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Short Communication

Catalytic potential of cauliflower (*Brassica oleracea*) bud peroxidase in decolorization of synthetic recalcitrant dyes using redox mediator

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ABSTRACT

We investigated the effect of cauliflower (*Brassica oleracea*) bud peroxidase along with redox mediators on decolorization of dyes (Reactive Red 2, Reactive Black 5, Reactive Blue 4, Disperse Orange 25 and Disperse Black 9). Results indicated that among the chosen mediators, 1-hydroxybenzotriazole (HOBT) followed by riboflavin were most effective for dye decolorization. At 0.36 EU/ml and with 0.8 mM HOBT in conjunction with 0.75 mM H_2O_2 the soluble cauliflower bud peroxidase proteins could decolorize the dyes. The enzyme worked in a broader range of pH and temperature and was adequately effective in batch processes when used with organic contaminants (dioxane and dimethylformide), detergents (Triton X-100 and sodium dodecyl sulfate), sodium chloride and heavy metals including zinc and cadmium chloride. Thus peroxidase from cauliflower bud is a better choice than other vegetable peroxidases as it is sufficiently thermostable, operates in a wide range of pH, economic and effective with low concentration of redox mediators in decolorizing recalcitrant synthetic dyes. Such peroxidases with better catalytic activity have potential of being used on a large scale.

1. Introduction

Peroxidases are oxidoreductases and require hydrogen peroxide to oxidize a wide variety of organic and inorganic substrates [1,2]. Dye overload in the industrial effluents adversely affects the biological communities. Not only these compounds form toxic products, their strong color causes turbidity and consequently disturbs the aquatic environment. These synthetic reactive dyes bond contains chromophoric groups like anthraquinone, azo, triarylmethane, etc. along with reactive group's viz., vinyl sulphone, chlorotriazine, trichloropyrimidine, etc. [3,4]. Conventional approaches in dye color removal from wastewaters are expensive, generate large volumes of sludge and require the addition of environmentally hazardous chemical additives [5–7]. Biodegradation appears to be a fruitful technology but, unfortunately the analysis of contaminated soil and water has shown persistence of toxic pollutants [4,8]. Decolorization of dye wastewater must employ environmentally friendly/green technologies. Enzymatic treatment of industrial pollutants has gathered much attention due to the potential of enzymes to remove pollutants from wastewater without creating harsh side effects which are associated with many other conventionally used procedures [8,9]. Enzymes have already been employed for the transformation of toxic compounds of industrial origin to preserve the quality of water [10,11].

Peroxidases and polyphenol oxidases participate in the catalysis of a broad range of substrate and interestingly operate even at very low concentration. Nevertheless, these oxidoreductases 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 [12–14]. Peroxidases ability in dye decolorization and degradation of polycyclic aromatic hydrocarbons is remarkable and dyes recalcitrant to decolorization show significant oxidation in the presence of redox mediators [15–17]. The present study was aimed at testing the competency of soluble peroxidase from cauliflower (*Brassica oleracea*) bud in decolorizing industrially important recalcitrant dyes which could not be effectively decolorized with *Trichosanthes dioica* peroxidase proteins. Its ability to decolorize dyes in the batch processes contaminated with organic solvents, detergents and heavy metals has also been examined.

2. Materials and methods

2.1. Chemicals

Reactive Red 2 (RR2), Reactive Black 5 (RB5), Reactive Blue 4 (RB4), Disperse Orange 25 (DO25), Disperse Black 9 (DB9); bovine serum albumin (BSA) and *O*-dianisidine HCl were procured from Sigma Chemical Co. (St. Louis, MO, USA). Dioxane and dimethylformide (DMF) were obtained from SRL Chemicals, Mumbai, India. All

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other chemicals were of analytical grade. The cauliflower buds were procured from local market of Faizabad, U.P., India.

2.2. Partial purification, protein quantitation and cauliflower bud peroxidase (CBP) activity assay

Fresh cauliflower (*B. oleracea*) buds were washed, drained, packed in polyethylene bags and stored at −80 °C until use. The homogenate preparation procedures for peroxidase proteins were adapted from the methods described by Sakharov et al. [18]. Partial purification of cauliflower bud proteins and measurement of peroxidase activity were done as described by Jamal et al. [19]. Protein was measured following the procedure of Lowry et al. [20].

2.3. Dye decolorization with SCBP in the presence of different redox mediators, varying pH, temperature and time

Each dye (0.5 mM; 7.0 ml) was incubated with soluble cauliflower bud peroxidase (SCBP) (0.36 EU/ml) in the presence of each redox viz., bromophenol, 2, 4-dichlorophenol, 1-hydroxybenzotriazole, riboflavin, m-cresol, quinol, (0.5 mM) and 0.8 mM H_2O_2 in 0.1 M glycine HCl buffer, pH 4.0 for 90 min at 37 °C. A similar set of experiment was performed to optimize the concentrations of HOBT (0.2 to 1.6 mM). Similarly each dye was incubated with increasing concentrations of CBP (0.02 to 0.57 EU/ml) and hydrogen peroxide (0.15 to 1.5 mM). The pH activity was studied using different buffers each of 100 mM and in the range of pH 2.0 to pH 10.0. For time activity plot individual dye was treated with SCBP (0.36 EU/ml) in the presence of 0.75 mM H_2O_2 in 0.1 M glycine HCl buffer, pH 4.0 at 37 °C for varying time intervals and with redox mediators. Each dye was incubated with SCBP (0.36 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 10 min. The absorbance of the dye solutions at the respective λ_{max} for each dye was recorded against untreated dye as control (100%). Five independent experiments were carried out in duplicate and the mean was calculated and rounded off to the nearest value.

