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Comparative modelling of 3D-structure of *Geobacter sp. M21* (a metal reducing bacteria) Mn-Fe superoxide dismutase and its binding properties with bisphenol-A, aminotriazole and ethylene-diurea

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Abstract

Superoxide dismutase play important roles in iron-respiratory bacteria such as Geobacteraceae as an antioxidant defense, and probably an effective enzyme of electron transfer network. Regarding the application of iron-respiratory bacteria in environmental biotechnology particularly biodegradation and bioremediation, understanding the mechanism of inhibition/induction of superoxide dismutase by any inhibitor or inducer will make a contribution to our understanding of biotechnological application and how to enhance the application of this enzyme. Bisphenol-A and aminotriazole were reported as inhibitor and potential inhibitor of superoxide dismutase respectively, while ethylene diurea was reported as a potential inducer of plants superoxide dismutase. In this paper, the mechanism by which bisphenol-A, aminotriazole and ethylene diurea interacts with superoxide dismutase of *Geobacter sp. M21* is investigated. The 3D structure of protein was predicted using Swissmodel, PS2, M4t and iTASSER servers and final model was provided by Modeler-V.9.13. AutoDock Vina and COACH server were used for prediction of cofactor (FeII) interaction site, and Molegro Virtual Docker used for prediction of potential active site pockets as well as docking process with ligands through MolDock and PLANTS scoring function. Ligandscout and Molegro were used for ligand/protein interaction visualization. The results showed that all ligands interact with protein at several different regions through hydrogenic, hydrophobic and/or electrostatic bonds. Understanding the exact effect of ligands needs in-vitro/in-vivo experimental investigations; however if bisphenol-A/aminotriazole may show inhibitory effects, the consequence is reduction in the yield of bioremediation of heavy metals and other pollutants, while ethylene diurea may lead to an increase in the yield.

Key words: *Geobacter strain M21*, Iron-Manganese superoxide dismutase, Comparative Modelling, Virtual Docking, Bisphenol-A (Diphenylpropane), Aminotriazole, Ethylene Diurea (EDU)

Highlights

- The 3D structure of Iron-Manganese superoxide dismutase of *Geobacter strain M21* was created with an efficient and acceptable Z-score.
- The cofactor site and active site region were predicted for the created 3D model.
- An in-silico investigation was done to understand the interaction mechanism of 3 ligands with the modeled protein.
- The in-silico docking analysis revealed that ethylene diurea, bisphenol-A (Diphenylpropane) and aminotriazole interacts with superoxide dismutase of *Geobacter strain M21* in at least two binding pocket through hydrogenic, hydrophobic and/or electrostatic bonds.
- The in-silico analysis revealed that interaction affinity of ethylene diurea is higher than bisphenol-A (Diphenylpropane) and aminotriazole

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Introduction

Mn-Fe superoxide dismutase (SOD) is a multifunctional protein in bacteria that its main role is a protective agent in heavy metal stresses, probably heavy metal adsorption (an important factor in bio-remediation and bio-reduction processes) and oxidative damages (1-7). Mn and Fe SODs are prevalent in bacteria and mitochondria. Mn SOD of *Escherichia coli* is more effective in DNA protection, while Fe-SOD is more effective in protecting cytoplasmic superoxide-sensitive components (6 and 8).

The Fe or Mn SODs are very similar and hard to recognize in sequence and structure. Fe-Mn SODs exist in dimer or tetramer forms and their cofactors can be reduced and reoxidized during interaction with oxygen radicals (2 and 6). The level of Fe-SOD in *E. coli* is increased when the bacteria is cultivated in iron-rich aerobic medium (2).

Geobacteraceae are among the most important bacteria in subsurface environment particularly iron-rich sites, and SOD is an important enzyme of this family to survive in this environment. In fact SOD is among those expressed genes in contaminated or iron oxide-rich environment such as Fe (III) rich environments, uranium-contaminated bioremediation sites, petroleum-contaminated aquifers (3, 5 and 9-11).

