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Involvement of Computational tools towards *In Silico* remediation - Synthetic textile dyes interacting with Azoreductase

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Abstract: Bacterial Azoreductase plays a critical role in breaking down the azo bond present in the textile azo dyes under aerobic or anaerobic conditions. Technology is delivering a number of bioremediation strategies till date for treating synthetic organics, but these technologies are unlikely to be implemented due to their technical snags in scaling up or during technology transfer to real time scenario. Computational approaches assist us before technology transfer by predicting the nature and toxicity of the interacting ligand with the target receptor protein. Molecular docking is expansively applied in all corners of applied sciences in optimizing the significance and interaction among protein-ligands. Forty azo dyes were selected, based on their wide application in the commercial units. Docking analysis was performed using AutoDock module present inbuilt in PyRx 0.8 version. The top ten dyes with higher affinity towards the target receptor protein is represented in the form of decreasing binding energy with Ponceau MX (-8.61)>Sudan IV (-7.82)> Methyl Yellow (-7.69)>Citrus Red 2(-7.6)>Scarlet GN(-7.38) > p-diazo violet (-7.19)> Acid Red 14 (-7.18)> Solvent Yellow 3 (-6.93)>Acid Blue 113 (-5.77)> Acid Red 88(-5.03). The findings obtained in the present study will pave avenue for system biologists to understand, comprehend and understand the theoretical mechanism and nature of interaction among azoreductase and the ligand.
Keywords: Azo dyes, System biology, Protein-ligand interactions, Binding energy and Hydrogen bonds.

Introduction

Currently there are about 70 million chemicals identified and playing a vital role in various textile, chemical, dyeing, paper making, cosmetics and other processing units[1]. Among the above, about a million chromogens are synthesized annually; of which 10-15% remain in textile spent wastewater after its application[2]. It is mandatory to treat/remove these spent wash released from various textile manufacturing and processing units, before being discharged into aquatic system[3]. Azo dyes are the diverse and largest group of textile dyes reporting low biodegradability and persistence of metabolites in the nature[4]. Azoreductase has involved in enzymatic reduction of azo bonds prior to mineralization of textile dyes[5]. The initial step in the biodegradation of azo dyes involves the reductive cleavage of azo bonds (–N=N–) with the help of azoreductase. Azoreductase reduce azo dyes under anaerobic conditions to colorless amines, which may be toxic, mutagenic and carcinogenic to humans and animals[6]. The effluents containing these dyes may/can cause adverse effects on livelihood such as allergic dermatitis, cancer and mutation[7]. Despite many physical, chemical and biological methodologies pervades in remediating textile effluents, mandatory strict regulations and measures are imposed on industries before discharging the effluents into ecosystem. But, on contrary, most of the methodologies are not eco-friendly, inefficient and are expensive, cementing with their own limitations and

advantages[8]. Various factors limit/inhibit the remediation processes such as structural complexity, transformation pathway and the micro-environment. Focus is towards enzyme based treatment methodologies, as enzymes play a pivotal role in developing an alternative or complementary biotechnological process towards treatment of colored wastewater/effluents[8,9,10]. Various reports on crude enzyme based methodologies utilizing laccase, peroxidase, azoreductase etc have been reported over past decade, nurturing the industrial needs spanning from synthesis to bioremediation[11,12,13,14]. *In-silico* biology has witnessed tremendous advancement over past two decades, leading to effective utilization of computational algorithms for high-throughput virtual screening of molecular interactions. Structure-based virtual screening is effective method to study protein-ligand interactions. Docking algorithms fits the generated poses into the target protein under investigation, thereby helps us to develop new metabolites[15,16]. AutoDock module generates energetically most favorable pose are evaluated based on its complementarity to the target and found to reproduce better results compared to DOCK, Flex and GOLD[17]. These computational methods help us to predict possible interactions and identify the hidden mechanisms involved in azo dye degradation. The objective of the current study is to study the relationships between receptor protein of investigation (azoreductase) and azo dye, thereby assisting in understanding the protein-dye interactions, as in-silico information related to azoreductase based-bioremediation is limited.

