Modulation of Banana Polyphenol Oxidase (PPO) Activity by Naturally Occurring Bioactive Compounds From Plant Extracts

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ABSTRACT

Introduction

Polyphenol Oxidase (PPO) (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 this study, various plant extracts like *Glycyrrhiza glabra, Rubia cordifolia, Hesperethusa crenulata* and oil from the seeds of *Hydnocarpus laurifolia* were observed to modulate the activity of banana PPO.

Method

In this study, various plant extracts were observed to modulate the activity of banana PPO at two different concentrations (0.4 and 40 µg/ml concentrations)

Result

Among these 4 plant extracts, *Glycyrrhiza glabra* and *Rubia cordifolia* were found to increase the activity of PPO up to 1.35- 2.7 fold at two different concentrations (4 and 40 µg/ml). Few other two samples like Chaulmogra oil (2 and 4 µl/ml) and the *Hesperethusa crenulata* plant extract (0.4 and 40 µg/ml concentrations), when used at low concentrations decreased the enzyme activity (38 %).

Conclusion

The novelty of this study is to screen their naturally occurring bioactive compounds from the plant extracts and their inhibitory activity against PPO.

KEYWORDS

Polyphenol Oxidase, *Musa paradisiaca*, plant extract, Inhibitory activity

INTRODUCTION

Polyphenol Oxidase (PPO), monophenol dihydroxy phenylalanine: oxygen oxidoreductase (E.C.1.14.18.1) is widely distributed in the plant kingdom. It is the enzyme responsible for catalyzing the discolouration of polyphenol-rich fruits and vegetables. PPO derived from *Musa paradisica* was used as a model for the study, since it is very rich in polyphenols and has a highly active PPO which leads to browning of the peel within a few minutes of peeling. This feature would thus make banana a very ideal candidate to study the effects of modulators. Now a day's, inhibition of PPO have become increasingly important in the field of medication[1] and in cosmetics field [2] by inhibiting enzymatic oxidation. Most studies on the modulation of PPO activity have been carried out to inhibit enzyme activity using compounds such as, oxyresveratrol, resveratrol, and kojic acid which show dose-dependent inhibitory effects on the mushroom tyrosinase activity[3].

A few natural molecules discovered to modulate PPO activity include chalcones and related compounds[4] . The most active compounds are glabridin, effective at around 1 µM and isoliquiritigenin, active at 8 µM[5]. Some flavanols active in the 50–100 µM range have been found, acting perhaps as copper chelators[6]. More recently, flavanones isolated from *Garcinia subelliptica* were found to inhibit tyrosinase[7]. Among the most interesting recent reports is that procyanidins can inhibit PPO from apples[8]. Based on the fundamental information about the enzyme activity has thus set the base for understanding the ideal experimental conditions for elucidating the effects of certain plant extracts.

The objective of this study was to identify inhibitory property of plant extracts. The plant extracts used were *Glycyrrhiza glabra, Rubia cordifolia* and *Hesperethusa crenulata*. Chaulmoogra oil from the seeds of *Hydnocarpus laurifolia.*

MATERIALS AND METHODS

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.

Polyphenol oxidase from Musa paradisiaca

The enzyme 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 rest 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.

Partial purification of PPO from Musaparadisiaca

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.

Assay of PPO

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, 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. The unit for enzymatic activity was defined as a change of 0.001 in the absorbance value under the conditions of the assay. All determinations were performed in triplicate and results averaged.

Effect of plant extract on enzyme activity

The enzyme assay was performed as described in materials and methods (ii. Assay of PPO) except that plant extract. In this method, the enzyme was taken in buffer and known quantity of the compound was added and the mixture was incubated for 30 minutes for reaction between the plant extract and PPO. After noting the time, the substrate was added to start the reaction and 15 minutes later TCA was added to stop the reaction. Absorbance was measured at 495 nm using a blank containing the plant extract. The plant extracts used were *Glycyrrhiza glabra*, *Rubia cordifolia* and *Hesperethusa crenulata*. Chaulmoogra oil from the seeds of *Hydnocarpus laurifolia* was used after suitable dilution and emulsification.

RESULTS AND DISCUSSIONS

Effect of Glycyrrhiza glabra and Rubia cordifolia on PPO activity

In the presence of these two plant extracts, there was an increase in the activity. When assayed with 4 µg plant extract per ml assay mixture, PPO activity increased by 2.7 fold with both *Glycyrrhiza glabra* (fig 1) and *Rubia cordifolia* (fig 2). However when the concentration was increased 10 fold, the increase in activity was only 1.35 fold and 1.93 fold respectively.

