5º CONGRESSO BRASILEIRO DE PESQUISA E DESENVOLVIMENTO EM PETRÓLEO E GÁS

TÍTULO DO TRABALHO:

COMPUTATIONAL SIMULATION STUDY TO OIL INDUSTRY: STRATEGY TO DIMINISH H2S PRODUCTION

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Abstract

Sulfur is a one of the most abundant elements of the Earth, including sea water. The microorganisms that reduce sulfate cause serious problem for industries, such as offshore oil industry. Sulfate reducing prokaryotes (SRP) are widespread in anoxic environments where they utilize hydrogen, simple organic acids, alcohols and hydrocarbons as electron donors for the reduction of sulfate. Sulfate is used for these microorganisms as the terminal electron acceptor for growth. This element is taken up as a nutrient and reduced to sulfide, which is then incorporated into sulfur-containing amino acids and enzymes. Dissimilatory sulfite reductase (D*sv*) is a key complex enzyme of sulfate and sulfite reduction pathway. The *dsv* gene products are part of an enzyme cascade in both the oxidative and reductive of dissimilatory sulfur metabolism. The δ-protobacterial sulfatereducers is one of the five branching phylogenetics lineages based on 16S rRNA and *dsv*AB gene biomarkers. It is important to study the energy metabolism of sulfate reducing prokaryotes due to its capability to biocorrosion of metallic materials, like in the refining oil process, because the sulfides produced have toxicity, corrosiveness and the ability to form insoluble metal sulfides that can lead to a loss of reservoir permeability and because the SRP can be utilized to development of bioremediation of sites contaminated with toxic metal ions, including oil products. This work aims to obtain a 3-D homology model of Dsv enzyme from *Desulfovibrio vulgaris* Hildenborough to investigate strategies to diminish the sulfide production in the complex through computational simulation approaches. There was performed 3-D modeling structures of Dsv protein complex using like reference the crystal template of dSir from *Archaeoglobus fulgidus* (DSM 4304T) (PDB:3c7b) with 2.00Å X-ray diffraction. Dsv is a heterotetramer formed with two asymmetric dimmers, both composed by α and β subunits. Computational simulation analysis shows that the similarity between the protein sequences was: DsvA and dSirA 54.21 %; DsvB and dSirB 55.47 %. The subunits DsvA and DsvB were modeled separately and after that the enzymatic complex was assembled. Based on this model, it is defined the interactions of SO3 and SO2 with the siroheme cofactor by docking simulations. The data obtained from **DsvABm** is utilized to discuss the role and localization of the substrate and product-binding pockets, respectively. It is clear that to diminish the production and promote the removal of sulfide is needed in view of health and safety concerns and to reduce the risk of pipeline corrosion and other negative effects. This computational simulation work contributes to understand the molecular events

on the sulfate metabolism in *Desulfovibrio vulgaris* Hildenborough applied to oil and gas industry aiming the biocontrol of sulfate reducing prokaryotes activities.

Introduction

Beyond methanogenesis, sulfate reduction is one of the anaerobic microbial processes predominant in metabolic pathways since the early stages of biogeochemical evolution in anoxic environments (Dhillon et al., 2005). Billions of tons of sulfur compounds per year are metabolized by various microbial species that use inorganic sulfur compounds as terminal electron donors or acceptors for the purpose of energy conservation (Schiffer et al., 2008).

Sulfate reducing prokaryotes (SRP) are widespread in anoxic environments where they utilize hydrogen, simple organic acids, alcohols and hydrocarbons as electron donors for the reduction of sulfate. The δ-protobacterial sulfate-reducers is one of the five branching phylogenetics lineages based on 16S rRNA and *dsv*AB gene biomarkers (Dhillon et al., 2006).

The dsv (or dsr) and the aSir are the best-studied members of the sulfite reductase gene family. Dissimilatory sulfite reductase is a key complex enzyme of sulfate and sulfite reduction pathway and they are conserved in archaeal and bacterial domains (Schiffer et al., 2008; Karkhoff-Schweizer et al., 1995). The dsv gene products are part of an enzyme cascade equally in the oxidative and the reductive of dissimilatory sulfur metabolism. Dsv (**DsvAB**) is a heterotetramer formed with two asymmetric dimmers, both composed by α and β subunits. In this way, sulfate is reduced to sulfite and, subsequently, to sulfide by dissimilatory sulfite reductase (**DsvAB**) in the final step of sulfate respiration, under participation of a unique magnetically coupled sirohaem–[4Fe–4S] Center (Mizuno et al., 2003; Schiffer et al., 2008).

