only PSIPRED and Predict Protein, two neural network based [18–19] were able to determine the secondary structures in the proteins correctly. Therefore the structurally conserved regions in the model sequence proposed according to the results of secondary structure predictions were obtained by PSIPRED and PHD.
With the help of the structure-based alignment and the prediction results the well conserved C-terminal region of CYP17 could be determined clearly. Characterisation of the substrate-binding-site however was rather tricky because of the high diversity in these parts of the compared sequences. To solve this problem we additionally performed a sequence alignment with sequences of CYP2B1 and CYP2B4, two cytochrome P450s which are involved in metabolism of steroid hormones [20, 21]. Their sequences show a much higher sequence homology to CYP17 in the postulated steroid-binding regions than do the crystal structures. In this way we obtained a rather good CLUSTALW [22] sequence alignment that enabled us to postulate helix B′ as a putative substrate-binding region.

2.3. Model construction

For model construction the n-terminal end indicated as membrane spanning region of CYP17 was omitted. After determination of the structurally conserved regions in the model sequence the structure of CYP17 was built using the Homology module of the MSI-software-package Insight II [23].
If more than one crystal structure is available for structure assignment two different strategies for model construction can be followed. Using the *consensus method* the crystal structures are superimposed and the coordinates of the average structure calculated are assigned to the model sequence. In the *replacement method* however stretches of the real coordinates of different crystal structures are used according to the correspondences in highest homology with the model sequence. Although nowadays the "consensus method" is preferred, the model presented here is built using the "replacement method". This decision is based on the following arguments. In the highly conserved regions the superposition of the different crystal structures shows rather small rms deviations, so calculating an average structure of these protein parts does not produce any further improvement. In the substrate-binding regions of the different P450 proteins however the variability is very high essentially depending on geometrical diversities of their natural substrates. Thus shape and volume of the active site in the crystal structures are determined by their particular substrates and one has to decide which crystal structure to refer to as template. For this region of the protein the use of an average structure would be even misleading.

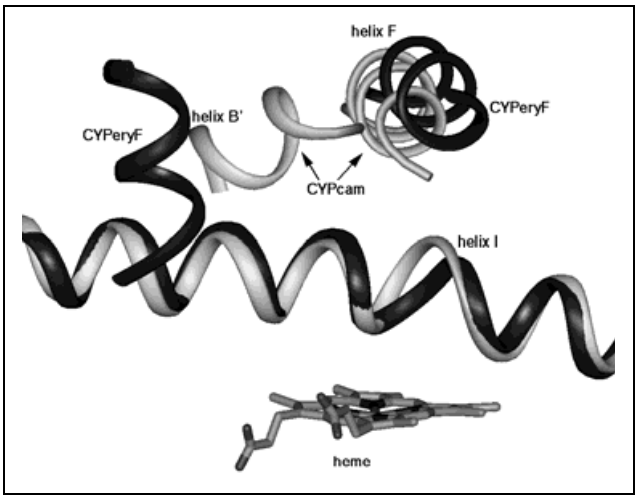

Fig. 1: The different positions of helices B′ and helix F in the active sites of CYPeryF (black) and CYPcam (light grey). The structures were superimposed over helix I

Two of the four P450 structures are crystallised in a substrate-bound conformation [7, 10]. In these proteins the substrate-access channel is closed and the substrate stays in a position favourable for catalytic conversion. In the substrate-free proteins and in the structure of CYPbm3 bound with palmitoleic acid [24–26], the substrate-access-channel is open. Since the aim of our work was to do docking studies with several ligands after modelling the protein structure, we decided to employ the substrate-bound proteins as template structures for model construction. Striking differences between CYPeryF and the CYPcam P450 crystals are found in the positions of helices B′ and F forming parts of the substrate-binding region in the active site (Fig. 1). Because of the demanding sterical requirements of the space-filling steroidal substrate we finally chose CYPeryF. Its substrate DEB6 is showing strong similarities in volume and shape to progesterone. In CYPeryF, differing from other CYPs, helix B′ is orientated almost perpendicular with respect to the heme plane which leads to a widening of the active site and enables the accommodation of larger ligands like DEB6 or progesterone.
CYPeryF was used as a template to assign the coordinates to the model sequence beginning with the structurally conserved regions (Fig. 2). Some insertions had to be introduced to the model sequence because the lengths of the two proteins differ in about one hundred amino acids. The insertions all located in variable random coiled segments were modelled using the loop search routine in HOMOLOGY module of INSIGHT II [23].
After construction of the rough model the splice points of different structural segments were adjusted by energy minimisation. Most parts of the structure were fixed, only a few residues near the splice points could move freely. In the next step the model was superimposed to the template structure of CYPeryF and the heme co-ordinates of CYPeryF were copied to the model structure. All following calculations of the complete protein model were done using a modified consistent valence force field of DISCOVER [23] with additional heme parameters developed by Kemmritz [27]. All calculations were done on Silicon Graphics workstations.