Materials and methods

Data structures

Forty azo dyes were selected based on the presence of azo linkage among the synthetic compound based on wide applications on dyeing industries. The azo dyes selected are as follows; Miketon Fast Orange 5R (A1), Amido Black (A2), Scarlet GN (A3), Aniline Yellow (A4), Methyl Yellow (A5), Solvent Yellow 3 (A6), Acid Red 2 (A7), Ponceau MX (A8), p-diazo violet (A9), Alizarine Yellow R (A10), Citrus Red 2 (A11), Bismarck Brown Y (A12), Basic Brown 4 (A13), Direct Blue 6 (A14), Amaranth (A15), Acid Red 14 (A16), Black B (A17),Blue RGB (A18), Chocolate Brown HT (A19), Direct Blue 14 (A20), Acid Red 88 (A21), Direct Red 28 (A22), Golden Brown RK-FQ (A23), Ingrain yellow (A24), Acid red 27 (A25), Direct Black 38 (A26), Brilliant Black BN (A27), Direct Blue 1 (A28), Acid red 2C (A29), Tartazine (A30), Solvent Black 3 (A31), Alcian Yellow (A32), Sudan IV (A33), Sudan III (A34), Solvent Red 23 (A35), Trypan Blue (A36), Allura Red (A37), Acid Blue 113 (A38), Direct Red 2 (A39) and Diethyl yellow (A40).

Ligand and receptor protein processing

Target protein (1NNI) was downloaded from protein data bank. Input file (target receptor protein) was generated by removing water molecules, ions, ligands and subunits from the original structure file. Kollman charges and polar hydrogen atoms are added into the receptor PDB file for the preparation of receptor protein in docking simulation. The dye structures were downloaded from PubChem in SDF format and converted in to standard MOL file format using ChemSketch 12.01 (Freeware version, ACD labs). Energy minimization of the ligands was performed using Open Babel software by steepest descent using both universal force fields, converted to PDBQT format[18].

Molecular docking

Molecular docking analysis was performed using Autodock module available in PyRx Version 0.8 software[19,20]. Blind docking grid size was increased to accommodate the entire protein inside the grid with dimensions 53, 55 and 50 A° (X, Y and Z). The Lamarckian Genetic Algorithm (LGA) was used for screening for best possible conformers. During molecular docking, a maximum of 10 conformers were considered for each compound to predict best conformers. The population size was set to 150 and the individuals were initialized randomly. Maximum number of energy evaluation was set to maximum of 2500000 energy evolutions, maximum number of top individual that automatically survived to 1, with a mutation rate of 0.02 and a crossover rate of 0.80. Later, results were analyzed with the help of Autodock tools 1.4.5. The interactions between the ligand and the target are given in figures.

Results and Discussion

Azo dyes are degraded by enzymatic cleavage of azo linkages with the aid of an azoreductase and an electron donor to produce carcinogenic aromatic amines[21]. Few parameters to be considered for decolorization of azo dyes are the specificity of azoreductase, electron acceptance and proper electron shuttling. Little

