Mini review

Active Sites of Cytochromes P450: What are They Like?

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Abstract

Although the x-ray crystallography is giving a relatively precise picture of spatial arrangement of the majority of protein structure, it is not able to give detailed information on the flexibility of the protein active site. The properties of active sites of cytochromes P450 (CYP) were supposed earlier to be relatively similar, reflecting the substrate specificities of the individual enzymes mainly by the amino acid residues present in the respective active site. On the other hand, the most recent experimental as well as theoretical results document that it is the flexibility of this part of protein which gives the active site the property to bind substrates correctly and to accommodate them. It is also the flexibility or plasticity of the structure which determines or limits the property of the protein to keep the conformation which guarantees the function of the enzyme.

Keywords: Cytochromes P450, active sites, protein flexibility, protein compressibility, molecular dynamics

1. Introduction

Cytochromes P450 (CYPs) are one of the most intensively studied enzymes thanks to their ability to act in many processes including biosynthesis of important regulatory molecules as e.g. steroid hormones or taking part in metabolism of foreign substances (xenobiotics) incl. drugs of human use.[1,2] As the main site of dug metabolism is the human liver, the focus here is to human liver microsomal CYP enzymes.[3]

Based on known structures of microbial, soluble CYP enzymes many predictions were made earlier, expecting the active sites of mammalian liver microsomal CYPs more or less similar to these of the bacterial ones with the amino acid residues aligned to the known structures. These studies were extremely useful helping to understand e.g. the docking of drug substates to the respective CYP binding sites.[4]

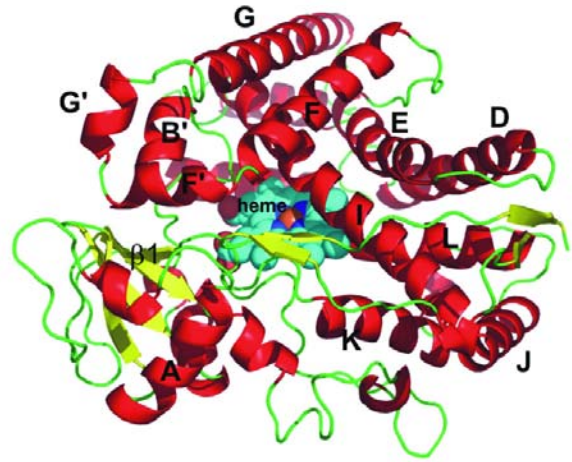

Figure 1: Common fold of typical mammalian CYP2C9 (from the 1OG2 structure[15]) is shown with labeled important secondary structure elements.

The known structures of liver microsomal CYP proteins published in the last six years show relatively conserved overall structure.[5] The "common fold" found in bacterial CYPs with alpha helices (labeled A to L) forming an orthogonal bundle with conserved F/G segment perpendicular to highly conserved I helix in a close vicinity to the heme (Fig. 1) is a typical structural feature of all cytochromes P450. Analyses of active site properties, namely, of the volume expected to be occupied by substrates, were performed with results which were in some cases not easily understood as the active site volumes were found to be hardly able to bind larger substrates.[6]

2. Examples of Active Sites of CYP Enzymes

2. 1. CYP3A4

The first crystallographic studies of this protein revealed an active site of approximately 520 Å^3 (Williams et al.[7]) or 1386 Å^3 (Yano et al.[8]). Whereas smaller substrate metyrapone was shown to bind to the active site without significant alteration of the active site structure, another substrate, progesterone, was found to be bound on the enzyme surface thus raising questions about the modes of substrate binding to this enzyme[7]. Also, the active site seemed to be relatively small to accommodate voluminous substrates as macrolide antibiotics, e.g. erythromycin or cyclosporin A with molecular volumes greater than 1000 Å^3. In fact, the active site volume calculated by Yano et al.[8] (1386 Å^3) was even smaller than this calculated for CYP2C8 (1438 Å^3) earlier [9] with much narrower substrate specificity. The third published X-ray structure analysis of CYP3A4 contained two relatively big substrates, ketoconazole and erythromycin, bound in the active site.[10] In this paper, the volume of the active site was estimated to be up to 2000 Å^3. The active site of this enzyme after binding of erythromycin is shown in Fig. 2 (based on the data deposited by Ekroos et al.[10]).

The apparent differences in the reported active site volumes are mostly due to (i) the selection, definition of the active site (e.g. how great is the part of channel(s) by which the substrate and solvent enter the active site) and (ii) to different methods of calculation as pointed out by Rydberg et al.[11] For example, the calculation of the solvent accessible volume which should not differ significantly from the cavity volume gives almost the same figure for the CYP3A4 structure with progesterone (1W0F according to Protein Data Bank[7]), namely, 554 and 520 Å^3. However, the same comparison for the substrate free structure of CYP3A4 (with a PDB code of 1TQN) yields the value of 512 Å^3 of the solvent accessible volume, but the cavity volume has been estimated as 1386 Å^3 in the original work.[8]

