Structural Characterization of the Rieske Oxygenase Complex from *Burkholderia fungorum* DBT1 strain: Insights from bioinformatics

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***Abstract*—Polycyclic aromatic hydrocarbons (PAHs) represent a class of organic compounds that negatively affect human health. These compounds are of toxicological concern because some of them have been identified as carcinogenic, mutagenic, and teratogenic. *Burkholderia fungorum* DBT1 is a bacterial strain, first isolated from an oil refinery discharge, which can utilize dibenzothiophene (DBT), phenanthtrene and napthalene as substrates for growth. This strain is capable of degrading DBT nearly completely through the "Kodama pathway" more efficiently than others. The work presented here is aimed at a structural characterization at the molecular level of the proteins involved in the first step of the PAH degradation pathway, i.e., the Rieske Oxygense (RO) complex. Thus, using state-of-the-art structural bioinformatics tools we have built the structural models of each of the members of the RO complex encoded in the *Burkholderia fungorum* DBT1 strain. The structural characterization combined with future molecular biology experiments may give important insights into the functioning of this particular strain.**

Keywords - biocomputing; *Burkholderia fungorum DBT1*; dibenzothiophene; modeling; PAHs.

I. INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are an ubiquitous class of hydrophobic organic compounds consisting of two or more fused aromatic rings. PAHs are widespread in the environment and persist over long periods of time: many polycyclic aromatic hydrocarbons (PAHs) are largely suspected to be mutagenic or carcinogenic [1], and their contamination in soil and aquifer is of great environmental concern.

Of the PAHs occurring in soils and groundwaters, about 0.04 - 5% (wt/wt) are sulfur heterocycles [2] among which dibenzothiophene (DBT) represents the prevailing compound. This is therefore taken into account as model chemical structure in studies dealing with either biodegradation of organo-sulfur contaminants by petroleum biodesulfurisation through the "4-S pathway"[3] or through the "Kodama pathway" [4] [5] [6]. The latter transforms the molecule to the final product 3-hydroxy-2-formylbenzothiophene (HFBT). Denome *et al.* [7] cloned the genes responsible for the Kodama pathway from *Pseudomonas fungorum*. These genes, that are organized in a single operon, encode enzymes of the upper naphthalene catabolic pathway, and belong to a group of genes showing a high degree of sequence identity with the *nah* genes from *Pseudomonas putida* G7 [8]. The *nah*-like class of genes, cloned from different microorganisms, are highly conserved and are involved in the transformation of molecules that constitute the low molecular weight fraction of PAHs, including DBT [9] [10] [11] [12] [13]. They are normally clustered in a single operon under the control of a single promoter.

A novel genotype for the initial steps of the oxidative degradation of dibenzothiophene was recently found in the bacterial strain *Burkholderia fungorum* DBT1 isolated from a drain receiving oil refinery wastewater [14]. *Burkholderia fungorum* DBT1 is a NON-PATHOGENIC strain capable of transforming DBT completely through the "Kodama pathway" with higher efficiency than other microorganisms. This strain shows a particular genomic organization for the initial steps of the oxidative degradation of PAHs when compared to previously described genes capable of PAHs catabolism. In fact the genes are organized in two operons instead of one: they are called pH1A-p46 and p51 respectively [14] (GenBank accession numbers AF380367 and AF404408 respectively). In DBT1genes involved in DBT transformation show only low similarity with the corresponding conserved isofunctional oxidative genes. The unusual gene organisation suggests the possibility of novel features of DBT transformation in natural context.

Rieske Oxygenase (RO) systems have been shown to catalyze the first step in the Kodama pathway [15]. ROs produce *cis*-dihydrodiols from a large variety of substrates and molecular oxygen (dioxygen).

RO systems use electrons from NAD(P)H to activate molecular oxygen, which is then used to oxidize the substrate. RO systems are composed of two or three components, including a reductase, a ferredoxin (not found in all systems), and an oxygenase (Fig. 1). The reductase component liberates electrons from NAD(P)H and transfers the electrons to the ferredoxin. The ferredoxin shuttles the electrons to the oxygenase, where they are used in catalysis. In systems where the ferredoxin is absent, the reductase transfers electrons directly to the oxygenase. The oxygenase component of these systems is responsible for catalysis. This component consists of an alpha subunit, which contains both a Rieske binding domain and a catalytic domain. In some cases, a beta subunit is present, which is believed to

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primarily function as a stabilizer for the alpha subunits. Rieske Oxygenase (RO) systems of *Burkholderia fungorum* DBT1 is called dibenzothiophene dioxygenase [14]. While structural studies have been performed on a number of ROs, no crystal structure exists for the DBT1 enzymes. Dibenzothiophene dioxygenase is able to degrade a wide spectra of molecules, including naphthalene, phenanthrene and DBT [16]. For its particular characteristics, *Burkholderia fungorum* DBT1 might be interestingly exploited in bioremediation protocols of PHA-contaminated sites. Ultimately, therefore, understanding the molecular basis of ligand-target interactions in this system may be fundamental for a complete characterization of the mechanism of action of the present strain and for future applications in bioremediation protocols of PHA-contaminated sites. Computational molecular biology (CMB) and protein structural bioinformatics approaches are keys to face these challenges, especially when, as for this particular case, no crystal structures exist for the different molecular components.

