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Probing the binding interaction of lysozyme-viologen herbicide

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ABSTRACT

The binding mechanism between ethyl viologen (EV) herbicide and lysozyme (Lys) was studied using spectroscopies and molecular docking. Apparent association constant (5.04×10^4 L/mol) was calculated using UV–Vis study and suggested the formation of a complex between Lys and EV. Fluorescence quenching of Lys occurred via a static quenching as confirmed by time-resolved data. Binding constant obtained using temperature dependent fluorescence quenching and strong binding affinity ($15.8 \pm 0.12 \times 10^4$ L/mol at 298 K) between Lys and EV has been observed. The mode of interaction studied using thermodynamic parameter, and weak force is responsible for the formation of Lys-EV complex. The binding distance between EV and Lys was found to be 1.57 nm indicating a non-radiative energy transfer process. There is no clear evidence of significant changes in the structure of Lys in the presence of EV. Also, experimental results for the Lys-EV interaction were in agreement with those finding of theoretical simulations.

1. Introduction

Herbicides are used in fields to remove selectively unwanted weeds without damaging the crops. Paraquat (1,1-dimethyl-4,4-bipyridinium dichloride) is a potent weed killer of highly water-soluble quaternary ammonium compound and it is a fast, non-selective contact herbicide. As a side effect, a wide range of activities can lead to acute respiratory distress syndrome [1]. Paraquat has an aliphatic detergent that enhances penetration into cells and increases toxicities [2]. Paraquat has been shown to be toxic to both humans and animals [3]. The action of paraquat has been attributed to lipid peroxidation [4] and to its ability to inhibit the synthesis of different macromolecules [5]. Another group of bipyridinium dichloride known to act as herbicides is viologens [6]. Viologens have been used in electrochromic devices, electron transfer catalysis, supercapacitors as novel dual redox shuttle [7,8]. Viologen, a potential herbicide, also contains a bipyridinium ring, which can cause toxicity and even side effects to various organs and is highly likely to be used as a herbicide [9].

Lysozyme (Lys) is a small globular monomeric protein, the most widely studied one following human/bovine serum albumin. Lys is composed of 129 amino acid residues with six tryptophan (Trp), three tyrosine (Tyr) residues and four disulfide bonds and is known as a highly functional protein in our body system [10]. Lys shows antibacterial action by hydrolyzing the bond in the middle of *N*-acetylglucosamine and *N*-acetylmuramic acid of the bacterial cell wall. Lys also has essential functions such as antibacterial, antivirus, detumescence, and drug carrier useful in pharmaceutical and food applications [11]. Several reports on interactions between serum albumin and small molecules are available but interaction with Lys is scanty [12]. Hen egg Lys is 60% sequence homologous to human Lys [13] and is therefore suitable for use as a model protein for studying protein-ligand interactions.

In recent days, the interaction between drug targets (Protein and DNA) and small synthetic organic compounds has received a lot of attention in the scientific community but not much report on the interaction of Lys with toxic material like herbicide. There are some reports on interactions between viologen derivatives and nucleosides and DNA [14–16]. However, the interaction between viologen and Lys has not been studied in depth so far. Hence the experimental and theoretical analysis of viologen and Lys interaction would be significant to understand the mechanism of viologen herbicide toxicity and its mechanism of action. The principal objective of the work was to investigate the interaction mechanism between Lys and ethyl viologen (EV) using a various spectroscopic technique like UV–vis absorption, steady-state fluorescence spectroscopic titration, time-resolved fluorescence, synchronous fluorescence, circular dichroism spectroscopy, and molecular docking methods.

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2. Experimental

2.1. Materials

Ethyl viologen diperchlorate (Scheme 1) and lysozyme (hen egg) were procured from Sigma-Aldrich (USA) whereas tris-base was obtained from Merck (Germany). Other chemicals used were of analytical reagent grade, and double distilled water used throughout the experiment.

2.2. Methods

2.2.1. Spectrophotometric measurements

The absorption spectra were obtained using a UV–vis spectrophotometer (Model: 100, Agilent Technologies). Tris-HCl (50 mM, pH 7.4) buffer was used to prepare a solution of Lys (1 μM). Spectral modifications of 0.1 μM Lys have been monitored in the presence of EV (0, 2, 4, 6, 8, 10, 12, 14 and 16 μM) by recording the light absorption at 230–330 nm.