The genetic and proteins sequence of SODs in Geobacteraceae is determined, but there is no available crystallography, NMR or even modelled structure. However the crystallography structure of SOD in some other bacteria such as Fe-Mn SOD of Deinococcus Radiodurans with PDB accession number of 1y67 is reported (available at http://www.rcsb.org). Regarding the important role of SODs in response to environmental changes, understanding the inhibitors and inducers of this protein as well as their binding properties is important particularly for Geobacteraceae as an important microorganism in environmental biotechnology especially in bioremediation processes.

Different inhibitors and inducers of SODs have been introduced such as SP72, SP13, Bisphenol -A (BPA) and Aminotriazole as potential inhibitors, and Ethylene Diurea and hydroxypropyl-β-cyclodextrin as potential inducers (12-16). However Bisphenol A and Aminotriazole are of much importance due to the fact that these two products and the related analogues are widely detected in environment. Bisphenol -A is a compound used widely in many consumer products that along with its analogues are present in environment and can be found almost everywhere and reported to be a non-biodegradable or readily biodegradable component (17). It was reported as an inhibitor of SOD function, for example it showed inhibitory effect on human and *Chlorella pyrenoidosa* superoxide dismutase (15 and 18), hence BPA and its analogues can affect the role of bacterial SOD in environment, as an example it can interfer the SOD role in electron transfer network of important bioremediator bacteria such as Geobacteraceae and as a

result ay interfere with bioremediation process. Aminotriazole is an herbicide with heterocyclic organic structure that is known as an inhibitor of catalase and imidazole glycerolphosphate dehydratase in microorganisms and might be a potential inhibitor of SODs (14 and 19). Since this chemical and its analogues like BPA can be found in the environment, hence it may interfere the SOD role. Ethylene Diurea was reported as an antiozonant component and an inducer of SOD in some species; and had been applied as a fertilizer and as an agent to enhance the resistance of some plants against oxidative attacks (16 and 20-22). However later research decline its direct effects on increasing the SOD level/function (23). There is no docking investigation available for the interaction of these three ligands with SOD of Geobacteraceae, however some researches done on other soices SODs and other ligands. For example Qui *et al* reported that hydroxypropyl-β-cyclodextrin interact with SOD mostly through hydrogenic interaction (12).

Superoxide dismutase has important functions in Geobacteraceae as well as other iron-respiratory bacteria such as an important factor of antioxidant defense, an effective enzyme of their electron transfer network and an important agent in interaction with heavy metal components. Regarding the application of iron-respiratory bacteria in environmental biotechnology particularly biodegradation and bioremediation, understanding the mechanism of inhibition or induction of SOD by any inhibitor or inducer can lead to an understating that aim to engineer a microorganism with more desirable features against environmental contaminants or for a better biotechnological use.

Geobacter sp. (strain M21) is an anaerobic, chemolithotrophic Gram-negative bacterium isolated from an in situ uranium bioremediation experiment in Rifle, Colorado, USA. Members of this genus are very interesting because of their novel electron transfer capabilities, impact on the natural environment, their application to the bioremediation of contaminated environments and harvesting electricity from waste organic matter. Geobacter species showed the ability to transfer electrons into the surface of electrodes, allowing the construction of microbial fuel cells which produce electricity out of waste organic matter. (Adapted from: http://www.ncbi.nlm.nih.gov/sites/entrez?Db=genomeprj&cmd=ShowDetailView&TermToSearch=20729).

Geobacter sp. M21. Complete genome sequence is available since 2009 from Lucas *et.al*. 2009 study (24).

Lineage: *Bacteria*; *Proteobacteria*; *Deltaproteobacteria*; *Desulfuromonadales*; *Geobacteraceae*; *Geobacter*; *Geobacter sp. M21* [Taxonomy ID: 443144].

We investigated the mechanism by which BPA, Aminotriazole and Ethylene Diurea interact with SOD of Geobacter strain M21. However the exact effect in an in-vitro or in-vivo conditions need experimental analysis.