The work presented here is aimed at a structural characterization at the molecular level of the proteins involved in the first step of the PHA degradation pathway, i.e., the RO system. We have built the structural models of each of the components encoded in the *Burkholderia fungorum* DBT1 strain. Although the work is in a preliminary phase, still several conclusions can already been drawn specially in the formation of protein complexes involved in the Kodama pathway.

II. MATERIALS AND METHODS

The sequence alignment of the different targets and their corresponding structural templates were extracted from the multiple sequence alignment considering the entire families of interest.

We then constructed the models for each of the members of the RO complex of *Burkholderia fungorum* DBT1 strain. This was calculated as follows: all sequences and those of their families were retrieved from the Uniprot [17] database using *ssearch* [18]. They were aligned with PROMALS [19]. This multiple sequence alignment was then used for the definition of the Hidden Markov profile (HMM) of each of the target sequences. The profiles were then funneled through the HHsearch [20] program to identify the most plausible homologous structural templates. Such procedure is currently one of the best ones as evaluated from CASP7 experiment [21]. The multiple sequence alignments obtained in this way were used as the reference for the structural prediction of the different targets by homology modeling. The models were then built up by the use of the program Modeller9v4 [22]. Superposition of the structures, protein visualization and figures were carried out using the program VMD [23].

III. RESULTS AND DISCUSSIONS

Ferredoxin reductase. The nucleotide sequence of ferredoxin reductase component of dibenzothiophene dioxygenase complex was taken from some recent studies (data not published) that indicates the presence of a putative ORF that probably encodes for this subunit. This component of the dibenzothiophene dioxygenase complex was modeled by standard homology modeling [24] [25] procedures by the use of the program HHpred [26] in a semi-automatic fashion, as described in the methods section. The subunit was modeled based on the corresponding subunit of Benzoate 1,2-Dioxygenase Reductase from *Acinetobacter fungorum* Strain ADP1 [27] (PDB code 1KRH). The sequence identity shared between the ferredoxin reductase subunit with its template is 24%. The sequence alignments are available as supplementary material (Fig. S1 [28]). The selected template was not co-crystallized with NADP ligand. So the functional structure was built by optimal superposition of the main chain of ferredoxin reductase with the main chain of the X-ray solved structure. To improve the quality of the model we modeled the enzyme in a putative catalytic conformation, that is, with NADP ligand in the putative binding cavity. The ligand was manually transferred to the model from the structure of Pea FNR Y308S mutant from *Pisum sativum* (PDB code 1QFY) following the procedure used by Deng *et al.* [28]. That is, using the program VMD, we superposed the main chain coordinates of our model to the main chain of 1QFY protein transferring NADP coordinates into our model.

Ferredoxin (Ac. N. AAK96190). This subunit of the dibenzothiophene dioxygenase was modeled by standard homology modeling using the same protocol and criteria of ferredoxin reductase (Fig. S2 [28]). The subunit model was built using as template the corresponding subunit from *Sphingomonas yanoikuyae* B1 ferredoxin [29] (PDB code 2I7F), which shears 50% the sequence identity. This operation was performed as described in the Methods section. Studies of the interaction of ferredoxin with the reductase and with the oxygenase will be carried in a near future by the use of protein-protein docking techniques and validated by experiments.

Oxygenase (Ac. N. AAK62353 and AAK62354). The oxygenase component (α and β subunits) of dibenzothiophene dioxygenase was modeled by standard homology modeling using the same protocol seen for the others components. The alpha/beta complexes were modeled based on the alpha/beta subunits of the Bifenyl 2,3-dioxygenase from *Sphingobium yanoikuyae* B1. The sequence identity shared between the alpha and beta subunits with their templates is 49 and 37 % respectively. The sequence alignments are available as supplementary material (Fig. S3, S4 [28]). The selected template was not

