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Purification And Characterization Of Peroxidase: A Review Of Studies Including Bitter Gourd

[1]Madhumitha M, [2]Dr T Indhumathi ,

[1]Student, [2]Professor,

Department of Biochemistry,

[1]Dr.N.G.P. Arts and Science college, Coimbatore, India

Abstract: Peroxidases, a versatile class of heme-containing enzymes, play critical roles in oxidative catalysis, plant defense mechanisms, and diverse industrial applications. Their isolation and purification have evolved significantly, integrating both classical and modern biochemical approaches. This review synthesizes the detailed methodologies discussed in Peter Goddard's work, highlighting the transition from conventional precipitation and dialysis techniques to advanced chromatographic and affinity-based strategies. Emphasis is placed on the choice of purification workflow, which is largely dictated by the physicochemical properties of the source material and the intended downstream application. Beyond the technical protocols, the review explores the challenges of maintaining enzyme activity during purification, scaling up for industrial use, and ensuring reproducibility across

KEYWORDS: Peroxidase, enzyme purification, isolation techniques, ammonium sulfate precipitation, gel filtration, chromatography, Temperature, enzyme activity

I. INTRODUCTION

Peroxidase (POD) is an enzyme belonging to the oxidoreductase group, responsible for catalyzing reactions in which hydrogen peroxide serves as the electron acceptor and another compound donates hydrogen atoms. These enzymes are widely distributed across nature, occurring in plants, microorganisms (both prokaryotic and eukaryotic), and mammalian cells. Among plant-derived peroxidases, the horseradish variety is the most studied, with more than 40 isoenzymes Identified.

Within plants, peroxidases carry out multiple important functions, including the synthesis and breakdown of lignin in cell walls, defense against disease-causing organisms, and repair of damaged tissues. They also help detoxify harmful compounds and reinforce cell walls, especially after injury.

In industrial applications, peroxidases are valued for their versatility. They are used for removing phenols and amines from industrial wastewater, bleaching dyes, breaking down lignin, producing fuels and chemicals from wood pulp, and facilitating various organic synthesis processes. The enzyme works by removing hydrogen atoms, often from alcohol groups, and combining them with hydrogen peroxide to form water and oxidized phenolic compounds.

Environmental studies have also highlighted the role of peroxidases in treating dye-contaminated wastewater. Research has shown that under various operating conditions, they can achieve 50–90% decolorization of azo dyes, with soybean peroxidase (SBP) frequently reported as highly effective.

Peroxidases contribute to many vital processes in plants, including the formation of lignin, the cross-linking of cell wall proteins, the breakdown of the plant hormone auxin, the enhancement of salt tolerance, and the regulation of aging (senescence). In some plants, like rice, specific peroxidase genes are activated by wounding, helping defend the plant against pathogens. Other peroxidases help break down excess hydrogen peroxide, which can build up under stress conditions such as intense light or UV radiation. Overall, these enzymes play a key role in enabling plants to cope with both biological (biotic) and environmental (abiotic) stresses.

In biotechnology, peroxidase genes and their promoters are valuable tools for molecular breeding to develop plants with desirable traits. Peroxidases are also widely used in diagnostic kits to measure substances such as uric acid, glucose, cholesterol, and lactose. In medical applications, horseradish peroxidase (HRP) has been employed to detect 8-dehydroxyguanosine in urine, an indicator of potential bladder and prostate cancer risk. Furthermore, these enzymes have potential in enzyme/prodrug cancer therapies, where they could serve as an efficient system for targeted anticancer treatment.

MATERIALS AND METHODS

The primary materials used in all the methods included bovine serum albumin, methyl α-D-Mannopyranoside, marker proteins, electrophoresis reagents, cysteine, Sephacryl S-100, and ABTS (Sigma Chemical Co., St. Louis, MO, USA). o-Dianisidine HCl was procured from IGIB, New Delhi, India. Concanavalin A–Sepharose and Freund's adjuvants were sourced from Genei Chemicals, Bangalore, India, while hydrogen peroxide was obtained from Merck, India. Ammonium sulphate, ascorbate, glucose, DTNB, guaiacol, and pyrogallol were purchased from SRL Chemicals, Mumbai, India. Fresh bitter gourd was obtained from the local vegetable market. All other chemicals and reagents used were of analytical grade and were utilized without further purification.

