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Application of a Pulsed Electric Field to Modify the Free Energy of the Active Site of an Azoreductase

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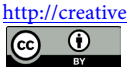

Abstract

The Gibbs free energy is strongly related to the stability and catalytic function of an enzyme through the energetic changes that occur in the chemical reactions the enzyme catalyzes. In this *in silico* study, a pulsed electric field was applied to an azoreductase, and its effect on the Gibbs free energy of molecular docking with two dyes was measured. We propose that certain stimuli from a pulsed electric field favor the structural stability of the enzyme by promoting an arrangement in the active site, potentially leading to an enhancement of enzymatic activity overall.

Keywords

Pulsed Electric Field, Molecular Dynamics, Docking, Azoreductase

1. Introduction

Preserving water resources or restoring them through treatment processes has become an urgent necessity to avert water scarcity in the near future. Furthermore, it is essential to develop cleansing methods that consider both treatment performance and financial perspectives [1]. In this context, azo dyes serve as a promising starting point to contribute to this goal. Due to their versatility, azo dyes are the most widely used synthetic colorants across various industries such as printing, textiles, food, and cosmetics [2]. The biodegradation of azo dyes by azoreductases proves highly efficient in the initial stages of wastewater treatment processes [3]. Consequently, there exists significant potential for enhancing the enzymatic properties of azoreductase, including its activity, stability, selectivity,

and specificity.

The azoreductases are enzymes capable of degrading azo dyes and are extensively employed in treating wastewater containing these dyes [4]. Azoreductase C (AzrC) is an enzyme found in *Bacillus* sp., encoded by 211 amino acids. It exhibits relative affinity to various dyes [5] [6]. There has been a suggestion of a growing potential for enhancing the enzymatic properties of this azoreductase [7].

The catalytic efficiency of the enzyme is associated with a thermodynamically favorable configuration [8]. Various approaches have been explored to induce structural alterations in enzymes, aiming to achieve more optimized configurations. Some of these approaches even aim to enhance enzymatic activity [9].

In a different approach, a Pulsed Electric Field (PEF) consists of brief pulses of voltage or short-duration electric discharges. This method has emerged as a potential means of modulating enzymatic activity [10]. Understanding the impact of PEF on enzyme conformation may provide valuable insights into modulating enzymatic activity and offer potential practical applications for this and other purposes. Therefore, we used molecular docking analysis to determine the effect of a pulsed electric field on the estimated Gibbs free energy at the active site of an azoreductase interacting with two azo dyes to propose the enhancement of its enzymatic activity.

2. Materials and Methods

The PDB file of AzrC for molecular dynamics and protein contact network construction was obtained from the Protein Data Bank (PDB) with the ID 3w7a. The 2D SFD molecular structures of the azo dyes were obtained from PubChem with the compound CIDs of 135465069 for Acid Red 88 (AR88) and 135513128 for Orange I (OI).

2.1. Molecular Dynamics

Molecular Dynamics (MD) simulations were performed using the GROMACS 2021.5 program (https://manual.gromacs.org/2021.5/). Briefly, the GROMOS 53A6 force field was used, and the protein was hydrated with the TIP3P water model. Chloride and sodium ions were added to neutralize the system. The NVT statistical ensemble and the Berendsen thermostat were implemented.

After equilibrating and minimizing the molecule, a production process of 1 ns was conducted, as during this simulation time, the system reached thermodynamic stability. The 1 ns simulations were conducted to obtain the final conformation of the azoreductase-dye complexes, AzrC-AR88 and AzrC-OI. The implementation of the pulsed electric field is described below.

2.2. Implementation of Pulsed Electric Field

In each complex, AzrC-AR88 and AzrC-OI, a Pulsed Electric Field at intensities of 0, 0.5 and 1.5 V/nm was implemented for 1 ns using GROMACS 2021.5 along

the x-axis according to Equation (1):

$$E(t) = E_0 \exp\left[-\frac{(t-t_0)^2}{2\sigma^2}\right]\cos\left[\omega(t-t_0)\right] \quad (1)$$

where E_0 is the field intensity, σ is the pulse amplitude and ω is the angular frequency.

We set an angular frequency of 150 ps^{-1}, an initial time of 5 ps, and an amplitude of 1 ps. The final conformation from these MD simulations was used for the docking analysis.

2.3. Molecular Docking

We utilized the final conformation derived from the MD simulations for the docking analysis aimed at identifying the binding region within the azoreductase-dyes complex. Docking calculations were executed using AutoDock 4.2 (https://autodock.scripps.edu/).