Materials and methods

Prediction of 3D model

Amino acid sequence of Geobacter sp. strain M21 SOD was obtained from Uniport web site at http://www.uniprot.org/uniprot/C6E886 in a FASTA format provided from Lucas *et.al*. 2009 study (24). It has 194 amino acid lengths.

The 3D structure was predicted using Swissmodel (25), PS^2 (26), M4t (27) and iTASSER (28 and 29) servers. All the webserver models introduced to Modeller V.9.13 and a comparative model was obtained (30 and 31).

Prediction of Cofactor and ligand binding sites

Autodock Vina (PyRx software) (32) Molegro Virtual Docker V 6.0., COFACTOR webserver (29) and COACH webserver (33 and 34) were used for prediction of cofactors, cofactor binding site and binding pockets of modeled protein.

Docking

Molegro Virtual Docker V 6.0.0 was used for preparation of protein and docking process with ligands. Both MolDock Scoring (GRID) function and PLANTS Scoring (GRID) function were used for docking process with ligands. Internal ES, Internal H-Bond and Sp2-Sp2 Torsions were applied for ligand evaluation with MolDock Scoring (GRID) function. Hydrogen was included in torsions terms for PLANTS Scoring (GRID) function process. For both scoring function MolDock SE was used as search algorithm at 100 runs, Max iterations of 1500 and Max Steps of 300, and the whole molecule surface and cavities was selected as docking space. Additionally, Energy minimization and Optimize H-Bonds functions were selected to run after docking. The software was adjusted to return the top 50 poses.

Analysis of Ligand-Protein interaction

After docking process the best poses were exported. The available interaction between ligands and protein were visualized and analyzed using Molegro Virtual Docker V 6.0.0/Ligand Map function, LigandScout V. 3.12/Pharmacofore function and Discovery Studio V. 4.0/ Receptor -Ligand Interactions function.

Results

Prediction and evaluation of 3D model of protein, Cofactor and ligand binding sites

The 3D structure was predicted using webservers, then introduced to Modeller to obtaine the final model. Fe II and its binding site was suggested by Coach web server, the AutoDock also suggest almost the same binding site for Fe II (data is not shown). The ProSA-web was used to evaluate the Z-score the generated model which showed the Z-score of -6.63 for the generated model (Fig. 1) (35). COACH server as well as Molegro were used to predict the ligand binding sites (Fig. 2).

Docking

All three ligands interacted with protein in several different positions. For each ligand the 50 poses were returned that the data of top 4 poses for each ligand provided in table 1. Both MolDock and PLANTS Grid scoring function are reported. Rerank score for both scoring function is also provided in table.

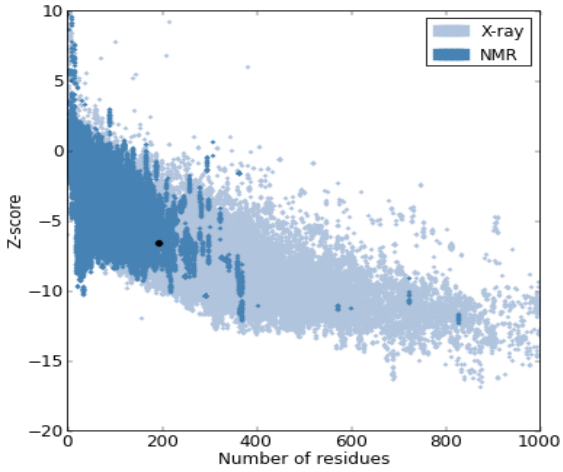

Fig. 1- Z-score plot of modelled SOD obtained from ProSA-web. The black dot is the modelled protein in the NMR and X-ray plot of all crystallography structures available at ProSA-web database. The Z-score of the modelled portion was -6.63.

