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Short Communication

Azo and anthraquinone dye decolorization in relation to its molecular structure using soluble *Trichosanthes dioica* peroxidase supplemented with redox mediator

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ABSTRACT

In this study we investigated the decolorizing ability of *Trichosanthes dioica*-derived soluble peroxidase on structurally complex azo and anthraquinone dyes in the presence of redox mediators. Our results show that riboflavin acted as a better redox mediator than antraquinone-2, 6-disulfonate (AQDS). Riboflavin served as an efficient electron transferor than AQDS in the reduction of azo dyes, by contrast with anthraquinone dyes. Although the extent of decolorization (expressed as percent dye decolorization) varied from one dye to the other, maximum decolorization was achieved for the case when suspensions containing 0.45 EU (Enzyme Units)/ml and 0.2 mM riboflavin at pH 5.0 were incubated for 2 h at 40 °C.

Redox mediator supplementation remarkably improved the decolorizing potential of *T. dioica* peroxidase. Azo dyes exhibited higher decolorization as compared to anthraquinone dyes in the presence of riboflavin. On the contrary, percent decolorization in the absence of redox mediators was inadequate and slow. Redox mediators studied herein facilitated decolorization of stable anthraquinone dyes. Therefore, the effect of redox mediators on *T. dioica* peroxidase-dye decolorization is affected by stability, thus the molecular structure.

1. Introduction

A large amount of unused dyes is released in the industrial effluents. Over ninety percent of textile dyes entering modern activated sludge sewage treatment plants pass through unchanged. These unbound dyes are generally resistant to degradation [1]. The release of dye-colored wastewaters in surface water is undesirable for esthetic reasons and the dyes' impact on aquatic plant photosynthesis and fish mortality, but also because many of these dyes are toxic and carcinogenic [2–4].

Azo dyes constitute the largest group of colorants used in industry [4]. They are characterized by the presence of one or more azo chromophores (N=N) and the bonds between two or more aromatic rings. Anthraquinone dyes constitute the second most important class of textile dyes, which have the chromophore groups, =C=O and =C=C=, forming an anthraquinone complex [4,5]. These dyes can be precipitated or adsorbed only in small amounts, while under anaerobic conditions they are cleaved by microorganisms forming potentially carcinogenic aromatic amines [5].

Dye characteristics such as resistance to biodegradation, toxicity and resistance to reductive processes, are related to the chemical structure of each dye. As such, due to the variety of dyes available in market, present in textile and other industrial wastewaters, color removal to be achieved in the wastewater treatment plants is still more complex [6,7]. Methods currently used to treat textile wastewaters have technical and economical limitations. Most of the physico-chemical methods that remove color from waters are expensive, produce large amounts of sludge and are inefficient for some soluble dyes. On the other hand, biological treatment of dyed-waters can be cheaper than physico-chemical treatment [8–10].

Decolorization of dye wastewater is an area where innovative treatment technologies need to be investigated. The focus in recent times has shifted towards enzyme based treatment of colored wastewater/industrial textile effluents. The peroxidase and polyphenol oxidases participate in the degradation of a broad range of substrate even at very low concentrations. Further, these peroxidases and polyphenol oxidases have been used for treatment of dyes but large scale exploitation has not been achieved due to their low enzymatic activity in biological materials and high cost of purification [11–13]. Bioresource technology is gaining importance due to its cost effective, environmental friendly approach and production of less sludge as compared to chemical and physical decomposition processes [14–17].

It has been shown that peroxidases catalyze a variety of oxidation reactions and importantly dyes recalcitrant to peroxidase show

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significant decolorization in the presence of redox mediators [18]. Redox mediated enzyme catalysis has wide application in degradation of polycyclic aromatic hydrocarbons which includes phenols, biphenyls, pesticides, insecticides etc. [18,19]. *T. dioica* commonly known as pointed gourd is widely planted in tropical areas. The present study is an investigation regarding functional competence of *T. dioica* proteins in decolorizing azo and anthraquinone dyes in the presence of redox mediators under varying experimental conditions of pH, temperature, time intervals and enzyme concentration on the basis of one-factor-at-a-time (OFAT) method. Also, decolorization of these dyes is correlated with structural complexity.

2. Experimental methods

2.1. Chemicals

All the azo (Congo Red, Reactive Red 2, Reactive Red 120 and Reactive Black 5) and anthraquinone dyes (Remazol Brilliant Blue R and Reactive Blue 4), ammonium sulfate, redox mediators (riboflavin and AQDS) and Tween-20 were procured from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals were of analytical grade. The pointed gourds were procured from the Narendra Dev University of Agriculture and Technology, Faizabad, U.P., India. The samples were aseptically transferred into sterilized plastic bags.

2.2. Partial purification of T. dioica peroxidase enzyme

Briefly, 100 g of pointed gourd was homogenized in 180 ml of 100 mM sodium acetate buffer, pH 5.6. The homogenate was filtered through multi-layers of cheese cloth and then centrifuged at the speed of 10,000 *g* on a Remi C-24 cooling centrifuge for 30 min at 4 °C. The clear solution thus obtained was used for salt fractionation by adding 10% to 80% (w/v) $(NH_4)_2SO_4$. The proteins were precipitated by continuously stirring at 4 °C overnight. The precipitate was collected by centrifugation at 10,000×*g* on a Remi C-24 cooling centrifuge, dissolved in 100 mM sodium acetate buffer, pH 5.6 and dialyzed against the assay buffer (0.1 M glycine HCl buffer, pH4.0) [20].