It is important to study the energy metabolism of sulfate-reducing prokaryotes due to its capability to biocorrosion of metallic materials and bioremediation of sites contaminated with toxic metal ions, including oil products (Heidelberg et al. 2004).

This work aims to obtain a 3-D homology model of **DsvAB** enzyme from *Desulfovibrio vulgaris* Hildenborough to investigate strategies to diminish the sulfide production in the complex through *in silico* approaches.

Metodology

A flowchart resume of steps of the methodology (Figure 1).

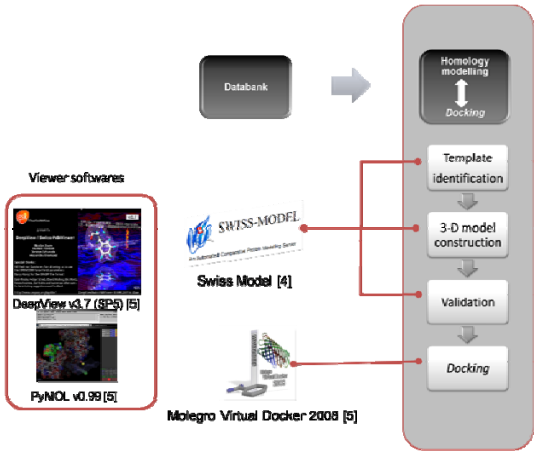

Figure 1: Steps of bioinformatics analysis in Dsv of *Desulfovibrio vulgaris* Hildenborough studies.

Sequences

Protein sequence of the **DsvAB** enzyme from *Desulfovibrio vulgaris* Hildenborough were obtained from NBCI-USA (GenBank Accession number: AAA70107 and AAA70108) (Karkhoff-Schweizer et al., 1995).

Crystallography template

The template used was the crystal of dSir from *Archaeoglobus fulgidus* (DSM 4304T) (PDB:3C7B http://www.ebi.ac.uk/pdbe-srv/view/entry/3c7b/summary) with 2.00Å X-ray diffraction, R-factor 19.101% and R-factor free 22.362%.

3-D modeling by Homology

The sequences were modeled in the Swiss-Model server and analyzed in DeepView v3.7 (SP5), PyMOL v0.99 and Molegro Virtual Docker 2008. The dimmer modeled was named **DsvABm** and the subunits **DsvAm** and **DsvBm**.

Docking

The docking simulation with the **DsvABm** model was performed with SO3, SO2 and sirohaem in Molegro Virtual Docker 2008 program. The patterns of the program were maintained. A total of 30 simulations were performed with each substrate and product. Resolution atomic precision 0,30 Å. The grid was adjusted with reference from Fe sirohaem enclosed atom until 2.5 Å radial distance.

Results and Discussion

Protein sequence of the DsvAB enzyme from *Desulfovibrio vulgaris* Hildenborough were obtained from GenBank (NBCI-USA). We used to define an *in silico* analysis of dSir protein sequences from *Archaeoglobus fulgidus.* The BLAST Two Sequences at NCBI analysis shows that the similarity between the protein sequences was: DsvA and dSirA 54.21 %; DsvB and dSirB 55.47 %. The DsvA and DsvB proteins from *Desulfovibrio vulgaris* Hildenborough were modeled using the 3-D structure (PDB: 3C7B) as template at the *on-line* Swiss-Model server to generate a 3-D model by homology strategy. The subunits **DsvAm** and **DsvBm** were obtained separately and after that the enzymatic complex was assembled. The model **DsvABm** were validated by Ramachandram plots and statistical comparative analysis between 3C7B dimmer chains AB from *Archaeoglobus fulgidus* and our model **DsvABm** from *Desulfovibrio vulgaris* Hildenborough (Figure 2). We use data from ProQRes, Anolea, Gromos, QMEAN, DFire, Whatcheck, Procheck, DSSP and Promotif software's also (data not showed).