To minimize the energy of the ligands, obtain their initial configuration, and generate the mol2 files, the molecular editor Avogadro 1.1.1 (https://avogadro.cc/) was used with the MMFF94 force field (for organic compounds).

The PDBQT formats for the ligands OI and AR88 and AzrC were obtained with AutoDockTools v 1.5.7. Briefly, to generate the PDBQT structure, Kollman charges were calculated for each atom of the AzrC molecule and Gasteiger charges were calculated for the ligands. The grid box dimensions over the ligand binding site were 126 × 126 × 126 Å with a spacing of 0. 269 Å for both the AzrC-OI complex and the AzrC-AR88 complex. The grid maps were calculated using AutoGrid from AutoDock.

The docking parameters were determined using the Lamarckian Genetic Algorithm (LGA) with 100 runs, an initial population of 300 random individuals, 2,500,000 energy evaluations, a mutation rate of 0.02, and a crossover rate of 0.8.

3. Results

We examined alterations in the binding sites of the AzrC-AR88 and AzrC-OI complexes under the influence of a pulsed electric field with intensities of 0, 0.5, and 1.5 V/nm using molecular docking. The findings are detailed in **Table 1**.

The residues in AzrC involved in hydrogen bond formation during interaction with the dye are primarily of an aromatic nature. In the case of the AzrC-Ar88 complex, residues Tyr156 and Phe105 are implicated in potential hydrogen bond formation within the active site region. Meanwhile, Trp103 is involved in the AzrC-OI complex, where the amino acid Val also plays a significant role.

The application of the pulsed electric field favors the conformational stability of the complexes (**Figure 1**). The Gibbs free energy (ΔG_b) decreases with the application of the pulsed electric field, potentially favoring their catalytic function.

Table 1. Molecular docking analysis.

Complex	ΔG_b[1]	Ki[2]	RMSD[3]	Hydrogen bond interactions		
				Donor	Acceptor	Distance[4]
AzrC-AR88						
0	−7.54	2.95	99.249	Lys192:HZ3	AR88:O	1.955
0.5	−9.08	.21982	79.192	AR88:H	Tyr156:O	2.225
				Tyr156:HN	AR88:O	2.218
1.5	−7.91	1.60	102.772	Phe105:HN	AR88:N, N	2.181
AzrC-OI						
0	−7.32	4.32	90.562	Val111:HN	OI:O	1.854
0.5	−8.01	1.35	101.393	Arg163:HE	OI:N	2.184
				Trp103:HE1	OI:N	2.091
1.5	−7.42	3.65	88.052	Val110:HN	OI:O	2.02
				Val111:HN	OI:O, O	2.098

[1]Estimated free energy of binding (kcal/mol); [2]Estimated inhibition constant (micromolar); [3]RMSD from reference structure (Å); [4]Average distance between hydrogen acceptor atom and hydrogen donor atom (Å).

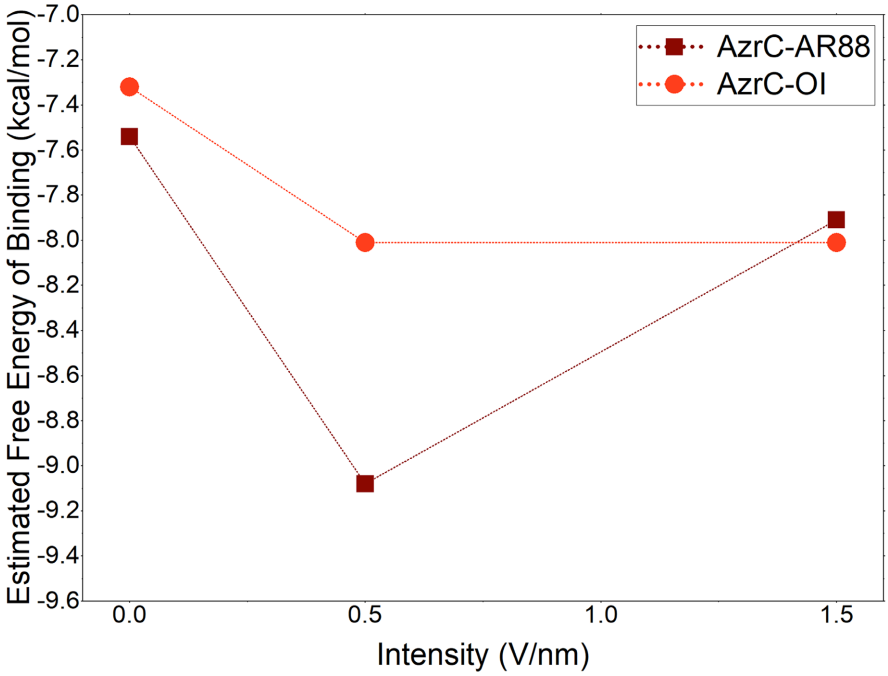

Figure 1. Effect of Pulsed Electric Field (PEF) on the Gibbs free energy of AzrC complexes. It is observed that the application of the pulsed electric field reduces the Gibbs free energy in the AzrC-AR88 complex (red squares); a PEF intensity of 0.5 V/nm more significantly enhances the stability of the complex. Meanwhile, in the AzrC-OI complex (orange circles), the PEF consistently promotes the conformational stability of the complex.

