Functional interpretation and structural insights of Arabidopsis lyrata cytochrome P450 CYP71A13 involved in auxin synthesis

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Abstract:
Cytochrome P450 CYP71A13 of Arabidopsis lyrata is a heme protein involved in biosynthesis of indole-3-acetonitrile which leads to the formation of auxin indolyl-3-acetic acid. It catalyzes a unique reaction: formation of a carbon-nitrogen triple bond and dehydration of indolyl-3-acetaldoxime. Homology model of this 57 kDa polypeptide revealed that the heme existed between H-helix and J-helix in the hydrophobic pocket, although both helixes are involved in catalytic activity, where Gly305 and Thr308, 311 of H helix were involved in its stabilization. The substrate indolyl-3-acetaldoxime was tightly fitted into the substrate pocket with the aromatic ring being surrounded by amino acid residues creating a hydrophobic environment. The smaller size of the substrate binding pocket in cytochrome P450 CYP71A13 was due to the bulkiness of the two amino acid residues Phe182 and Trp315 pointing into the substrate binding cavity. The apparent role of the heme in cytochrome P450 CYP71A13 was to tether the substrate in the catalysis by indolyl-3-acetaldoxime dehydratase. Since the crystal structure of cytochrome P450 CYP71A13 has not yet been solved, the modeled structure revealed mechanism of substrate recognition and catalysis.

Keywords: Cytochrome P450 CYP71A13, indolyl-3-acetic acid and homology model.

Background:
Indole-3-acetic acid (IAA) is the main endogenous auxin in higher plants which plays role in plant growth including initiation and emergence of lateral roots, patterning of the root apical meristem, gravitropism, root elongation and development [1-4]. Plants and some plant pathogens can produce IAA to modulate plant growth. Plants can produce IAA from both Trp-dependent and Trp-independent pathways [5-6]. In Trp-dependent pathway, cytochromes CYP79B2 and CYP79B3 of Arabidopsis have been shown to metabolize tryptophan to indole-3-acetaldoxime (Figure 1) which is a precursor for the production of indole-3-acetonitrile in IAA synthesis as well as the precursor for the thiohydroximates in glucosinolate biosynthesis [7-9]. Cytochrome P450 CYP71A13 is involved in the conversion of indole-3-acetaldoxime to indole-3-acetonitrile which is subsequently converted to IAA by nitrilases [10, 11-29]. Cytochrome P450 CYP71A13 is a heme protein with protoheme as the prosthetic group to catalyze a unique reaction: formation of a carbon-nitrogen triple bond and dehydration of indolyl-3-acetaldoxime. Therefore, it is of interest to analyze the predicted structural model of cytochrome P450 CYP71A13 from Arabidopsis lyrata for further docking procedures with suitable ligands.

Methodology:
Amino acid sequence analysis, template searching and sequence alignment
Amino acid sequences of all known cytochrome P450 CYP71A13 was retrieved from Expert Protein Analysis System (http://www.expasy.ch) i.e. the proteome server of Swiss Institute of Bioinformatics [12] and retrieved amino acid sequences were aligned using multiple alignment fast fourier transform (MAFFT version 6). A protein BLAST search was performed against PDB (Protein Databank) to retrieve the
corresponding template for the enzyme [13]. A BLAST search of the Protein Databank demonstrated strongest similarity with human cytochrome P450 PDB code 3QM4 [14]. This similar protein was used as template for modeling, after alignment with the MAFFT program [15].

### Homology modeling

The model building of cytochrome P450 CYP71A13 using human cytochrome P450 (PDB code 3QM4) was achieved using MODELLER 9.11 program [16, 17]. The model was built including the coordinates of heme group from 3QM4 during the modeling. The energy of the model was optimized by several rounds of energy minimizations with Swiss-PdbViewer [18], monitored manually to avoid major rearrangements of the structure. Bond lengths and angles were verified and optimized by consultation of Ramachandran maps followed by energy minimizations.
Results & discussion:
The alignment used for building the homology model is shown in Figure 2 the amino acid sequence identity of cytochrome P450 CYP71A13 was 27% to 3QM4. Based on sequence alignment as an input, the MODELLER 9.11 software generated a large number of spatial restraints from the template structure and constructed a molecular model of cytochrome P450 CYP71A13. The possible applications of generated model depend mainly on the quality of the obtained model. The quality of generated model of cytochrome P450 CYP71A13 was evaluated by PROCHECK [28] which indicated that more than 98.5% of residues ϕ-ψ angles were in favoured and additional allowed region of Ramachandran plot (Figure 3). Again it indicated that the finally obtained 3D model of cytochrome P450 CYP71A13 was satisfactory with respect to Ramachandran plot where only two residues were in disallowed region and four (1.0%) were in generously allowed regions. Residues located in unfavourable region were far from substrate binding domain indicating that these residues may not affect the ligand protein binding simulation. Analysis of physiochemical properties revealed that cytochrome P450 CYP71A13 had higher content of negatively and positively charged amino acids (62, 69 respectively) resulting in increased hydrophilic character Table 1 (see supplementary material). The values of pl showed that plant cytochrome P450 CYP71A13 and cytochrome P450 CYP71E1 was stable at higher pH as compared to human cytochrome P450. The final models of cytochrome P450 CYP71A13 contained α+β structure consisting of ten major α-helices that form the conserved 3D fold of CYP proteins and seven β- sheets (Figure 4). The heme existed between H-helix and J- helix in the hydrophobic pocket where Gly305 and Thr308, 311 of H- helix were involved in its stabilization (Figure 5D). Analysis of the surface of the structural model of cytochrome P450 CYP71A13 identified surface exposed hydrophilic domains in the region positioned near the N-terminal region while a hydrophobic core was present near the C-terminal region (Figure 5A). The hydrophobic core was formed by Leu218, 370, 364, 452, Phe338, Pro446, Ile448 and Ala451 amino acids near the C-terminal region. This region seemed to be most likely the binding site for substrate.

