Available online at www.sciencedirect.com

ScienceDirect

Journal of Taibah University for Science 10 (2016) 621–630

Journal of Taibah University for Science

www.elsevier.com/locate/jtusci

Comparative modelling and molecular docking of nitrate reductase from *Bacillus weihenstephanensis* (DS45)

R. Seenivasagan [a], R. Kasimani [b], S. Rajakumar [c], R. Kalidoss [d], P.M. Ayyasamy [a,*]

[a] *Department of Microbiology, Periyar University, Salem 636 011, Tamil Nadu, India*
[b] *Department of Biotechnology, Kalasalingam University, Srivilliputtur 626 126, India*
[c] *Department of Marine Biotechnology, Bharathidasan University, Tiruchirappalli 620 024, Tamil Nadu, India*
[d] *Department of Molecular Signal Processing, Leibniz Institute of Plant Biochemistry (IPB), Halle 06120, Saxony-Anhalt, Germany*

Received 25 November 2015; received in revised form 9 February 2016; accepted 26 February 2016
Available online 15 March 2016

Abstract

Nitrate reductase catalyses the oxidation of NAD(P)H and the reduction of nitrate to nitrite. NR serves as a central point for the integration of metabolic pathways by governing the flux of reduced nitrogen through several regulatory mechanisms in plants, algae and fungi. Bacteria express nitrate reductases that convert nitrate to nitrite, but mammals lack these specific enzymes. The microbial nitrate reductase reduces toxic compounds to nontoxic compounds with the help of NAD(P)H. In the present study, our results revealed that *Bacillus weihenstephanensis* expresses a nitrate reductase enzyme, which was made to generate the 3D structure of the enzyme. Six different modelling servers, namely Phyre2, RaptorX, M4T Server, HHpred, SWISS MODEL and Mod Web, were used for comparative modelling of the structure. The model was validated with standard parameters (PROCHECK and Verify 3D). This study will be useful in the functional characterization of the nitrate reductase enzyme and its docking with nitrate molecules, as well as for use with autodocking.

Keywords: Nitrate reductase; Molecular modelling; Autodock; *Bacillus weihenstephanensis*

1. Introduction

Human excretion, agricultural activities and the industries producing fertilizer, meat and food, milk, and detergents are the main sources of nitrogenous wastes. The ground water nitrate (NO_3) concentration has increased globally [1,2]. Nitrate is transformed into nitrite in the digestive system of humans, which causes the condition known as methemoglobinemia, also called Blue Baby Syndrome [3]. Proper handling can prevent nitrate contamination in the ground water and soil, and if it is not handled correctly, it can create several hazards. The increase in the pollution of natural sources of drinking water demands proper attention for the development of technologies for water remediation [4]. For traditional physical and chemical techniques, such as ion exchange, reverse osmosis, catalysis and electro-dialysis, the costs

* Corresponding author. Tel.: +91 427 2345779x226; fax: +91 0427 2345124.
E-mail address: pmayyasamy@gmail.com (P.M. Ayyasamy).
Peer review under responsibility of Taibah University.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.jtusci.2016.02.006

of operations are high, and the methods are inefficient in removing waste brines from large volumes of waste [5,6]. Biological denitrification (alternative technology) methods are efficient methods and produce no secondary waste products [7,8]. Yan et al. [9] reported that in aquatic systems, nitrate and nitrite can be reduced to ammonium, which can then be used for the synthesis of amino acids and the regulation of other biological pathways.

The last step of complete heterotrophic denitrification is the final conversion of nitrogen gas (N_2), which is made by the sequential reductive reactions of nitrate (NO_3) to nitrite (NO_2), nitric oxide (NO) and nitrous oxide (N_2O). Enzymes such as nitrate reductase (Nar), nitrite reductase (Nir), nitric oxide reductase (Nor) and nitrous oxide reductase (Nos) play a role in this process [10].