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co-crystallized with ligands. The functional hexamer (three alpha and three beta subunits) was built by optimal superposition of the main chain of the alpha/beta complexes with the main chain of the X-ray solved structure. To test the validity of our models we modeled the hexamer in a putative catalytic conformation, that is, with the ligands bound in the putative binding cavity. The moiety of dioxygenase with their cognate ligands were solved for a variety of members of the family, nevertheless, two structures were co-crystallized with ligands that also bind to dibenzothiofene, i.e., naphthalene and phenanthrene. These structures are the Naphthalene 1,2-Dioxygenase from *Pseudomonas* sp. strain NCIB 9816-4 [30] (PDB code 2HMK) and Naphthalene 1,2-Dioxygenase [31] (PDB code 1O7G) from *Pseudomonas putida*. Therefore, using the program VMD, we superposed the main chain coordinates of our model to the main chain of 2HMK and of 1O7G protein. We then transferred naphthalene and phenanthrene coordinates respectively, into our hexamer model. In the supplementary material (Fig. S5 [28]), the high structural conservation of the binding sites can be appreciated, showing the full conservation of the latter, although the ligands were transferred from independent crystal structures belonging to different species. The conservation of all the interacting residues, albeit the low sequence identity between templates and target, provide an initial, although non definitive, validation of the reliability of our models. Indeed, future work will include the virtual docking of dibenzothiofene into the binding cavity combined with experimental validation. The final modeled RO complex members can be appreciated in Figure 1.

IV. CONCLUSION

In the present work, we aimed at the modeling of the initial step in the Kodama pathway for the degradation of PAHs by the *Burkholderia fungorum* DBT1 strain. The availability of several templates covering the entire RO complex gave us the possibility of building not only the structural models of the isolated components but also to gain insight into the big protein complexes involved in the process. Although, the obtained results are preliminary and correspond to the first step of a lengthy iterative process of experimental and computational work, the possibility of modeling one of the most important proteins complexes and its validation with experiments extracted from literature, prompted us to hypothesize that more refined models will offer more a important overview of the system under study and may allow the full characterization of the entire pathway. Moreover, at the present stage site directed mutagenesis experiments can be already designed and proposed from the models. The extremely high three-dimensional conservation observed in the binding cavities will allow the production of targeted mutants that may permit a deeper characterization of the enzymatic mechanisms. Our analysis and modelling procedures allowed us to find, in the alpha subunit of RO complex of *Burkholderia fungoru*m DBT1 strain, an insertion of three amino acids very close to the active site that seems to be a duplication. This insertion is present only in *Burkholderia fungoru*m DBT1 strain and could represent a peculiar characteristic of DBT1 strain for substrate degradation efficiency. We are also involved in modelling of the complex between the alpha subunit of RO and the ferredoxin component and the transient complex between the ferredoxin and ferredoxin reductase subunit in different activation states. This work, will be extended to the entire Kodama pathway with the aim of characterizing such an interesting and efficient PAH-contaminated degradation organism.

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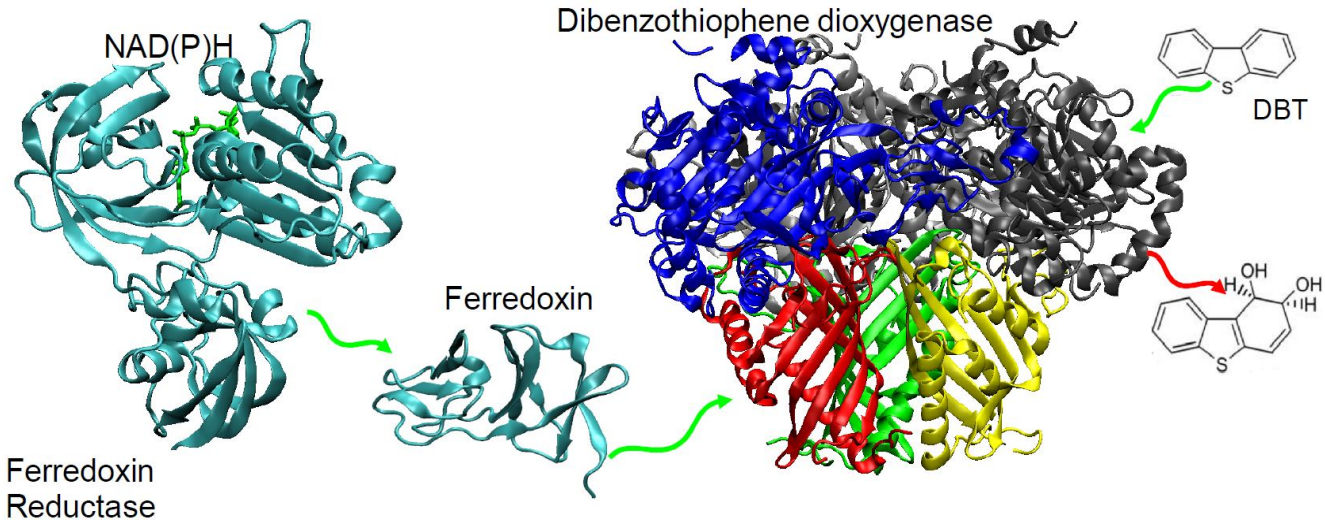

Figure 1. Schematic description of the oxidation process of polycyclic aromatic hydrocarbons (PAHs) catalyzed by Dibenzothiophene dioygenase complex (Rieske oxygenase system). The enzymes depicted were modeled using the protocol described in this article.

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