2.2.2. Fluorescence measurements

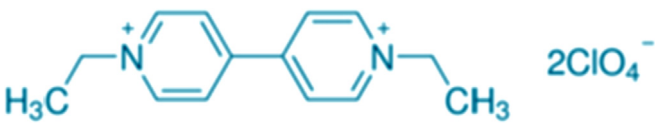
Emission spectra were taken using a Fluoromax-4p Spectrofluorimeter (Horiba Jobin Yvon, Model: FM-100) with an attached temperature controller. Time-resolved studies were carried out in a picosecond time-correlated single photon counting (TCSPC) system (Horiba Yovin, Model: Fluorocube-01-NL). The emission of Lys has observed at the excitation wavelength of 280 nm. The experiments have conducted after adding varying amounts of EV (0, 4, 8, 12, 16, 20, 24, 28 and 32 μM) in Lys solution. The experiments repeated at varying temperature 298, 305, and 312 K. In case of time-resolved studies, a picosecond time-correlated single photon counting (TCSPC) system used. To a solution of Lys varying amount of EV added (0, 2, and 4 μM). After performing the experimental data have been analyzed using IBH DAS (version 6) decay analysis software.

2.2.3. Circular dichroism spectroscopy

Circular dichroic spectra were examined using a Jasco CD spectrometer (Model: J-815). In Lys solution varying amounts of EV (0, 10, and 20 μM) added to conduct CD experiment. CD spectra are recorded in 0.1 cm path length cell using a step size of 0.5 nm, bandwidth of 1 nm with the scan rate of 20 nm/min.

2.2.4. Molecular docking

The molecular modeling study was performed on AutoDock 4.2 suite of programs. Lamarckian genetic algorithm (LGA) available in AutoDock 4.2 is used to study the interaction between Lys and EV. The Lys structure acquired from the Protein Data Bank (PDB ID 6LEV). For docking study of Lys in the presence of EV ligand, ten independent docking runs were carried out. Visualization of the docked position has been done using the discovery digital studio molecular graphics program.



Scheme 1. Structure of EV.

3. Results and discussion

3.1. UV–vis spectroscopy

To discover the molecular interaction between EV and Lys, the absorption spectra of Lys were checked in the absence or presence of EV. Fig. 1 shows UV–vis absorption spectra of Lys in the presence of EV (0–16 μM).

Addition of EV increased absorption of Lys step by step with a blue shift in absorption maxima. The blue shift observed was due to the signatures of electronic transitions (band-band transitions or HOMO-LUMO transitions for Lys molecules). Due to the blue shift of Lys UV band of Lys in the presence of viologen herbicide, the electronic structure has been modified, and there is the possibility of structural changes in Lys.

So, the increase in absorption of Lys in the presence of EV may be due to the creation of the ground state complex between Lys and EV which is given in Eq. (1) [17].

$$\mathrm{Lys} + \mathrm{EV} \overset{K_{app}}{\Leftrightarrow} \mathrm{Lys}.....\mathrm{EV} \tag{1}$$

To know about the magnitude of interaction between Lys and EV, the apparent association constant (K_{app}) have been calculated according to the available works [18,19]. Using UV–vis data the K_{app} value can be calculated using Eq. (2) [26].

$$\frac{(A_C - A_0)}{(A_{obs} - A_0)} = 1 + \frac{1}{K_{app}[EV]} \tag{2}$$

where A_{obs} denotes absorbance of Lys with varying concentrations of EV at 280 nm, A_0 and A_C denote absorbance of Lys and the complex, respectively, and [EV] is the concentration of EV in mol/L. $(A_C - A_0)/(A_{obs} - A_0)$ vs. 1/[EV] plotted in Fig. 2 which yields a straight line and from this K_{app} have been calculated to be 5.04×10^4 L/mol ($R^2 = 0.9998$). The obtained K_{app} point out the establishment of a moderate complex between Lys and EV. So, these results suggest the creation of ground state complex by the intermolecular interactions between Lys and EV [20]. Hence, the details interaction studies need to carry out for a more beneficial understanding of the Lys-EV interaction.

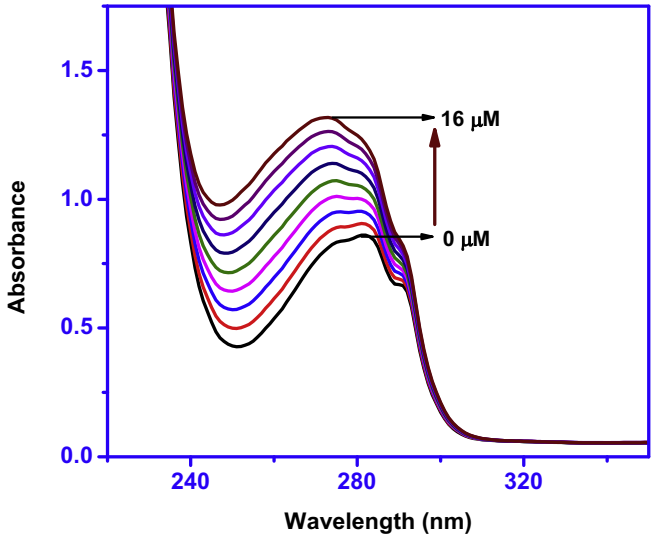


Fig. 1. Light absorption of lysozyme interacted with various concentration (0, 2, 4, 6, 8, 10, 12, 14 and 16 μM) of ethyl viologen.