In the light of these discrepancies, we feel that it is the flexibility of the enzyme active site which should be taken into account when a comparison of the abilities of the active sites to bind the substrates and convert them to the respective products is made. As it has been shown earlier, the CYP3A4 enzyme is one of the CYP enzymes exhibiting the most compressible heme active site (Anzenbacherova et al.[12]). This conclusion has been obtained by a direct experimental method, i.e. by measuring the red shift of the heme absorption band with pressure. The second spectroscopic method which gives information on the flexibility of the active site is resonance Raman spectroscopy. Vinyl side chains of the heme *b* can be present in an in-plane or out-of-plane conformations. These two conformations are characterized by two stretching modes in the vibrational spectrum (Kalsbeck et al.[13]): The in-plane conformer vibrates at 1620 cm^{-1}, whereas the out-of-plane conformer exhibits its stretching vibration at about 1630 cm^{-1}. Presence of both vibrational modes in the spectrum

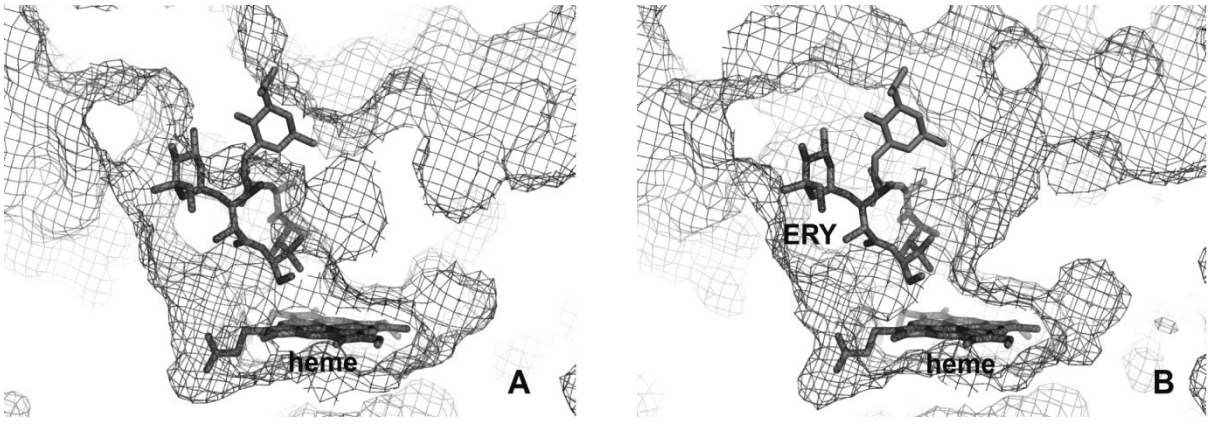

Figure 2: A, Active site (represented by mesh) of free CYP3A4 (1TQN structure) is not large enough for binding of bulky substrates like erythromycin (represented in sticks) or cyclosporins. **B,** Large conformational change in the F/G segment enables binding of these large substrates as shown in the structure of CYP3A4 with bound erythromycin (represented in sticks). The conformational change leads to significant increase in the active site volume due to opening of the distal side, on the other hand, the heme proximity is well preserved.

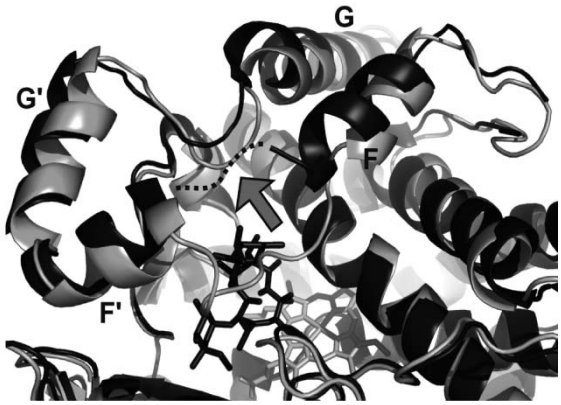

Figure 3: Conformational changes in the CYP3A4 structures (between free CYP3A4 in gray and CYP3A4 in complex with erythromycin in black) induced by binding of a large substrate (erythromycin, in sticks) are localized to the F/G-segment. The shift of the F/F' loop lead to the dramatic increase in the active site volume (*cf.* Fig. 2)

apparently reflects higher degree of freedom of the heme side chains, whereas the presence of a single mode (usually the in-plane mode at about 1620 cm^{-1}) means a fixation of the side chains in a more rigid moiety. As evidenced for CYP3A4, both vibrations are present in resonance Raman spectrum which is indicative of a less conformationally restricted heme in the active site.[14]

Taken together, it can be summarized that the CYP3A4 protein possesses a relatively voluminous and flexible active site, which is in line with its ability to bind substrates of variable size. This can be also nicely documented by expressing the changes in the F/G segment after binding of a substrate (Fig. 3). The figure shows that the most prominent changes are localized in the coordinates determining the properties of the active site, namely, of its ability to accommodate the relatively great substrates of the CYP3A4.