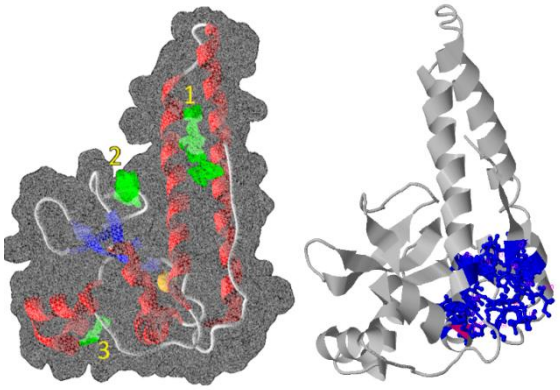

Fig. 2- The left picture is the detected cavities (the green color areas) by MVD; and the right picture is a binding site predicted by COACH web server (the blue color area) that was not detected as a cavity by MVD. The cavities number 1 and 2 detected by MVD were also predicted by COACH webserver as a binding site.

Table 1- Top four available poses for Aminotriazole, BPA and Ethylene Diurea using PLANTS and MolDock scoring function. Based on scoring function Ethylene Diurea poses showed higher affinity to the predicted protein pockets and cavities than BPA and Aminotriazole which their poses showed weaker affinity.

Aminotriazole							
	PLANTS Grid scoring function				MolDock Grid scoring function		
Pos #	PLANTS Score	MolDock Score	Rerank Score	Pos #	MolDock Score	Rerank Score	H-Bond energy
00	-38.6893	-31.241	-25.9263	00	-50.9647	-41.979	-2.26
01	-36.9453	-31.3412	-26.0986	02	-44.3742	-37.5174	-5.16
02	-36.7948	-31.8195	-26.7791	04	-43.5072	-18.4454	-4.26
03	-36.0511	-41.7118	-34.9965	06	-43.2542	-36.1465	-4.06
BPA							
00	-66.4758	-61.1285	-49.4541	00	-78.0804	-59.5961	-4.86
01	-66.2206	-63.717	-51.0667	03	-72.5635	-58.3855	-2.5
02	-64.2301	-76.1306	-59.8422	04	-72.3111	-59.4193	-2.49
03	-63.0357	-68.3332	-52.9244	14	-71.812	-59.368	-2.5
Ethylene Diurea							
00	-74.025	-97.2458	-78.7985	07	-101.432	-80.8738	-4.79
01	-73.9485	-88.9447	-80.7496	00	-101.126	-58.9016	-4.76
02	-71.0033	-84.4302	-67.6053	13	-100.481	-61.1375	-4.73
03	-69.3477	-88.8339	-74.817	01	-97.447	-74.575	-3.99

Discussion and conclusion

Evaluation of 3D model of protein

ITASSER analysis for comparative modelling showed that *Mycobacterium tuberculosis* iron-superoxide dismutase with PDB id of 1IDS-D has the top identified structural analog in PDB with TM-score of 0.969. TM-score is a measure of global structural similarity between query and template protein. *Sulfolobus solfataricus* iron-superoxide dismutase with PDB id of 1WB7-A has the best similarity in binding site with the query amino acid sequence with C-score^{LB} of 0.78. C-score is the confidence score of predicted binding site. C-score values range in between (0-1); where a higher score indicates a more reliable prediction. The Swiss model was also provided using 1WB7-A crystal structure. COFACTOR and COACH webservers suggested that iron (Fe II) is the probable cofactor.

The z-score indicates overall model quality and measures the deviation of the total energy of the structure with respect to an energy distribution derived from random conformations (35-37). Regarding the number of residues of SOD and in comparison with the Z-score of 1IDS-D (-6.74) and 1WB7-A (-8.28), the score of -6.63 is acceptable and in the area of NMR quality.