2.4. Model refinement

The adjustment of the backbone-geometry was followed by modelling of the side-chain conformations. In the preliminary model coordinates of non-identical side-chains were assigned in an extended conformation which often leads to some steric clashes or unfavourable geometries. So the conformations had to be modified and refined using the library of Ponder and Richards which contains the side-chain conformations most frequently observed in protein X-ray [28].
For further refinement of protein geometry the model structure was subjected to intensive energy minimisations using steepest descent and conjugate gradient procedures.

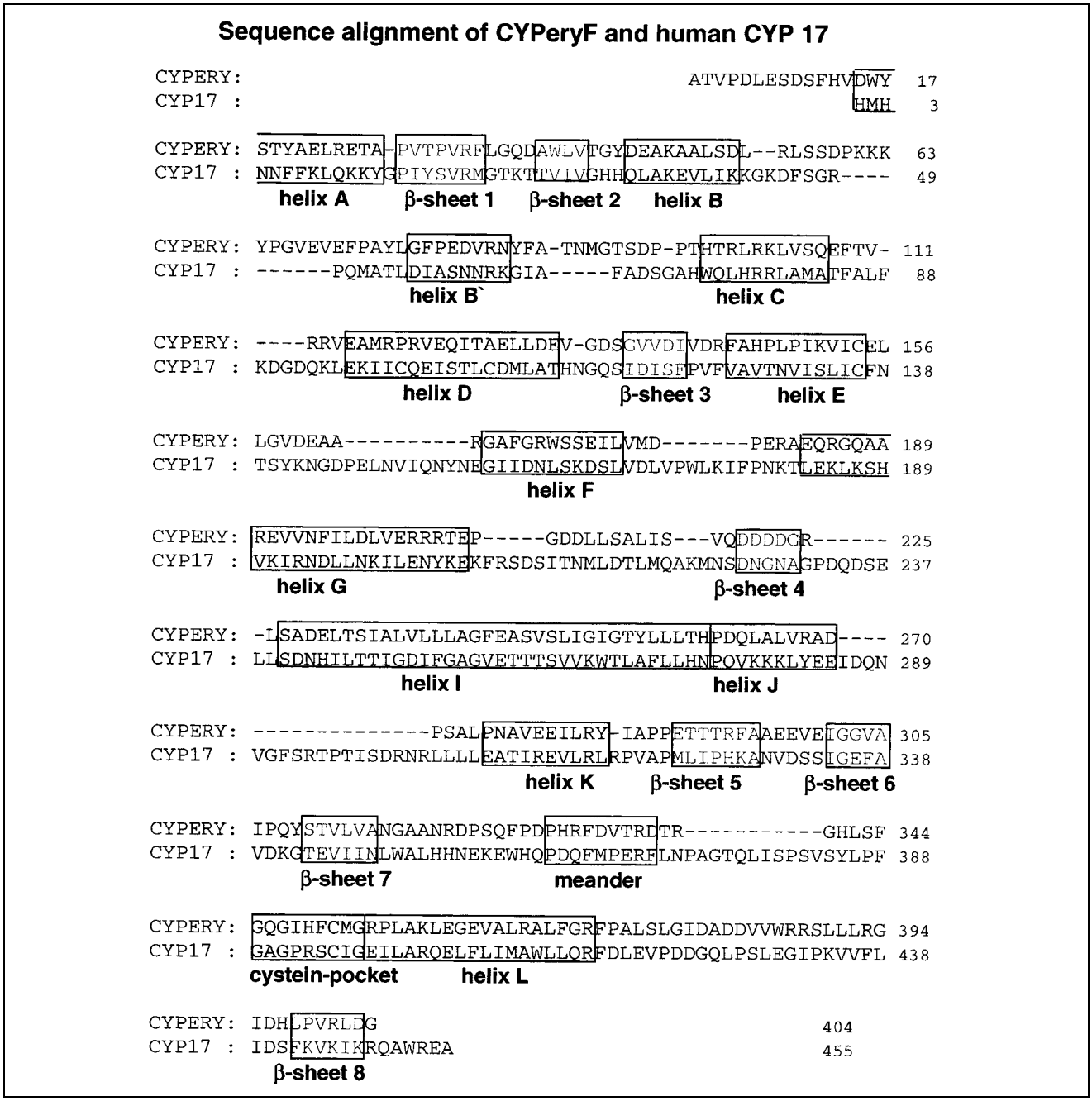

Fig. 2: Sequence alignment of CYPeryF and human CYP17. The observed secondary structures used for construction of the CYP17 model are indicated

During the minimisation process the protein backbone was tethered beginning with a tethering force of 100 kcal/mol followed by a stepwise decrease of the force until the whole structure was allowed to move freely.

2.5. Evaluation of protein geometry

The quality of protein geometry was checked employing PROCHECK [29]. This program checks all important parameters characterising the quality of a protein (like bond-length, bond-angle, φ/ψ-angle-distribution (Ramachandran-plot), χ_1/χ_2-angle-distribution, bad contacts, h-bonding energy, folding-quality) and compares the data with X-ray structures at a defined resolution level. According to PROCHECK the optimised model shows a relatively good protein geometry, with most of the quality parameters being better than average or in between the range of tolerance (thresholds). Only the values of the ω-angles are worse than average. One reason for the unnatural deviation from planarity may be the insufficient force field parameters describing the peptide-bonds, because bad ω-angle geometry is frequently observed in force field calculation.