2.3. Protein estimation and measurement of T. dioica peroxidase activity

Protein concentration was estimated by taking BSA as a standard protein and following the procedure of Lowry et al. [21]. Peroxidase activity was determined by a change in the optical density (A_{460} nm) at 37 °C by measuring the initial rate of oxidation of 6.0 mM o-dianisidine HCl in the presence of 18.0 mM H_2O_2 in 0.1 M glycine-HCl buffer, pH 4.0, for 20 min at 37 °C. One unit of activity was defined as the amount of enzyme that transformed 1 μmol of o-dianisidine HCl as substrate per min.

2.4. Preparation of dye solutions and calculation of percent decolorization

Azo and anthraquinone dyes (0.4 mM) were solubilized in 100 mM glycine HCl buffer, pH 4.0. Each dye was independently incubated with pointed gourd peroxidase (PGP) (0.45 EU/ml) in 100 mM glycine HCl buffer, pH 4.0 in the presence of 0.80 mM H_2O_2 for varying times at 37 °C. The reaction was stopped by boiling at 100 °C for 7 min. Dye decolorization was monitored by measuring the difference at the maximum absorbance for each dye as compared with control experiments without enzyme on UV–visible spectrophotometer (JASCO V-550, Japan). Untreated dye solution (inclusive of all reagents except the enzymes) was used as control for calculation of percent decolorization. The dye decolorization was calculated as the ratio of the difference of absorbance of treated and untreated dyes to that of treated dye and converted in terms of percentage. Five independent experiments were carried out in duplicate and the mean was calculated.

2.5. Decolorization of azo and anthraquinone dyes in the presence of redox mediators

Each of the azo and anthraquinone dyes was incubated with PGP (0.45 EU/ml) in the presence of two different redox mediators viz., riboflavin and AQDS at varying concentrations (0.05–1.0 mM) along with 0.8 mM H_2O_2 in 100 mM glycine HCl buffer, pH 4.0 for 2 h at 37 °C. The final reaction volume was kept at 20 ml.

In the following sets of experiments of this section the reaction was stopped by boiling the sample at 100 °C for 7 min. The absorbance of the dye solutions at the respective λ_{max} for each dye was recorded against untreated dye as control (100%).

2.6. Effect of T. dioica peroxidase concentration on decolorization of azo and anthraquinone dyes

Each of the two categories of individual dyes was incubated with increasing concentrations of PGP (0.065 to 0.50 EU/ml) in 100 mM glycine HCl buffer, pH 4.0 in the presence of 0.8 mM H_2O_2 for 2 h at 37 °C. Riboflavin and AQDS were used as redox mediators, each at 0.2 mM concentration.

2.7. Effect of pH on decolorization of azo and anthraquinone dyes

The dye solutions were made in different buffers each of 100 mM and in the range of pH 2.0 to pH 10.0. The buffers were glycine-HCl (pH 2.0, 3.0 and 4.0), sodium acetate (pH 5.0), sodium phosphate (pH 6.0, 7.0 and 8.0), and Tris–HCl (pH 9.0 and 10.0). Each of the azo and anthraquinone dyes was treated with PGP (0.45 EU/ml) in buffers of varying pHs, in the presence of 0.8 mM H_2O_2 and riboflavin at 0.2 mM.

2.8. Effect of temperature and time on decolorization of azo and anthraquinone dyes

Each of the dyes was incubated with PGP (0.45 EU/ml) at different temperatures (20 °C to 90 °C). Other reaction conditions were common. The reaction was stopped by boiling the sample at 100 °C for 7 min. The individual textile dye was treated with PGP (0.45 EU/ml) in the presence of 0.8 mM H_2O_2 in 0.1 M glycine HCl buffer, pH 4.0 at 37 °C for varying time intervals. Riboflavin was used as a redox mediator as mentioned earlier.

3. Results

3.1. Effect of redox mediator on decolorization profile of azo and anthraquinone dyes

The effect of different redox mediators (riboflavin and AQDS) on the dye decolorization by PGP is shown in Table 1. Out of the two different redox mediators studied for dye decolorization, riboflavin was more effective in decolorizing these dyes. The extent of decolorization in the presence of riboflavin was in the range of 98.6% to 74.2% for the azo dyes whereas for anthraquinone dyes the decolorization range was 92.4% to 62.5%. The effective riboflavin concentrations were 0.2 mM for azo dyes and 1.0 mM for anthraquinone dyes. The dye decolorization with AQDS was lower for both types of dyes at similar concentrations. Although, with increasing concentration of riboflavin or AQDS there was substantial increase in the extent of decolorization at 0.2 mM but percent color removal was more profound with riboflavin. Among the azo dyes, the percent decolorizations of RR2 and RB5 were lower than CR, RR120 and RO16. The anthraquinone dyes RB4 and RBBR decolorized at five fold higher concentrations of both the redox mediators.