The generated 3D-model of Fe-SOD of *Geobacter sp. M21* with Modeller V.9.13 was introduced to MVD V 6.0.0. The import preparation was the default preference of MVD. The probable binding sites were predicted by using COACH web server and MVD (Fig. 2). Three available binding sites (cavities) were detected by MVD and COACH web server. Cavity one and two of MVD were also suggested by COACH. The main binding site predicted by COACH was not detected by MVD. Regarding the docking results (as shown in Fig. 3 this area showed no role in interaction with ligands) and based on our analysis for symmetry interaction for probable homo dimer and tetramer structure of the modeled SOD, this site might be a probable area for dimer and tetramer interactions (data is not shown).

Analysis of docking results and ligand-protein interactions

The docking search space was set to be the whole available surface of the protein as well as all the cavities. It found that BPA, Aminotriazole and Ethylene Diurea interact with several locations of the surface and protein pockets (cavities) of SOD. The protein pockets and cavities and the involved residues are almost common between all three ligands. However, based on scoring function of Docking results, the affinity order of ligands is as follow: Ethylene Diurea> BPA >Aminotriazole (Table 1).

With regard to previous studies BPA and Aminotriazole had been reported as SOD inhibitor in other species like human SOD, while Ethylene Diurea had been reported as an inducer of plants SOD. Table one shows the top 4 obtained results (poses) of docking process for all ligands. As it shown, BPA can interact with a larger surface area and has a higher affinity to the binding sites in comparison with Aminotriazole (Table 1 and Fig. 3). The lower affinity of Aminotriazole probably is the reason of the observed fluctuation of its inhibitory effect on SOD the *Antheraea mylitta* (14). Ethylene Diurea was previously reported as a probable inducer of SOD. The docking results showed that the affinity of Ethylene Diurea to the protein binding pockets is much more than

BPA and Aminotriazole. For example the highest MolDock score for Ethylene Diurea (pose 07) is -101.43 while it is 78.80 and -50.96 for BPA (pose 00) and Aminotriazole (pose 00) respectively). As it is clear from Fig. 3 BPA can interact with a more extensive area of SOD (binding site residues and non-binding site residues) compared to Aminotriazole and Ethylene Diurea., however Ethylene Diurea affinity is somehow focused on the available protein binding pockets (cavities), in other word it is more specific to binding site residues.

The ligands interact with the protein residues with different values of hydrogenic, hydrophobic and electrostatic bonds which are responsible for the level of affinity of ligand to the protein. For example for one poses that was almost common in all ligands, ASP41 and THR45 of the protein have interaction with functional groups of the ligands like hydroxyl (OH) groups (Fig. 4). As it shown in Fig. 4, hydrogenic bonds are the main interaction in between ligands and protein residues.