Forced by the low sequence identity below 20% between the P450 template and the CYP17 model many substitutions of completely different residues were necessary for model construction. Because of the many changes an excellent geometry of the model structure cannot be expected. Nevertheless the model has good overall G-factors, which represent the sum of all bonding- and protein folding quality parameters. The distribution of φ/ψ-angles (Ramachandran-plot) is reasonable, more than 95% of the residues are located in the two "core" (favoured and most favoured) regions (Fig. 3). Only three amino acids are found in "disallowed" regions, but they are located in the outer bounds of the protein far away from the active site (Fig. 4).

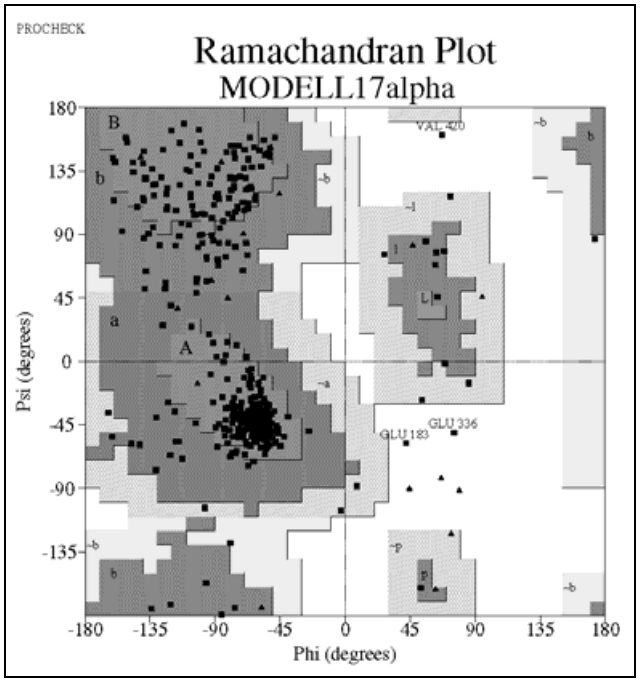

Fig. 3: Ramachandran-plot of the CYP17 model. The most favoured regions (A, B, L) are shadowed medium grey, the favoured regions (a, b, l) are marked dark grey, additionally allowed regions (∼a, ∼b, ∼l) are shadowed in light grey. The amino acids located in the disallowed region are labelled by type and sequence number

The few segments of minor quality were all found in loop regions commonly known as being the most ambiguous, uncertain and critical parts of a protein structure.

For further model refinement a molecular dynamics simulation was performed for a period of 120 ps in order to relax the whole structure including the side-chains. The dynamics simulation was done at physiological temperature of 310 K with a distant-dependent dielectric constant. The heme molecule was fixed, the protein backbone was tethered using a tethering force of 200 kcal/mol. After 20 ps of equilibration, every 10.000 fs (10 ps) the actual frame was stored and minimised to reduce the internal energy and to diminish distortions in the structure. The average structure (calculated over the last 100 ps of the dynamics simulation) was checked for protein geometry and folding reliability. The results demonstrate that the dynamical treatment led to relaxation and improvement of protein geometry. The rms deviation of 1.2 Å calculated for the backbone atoms after superposition of the starting and average structure was comparable to the structural movements determined in the molecular dynamics simulation of CYPeryF which was treated accordingly (rms deviation 0.8 Å). The reliability of protein folding appears to be good because the secondary structures are stable, major structural changes are observed only in the random coiled loop regions.

2.6. Stability of the structure

The model was investigated in more detail in continued molecular dynamics simulations using the program GROMACS [30]. In contrast to the preceding simulations this time the calculations were performed under almost physiological conditions using a waterbox, at physiological pH 7.4 and physiological temperature of 310 K. The protein was embedded in a box filled with water molecules (Fig. 5). Periodic boundary conditions were used to avoid perturbations etc. at the edges of the box. The dimensions of the box were about 70–60–68 Å. Because the parameter set describing the heme molecule in the original GROMACS force field is insufficient, new parameters were added according to the values developed by Wade and Helms [31].

After 100 ps of water equilibration where the structure was fixed, a free dynamics simulation was performed for a period of 300 ps. During the simulation every 1.000 fs the actual frame was stored. The trajectory file was visualised and analysed with the Insight II software, the average structure calculated from the equilibrated trajectory system was evaluated for quality of protein geometry and structure folding reliability. For evaluation of folding reliability the stability of the secondary structures was analysed using the structure analysis program STRIDE [32]. Even after 300 ps of free molecular dynamics no dissolution effects could be observed, the protein folding is unchanged and all secondary structures remain stable in their full lengths. The simulation yielded a relaxed structure showing improved geometrical parameters including the ω-angles. In order to allow for a better assessment of the