2. 2. CYP2C9

The second CYP enzyme discussed here is the CYP2C9, which was in fact the first human liver microsomal CYP enzyme of known crystal structure.[15] Here, the preliminary experiments with the CYP2C9 protein under high pressure indicate also a highly compressible active site with flexibility comparable to CYP3A4. Also, the resonance Raman data show the presence of both conformers which can be interpreted again in terms of more loose active site (Anzenbacher et al., unpublished). On the other hand, the active site volume of the substrate free structure (PDB code 1OG2) has been reported to be 470 $Å^3$; the solvent accessible volume was estimated again by Rydberg et al.[11] and found to be 484 $Å^3$. Interestingly, substrate binding (structure 1R9O, with flurbiprofen bound, Wester et al.[16]) leads to a modest increase of the solvent accessible volume to 819 $Å^3$ as calculated by Rydberg et al.[11] In the light of the flexibility of the active site assessed by the experimental method, it seems that the active site of the CYP2C9 is rather flexible which is again in an accordance with known relatively broad substrate specificity of this protein (see e.g. reviews of Anzenbacher and Anzenbacherova[2] or Guengerich[17]). The comparison of CYP2C9 active sites based on published structures is displayed in Fig. 4. It may be expected here that further progress in solving the crystal structures (namely with lar-

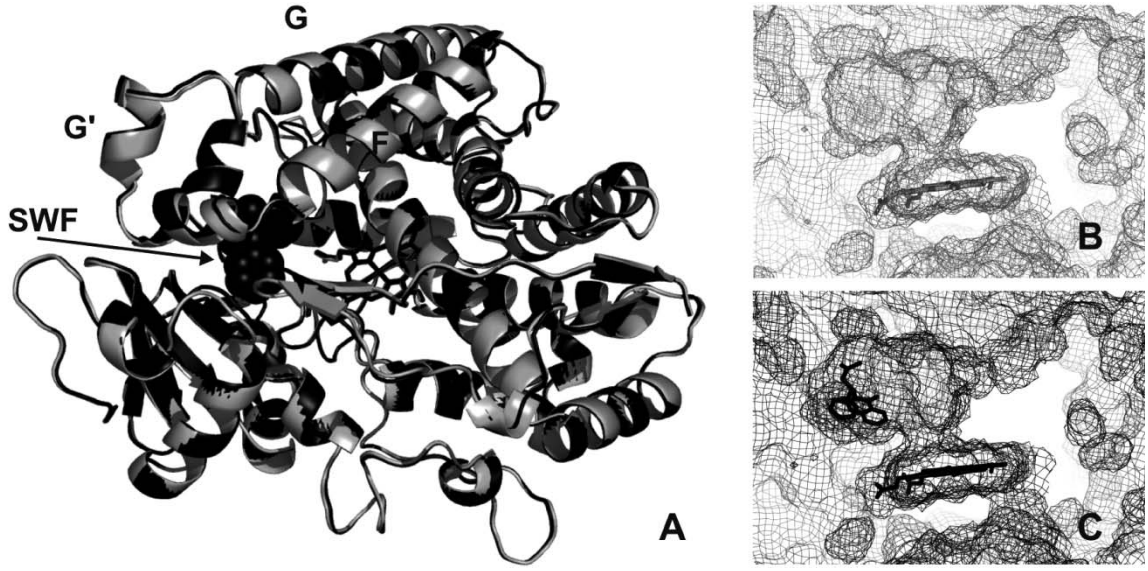

Figure 4: A, Both structures of CYP2C9, the free enzyme (gray) and the complex with S-warfarin (black, S-warfarin abbrev. SWF is represented by black spheres) do not show any large difference in the structures. The active site volume (Figs. **B**, **C**) is highly similar in both structures. Nonetheless, the hardly observable conformational changes induced by ligand binding should not directly imply a low conformational plasticity of CYP2C9 because bulky substrates may induce larger conformational changes (as in case of CYP3A4, *cf.* Ekroos and Sjögren[10]).

ger substrates) will confirm the flexibility of the active site found by the experimental methods.

3. Conclusions

In a summary, it is necessary to point out that our knowledge of active site properties of human liver microsomal cytochromes P450 is far from complete. The flexibility of the active site is necessary experimental information as the crystal structures are representing by their nature only a static picture. The information on the flexibility or plasticity of the structure can be obtained by experimental methods (an example of the spectroscopic studies at higher hydrostatic pressure was given here) or by molecular dynamics.[5,11]

4. Acknowledgment

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5. References

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Povzetek

Čeprav rentgenska kristalografija omogoča dokaj natančen vpogled v prostorsko ureditev večjega dela strukture proteina, ta metoda ne da podrobnih informacij o fleksibilnosti aktivnega mesta proteina. Najprej so domnevali, da so lastnosti aktivnih mest različnih citokromov P450 (CYP) dokaj podobne ter se substratna specifičnost posameznih encimov odraža predvsem v prisotnosti različnih aminokislinskih ostankov v aktivnem mestu. Po drugi strani najnovejši eksperimentalni in tudi teoretični rezultati dokazujejo, da je fleksibilnost aktivnega mesta tista, ki omogoča pravilno vezavo in umestitev substratov. Fleksibilnost oziroma plastičnost strukture tudi določa ali pa omejuje zmožnost proteina, da ostane v konformaciji, ki zagotavlja delovanje encima.