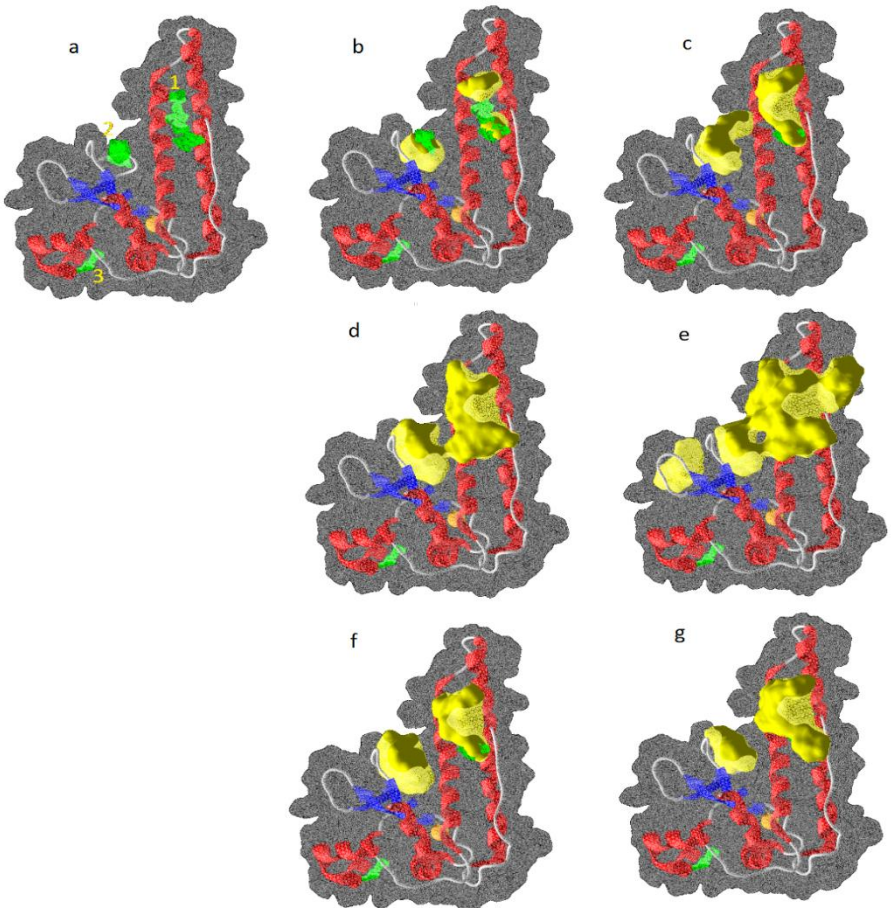

Fig. 3- The available interaction surface for ligands (produced from all poses from docking results-yellow color); **a**. 3D surface of the monomer form of SOD with the predicted pockets (cavities) by MVD in green color, numbered from 1 to three from the biggest to the smallest pocket. the b to g are the available surfaces for docked ligand as follow: **b**. MolDock algorithm-Aminotriazole, **c**. PLANTS algorithm-Aminotriazole, **d**. MolDock algorithm-BPA, **e**. PLANTS algorithm-BPA, **f**. MolDock algorithm-Ethylene Diurea, **g**. PLANTS algorithm-Ethylene Diurea. Cavity number 3 has no role in interaction with ligands.

