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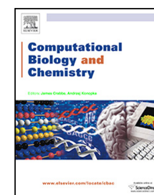

Molecular characterization, modeling and docking of CYP107CB2 from *Bacillus lehensis* G1, an alkaliphile

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ABSTRACT

Cytochrome P450s are a superfamily of heme monooxygenases which catalyze a wide range of biochemical reactions. The reactions involve the introduction of an oxygen atom into an inactivated carbon of a compound which is essential to produce an intermediate of a hydroxylated product. The diversity of chemical reactions catalyzed by cytochrome P450s has led to their increased demand in numerous industrial and biotechnology applications. A recent study showed that a gene sequence encoding a CYP was found in the genome of *Bacillus lehensis* G1, and this gene shared structural similarity with the bacterial vitamin D hydroxylase (Vdh) from *Pseudonocardia autotrophica*. The objectives of present study was to mine, for a novel CYP from a new isolate *B. lehensis* G1 alkaliphile and determine the biological properties and functionalities of CYP in this bacterium. Our study employed the usage of computational methods to search for the novel CYP from CYP structural databases to identify the conserved pattern, functional domain and sequence properties of the uncharacterized CYP from *B. lehensis* G1. A computational homology model of the protein's structure was generated and a docking analysis was performed to provide useful structural knowledge on the enzyme's possible substrate and their interaction. Sequence analysis indicated that the newly identified CYP, termed CYP107CB2, contained the fingerprint heme binding sequence motif FxxGxxxCxG at position 336-345 as well as other highly conserved motifs characteristic of cytochrome P450 proteins. Using docking studies, we identified Ser-79, Leu-81, Val-231, Val-279, Val-383, Ala-232, Thr-236 and Thr-283 as important active site residues capable of stabilizing interactions with several potential substrates, including vitamin D_3, 25-hydroxyvitamin D_3 and 1α-hydroxyvitamin D_3, in which all substrates docked proximally to the enzyme's heme center. Biochemical analysis indicated that CYP107CB2 is a biologically active protein to produce 1α,25-dihydroxyvitamin D_3 from 1α-hydroxyvitamin D_3. Based on these results, we conclude that the novel CYP107CB2 identified from *B. lehensis* G1 is a putative vitamin D hydroxylase which is possibly capable of catalyzing the bioconversion of parental vitamin D_3 to calcitriol, or related metabolic products.

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1. Introduction

Cytochromes P450s are a superfamily of heme monooxygenases which are present in a wide variety of organisms in nature (Bernhardt, 2006; Zurek et al., 2006). They catalyze a broad range of biochemical reactions and play an essential role in the assimilation of carbon source. Prototypical reactions catalyzed by CYPs include fatty acid hydroxylation, secondary metabolite biosynthesis and drug metabolism (Schallmey et al., 2011; Sono et al., 1996).

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CYPs catalyze the molecular insertion of oxygen into a wide range of substrates, making them to be promising biocatalysts for the synthetic industry (Grogan, 2011; Urlacher and Eiben, 2006).

The biotransformation of vitamin D_3 to 1α,25-dihydroxyvitamin D_3 is one of the most successful applications of cytochrome P450 in biotechnology. The product has been used to treat numerous diseases such as osteoporosis, chronic renal failure and hypothyroidism (Sakaki, 2012; Yasutake et al., 2009). Vitamin D_3 is a biologically inactive compound that requires one or more CYPs to catalyze the formation of the most active form of vitamin D hormone, 1α,25-dihydroxyvitamin D_3 or calcitriol. The major function of calcitriol is to maintain safe levels of calcium and phosphorus in the blood, by regulating the absorption of these ions in the intestine, bone and kidney. In addition, calcitriol is also recognized as an important anti-proliferative factor for dividing cells and tissues, as vitamin D deficiency is now linked to over 20 forms of cancer (Guyton et al., 2003). The chemical synthesis of 1α,25-dihydroxyvitamin D_3 requires complex procedures, including almost 20 reactions steps with low production yields. Therefore, development of an efficient and simplified production process for calcitriol remains an important area of investigation (Sakaki et al., 2011; Zhu and Okamura, 1995). Only a few studies have reported on the usage of bacterial cytochrome P450s for the production of calcitriol in which vitamin D_3 was converted to 1α,25-dihydroxyvitamin via 25-hydroxyvitamin D_3.

Since, there is an increasing industrial demand to exploit cytochrome P450 as a valuable biocatalyst, considerable attempts have been devoted to search for novel enzymes with unique metabolic properties. This strategy was attempted in this study, where a novel CYP from the alkaliphilic bacteria, *Bacillus lehensis* G1, was mined. This bacterium dwells in soil with the capability to thrive at high pH up to 11. A survey on the complete *B. lehensis* G1 genome revealed a single candidate gene that potentially coded for a functional CYP enzyme.