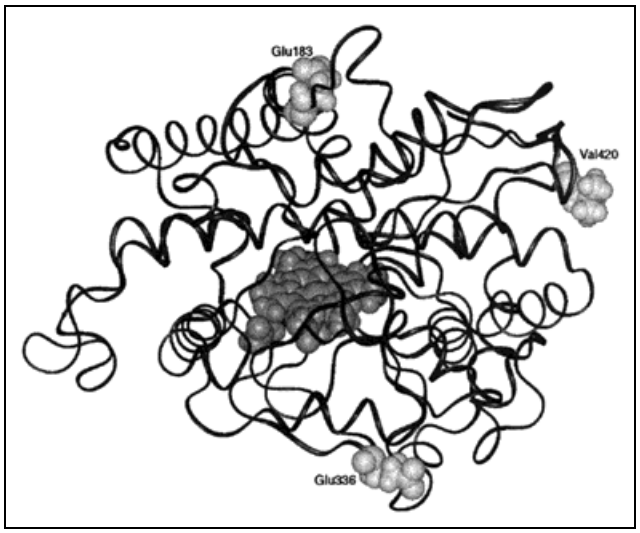

Fig. 4: Ribbon model of the trace of CYP17. The three amino acids (shown as CPK models) of the disallowed regions are all located in the outer bounds of the protein

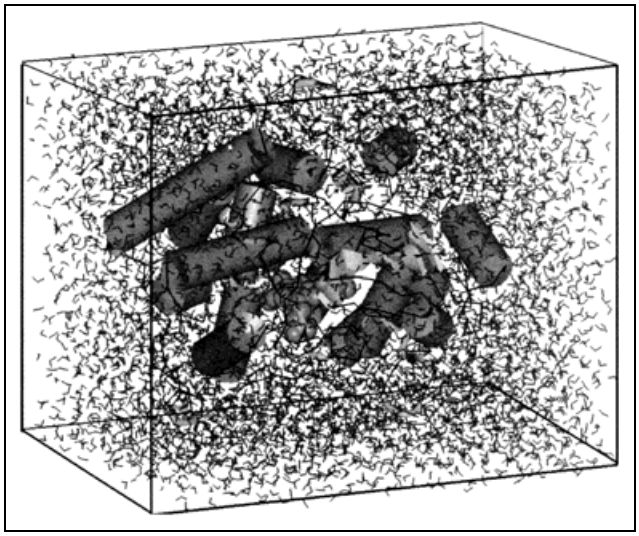

Fig. 5: Model of human CYP17 embedded in the waterbox

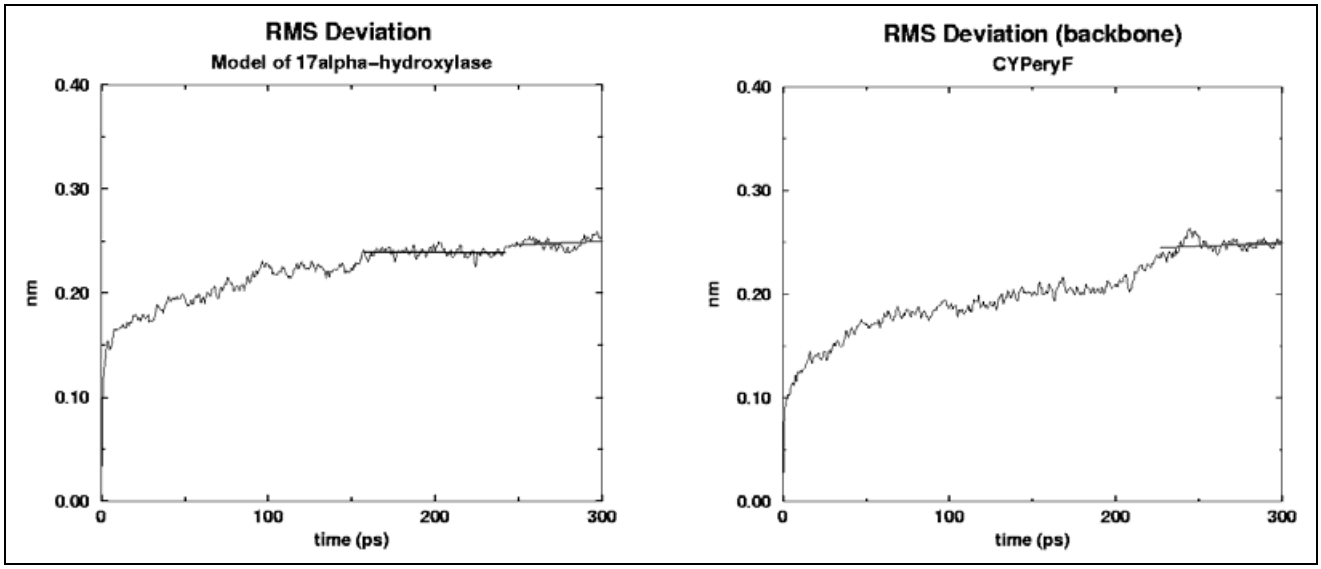

Fig. 6: The RMS deviation of the backbone atoms during 300 ps of molecular dynamics simulation in GROMACS are shown for CYPeryF (left) and for the CYP17 model (right)

results simultaneous molecular dynamics simulations of the CYPeryF crystal structure were performed using the same parameters and conditions.
Subsequently the dynamic behaviour and the structural changes of the proteins were analysed and compared in detail. A commonly used parameter indicating the structural movements is the rms deviation. Both proteins undergo comparable structural movements and changes during the dynamics simulations. The rms values of the CYP17 model and the CYPeryF crystal structure calculated over the backbone atoms are 2.5 Å and 2.3 Å, respectively. In summarising the results demonstrate that the model structure has equivalent stability and equally good protein quality when compared to the CYPeryF crystal structure (Fig. 6).

