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Data Article

Spectrophotometric determination of peroxidase using N, N-diethyl-p-phylenediamine sulphate and 3-Aminophenol as a chromogenic reagent: Application of the method to seeds of some fruits

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ABSTRACT

A biocatalatic pathway for the determination of peroxidase using N, N–diethyl–p-phylenediamine sulphate (DPD) and 3-amino phenol (3-AP) is presented. The assay is based on the enzymatic consumption of hydrogen peroxide using DPD- 3AP system to give an intense blue colored compound with absorbance maxima at 660 nm. The increase in absorbance is proportional to the concentration of peroxidase in the range from 1.5–15.15 nM and 0.47 and 15.15 nM from rate and fixed time method respectively. The assay was adapted for the measurement of H_2O_2 at concentrations of 3.5–120 µM. The kinetic parameters like catalytic power, catalytic efficiency, catalytic constant (k_{cat}) and specificity constant (k_{cat}/K_m) was found to be 9.78×10^{-5} μM^{-1} min^{-1}, 1.483×10^{-3} min^{-1},

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0.0245×10^3 min^{-1} and 0.0445 μM^{-1} min^{-1} respectively. The applicability and thermal properties of the method has been tested in different seeds of fruits extracts that showed peroxidase activity.

Specifications table

Subject area	*Analytical Biochemistry*
Compounds	–
Data category	*Analtical procedure, Spectrophotometric data*
Data acquisition format	*UV visible spectrophotomeric, Kinetic measurement*
Data type	*Analyzed*
Procedure	*Development of rate and kinetic method*
Data accessibility	*Data is with this article*

1. Rationale

Peroxidase (E.C.1.11.1.7) is a typical heme enzyme found in many plants. Peroxidase is essential to many biochemical assays for their application in biomedicine or environmental monitoring. It contains glycoprotein with approximately 18% of its weight due to the covalently bound carbohydrate moiety. The native enzyme consists of single polypeptide chain with 308 amino acid residues; the relative molecular mass of POD is 44,000 g/mol [1–4]. They oxidize a wide range of substrates and are implicated in various physiological processes including pathogen defense, stress response and lignin polymerization.

POD is commonly occurs in animals, plants, fungi and microorganisms that act as oxidoreductase. It is a is major H_2O_2 decomposing enzymes which catalyze the oxidation of wide range of substrates at the expense of H_2O_2 [5], only a few reports of peroxidase from actinomycetes are available [6–9]. The mechanism of peroxidase is based on the formation of enzyme–hydrogen donor complexes [10]. It catalyses the oxidation of many aromatic compounds by hydrogen peroxide through on oxidation process that involves a cycle of changes in the oxidation state of an iron atom located at the catalytic site of the enzyme [11]. POD is considered as one of the thermal stable enzyme used as index of blanching. Peroxidase usually having heat resistant and heat labile isoenzymes, due to this it loses its activity in two phases. Owing to this property it is used in polymer synthesis especially for phenolic resin synthesis, nucleic acid analysis, biosensors, bioremediations, and other biotechnological processes [12–17]. POD can act on many hydrogen donors including chromogenic and luminescent compounds. This leads to its widespread use in detection and probe systems [18]. Due to potential application in wide spread areas, it is necessary to find and quantify the amount of peroxidase. The literature survey shows that several analytical techniques are available to quantify the HRP, such as fluorescence [19], Chemiluminescence [20], Electrochemical [21], Magneto elastic sensors [22], Amperometric [23], Flow injection analysis [24], Potentiometric assay [25], Radiometric assay and Coulometric biosensor techniques [26]. The instruments used in these are either very pricey, less flexible, use of radioactive substances and they are susceptible to interference from compounds that either absorb light in the excitation or emission range of the assay or that are themselves fluorescent resulting in false negatives [27]. In electrochemical assay enzyme activity (15%) gets reduces and resulting in the spoil of expensive biocatalyst [28].

To overcome the above drawbacks, we designed an innovative spectrophotometric method for the quantification of HRP and H_2O_2 using DPD and 3- AP. Spectrophotometers are economical, easy to handle and the co- substrates used are generally less pricey, water soluble and stable under lab conditions. The narrow linearity for the assay of H_2O_2 makes the method more significant in the assay of peroxidase. The proposed method is rapid, selective, and highly sensitive. Moreover, the absorption at longer wavelengths allows it to avoid the background interference caused by the biological constituents.