Following sample preparation, ammonium sulfate precipitation, gel filtration, and affinity chromatography were employed for protein purification. The choice of chromatographic technique was determined based on the properties and requirements of sample.

AMMONIUM SULPHATE FRACTIONATION

Bitter gourd was homogenized in sodium acetate buffer (pH 5.6) using a blender. The homogenate was centrifuged in a cooling centrifuge, and the resulting supernatant was subjected to ammonium sulfate fractionation. The mixture was stirred at low temperature to ensure maximum precipitation. The precipitate obtained was collected by centrifugation, redissolved in sodium acetate buffer (pH 5.6), and dialyzed extensively against the same buffer.

PURIFICATION OF BGP BY GEL FILTRATION

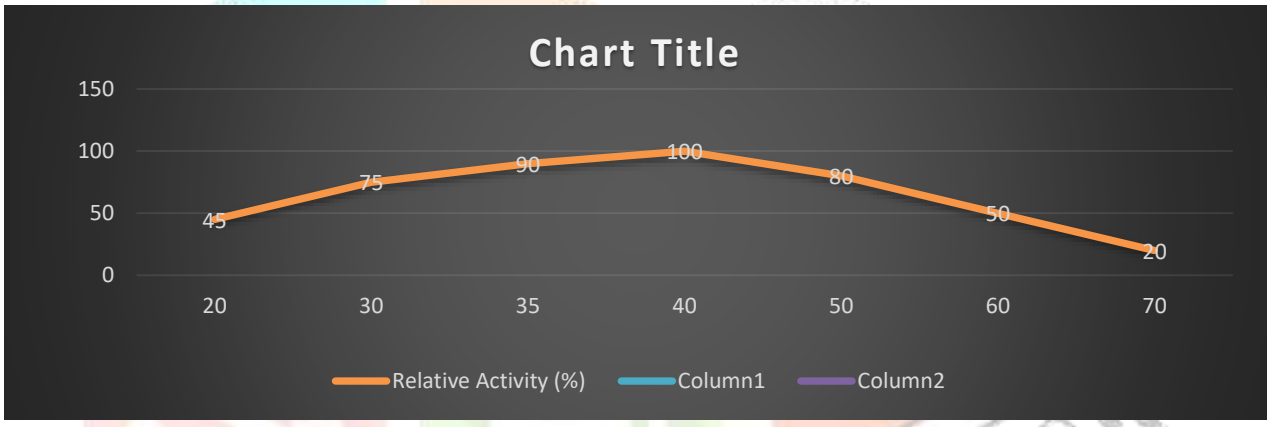
The salt-fractionated and dialyzed BGP was first filtered through Whatman filter paper and concentrated. The concentrated enzyme was applied to a Sephacryl S-100 gel filtration column, pre-equilibrated with sodium acetate buffer (pH 5.6). Fractions were collected in the same buffer, and each fraction was analyzed for protein content and peroxidase activity. Fractions corresponding to the main activity peak were pooled and subjected to affinity purification using a Con A–Sepharose column, pre-equilibrated with sodium acetate buffer (pH 5.6) containing calcium chloride, magnesium chloride, and sodium chloride. Bio affinity-bound proteins were eluted using sodium acetate buffer (pH 5.6) supplemented with methyl α-D-mannopyranoside. Chromatography techniques are commonly used for peroxidase purification, but the specific method should be chosen according to the properties of the sample.

OPTIMUM TEMPERATURE AND PH OF POD ACTIVITY

Analysis of these studies revealed that the optimum temperature for peroxidase activity was consistently 40 °C. This consistency, despite differences in purification protocols, indicates that the enzyme maintains its highest catalytic efficiency at moderate temperatures. Furthermore, the enzyme retains substantial activity even at elevated temperatures, suggesting potential for industrial and biotechnological applications. Based on the reported studies, the optimum pH for bitter gourd peroxidase activity is around 6, suggesting that the enzyme maintains maximum efficiency under mildly acidic conditions.