2.7. Characterisation of the active site

2.7.1. Binding properties

In the next step the binding-potential and the sterical properties of the active site of the model were established and a putative substrate-binding site was identified. For this purpose firstly interaction fields were computed employing the program GRIN/GRID [33, 34]. The protein is set into a 3D grid-cage, where each grid point is loaded with an atomic probe of predefined electronic and sterical properties. Then the interaction energy between the protein and the atomic probe at each grid point is calculated. The interaction energy is attractive (negative sign) or repulsive (positive sign) depending on the sign of the energy value. The interaction energies can be visualised at a defined energy level to identify and locate the regions of attractive or repulsive atomic interactions. Three different atomic probes, the methyl probe, hydroxyl probe and the water probe, were used in order to identify appropriate regions for accommodation of a large substrate like a steroid, constituted by a big hydrophobic corps with only small hydrophilic parts at the "edges" of the molecule. The computed interaction fields reveal the following characteristics of the active site cavity. The active site is relatively large and consists of two regions of hydrophobic character. One is located in almost parallel orientation above the heme plane, the other extends from the heme plane in orthogonal direction. The endpoints of both hydrophobic fields are marked by small hydrophilic regions (Fig. 7).

2.7.2. Geometrical properties

The geometrical properties of the active site of our model present all the well known characteristic features of the P450 systems. The heme molecule in the inner core is embedded sandwich-like between helix I and helix L, that span over the width of the entire protein. The heme position is fixed by salt-bridges between the propionate-groups and basic residues His326 in β-sheet 5 and Arg78 in helix C. The whole protein topology is stabilised by interhelical contacts which can be observed between Glu258 (helix I) and Ser163 (helix F) and Glu404 (helix L) and Thr260 (helix I).
The hydrogen bonding pattern of helix I shows an abnormality which is typical for P450 proteins. In the mid-

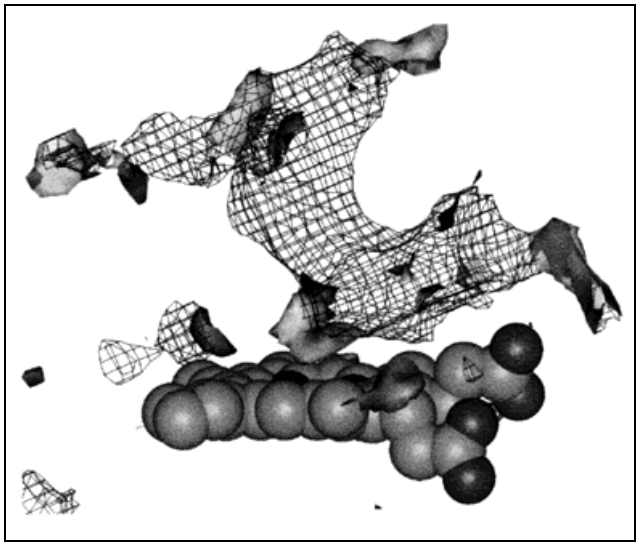

Fig. 7: Presentation of the heme molecule (solid surface) with the interaction fields of the active site of CYP17 calculated with the program GRID. In the shadowed fields (provoked by the methyl probe) favourable hydrophobic interactions are indicated. The smaller fields computed with the water probe (solid dark) and the carbonyl probe (solid light) show regions of preferred hydrophilic interactions

dle of helix I nearby the highly conserved threonine the usual hydrogen bonding of the backbone atoms is disrupted. Instead of forming the regular hydrogen bonds between backbone atoms of residues one and four, alternative hydrogen bonds are generated with the side-chains of threonine and serine residues. This results in a widening of the helix and the formation of a cleft for the accommodation of molecular oxygen. Two residues remain unbound and thus are free to interact with the catalytic molecular oxygen forming hydrogen bonds.

The 300 ps molecular dynamics of the solvated CYP17 model illustrates that a few water molecules penetrate the inner core and stabilise the hydrogen bonding pattern in the active site. One water molecule coordinates with the heme-iron as sixth ligand, while the others form a small water cluster. Like in the crystal structure of CYPeryF these water molecules also participate in the characteristic geometrical pattern of helix I and replace the missing hydrogen bonding network.

The almost invariant Thr259 in helix I is required for catalysis by donating protons to the activated catalytic molecular oxygen. It has been proven by experimental mutation studies that also Asp251 located in the vicinity of Thr259 is essential for enzyme activity of CYP17. Each mutation of this acidic residue yielded in a complete loss of enzyme activity. Like Thr259 the Asp251 is assumed to take part in proton transfer during the catalytic cycle [35]. The results of the dynamics simulation correspond well with these experimental data. In the average structure of our CYP17 protein model the acidic side-chain of Asp251 points into the direction of the catalytic heme-iron and is part of the small cluster of water molecules mentioned above.