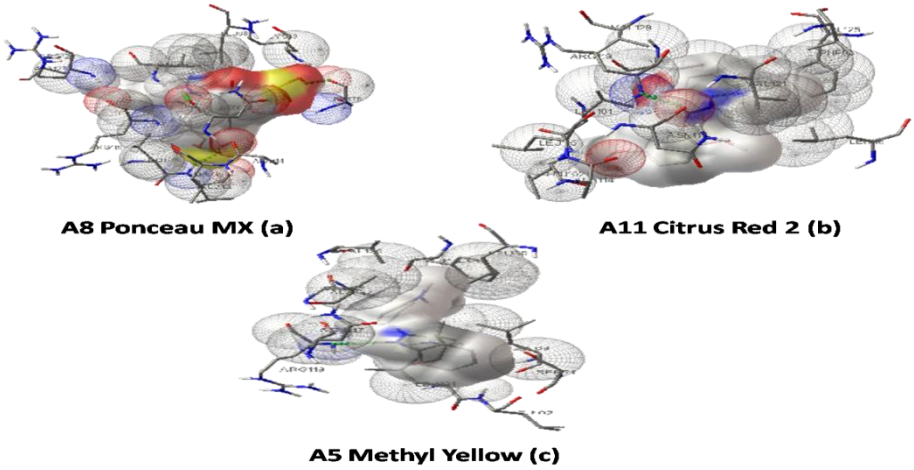
information is available about the mode of interaction of azo dyes with the proteins (Azoreductase, Laccase, Peroxidases etc) involved in bioremediation. So screening and investigation of protein-ligand interactions against potential receptors involved in bioremediation remains critical. There is an exponential production of synthetic organic compounds and the breakdown metabolites are released into environment after application. As quoted above, remediation of these azo dyes containing spent wash are requires cost effective and eco-friendly processes, because most of these dyes escape conventional wastewater treatment processes[22]. Many literatures on azoreductase-based bioremediation by bacterial species have been reported in past decades, but in-silico data is very limited. Receptor-based virtual screening and molecular docking predicts the conformation and binding affinity of small molecules[23]. Autodock in PyRx 0.8 module was found to be useful to study ligand comparison, assisting in binding analysis studies. For molecular docking studies, a set of forty ligands (Azo dyes-pollutants) were selected from Pubchem based on literature and wide application in various processing and finishing industries. Energy minimization was done using universal force field and forty ligands were subjected molecular docking using AutoDock 4.0 inbuilt in PyRx 0.8 software[19,20, 24]. Out of forty ligand sets, ten azo dyes have been found to show a high negative binding energy, suggesting that azoreductase might be able to reduce these pollutants based on their binding energies; moreover, in few azo dyes experimental literatures are available to support this theoretical hypothesis. (Table- 1) represents the lead compounds binding energy and intermolecular hydrogen bonding. Binding energy was used as a tool to correlate the interaction between enzymes and various synthetic organics, among the ten ligands, A8 found to show highest negative binding energy (-8.61), whereas A21 reports least binding energy (-5.03). The decreasing order of affinity of the textile dyes towards the receptor protein is as follows; A8>A11>A5>A7>A3>A9>A16>A6>A38>A21. A8 represents strongest interaction among the ten ligands, hydrogen bond plays an important role in stabilizing protein-ligand interactions[25]. It is hypothesized that, based on number of hydrogen bonds, it is recommended that A8 may interact effectively with azoreductase and might undergo degradation. Similarly decreasing order of intermolecular hydrogen bonds are; A8>A9/A6/A19>A11/A3/A16/A4/A21. (Figure- 1) represents the interactions of top three azo dyes with the azoreductase, where the intermolecular hydrogen bondings are represented as green spheres (Top three azo dyes representing highest binding, (Figure- 1(a)) represents molecular interactions of Azoreductase protein with Ponceau MX, (Figure- 1(b)) represents Azoreductase with Citrus Red 2 and (Figure- 1(c)) represents Azoreductase with Methyl Yellow).

Table 1: Top Ten Azo Dyes Representing Binding Energy and Intermolecular Hydrogen Bonds

S.No	Azo Dye	Ligand Structures	Binding Energy	Intermolecular Hydrogen No. of Bonds
1	Ponceau MX	A8	-8.61	3 (SER 79, LEU 102, ASN117)
2	Citrus Red 2	A11	-7.82	1(ARG 119)
3	Methyl Yellow	A5	-7.6	1(ARG 119)
4	Acid Red 2	A7	-7.69	1(SER 71)
5	Scarlet GN	A3	-7.38	1(SER71)
6	p-diazo violet	A9	-7.19	2(ARG 119, LEU 115)
7	Acid Red 14	A16	-7.18	1(ASN 117)
8	Solvent Yellow 3	A6	-6.93	2(ARG 119, ARG 123)
9	Acid Blue 113	A38	-5.77	2(GLY170, GLN 55)
10	Acid Red 88	A21	-5.03	1(LYS 97)

Present study strongly recommend that azoreductase has a strong hold in environmental bioremediation, so prior information about the ligand-protein interactions might provide mechanistic information of reactions at molecular level. Even though, with this theoretical information it is equivocal to recommend a strong mechanism of azoreductase with azo dye at this point, requires a strong wet lab experimental data. Despite success, molecular docking requires validations with experimental methodologies because due to variations among *in-silico* data with real time environments/bench scale lab data, due to structural/functional variations among the receptor protein of investigations.

Figure 1: Molecular Docking of Top three Azo Dyes with Azoreductase Protein



Conclusion

Bioinformatics approach can be used to mimic, investigate the insights of molecular interactions due to their inexpensiveness, less time consuming, higher reproducibility with low compound synthesis requirements and have the potential of reduced utilization of animals, thereby assisting us by providing mechanistic information ligand-protein interactions[26,27]. The present study involved a set of forty azo dyes interaction with azoreductase receptor protein, suggesting that microbes secreting azoreductase play a very important role in the mineralization of pollution.

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