Figure 1.Optimum temperature for POD activity, Data adapted from Koksal et al., 2012.

TEMPERATURE (°C)	RELATIVE ACTIVITY (%)
20	**45**
30	**75**
35	**90**
40	**100**
50	**80**
60	**50**
70	**20**



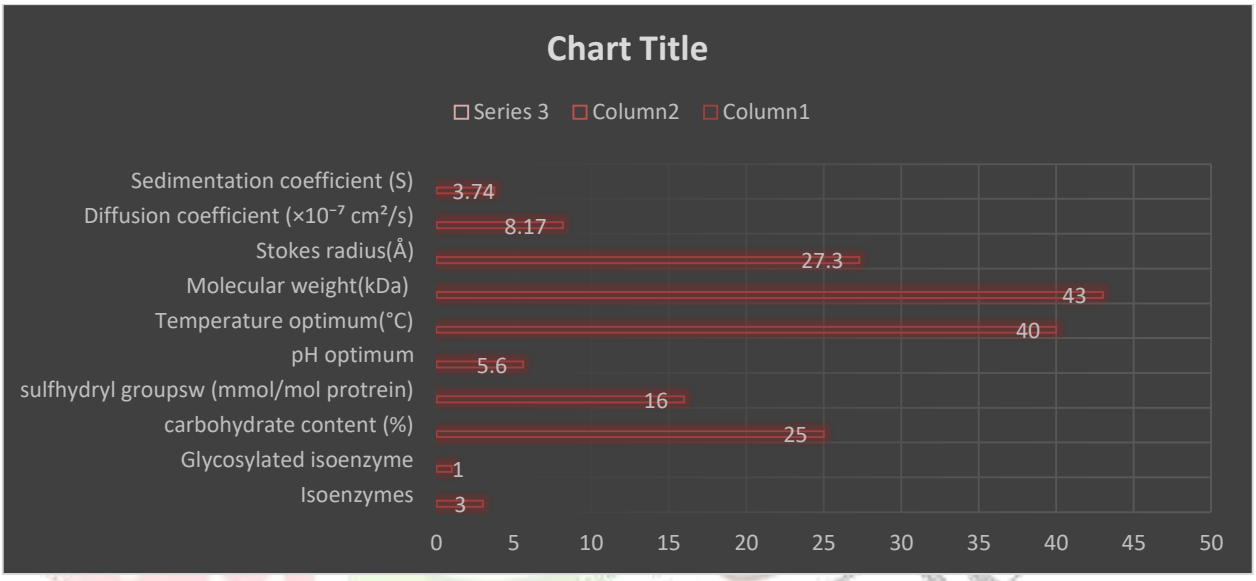
Peroxidase from Cucurbita moschata exhibits a specific optimum temperature for activity, as shown in Figure 1 (Koksal et al., 2012). Based on a review of 5–6 studies, the enzyme activity under these conditions reaches approximately 40%, indicating this as a general consensus for optimal peroxidase activity.

PHYSICOCHEMICAL PROPERTIES OF PURIFIED PEROXIDASE

The purified peroxidase behaves like a finely tuned molecular machine, sculpted for precision catalysis. Its architecture appears to balance rigidity and flexibility — a compact protein core acting as a thermal spring, storing stability until the optimal 40 °C "activation point" is reached. Instead of simply tolerating heat, it seems to use temperature as a structural cue, subtly reshaping its active site for maximal efficiency. The enzyme's surface likely bears a mosaic of charged and hydrophobic regions, functioning like docking pads for substrates and co-factors, while a hidden network of hydrogen-bonded tunnels could channel electrons and protons with remarkable speed. Its glycosylation might act not only as a stabilizing ornament but as a hydration shield, maintaining the protein's dynamic dance even in stressful conditions. This harmony between shape, charge, and motion suggests peroxidase is less a static tool and more a responsive nanobot — one capable of adapting its chemistry to the ever-changing biochemical landscape.