Two other experimentally observed point mutations dealt with the two lipophilic amino acids Met322 and Ile324. Each alteration of the lipophilic character and size in these positions results in a decrease of enzyme activity [36]. Both residues are located in the active site flanking the hydrophobic pocket in parallel direction above the heme. Substitution by larger and more voluminous side-chains reduces the width of the hydrophobic pocket. The downscaled active site then offers not enough space to accommodate the large and rigid steroidal substrate. Thus the low enzyme activity can be nicely explained on the basis of the model presented here.

2.7.3. Docking of the substrate

After generation of a reasonable and stable CYP17 model and after having gained an instructive image of the interaction potential of its active site, further investigations dealt with the construction of a substrate enzyme interaction complex.

Based on the information about the size of the active site and the geometrical form of the lipophilic interaction fields as well as the existence of polar interaction fields in suitable positions, the physiological substrate, pregnenolone, can be docked in the active site in two different orientations located orthogonal or parallel with respect to the heme.

In order to be able to decide, which of the two is preferred over the other, both geometries of interaction were tested in molecular dynamics simulations using GROMACS. For this purpose the heme molecules of the protein model CYP17 and the template structure of CYPeryF were superimposed. Then the steroid pregnenolone was mapped onto the substrate DEB6 (6-desoxyerythronolide B) of the crystal structure taking into account the interaction fields and also considering the substrate-iron orientation (the hydrogen atom to be replaced always is orientated in direction to the heme-iron) and the distance (4.8 Å) found in the template CYPeryF. In both orientations, parallel as well as orthogonal, the active site offers enough space for accommodation of the steroid. In order to find out, which orientation is the preferred one for the catalytic transformation, further molecular dynamics simulations of the substrate-protein-complex using the two different starting positions of the steroid were performed.

For 100 ps of water equilibration the protein-complex was fixed and the complete system was heated to 310 K, afterwards the whole system was allowed to move freely for 300 ps. In the orthogonal orientation of pregenolone, the steroid changes this position dramatically during the dynamics simulation. The distance between the iron-atom and the C17 of the steroid increases from 4.8 Å up to 7.8 Å. The steroid finds a new interaction partner in helix I by forming a hydrogen bond with Glu258. Most surprisingly the molecule turns completely about 180°, so that the C17 position which is to be hydroxylated now points away from the iron-atom into the opposite direction. Therefore the substrate is prevented from C17-hydroxylation. This behaviour allows the conclusion that the orthogonal orientation of the substrate seems to be not favoured for substrate transformation (Fig. 8).

In the starting geometry for parallel orientation of the substrate pregnenolone forms two hydrogen bonds. The carbonyl atom of the acetyl-group interacts with the hydroxyl-group of Thr259 in helix I, whereas the 3-hydroxyl-group in ring A interacts with Ser70 located in the turn between helices B′ and C. During the dynamics simulation the position of the steroid only changes to a small extent, the C17–iron distance increases from 4.8 Å to 5.8 Å. Most important however is that the relative orientation of pregnenolone to the catalytic heme-iron is always maintained. The substrate is stabilised in this position

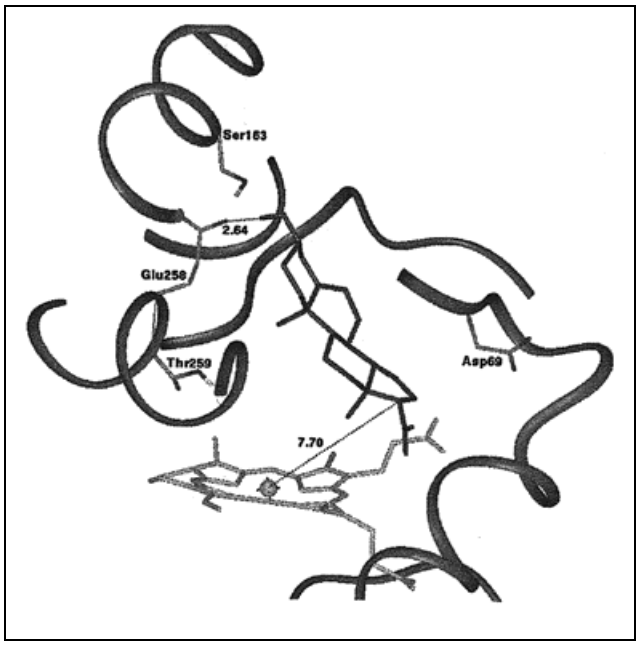

Fig. 8: Average structure of the active site of the CYP17 model (300 ps dynamics simulation). Pregnenolone is docked in orthogonal orientation to the heme plane. The trace is shown as ribbon, some amino acids and the heme molecule are shown in capped sticks. The steroid forms a hydrogen bond to Glu258. The Fe-C17-distance of 7.7 Å is labelled

forming two hydrogen bonds: the carbonyl-group in the side-chain still interacts with the hydroxyl-group of Thr259 in helix I, whereas the hydroxyl-group of ring A changes the interaction partner and interacts with Asp69 of the B′–C-turn (Fig. 9). These results allow us to conclude that only the parallel orientation of the substrate seems to be the preferred geometry for the catalytic reaction to proceed.