Cytochrome P450 CYP71A13 converts indole-3-acetaldoxime to indole-3-acetonitrile. The substrate indole-3-acetaldoxime was docked into the substrate binding pocket so that the atoms involved in the initial dehydration reaction were positioned above the heme. The smaller size of the substrate binding pocket in cytochrome P450 CYP71A13 was due to the bulkiness of the two amino acid residues Phe182 and Thr315 pointing into the substrate binding cavity. Indole group of the oxime was positioned slightly closer to the heme iron and O-atom towards the Ser369 and His367 respectively with a final distance of 2.1 Å and 4.3 Å (Figure 5B). The substrate indole-3-acetaldoxime was tightly fitted into the substrate pocket with the aromatic ring being surrounded by residues creating a hydrophobic environment. Leu312, 370 and Pro368 from the C-terminal were positioned favourably for interaction with the indole group of the substrates in the proximal end of the pocket, while Ser369 in the heme distal end of the substrate pocket was positioned to interact with the leaving group of indole-3-acetaldoxime (Figure 5C). The apparent role of the heme in cytochrome P450 CYP71A13 was to tether the
substrate in the catalysis by indole-3-acetaldoxime dehydratase. This homology model revealed that hydrogen bonding between the OH group of indole-3-acetaldoxime and the side chains of Ser369 and His367 controls the specific orientation of the heme-bound substrate suitable for the elimination of the OH group of indole-3-acetaldoxime and that these residues and the heme created a prefixed site for substrate recognition and binding (Figure 5C).

Figure 5: Active site of cytochrome P450 CYP71A13 from Arabidopsis lyrata A) Surface representation of amino acid hydrophobicity in cytochrome P450 CYP71A13 with a color scale that varies from blue to white, representing dodger blue for the most hydrophilic, to white for the most hydrophobic residues; B and C show features of the substrate indole-3-acetaldoxime binding pocket of cytochrome P450 CYP71A13 and D represents amino acids involved in stabilization of heme in cytochrome P450 CYP71A
Conclusion:
We describe structural model of Arabidopsis lyrata cytochrome P450 CYP71A13 involved in auxin synthesis generated using molecular modeling techniques. The model was further docked with indole-3-acetaldoxime to explore structural features and binding mechanism. Analysis of active site and binding specificities of indole-3 acetaldoxime to the enzyme show two amino acid residues (His367 and Ser369) for catalytic activity and substrate binding. The model complex provide insights for the understanding of mechanism of substrate recognition and its conversion to corresponding nitrile by cytochrome P450 CYP71A31.

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### Supplementary material:

**Table 1**: Analysis of physiochemical properties of human cytochrome P450, cytochrome P450 CYP71A13 and cytochrome P450 CYP71E1

| Parameters                      | Homo sapiens cytochrome P450 | Arabidopsis lyrata cytochrome P450 CYP71A13 | Sorghum vulgare cytochrome P450 CYP71E1 |
|---------------------------------|------------------------------|---------------------------------------------|------------------------------------------|
| Uniprot accession number       | P10635                       | D7LC87                                      | O48958                                   |
| Theoretical pI                  | 6.77                         | 8.94                                        | 8.76                                     |
| Negatively charged residues (Asp + Glu) | 53                           | 62                                          | 60                                       |
| Positively charged residues     | 51                           | 69                                          | 66                                       |
| Extinction coefficient (M⁻²·cm⁻¹·at 280nm) | 47815                       | 51910                                       | 55850                                    |
| Instability index               | 44.63                        | 38.66                                       | 36.81                                    |
| Aliphatic index                 | 95.15                        | 99.01                                       | 89.83                                    |
| GRAVY                           | -0.031                       | -0.179                                      | -0.147                                   |