Title : Factors affecting the catalysis of castor plant peroxidase

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ABSTRACT

Peroxidase is a group of enzymes that catalyze the oxidation of various substrates using hydrogen peroxide (H_2O_2) as an electron acceptor. This enzyme is commonly found in plants and is known for its versatility in catalyzing a wide range of reactions. The objectives of this study were to extract peroxidase from castor leaves using an aqueous two-phase system (ATPS) and to investigate factors affecting its catalytic activity, including pH, temperature, and metal ions. The extraction process was performed using an aqueous two-phase system consisting of polyethylene glycol (PEG 1500) and ammonium sulfate in a ratio of 1.4 g to 1.2 g in a total volume of 10 ml of crude enzyme extract. Results revealed that the total enzymatic activity in the bottom phase containing the salt solution was higher than that in the top phase, indicating that the lower phase retained most of the active peroxidase. The purification fold of the enzyme was 3.77. The enzyme exhibited maximum activity at pH 5.0 in a temperature range of 30°C to 50°C. The enhanced activity at mildly acidic pH is attributed to the stability of the heme group under these conditions, which maintains the active site's structural integrity, allowing efficient electron transfer during catalysis. Metal ions including $FeCl_3$ (74%), $ZnCl_2$ (49%), $CoCl_2$ (40%), $MgCl_2$ (37%) and $CaCl_2$ (31%) were found to inhibit enzymatic activity from the most to the least. This inhibition was likely due to the interaction of metal ions with amino acid side chains, resulting in conformational changes that disrupt the active site, thereby preventing substrate binding. The properties of castor peroxidase reported here are useful for future applications of this enzyme in industrial and biotechnological processes such as bioremediation, the synthesis of bioactive compounds and analytical biochemistry.

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