Recently Cupp-Vickery et al. succeeded in crystallising the cytochrome P450 CYPeryF bound with androstendione [37]. These experimental data for the first time provide information on the exact location of the substrate-binding site and the position of a steroidal ligand in the active site of a P450 system. The steroid is oriented in almost parallel direction to the heme plane between rings A and D of the porphyrin corps. The closest distance of the carbon atom C6 is about 3,9 Å from the heme-iron and fits well to the location of the natural substrate DEB6. The carbonyl-group in steroid-ring D is hydrogen-bonded with the NH-group of Ala245 in helix I, which stabilises the position of the steroid. The other hydrophilic partial structure in ring A the 3-oxo-group however remains unbound. CYPeryF is the only P450 protein having an alanine residue instead of the almost invariant threonine in the middle of helix I. The hydroxyl function in this case is taken over by three water molecules bound to amino acids nearby Ala245 [10].

Although the ligand in the crystal structure stays in closer distance to the heme-iron the relative position of pregnenolone in our average-model is comparable and the parallel orientation of the steroid is evident. These experimental results strongly support our suggestion of a preferred parallel orientation of pregnenolone in the active site, which were based exclusively on molecular dynamics simulation. The whole model generation procedure thereby gains encouraging confirmation.

2.8. Conclusion

The 17α-hydroxylase-17,20-lyase has become an interesting research target in prostate cancer therapy because of its importance in hormone physiology. The 3D-structure of the enzyme is still unknown. In the last years two models of CYP17 have been proposed on the basis of different template crystal structures [38, 39]. In our opinion the accuracy of these models is especially in the active site-region not sufficient for the determination of a reasonable substrate-binding geometry. Therefore the major goal of our work was to evaluate the structural characteristics and requirements in the active site of the CYP17 protein as reliably as possible so that the derived model can serve as a basis for the construction of ligand-enzyme complexes. The proposed CYP17 protein was modelled on a structure-based approach rather than a sequence-derived homology modelling because similarity in structure and 3D-protein folding is the striking characteristic of all P450 proteins resolved up to now. Since the structural features of the active site regions of the CYPs are mainly influenced by volume and shape of the substrates, we used important (structural) information obtained from the crystallised P450 protein of CYPeryF because its large active site offers the possibility to accommodate big ligands like steroids.

After model construction and structure refinement by energy minimisation and molecular dynamics simulations the protein quality of the modelled structure was evaluated. In view of a rather low sequence homology and the discrepancy in sequence length between template CYPeryF and CYP17 the accuracy of the constructed model is high. The protein shows good geometry and quite reasonable protein folding that can be compared with the results calculated for the CYPeryF crystal structure.

Further improvement of protein quality was achieved by long running molecular dynamics simulations under almost physiological conditions with the protein being embedded in a waterbox. The stabilising effect of the solvent supported protein folding and led to an even better relaxation of the entire structure. The calculation of interaction fields revealed a large and mainly hydrophobic active site with smaller hydrophilic parts at the outer bounds. The interaction fields allow to dock the physiological substrate in two different ways in the active site. Hence further molecular dynamics of the protein-substrate-complexes had to be performed in order to determine the preferred binding position of the substrate. Different starting positions of the protein-substrate-complexes were generated according to the interaction fields and also taking into account the heme-substrate-distances observed in the P450 crystal structures. Also the protein-complexes were subjected to extended molecular dynamics. These simulations result in a clear preference of the substrate-binding in almost parallel direction to the heme. Only in this orientation the substrate can be converted into the 17α-hydroxylated product. The recently resolved protein-ligand-complex of CYPeryF bound with the steroidal ligand androstendione in parallel orientation to the heme plane strongly supports our suggestions for the interaction geometry in the substrate-binding-site in CYP17.

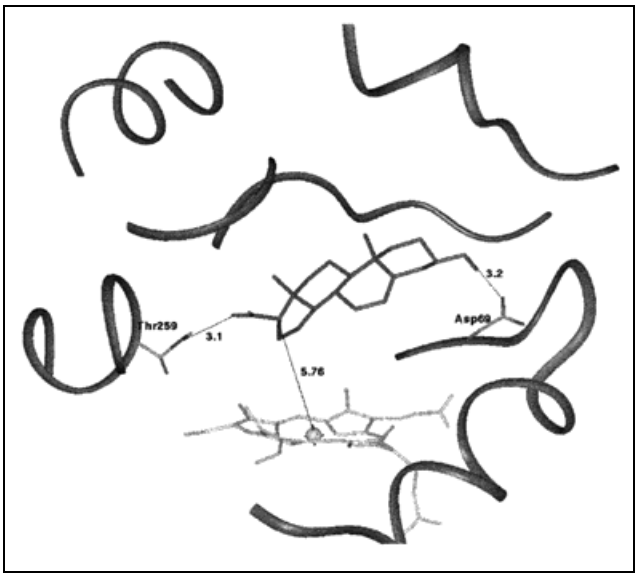

Fig. 9: Average structure of the active site of the CYP17 model (300 ps dynamics simulation). Pregnenolone is docked in parallel orientation to the heme plane. The trace is shown as ribbon, some amino acids and the heme molecule are shown in capped sticks. The two hydrogen bonds formed by the steroid to Thr259 and Asp69 are indicated. The relative position of the steroid to the heme is maintained, the Fe-C17-distance is about 5.8 Å