Cupriavidus necator produces periplasmic nitrate reductase (NapAB), a heterodimeric protein that belongs to the dimethyl sulfoxide reductase family of mononuclear Mo-containing enzymes and catalyses the reduction of nitrate to nitrite. The nitrate reductase from *Desulfovibrio desulfuricans* ATCC 27774 (DdNapA), a monomeric protein of 80 kDa, harbours a bis(molybdopterin guanine dinucleotide) active site and a (4Fe-4S) cluster. DdNapA was co-crystallized with its substrates and inhibitors, and the corresponding structures were solved at resolutions ranging from 1.99 to 2.45 Å [11]. Einsle [12] stated that the final product of an ammonium ion was produced and bound in coordination with the substrate nitrite to the active site of the heme iron, though the free electron pair at the nitrogen atom was reduced by the transfer of an electron and a proton in the series. While no intermediate reactions were found, NrfA was able to reduce various other nitrogen oxides, such as nitric oxide (NO), hydroxylamine (H_2NOH) and nitrous oxide (N_2O). Sulfite is the only known direct link between the nitrogen and sulfur cycles.

The molecular docking of the protein was performed to directly compare the predicted data with the experimental data and to determine the potential modes of action of the nitrate reductase enzymes. The combined data help us to understand the structure–activity relationships between the selected proteome and the binding sites for the nitrate molecule. Most protein families have three-dimensional structures, and it is likely to identify a homolog of a known structure from sequence database searches. Entrez's 3D-structure database has made it easy to access structural information and the functional annotation of proteins. Gavanji et al. [13] showed that the important cellular enzyme nitrate reductase (Nar) plays a major part in cell activity. In the present study, the DiANNA 1.1 web server, Molegro Virtual Docker (MVD) and MetalDetector v2.0 software were used. The results obtained from docking showed the best pose, which was derived from the MolDock score for Catalase-peroxidase 30.7299 with a re-ranking score equal to 35.1088. However, a study by Georrge and Umrania [14] utilized an *in silico*-based approach for the identification of drug targets. A comparison of the proteomes of the causal organism and humans was made to screen out non-homologous proteins. Different databases were used to find novel drug targets, and various tools were used for the prediction of sub-cellular localization and membrane proteins.

In the present study, an effort was made to generate the three-dimensional (3D) structure of the nitrate reductase (A9VSW1, A9VSW2, A9VSW3 and A9VSW4) from *Bacillus weihenstephanensis*. Six different methods, namely Phyre, RaptorX, M4T Server, HHpred, SWISS MODEL and Mod Web, were used for the comparative modelling. The model was validated with standard parameters (PROCHECK and Verify 3D).

2. Materials and method

2.1. Software and hardware

Automated comparative modelling was performed by Phyre 2 [15], RaptorX [16], M4T Server [17], HHpred [18], SWISS model [19] and Mod Web [20], and the resulting models were evaluated by PROCHECK [21] and Verify 3D [22]. Docking studies were carried out in the Autodock V 4.0 Workspace [23]. Interactive visualization and analyses of the molecular structures were carried out in Pymol Viewer.

2.2. Analysis of proteome for Bacillus weinstephnisis (DS45)

The genomes of the effective nitrate-reducing bacterial strains DS45 were sequenced by 16S rRNA using NCBI-BLAST. The genome sequences of the DS45 strains were similar to the dissimilatory nitrate reducer *B. weihenstephanensis*. The proteome of *B. weinstephnisis* (Taxon Identification: 272620) was downloaded from UniProt (www.uniprot.org). The *B. weihenstephanensis* proteins were analyzed using CD-HIT to identify the paralogous or duplicate proteins. The sequence identity cut-off was kept at 0.6 (60% identity), and the global sequence identity algorithm was selected for alignment of the amino acids. A bandwidth of 20 amino acids and default parameters for alignment coverage were used. These proteins were subjected to screening for fragment