The purified peroxidase revealed a distinctive molecular identity, existing as three dominant isoenzymes, one of which carried a unique glycosylation pattern contributing 25% carbohydrate content to its structure. Its catalytic personality thrived in a mildly acidic microenvironment, with an optimal pH of 5.6, and displayed peak thermal efficiency at 40 °C, maintaining notable stability even beyond this point. Structurally, the enzyme resembled a tightly folded, globular entity weighing 43 kDa, with a compact Stokes radius and exceptional mobility in solution, as evidenced by its high diffusion coefficient. The presence of 16 reactive sulfhydryl groups suggested potential for redox flexibility, while specific inhibitors—azide, sulfide, and L-cysteine—acted as molecular "off-switches," offering insights into its regulatory mechanisms. This unique combination of structural precision and functional adaptability underscores peroxidase's versatility in biological and industrial contexts.

Figure 2. Physicochemical properties of purified POD. Data adapted from Koksal et al., 2012.



SIGNIFICANCE OF POD

The purified peroxidase from bitter gourd (Cucurbita moschata) demonstrates distinct physicochemical properties and optimal activity conditions, making it a highly versatile enzyme for various applications. Its optimum temperature and pH, combined with inherent stability, suggest that it can perform efficiently under controlled industrial conditions. These properties make bitter gourd peroxidase suitable for applications in the food industry, such as improving nutritional quality, preventing browning, and enhancing flavor. Additionally, its oxidative capabilities offer potential in environmental bioremediation, including the degradation of phenolic pollutants and dyes. In the field of biotechnology, peroxidase can be utilized in biosensors, diagnostic kits, and bioengineering applications. Understanding these properties not only expands our knowledge of plant enzymology but also opens avenues for innovative uses, encouraging further research into enzyme engineering, stability enhancement, and novel industrial applications. Overall, the unique characteristics of bitter gourd peroxidase highlight its potential as a multifunctional enzyme with wide-ranging scientific and practical significance.

CONCLUSION

Peroxidase from bitter gourd (Cucurbita moschata) exemplifies the elegant interplay between structure and function in plant enzymes. Its optimum temperature and pH, along with distinct physicochemical properties, reveal a finely tuned biological catalyst capable of thriving under specific environmental conditions. Beyond its biochemical characterization, this enzyme represents a bridge between natural plant defense mechanisms and cutting-edge industrial applications. By leveraging its stability and activity, researchers and industries can explore novel avenues—from sustainable food processing to eco-friendly bioremediation and innovative biosensing technologies. Ultimately, the study of bitter gourd peroxidase is not merely an exploration of an enzyme's characteristics, but a gateway to unlocking nature-inspired solutions that integrate science, industry, and sustainability in unprecedented ways.

REFERENCES

[1] Koksal, Ekrem, et al. "Purification and characterization of peroxidase from sweet gourd (Cucurbita Moschata Lam. Poiret)." International journal of food properties 15.5 (2012): 1110-1119.

[2] Koksal, E., Bursal, E., Aggul, A. G., & Gulcin, I. (2012). Purification and characterization of peroxidase from sweet gourd (Cucurbita Moschata Lam. Poiret). International journal of food properties, 15(5), 1110-1119.

[3] Basu, S. 1997. The Investment Performance of Common Stocks in Relation to their Price to Earnings Ratio: A Test of the Efficient Markets Hypothesis. Journal of Finance, 33(3): 663-682.

[4] Bhatti, U. and Hanif. M. 2010. Validity of Capital Assets Pricing Model.Evidence from KSE-Pakistan.European Journal of Economics, Finance and Administrative Science, 3 (20).

[5] Yoshida, K., Kaothien, P., Matsui, T., Kawaoka, A., and Shinmyo, A. (2003) Appl. Microbiol. Biotechnol., 60, 665-670.

[6] Regalado, C., Garcia-Almendarez, B. E., and Duarte-Vazquez, M. A. (2004) Phytochem. Rev., 3, 243-246.

[7] Chiou, C. C., Chang, P.Y., Chan, E. C., Wu, T. L., Tsao, K.C., and Wu, J.T. (2003) Clin. Chim. Acta 334, 87-94.

[8] Greco, O., Rossiter, S., Kantbou, C., Folkes, L.K., Wardman, P., Tozer, G.M., and Dachs, G.U. (2001) Mol. Can. Ther., 1, 151-160.