References

1 Lewis, D. F. V.; Watson, E.; Lake, B. G.: Mutation Res. **410**, 245 (1998)
2 Lewis, D. F. V.; Cytochromes P450 – Structure, Function und Mechanism, Taylor & Francis Ltd. London 1996
3 Wang, M.; Roberts, D. L.; Paschke, R. et al.: Nat. Acad. Sci. (USA) **94**, 8411 (1997)
4 Akhtar, M.; Njar, V. C. O.; Wright, N. J.: J. Steroid. Biochem. Molec. Biol. **44**, 375 (1993)
5 Akhtar, M.; Wright, N.; Shyadehi, A. Z.: Biochemistry **22**, 4410 (1994)

6 Njar V. C. O.; Brodie, A. M. H.: Current Pharmaceutical Design **5**, 163 (1999)
7 Poulos, T. L.; Finzel, B. C.; Howard, A. J.: J. Mol. Biol. **195**, 687 (1987)
8 Ravichandran, K. G.; Boddupalli, S. S.; Haseman, C. A.; Peterson, J. A.: Science **261**, 731 (1993)
9 Haseman, C. A.; Ravichandran, K. G.; Peterson, J. A. et al.: J. Mol. Biol. **236**, 1169 (1994)
10 Cupp-Vickery, J. R., Poulos, T. L.: Nat. Struct. Biol. **2**, 144 (1995)
11 Poulos, T. L.; Raman, C. S.; Huiying Li: Structure **6**, (1998)
12 Peterson, J.; Graham, S.: Structure **6**, 1079 (1998)
13 Haseman, C. A.; Kurumbail, R. G.: Structure, **3**, 41 (1995)
14 Holm, L; Ouzounis, C. et al.: Protein Science **1**, 1691 (1992)
15 Frishman, D.; Argos, P.: PROTEINS- Struct. Funct. Genet. **27**, 329 (1997)
16 Garnier, D. J.; Osguthorpe, J.; Robson, B.: J. Mol. Biol. **120**, 97 (1978)
17 Salamov, A. A.; Solovyev, V. V. J.: Mol. Biol. **247**, 11 (1995)
18 Sander, C.; Schneider, R.: Proteins **9**, 56 (1991)
19 Altshul, S. F.; Nucleic Acids Res. **25**, 3389 (1997)
20 Szklarz, G. S.; He, Y. A.; Halpert, J. R.: Biochemistry **34**, 14312 (1995)
21 Chang, Y.-T.; Stiffelman, O. B.; Loew, G.; et al: Protein Eng. **10**, 119 (1997)
22 Thompson, J. D.; Higgins, D. G.; Gibson, T. J.: Nucleic Acids Res. **22**, 4673 (1994)
23 INSIGHT II, DISCOVER, HOMOLOGY, Biosym MSI, 9685 Scranton Road San Diego, CA 92121–3752
24 Poulos T. L.: Nat. Struct. Biol. **3**, 401 (1996)
25 Li, H.; Poulos, T. L.: Nat. Struct. Biol. **4**, 140 (1997)
26 Roberts, G. C. K.; Modi, S.; Sutcliffe, M. J.: Nat. Struct. Biol. **3**, 414 (1996)
27 Kemmritz, K.: Dissertation, Freie Universität Berlin 1994
28 Ponder, J. W.; Richards, F. M.: J. Mol. Biol. **193**, 775 (1987)
29 Laskowski, R.: J. Appl. Cryst. **26**, 283 (1993)
30 BIOSON Research Institute and Laboratory of Biophysical Chemistry, University of Groningen, 9747 AG Groningen, The Netherlands
31 Wade, R.; Helms, V: Biophysical Journal **69**, 810 (1995)
32 Frishman, D.; Argos, P.: Proteins **23**, 566 (1995)
33 Goodford, P.: J. Med. Chem. **28**, 849 (1985)
34 GRID, Version 14, Molecular Discovery Ltd., Oxford, U.K.
35 Miller, W.; Auchus, R. J.; Geller, D. H.: Steroids **62**, 133 (1997)
36 Lin, D.; Black, S. M.; Nagahama, Y.: Endocrinology **132**, 2498 (1993)
37 Cupp-Vickery, J.; Anderson, R.; Hatziris, Z.: Proc. Natl. Acad. Sci. (USA) **10**, 1073 (2000)
38 Laughton, C. A.; Neidle, S.; Zvelebil, M.; J. J. M.; Sternberg, M. J. M. E.: Biochem. and Biophys. Res. Commun. **171**, 3, 1160 (1990)
39 Burke D. F., Laughton, C. A.; Neidle, S.: Anti-Cancer Drug Design **12**, 113 (1997)

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