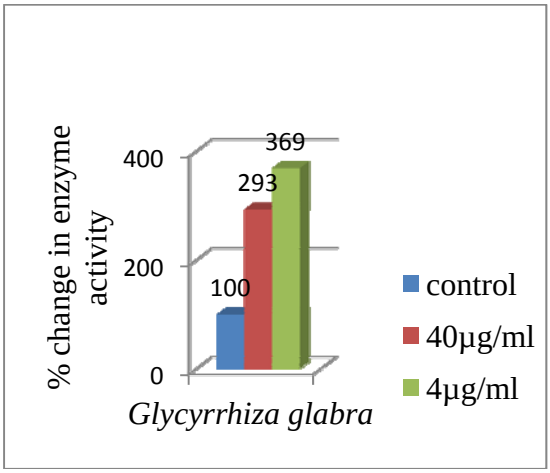

Figure 1 Effect of *Glycyrrhiza glabra* on PPO activity

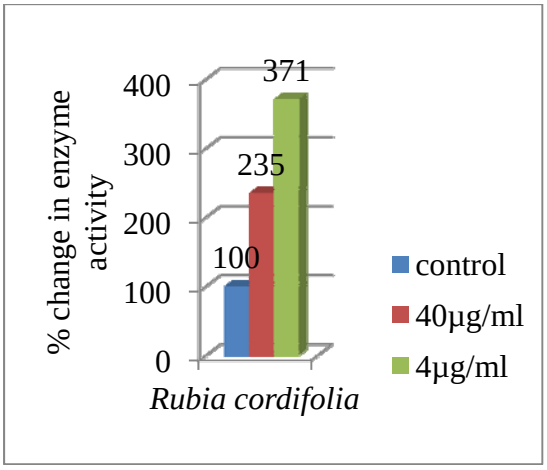

Figure 2 Effect of *Rubia cordifolia* on PPO activity

Effect of Chaulmogra oil and *Hesperethusa crenulata* on PPO activity

At low concentrations, chaulmogra oil did not influence PPO activity while at higher concentrations there was a 10 fold activation (fig 3). When assayed with low concentrations of *Hesperethusa crenulata* extract there was inhibition of activity by 38%. At higher concentrations of 40 µg of Thanaka per ml of assay mixture there was activation of PPO by 82% (fig 4).

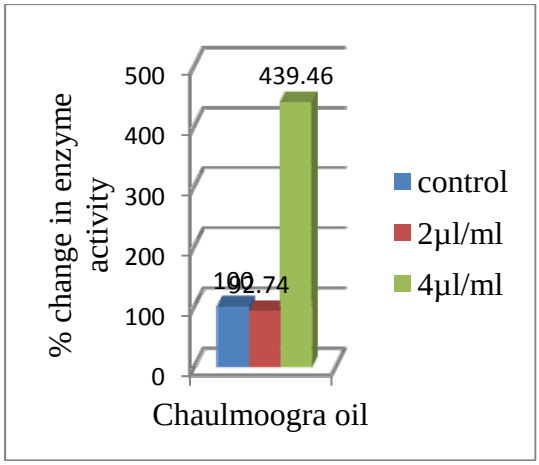

Figure 3 Effect of chaulmoogra oil on PPO activity

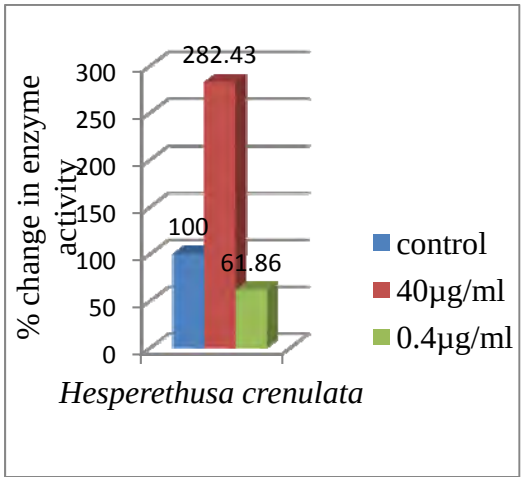

Figure 4 Effect of *Hesperethusa crenulata* on PPO activity

The activity of the enzyme PPO used in this study is activated to different extents by different compounds giving it sufficient versatility to be activated as the situation demands. For enzyme inhibition study, preincubation of the enzyme with inhibitors without substrate did not influence enzyme activity significantly,

suggesting that these compounds are inhibitors rather than inactivators of the particular enzyme under reaction condition[9] Activation of an enzyme can be brought about by changes in the tertiary structure of the protein molecule. It needs to be emphasized here that the plants are effective in increasing activity from 50 to 1411 % over control and the most suited extract could be used depending on the specific application. In the case of chaulmogra oil, atleast 4 µl of oil per ml of assay mixture was required to elicit an effect on polyphenol oxidase activity. At this concentration, polyphenol oxidase activity increased 10 fold justifying its use as a base in creams formulated for treatment of Vitiligo/Leucoderma.