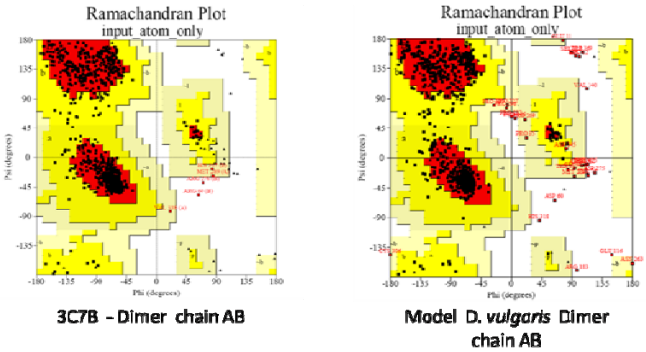

Figure 2: Ramachandram plots of dimmer chains AB from *A. fulgidus* and **DsvABm** from *D. vulgaris*.

The 3-D model **DsvABm** obtained in this work was based on analysis of 118 structures of resolution of at least 2.0 Angstroms and R-factor no greater than 20%. A good quality model would be expected with over of 90% in the most favored regions (Figure 2 and Table 1).

Table 1: Ramachandran plot statistics of 3C7B and *D. vulgaris* model (Dsv protein – AB chains).

AB chain	Residues in most favoured regions [A,B,L]	Residues additional allowed regions [a,b,l,p]	Residues in generously allowed regions [~a,~b,~l,~p]	Residues disallowed regions	Number of non-glycine and non-proline residues	Number of end-residues (excl. Gly and Pro)	Number of glycine residues (shown as triangles)	Number of proline residues	Total number of residues
3C7B *A. fulgidus*	**592 (88.8%)**	**70 (10.5%)**	**3 (0.4%)**	**2 (0.3%)**	**667 (100%)**	**4**	**63**	**46**	**780**
***D. vulgaris* Model**	**549 (82.8%)**	**93 (14.0%)**	**9 (1.4%)**	**12 (1.8%)**	**663 (100%)**	**4**	**66**	**47**	**780**

It is notable the similarity of Dsv enzymatic complex between *Archaea* and *Bacteria* domains, suggesting the conservation of dissimilatory sulfite reduction, a key bioprocess, indispensable to maintain the sulfur cycle on many environments (Muyzer & Stams, 2008). In the Figure 3, could be visualized the similarity through the fitting comparing (100%), both in archaeal and bacterial chains (Deep View Alignment software).

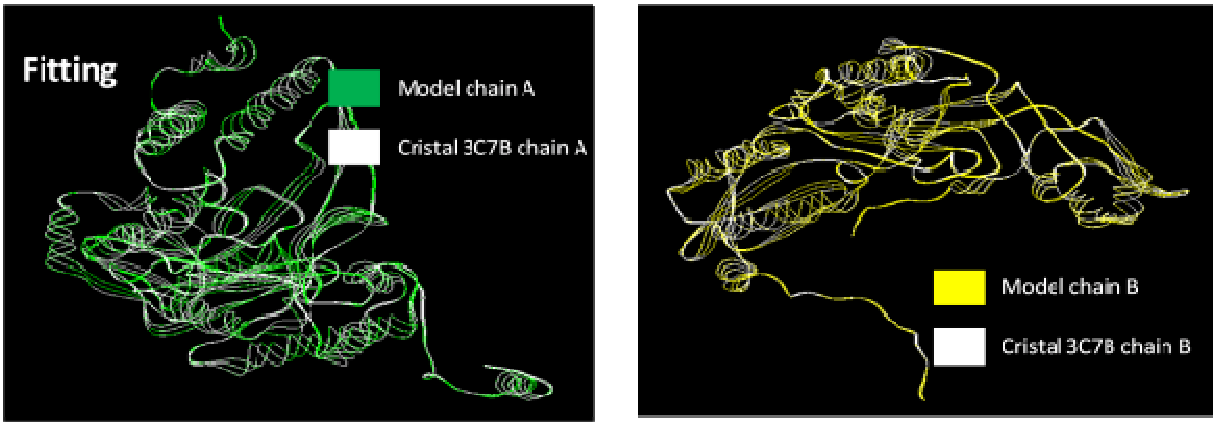

Figure 3: Fitting showing the similarity (100%) between the **DsvAm** and **DsvBm** chains of *D. vulgaris* with equivalents chains from the crystal 3C7B of *A. fulgidus*.