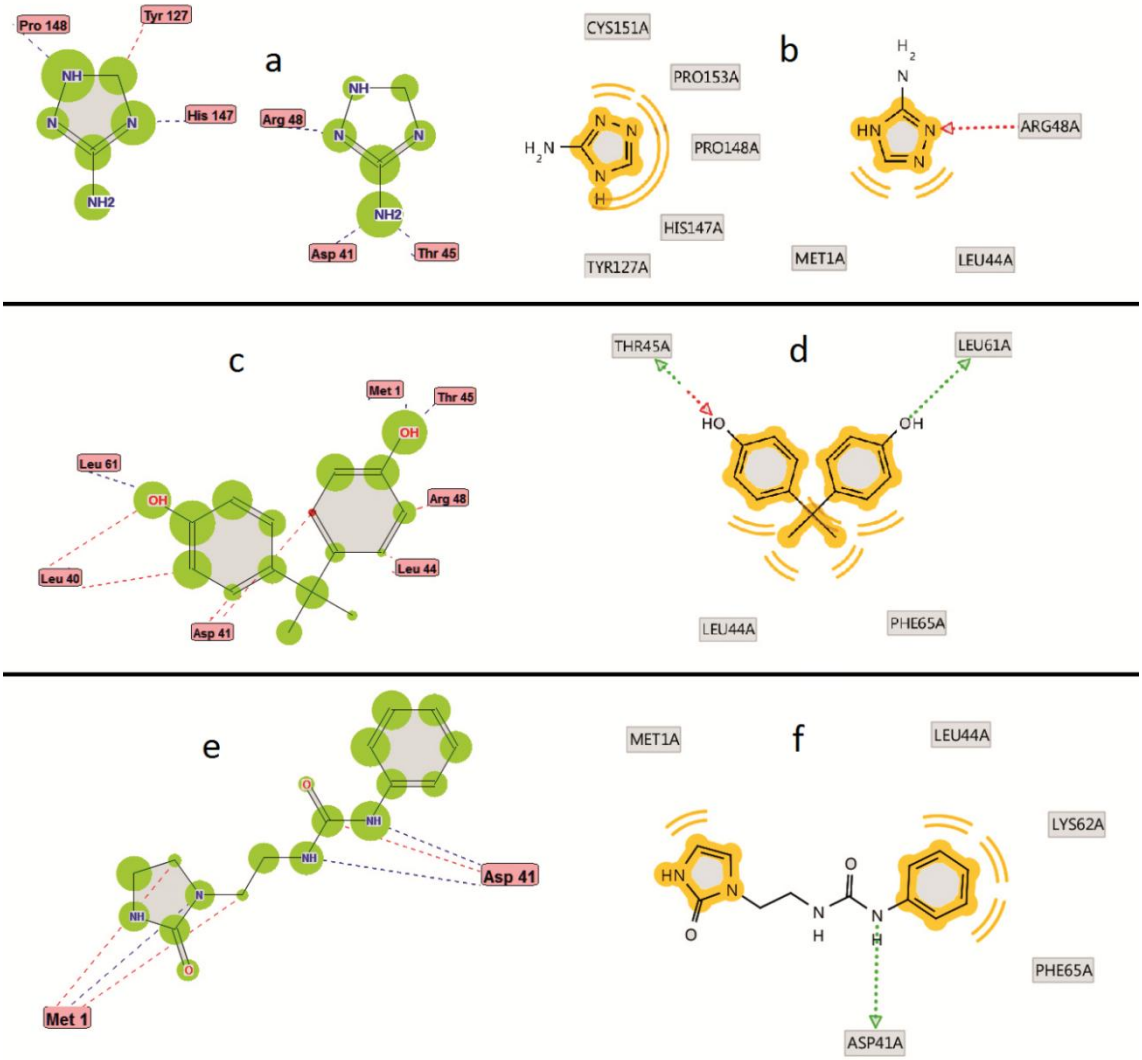

Fig. 4- Visualization of Aminotriazole, BPA and Ethylene Diurea interactions with iron/manganese superoxide dismutase of Geobacter sp. (strain M21). The picture display the schematic interactions between a ligand and the residue of SOD depicted by MVD visualization (blue lines= hydrogenic bonds, brown bonds= steric interaction), and LigandScout visualization.
Two pose of Aminotriazole with SOD **a**. MVD visualization, **b**. LigandScout visualization
One pose of BPA with SOD **c**. MVD visualization, **d**. LigandScout visualization
One pose of Ethylene Diurea with SOD **e**. MVD visualization, **f**. LigandScout visualization

Finding the exact effect of BPA, aminotriazole and ethylene diurea on SOD of *Geobacter sp. M21* needs experimental investigations. The ability of interaction of ligands with SOD in different areas (poses) and with different residues suggest that probably the ligands inhibitory/inducery effects is not only from interaction of one single molecule of ligand, rather a consequence of interaction with several molecules. Furthermore, since the interactions between ligand-protein are not covalent the interaction is revisable and as a result the effects are reversible too.

Moreover, If BPA and Aminotriazole would show inhibitory effect on SOD, with regard to the docking results that these two ligands interact with several binding sites and residues, no mutation can be done to improve the structure of SOD against these potential inhibitors. However, if Ethylene Diurea would be an inducer, some mutation can be done at some specific residues in order to enhance the affinity of Ethylene Diurea to SOD, and as a result improve the inducery effect of this ligand on SOD function.

