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Modulation of Banana Polyphenol Oxidase (PPO) Activity by Naturally Occurring Compounds

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Abstract

Polyphenol Oxidase (E.C number 1.14.18.1) was extracted from banana (*Musa paradisiaca*) and partially purified by acetone precipitation. The enzyme was found to have high affinity towards its substrate, catechol. In the present work, purified compounds like Curcumin, Psoralen were observed to modulate the activity of PPO. The results obtained on the effect of Curcumin even at lower concentration (0.8µg/ml), it inhibits the enzyme activity up to 50 %, and the concentrations of (800 ng/ml) of curcumin, there was activation of PPO by 50%. Similarly, psoralen on PPO activity is proportional increase in PPO activity by 7 fold and 15 fold in the presence of 30.8µg/ml and 61.6µg/ml of Psoralen per ml of assay mixture. The novelty of this study is to screen their naturally occurring bioactive compounds for their inhibitory activity against PPO.

Key words: *Musa paradisiacal, PPO, Enzyme activity, Curcumin, Psoralen*

1. Introduction

Banana is a nutritious fruit with a pleasant flavour that is widely consumed throughout the world. Browning is mainly attributed to oxidation of phenolic compounds by Polyphenol Oxidase. This enzyme widely distributed in the plant kingdom. It is the enzyme responsible for catalyzing the discolouration of polyphenol-rich fruits and vegetables. Previously, the various sources of Polyphenol Oxidase are extracted and studied with different physiochemical conditions such as pH, Temperature, substrate and substrate concentration and optimize the condition for maximum enzymatic activity. This PPO was isolated from various fruits such as plum [1], Litchi [2], Apple [3], Mango [4], Grape[5]. Apart from various plant sources, the polyphenol Oxidase seems to be of almost universal distribution in animals, fungi and bacteria, when compared to other eukaryotes, it is widespread in prokaryotes [6].

Now a days, various research groups are mainly focused on three main aspects: (i) its antiviral and antioxidant potential and protect the damage from UV radiation [7] , (ii) it prevent the browning of damaged tissues of various fruits and vegetables at the time of storage and processing [8], (iii)PPO play a main role in in the betalain biosynthetic pathway, in which the PPO catalyses two different reactions: hydroxylation of tyrosine to form L-DOPA and oxidation of the DOPA to produce dopaquinone [9,10]. Very few research groups have focused on the activation of PPO, wherein chemical agents such as SDS, erythrulose, dihydroxy acetone, sodium azide have been used to activate the enzyme. In certain studies, chemical agents such as detergents (SDS), amino acids such as cysteine and also some chemicals like sodium azide, erythrulose and dihydroxyacetone have been used [11]. Undoubtedly these compounds cannot be used on the skin. While the above-mentioned compounds are chemicals, the search for natural plant-derived compounds still continues, and this study is an attempt towards this goal. Various metal ion can inhibit the activity of PPO, ions such as azide [12] and cyanide [13,14,15] complex with the copper ions in the active site and

cause their loss from the protein. Removal of the metal ion inhibitor results in at least partial recovery of activity, since the copper can be recombined with the apoenzyme. Similarly, the anti-thyroid drug methimazole, also inhibits PPO activity, which was isolated from mushroom polyphenol oxidase, by chelating the copper ions [16,17].

Curcumin is a yellow pigment found in turmeric *Curcuma longa* and *Curcuma aromatica*. Turmeric is primarily used as a spice but it is a traditional healing remedy as well. It also has the ability to neutralize free radicals, reduce inflammation, modulate abnormal cell growth, reduce UV damage, and inhibit accumulation of age-related pigments. Curcumin treatment also reduced wound-healing time, improved collagen deposition and increased fibroblast and vascular density in wounds thereby enhancing both normal and impaired wound-healing. Hence it acts as a potent non-toxic agent for treating skin diseases.

Psoralen (also called psoralene) is the parent compound in a family of natural products known as furocoumarins. Psoralen occurs naturally in the seeds of *Psoralea corylifolia*, as well as in the common fig, celery, parsley and West Indian satinwood. Psoralen has been used in the treatment of vitiligo patches or white spots. It is capable of high Ultraviolet (UV) absorbance. It is the UV part of sun light that does the tanning magic (repigmentation of skin). It also improves circulation of blood that increases chances of re-activating the melanin-producing cells in the skin.

Enzyme PPO was isolated from fruit peels of *Musa paradisiaca*. After identifying the ideal reaction conditions, modulation of enzyme activity has been studied with the pure biochemical compounds Curcumin and Psoralen. It is envisaged that this study would help to identify plants and pure biologicals capable of altering enzyme activity which would be of help for various cosmetic and therapeutic applications involving PPO.

2. Material and methods

2.1. Plant materials

For preparation of the PPO extract, fresh Banana peel was used. Fresh banana fruits (Poovan) were collected from local market of Coimbatore district and washed twice with autoclaved distilled water, and cut into small pieces (1-2 cm long) and processed for enzyme extraction immediately.

2.2. Extraction of PPO from *Musa paradisiaca*

Polyphenol oxidase was extracted from the fruit of banana, which was sonicated using a Labsonic Sonicator (B Braun) with a variable power input of up to 200 W and a frequency of 15 KHZ. The sample was suspended in 50 mM Sodium phosphate buffer (pH 8) and sonicated for a total of 30 min. with 2 minutes burst followed by 5 min incubation on ice. The ground mass was centrifuged at 10,000 rpm for 15 min. and the supernatant was used for further processing. All these procedures were carried out at 4-6ºC.