Just the valuation of A and B chains was necessary. They are equal to D and E chains, when compared separately with ABDE crystal chains in multiple alignments in ClustalW on line software.

The 3C7B crystal of *A.fulgidus* template was the reference for comparisons, which left to speculate the positions of the prosthetic group in **DsvABm** and the interactions with substrates and products. Figure 4 shows the secondary structures and prosthetic groups (A) and dimmer surface with prosthetic groups (B) of **DsvABm** of *D. vulgaris*. The 3C7B 3-D of *A.fulgidus* is showed in Figure 5, emphasizing the surface of the tetramer (C). In evidence, the **DsvABm** dimmer and the 3C7B tetramer are superposed (D), exhibiting high resemblance in topology and closely the same prosthetic group position. The Figure 4 and 5 shows a possible transit region for SO3 and SO2, suggesting preferential catalytic centers.

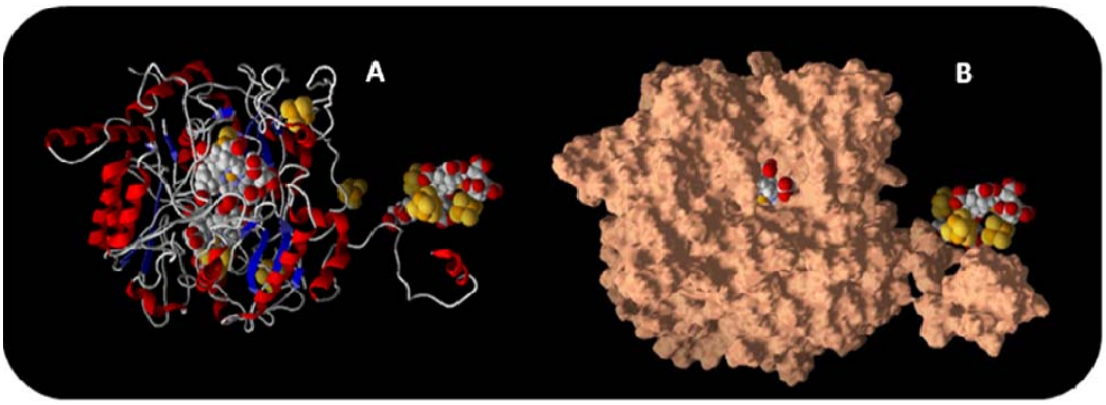

Figure 4: Secondary structures of the dimmer chain **DsvABm** of *D. vulgaris* (A) and dimmer surface of the same model (generated by Molegro Virtual Docker).