To sum up, SOD is an important enzyme in Geobacteraceae as the significant bacteria of subsurface microbial communities and an important bacterium for microbial bioremediation and bio-reduction of heavy metal polluted environments. The data showed that BPA, Aminotriazole and Ethylene Diurea can interact with SOD at protein binding sites residues and non-protein binding site residues mostly through hydrogenic and electrostatic bonds. An in-vitro or in-vivo experiment needs to be done to understand the exact effect of this product on the roles of SOD in this bacterium. If in the experimental test BPA and Aminotriazole showed to be inhibitors of SOD and Ethylene Diurea would be an inducer, these two products may also interfere with the bioremediation capability of this bacteria. Hence, for an in-situ bioremediation process the utilized water and soil should be BPA free in order to achieve a higher yield in bioremediation. However, on the other hand supplementation of *Geobacter sp. M21* medium/environment with Ethylene Diurea not only may enhance the SOD function, but also as a results of its higher affinity with SOD amino acid residues, it can prevent the interaction of SOD and BPA or Aminotriazole. In that case for an in-situ bioremediation in a site with BPA polluted water and soil, supplementation of soil and/or water with Ethylene Diurea can inhibit the BPA effect on SOD and as a result increase the bioremediation yield with Geobacter.

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مدل‌سازی مقایسه‌ای ساختار سه بعدی سوپراکسید دیسموتاز منگنز-آهن باکتری ژئوباکتر سویه *M21* (یک باکتری کاهنده آهن) و ویژگی‌های اتصالی آن با بیس‌فنول-آ، آمینوتریازول و اتیلین دی‌اوره

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چکیده

سوپراکسیددیسوموتاز نقش مهمی در دفاع آنتی‌اکسیدانی داشته و آنزیم مهمی در شبکه انتقال الکترون باکتریای‌های تنفس کننده آهن مانند خانواده ژئوباکتریاسه دارد. با توجه به نقش مهم باکتری‌های تنفس کننده آهن در بیوتکنولوژی محیط زیست به ویژه در پالایش و تجزیه زیستی، درک مکانیسم مهار یا تحریک سوپراکسید دیسموتاز توسط عامل مهارگر یا محرک، به درک کاربردهای بیوتکنولوژیک آنزیم و چگونگی ارتقای کاربردها کمک خواهد کرد. پیش از این، بیس‌فنول-آ و آمینوتریازول به ترتیب به عنوان مهارگر و مهارگر احتمالی سوپراکسید دیسموتاز گزارش شده‌اند، و اتیلن دی-اوره به عنوان یک القاگر احتمالی سوپراکسید دییسموتاز در گیاهان گزارش شده است. در این پژوهش، مکانیسم میان کنش بین بیس‌فنول-آ، آمینوتریازول و اتیلن دی-اوره با سوپراکسید دیسموتاز *Geobacter sp. M21* بررسی شده است. ساختار سه بعدی پروتئین توسط سرورهای Swissmodel، PS2، M4t و iTASSER پیش‌بینی شده و ساختار نهایی توسط نرم‌افزار Modeler-V.9.13 ساخته شد. محل اتصال کوفاکتور (Fe II) با نرم‌افزار AutoDock Vina و سرور COACH پیش‌بینی شد. نرم‌افزار Molegro Virtual Docker برای تعیین محل جایگاه فعال، الگوهای MolDock و PLANTS scoring function برای فرآیند داکینگ و همچنین، نرم‌افزارهای Ligandscout و Molegro برای بررسی میانکنش‌های میان لیگاند و پروتئین استفاده شد. نتایج نشان داد که همه لیگاندها در چند محل متفاوت و از طریق پیوندهای هیدروژنی، هیدروفوبیک و الکترواستاتیک با پروتئین میانکنش دارند. درک آثار واقعی لیگاندها نیازمند آزمایش‌های عملی در آزمایشگاه یا محل آلودگی دارد. اگر چه، اگر مشخص شود بیس‌فنول-آ یا آمینوتریازول آثار مهارکنندگی دارند، پیامد این اثر، کاهش بازده زیست‌پالایی فلزات سنگین و سایر آلاینده‌ها خواهد بود، در حالی که اتیلن دی- اوره ممکن است سبب افزایش بازده این فرآیند شود.

واژه‌های کلیدی: *Geobacter strain M21*، سوپراکسید دیسموتاز منگنز- آهن، مدل سازی مقایسه‌ای، داکینگ مجازی، بیس فنول-آ، آمینوتریازول، اتیلن دی- اوره

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