2.3 Partial purification of PPO from *Musa paradisiaca*

For partial purification, samples were homogenized in 50 mM Sodium phosphate buffer (pH 8), then ground mass was centrifuged at 10,000 rpm for 15 min and supernatant was treated with acetone precipitation. The samples were then centrifuged at 12,000 rpm for 15 min. and the pellet was dissolved in 0.1 N NaOH. All these procedure were carried out at 4-6ºC.

2.4 Assay of PPO activity

Enzymatic activity was measured by the rate of change in absorbance every 15 second, in a UV/VIS spectrophotometer (Shimadzu Corp., Tokyo, Japan) till no further change in O.D. was observed. In a 3.00 ml reaction mix, the final concentrations were 50 mM potassium phosphate (pH 8), 0.17 mM catechol, 0.070 mM L-citric acid, 0.0022 mM EDTA, and 50 - 100 units of catechol oxidase freshly prepared in 0.05 M sodium phosphate buffer at pH 6.5. The reference cuvette contained only the substrate solution. The reaction was conducted at 25ºC. All determinations were performed in triplicate.

2.5. Effect of Curcumin and Psoralen on PPO activity

Effect of two purified naturally occurring compounds such as Curcumin and Psoralen were prepared with two different concentrations. For curcumin, 0.8 µg /ml and 800 ng per ml reaction mixture were prepared, where in case of Psoralen, 30.8µg and 61.6µg / ml of assay mixture were prepared. For enzymatic assay, the entire procedure were described in previous materials and method 2.4

3. Results and discussion

The enzyme PPO was isolated and partially purified from the peels of *Musa paradisiaca* as described in

Materials and Methods and the results presented below

3.1 Effect of curcumin concentration on Banana PPO activity

The results obtained on the effect of concentration of psoralen on PPO activity are presented in the figure 1. When assayed with low concentrations of (0.8 µg /ml) curcumin , there was inhibition of PPO enzymatic activity (55%). At higher concentrations of 800 ng per ml of assay mixture with curcumin, there was activation of PPO enzymatic activity up to 50%. Curcuma aromatic and *Curcuma longa* (from which curcumin is isolated) have been used by Indian women since ancient times as a home remedy for maintaining skin health. In the present study, it was observed that the concentration of curcumin appeared to play a very critical role since trace amounts were able to inhibit the enzyme (which would mean fairer skin), while higher concentrations were enhancing the PPO activity.

3.2 Effect of Psoralen concentration on Banana PPO activity

The results obtained on the effect of concentration of psoralen on PPO activity are presented in the figure 2. The addition of Psoralen to the assay mixture resulted in a proportional increase in PPO activity by 7 fold and 15 fold in the presence of 30.8µg and 61.6µg of Psoralen per ml of assay mixture. Psoralen could increase the polyphenol oxidase activity at any concentration. So it could be used in the treatment of Vitiligo and Leucoderma by induction of melanisation in the white patches.

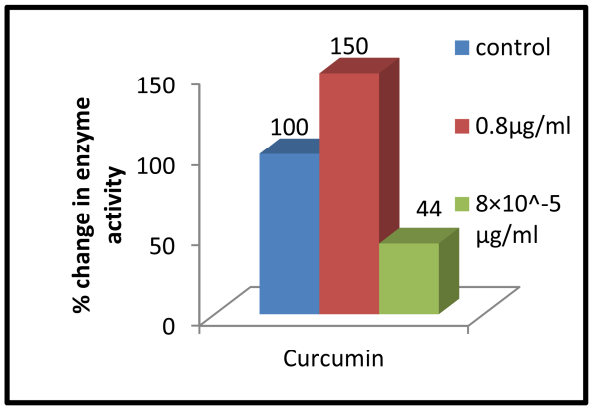

Fig. 1: Effect of curcumin concentration on Banana PPO activity

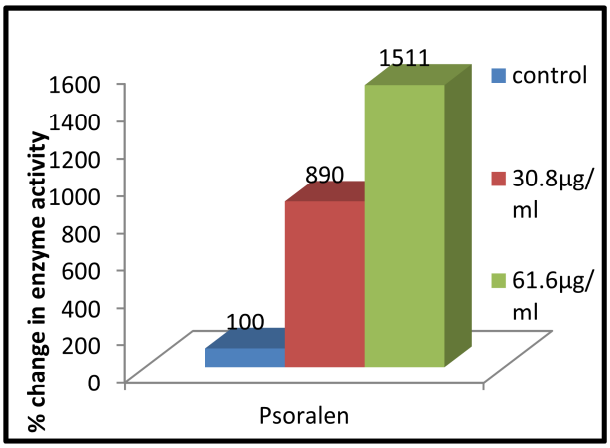

Figure 2: Effect of Psoralen concentration on Banana PPO activity

4. Conclusion

Polyphenol Oxidase (PPO), one of the most abundantly occurring enzyme in nature was isolated from banana peel for this study and their modulation in activity was tested using two naturally occurring compounds. The purified compound Curcumin, alter the enzymatic activity up to 50% but incase of Psoralen were found to increase the activity of PPO at any concentrations. The enzyme activated using these activators could be used in the treatment of certain skin diseases like Vitiligo and Leucoderma as it could improve the melanin synthesis of skin. The inhibited enzyme could be used in the formulation of fairness creams to enhance the skin colour. The same compounds were found to activate the enzyme at high concentrations. In conclusion, Polyphenol Oxidase or Tyrosinase is an exceptionally versatile enzyme and more investigations are needed for a better understanding of its physiological importance and to further define its great biotechnological potential. The results obtained in the present study reveal that a search for naturally occurring

modulators can lead to the discovery of a number of active compounds.

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