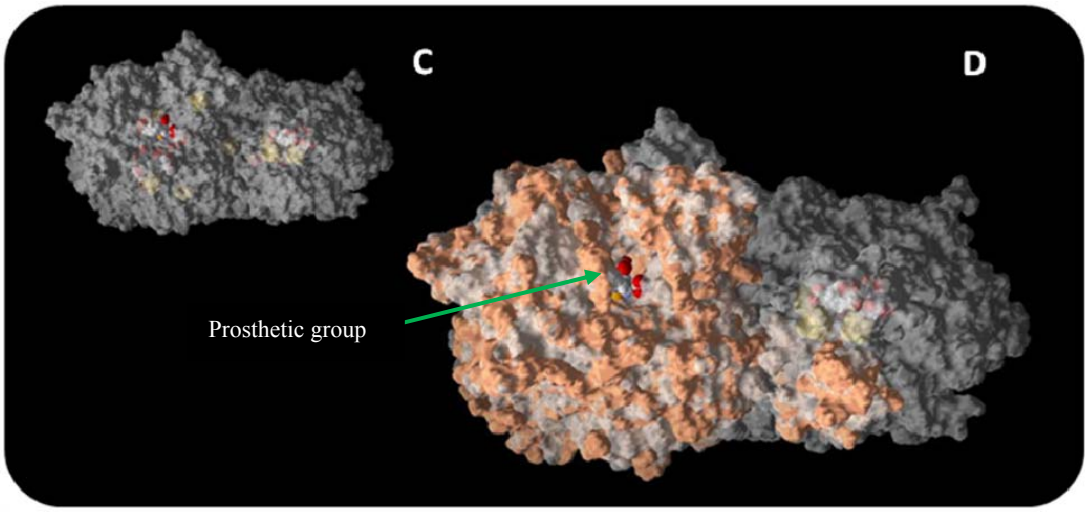

Figure 5: The 3C7B crystal heterotetramer surface of *A. fulgidus* (C) and **DsvABm** dimmer (salmon) superposed to 3C7B crystal (grey) in D (generated by Molegro Virtual Docker).

Based on **DsvABm** model, there are defined the SO3 and SO2 interactions with the sirohaem prosthetic group by docking simulations. Docking with sirohaem prosthetic groups and FeSO4 were realized in **DsvABm** and the positions selected with reference to prosthetic groups of 3C7B (Figure 6). Docking with SO3 and SO2 were realized simultaneously in the crystal dimer 3C7B and in **DsvABm** (Figure 6). Comparisons of substrates and products positioning were analyzed in 3C7B and **DsvABm** and suggests SO3 and SO2 trajectories.

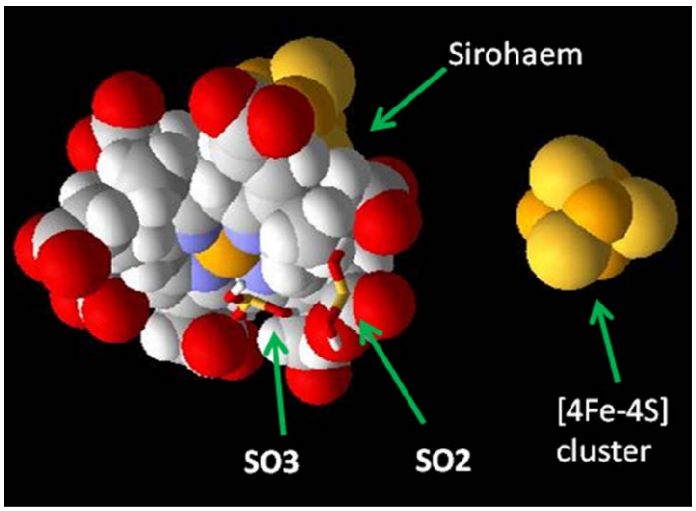

Figure 6: Prosthetic group of **DsvABm** *D. vulgaris* model. Showing sirohaem and 4Fe-4S cluster, beyond SO3 and SO2 nears sirohaem.

The data obtained from **DsvABm** of *D. vulgaris* is utilized to understand the role and localization of the substrate and product-binding pockets, respectively.

If the biochemistry and genetic diversity are known, microbiology tools have greater potential for accurately evaluating bacterial degradative potential. Sulfate-reducing bacteria play a key role in anaerobic corrosion, oil degrading and souring in oil and gas fields, and elucidating their modes of action is important to oil companies (Van Hamme et al., 2003). Hence, the bioinformatics studies based on enzymatic functions in *D. vulgaris* and *A. fulgidus* models, allows to turn sulfate-reducing metabolic processes most compreensives and helps to investigate the circumstancial transformations that left to oil outlay, contributing to a more detailed characterization of bacterial influence in oil degrading, souring and biocorrosion.

Conclusions

This *in silico* work based on **DsvABm** *of Desulfovibrio vulgaris* Hildenborough contributes to understand the molecular events on the sulfate metabolism. It is clear that to reduce the risk of pipeline corrosion and other negative effects is necessary to diminish the production and promote the removal of sulfide in the environments, supporting the view of health and safety concerns. This computational simulation work contributes to understand the molecular events on the sulfate metabolism in *Desulfovibrio vulgaris* Hildenborough applied to oil and gas industry aiming the biocontrol of sulfate reducing prokaryotes activities.

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