2.4. Effect of detergents, organic solvents, salt and heavy metals on dye color removal by SCBP in batch processes

SCBP were incubated with 10–80% (v/v) of water-miscible organic solvents; dioxane and DMF prepared in 100 mM sodium acetate buffer (pH 5.6) at 37 °C for 90 min. In another experiment SCBP were incubated with increasing concentrations of sodium dodecyl sulfate (SDS) and Triton X-100 (0.5–6.0%, v/v) prepared in 100 mM sodium acetate buffer (pH 5.6) at 37 °C for 90 min. Catalytic activity was monitored at all the indicated detergent concentrations. The decolorization of dye solutions was independently conducted in the presence of 0.5 M NaCl, 0.5 mM $ZnCl_2$ and 0.5 mM $CdCl_2$ for 90 min. The reaction was terminated and absorbance was recorded. Untreated dye solution was considered as control (100%) for the calculation of percent decolorization.

3. Results

3.1. Effect of varying concentration of redox mediator, SCBP and hydrogen peroxide on dye decolorization

Among the six different redox mediators tested for their effectiveness in conjunction with SCBP, HOBT was the most effective in decolorizing these dyes (Fig. 1a). Decolorization achieved with HOBT was 78%, 83%, 76% for RR2, RB5, RB4 and 72%, 73% for DB9 and DO25, respectively. Other redox mediator was relatively less effective; however at the same concentration comparable levels of decolorization was shown by riboflavin. On increasing the concentration of HOBT there was an increase in the extent of decolorization of all dyes (Fig. 1b). Decolorization of >85% was observed with 0.8 mM HOBT, whereas above this value there was a slight decrease or no effect in percent decolorization of the dyes.

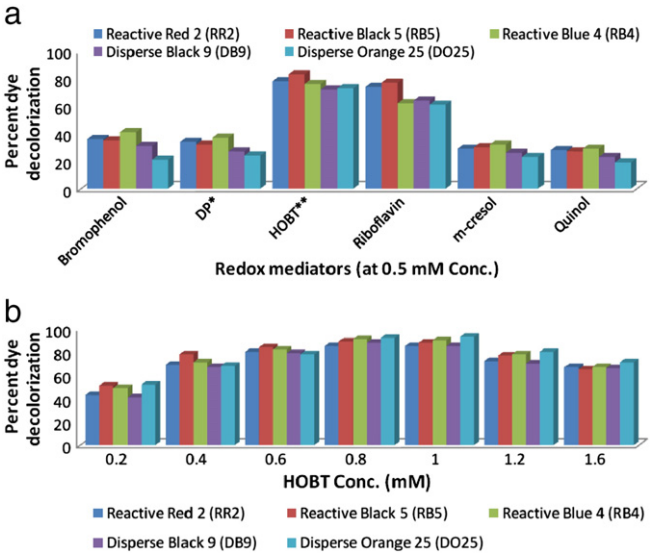

Fig. 1. a: percent dye decolorization as a function of different redox mediators. The dye solutions were incubated independently with SCBP (0.36 EU/ml) in the presence of 0.5 mM concentration of each redox mediators; other conditions were 0.75 mM H_2O_2, 100 mM glycine HCl buffer, pH 4.0 for 90 min at 37 °C. (λ_{max} for each dye is shown in Table 1). The color code of each dye is kept uniform in all the figures. b: percent dye decolorization as a function of different concentration of redox mediator HOBT. The dye solutions were incubated independently with SCBP (0.36 EU/ml) in the presence of varying concentration of HOBT (0.2 to 1.6 mM); other conditions were 0.75 mM H_2O_2, 100 mM glycine HCl buffer, pH 4.0 for 90 min at 37 °C. (λ_{max} for each dye is shown in Table 1). *DP (2, 4-dichlorophenol), **HOBT (1-hydroxybenzotriazole).

At an enzyme concentration of 0.36 EU/ml maximum decolorization achieved with RR2, RB5, RB4 was 88%, 91%, 95% and 89%, 96% for DB9 and DO25, respectively (Table 1). Maximum decolorization (RR2, RB5, RB4 was 87%, 90%, 94% and 89%, 90% for DB9 and DO25, respectively) was observed at a concentration of 0.75 mM H_2O_2, whereas concentrations of H_2O_2 above 0.75 mM had a negative effect on the extent of decolorization (Fig. 2a).

Table 1
Dye decolorization (percentage) as a function of SCBP enzyme (EU/ml). (λ_{max} for each dye is shown in Table 1).

Dyes (λ_{max})	Percent dye decolorization by SCBP as a function of different concentration of enzyme (EU/ml) supplemented with 0.8 mM HOBT/0.75 H_2O_2										
	0.03	0.06	0.09	0.12	0.15	0.24	0.30	0.33	0.36	0.45	0.57
Reactive Red 2 (RR2) (538 nm)	38	43	49	56	66	73	76	80	88	87	86
Reactive Black 5 (RB5) (597 nm)	41	46	53	59	64	69	71	79	91	91	90
Reactive Blue 4 (RB4) (595 nm)	43	48	56	62	66	72	79	86	95	95	94
Disperse Black 9 (DB9) (464 nm)	41	47	54	64	67	76	80	84	89	89	87
Disperse Orange 25 (DO25) (457 nm)	50	56	67	75	79	82	88	91	96	95	95