In both cases, an intensity of 0.5 V/nm yields better results.

4. Discussion

There is a significant opportunity to enhance the stability and function of proteins by understanding thermodynamic activation variables. It is imperative to understand the factors influencing protein stability in computational molecular biology [8].

We conducted an *in silico* analysis to simulate the application of a Pulsed Electric Field (PEF) at intensities of 0, 0.5, and 1.5 V/nm for 1 ns. The final conformation obtained from the simulation process was utilized to construct the AzrC-AR88 and AzrC-OI complexes, both of which underwent an energy minimization process. While other studies on AzrC have characterized the active site [3] [5] [6], and suggested potential improvements in enzymatic activity through mutations [7], none have specifically focused on enhancing enzymatic activity as an effect of a PEF to Modify the Free Energy of the Active Site of the AzrC.

The molecular docking results revealed specific interactions between azoreductase and the dyes. It was observed that these interactions are modulated by the effect of the PEF. In the AzrC-AR88 complex, with a PEF intensity of 0.5 V/nm, two hydrogen bonds formed between the dye and the Tyr156 residue with a Ki of 0.21982 μM (**Table 1**). This suggests a stronger interaction compared to when a PEF of 1.5 V/nm is applied, where a single hydrogen bond forms between the AR88 dye and the Phe105 residue. A study by Haghshenas and colleagues (2016) mentions that AR88 does not have a significant hydrogen bond network [7]; however, our results suggest the opposite when a PEF of 0.5 V/nm is applied. On the other hand, although there is a low relative specificity between the OI dye and azoreductase [6], the interaction between the AzrC-OI complex improves when a PEF of 0.5 V/nm is applied. Val111, Arg163 and Trp103 are involved in hydrogen bond formation. Nevertheless, the network of bonds is not as significant. These differences in interactions could influence the conformational stability of the enzyme and, therefore, its catalytic activity as explained below.

The structural stability and catalytic function of an enzyme are largely explained by enthalpy and Gibbs free energy [11] [12]. Negative ΔG values indicate thermodynamically favorable reactions, meaning that the enzymatic reaction occurs more easily and efficiently. The presented results provide evidence that the application of the PEF appears to favorably modify the free energy of the AzrC active site, since the application of PEF decreases Gibbs free energy. When a PEF of 0.5 V/nm intensity is applied, the Gibbs free energy changes from −7.54 to −9.08 and from −7.32 to −8.01 in of the AzrC-AR88 and AzrC-OI complex respectively. These changes could be interpreted as a better tertiary conformation, which in a biological context would imply that the application of a PEF enhances stability. This, in turn, might indicate a greater affinity in the active site towards the AR88 and OI dyes, promoting improved catalytic function, *i.e.* enhanced degradation of azo dyes.

There is evidence from research indicating that certain intensities of an electric field enhance the enzymatic activity of certain enzymes [9]; thus, it can be inferred that these *in silico* results will likely yield favorable outcomes upon direct application in azoreductases.

5. Conclusions

A pulsed electric field intensity of 0.5 V/nm promotes the structural stability of Azoreductase C and potentially enhances its enzymatic activity by facilitating the arrangement of active residues and their interaction with the azodyes Acid Red 88 and Orange I. The increased affinity of the active site towards these azo dyes can be interpreted as indicative of greater efficiency in degrading these compounds. These results present a potential method for enhancing the enzymatic properties of azoreductase and could have significant implications in bioremediation and wastewater treatment applications.

Although *in silico* analyses provide valuable results in scientific research, experimental validation of these findings is necessary. Incorporating other types of azo dyes and conducting a detailed kinetic analysis—also other methods for calculating Gibbs free energy—could offer a more profound understanding of the underlying mechanisms, enabling the proposed methodology to be applicable to other enzyme types. Moreover, we do not overlook mentioning the potential application of the pulsed electric field to modulate the enzymatic activity of other enzymes of interest in food, medical, or industrial settings.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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