Sequence analysis showed the protein sequence of the cytochrome P450 from *B. lehensis* G1 exhibited sequence identity of 44% with vitamin D_3 hydroxylase (Vdh) from *Pseudonocardia autotrophica* which had been loosely grouped into the CYP107 family of enzymes (Fujii et al., 2009; Yasutake et al., 2009). However, the lack of structural information for the putative CYP from *B. lehensis* G1 prevented a more detailed characterization of its biological role. Therefore, in this study, the conserved pattern, functional domain and sequence properties of cytochrome P450 from *B. lehensis* G1 were analyzed and subsequently, a structural model of the enzyme was constructed. Docking analysis was performed to provide valuable insight regarding the architecture of its active site and ligand binding interactions. All results pertaining to the possible structure and sequence properties of cytochrome P450 from *B. lehensis* G1, now referred to as CYP107CB2, are duly reported and discussed.

2. Materials and methods

2.1. Molecular analysis of P450

The search for gene sequence encoding cytochrome P450 from the genome of *B. lehensis* G1 was performed through the BLASTN program provided by National Center for Biotechnology Information (NCBI). The conserved domain of cytochrome P450 was determined through conserved domain database (CDD) provided by NCBI (http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi). Protein molecular weight and theoretical isoeletric point were computed with ExPasy compute pI/Mw tool (http://web.expasy.org/compute_pi/). Gene ontology annotation was performed by using ScanProSite program (http://www.ebi.ac.uk/Tools/pfa/iprscan/). Protein family and functional domain were determined through Pfam server (http://pfam.sanger.ac.uk/).

2.2. Multiple sequence alignment

In order to investigate the conserved motif of CYP107CB2, three bacterial protein sequences from the CYP107 superfamily of enzymes include Vdh (PDB: 3A4G) from *P. autotrophica*; PikC (PDB: 2BVJ) from *Streptomyces venezuelae*; and EryF (PDB: 1EGY) from *Saccharopolyspora erythraea* were obtained from the Protein Data Bank (PDB) and aligned by using ClustalW (Thompson et al., 1994). Sequence identity was analyzed using PSI-BLAST (Position-Specific Iterated BLAST) against sequences in the PDB.

2.3. Homology modeling of CYP107CB2 from B. lehensis *G1*

The amino acid sequence of CYP107CB2 was submitted to NCBI BLAST server through PDB using PSI-BLAST for homologous template selection. The template having the closest match (highest similarity) for all retrieved query sequences was chosen for the construction of three dimensional structural model of cytochrome P450 of *B. lehensis* G1. An automated homology modeling program SwissModel (Arnold et al., 2006) was used to generate the model. Cytochrome P450 vitamin D_3 hydroxylase (Vdh) from *P. autotropica*'s (protein data bank code: 3A4G; resolution: 1.75 Å) was used as a template.

2.4. Validation of the CYP107CB2 model

The constructed model (CYP107CB2) was subjected to quality assessment and structure validation with respect to its geometry and energy aspect using PROCHECK (Laskowski et al., 1996) and ProSA (Wiederstein and Sippl, 2007). Root mean square deviation (RMSD) between the homologue model and its template was calculated by structural superimposition of both structures by using MUSTANG method (Konagurthu et al., 2006) in YASARA (Yet Another Scientific Artificial Reality Application) program Version 11.3.22 (Krieger et al., 2002).

2.5. Molecular docking of CYP107CB2 with vitamin D_3, 25-hydroxyvitamin D_3 and 1α-hydroxyvitamin D_3

To predict the potential substrates for CYP107CB2, the built model was subjected to *in silico* docking experiment using AutoDock and its functionality which is embedded into the YASARA Structure package. For this purpose, vitamin D_3, 25-hydroxyvitamin D_3 and 1α-hydroxyvitamin D_3 were focused and docked into the region of active site. The structures of the substrates (ligands) were obtained from NCBI PubChem. Lamarckian genetic algorithm using 0.2 Å translational step size; 5 Å orientational and torsional step size was applied in docking experiment (Morris et al., 1998). The grid size of $19 \times 21 \times 19$ Å^3 with grid point spacing 0.375 Å was set in the built model for ligands docking. The other docking parameters used in this analysis were: 25 docking runs; 25,000,000 energy evaluation; AMBER03 force field; 150 population size; the number of generation was 27,000. After docking simulation, the binding energy calculated by AutoDock was obtained from the summary of log file. The data were sorted by the positioned distances of oxidizable carbon atom and binding energy, where shorter distance and positive energy indicated stronger binding.

2.6. Expression of recombinant CYP107CB2 protein

Gene sequence of *CYP107CB2* was cloned into pET102/D-TOPO vector and the expression of recombinant cytochrome P450

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