Previously, Various gallic acid derivatives have been isolated from green tea[10] and *Galla rhois*[11-12]. With respect to mode of action, *p*-coumaric acid inhibited both monophenolase and diphenolase activities and a polar hydroxy group at the para position increased the monophenolase inhibitory activity, whereas it decreased diphenolase inhibitory activity[13]. Strong tyrosinase inhibitory activity was reported by oxyresveratrol[14], due to the presence of a maximum number of hydroxy groups in the ring.

Thus, plant extracts such as *Glycyrrhiza glabra*, *Rubia cordifolia* and chaulmoogra oil are effective in enhancing enzymatic catalysis, which would make them suitable for enhancing melanin synthesis. Other extract such as those obtained from *Hesperethusa crenulata* moderate in enhancement of activity. It is envisaged that this study would help to identify plants and pure biologicals capable of altering enzyme activity and further development of biochemical understanding and PPO kinetic relationship with specific substrates which would be of help for better understand of PPO and their mode of action and inhibitory activity with specific inhibitors.

CONCLUSION

Polyphenol oxidase, one of the most abundantly occurring enzyme in nature was isolated from banana peel for this study and their modulation in activity was tested with various plants extract like *Glycyrrhiza glabra*, *Rubia cordifolia*, *Hesperethusa crenulata* and oil from the seeds of *Hydnocarpus laurifolia*. Among these 4 plant extracts, *Glycyrrhiza glabra* and *Rubia cordifolia* were found to increase the activity of PPO at any concentrations. Few other compounds like Chaulmogra oil and the *Hesperethusa crenulata* extract, when used at low concentrations decreased the enzyme activity which results in the lowering of melanin production of the 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. Atleast 4 natural sources have been identified in this short-term study and further intense studies could help to identify more such compounds which are safe to use on the skin.

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REFERENCES

[1] Seo, S.Y., Sharma, V.K., Sharma, N., Mushroom tyrosinase: recent prospects. J. Agric. Food Chem.2003, 51, 2837–2853.
[2] Maeda, K., Fukuda, M., In vitro effectiveness of serval whitening cosmetic components in human melanocytes. J. Soc. Cosmet. Chem. 1991, 42, 361–368.
[3] Kim, Y. M.; Yun, J.; Lee, C. K.; Lee, H.; Min, K. R.; Kim, Y. Oxyresveratrol and hydroxystilbene compounds. Inhibitory effect on tyrosinase and mechanism of action. J. Biol. Chem.2002, 277, 16340-16344.
[4] Nerya, O., Musa, R., Khatic, S., Tamir, S., Vaya, J., Chalcones as potent tyrosinase inhibitors: the effect of hydroxyl positions and numbers. Phytochemistry, 2004, 265, 1389–1395.
[5] Nerya, O., Vaya, J., Musa, R., Izrael, S., Ben-Arie, R., Tamir, S., Glabrene and isoliquiritigenin as tyrosinase inhibitors from licorice roots. J. Agric. Food. Chem.2000, 51, 1201–1207.
[6] Kubo, I., Kinst-Hori, I., Chaudhuri, S.K., Sanchez, Y., Ogura, T., Flavonols from Heterotheca inuloides: tryrosinase inhibitory activity and structural criteria. Biorg. Med. Chem.2000, 8, 1749–1755.
[7] Masuda, T., Yamashita, D., Takeda, Y., Yonemori, S., Screening for tyrosinase inhibitors among the extracts of seashore plants and identification of potent inhibitors from Garcinia subelliptica. Biosci. Biotechnol. Biochem.2005, 69, 197–201.
[8] Le Bourvellec, C., Le Quere, J.-M., Sanoner, P., Drilleau, J.-F., Guyot, S., Inhibition of apple polyphenol oxidase activity by procyanidins and polyphenol oxidation products. J. Agric. Food Chem.2004, 52, 122–133.
[9] Khan, V.; Andrawis, A. Inhibition of mushroom tyrosinase by tropolone. Phytochemistry,1985, 24, 905-908.
[10] No, J.K., Soung DY, Kim YJ., Inhibition of tyrosinase by green tea components. Life Sci. 1999,65: 241–246.
[11] Kim JH, Sapers GM, Choi SW., Identification of tyrosinase inhibitor from Galla rhois. Food Sci Biotechnol.1998, 7: 56–59.
[12] Parvez S, M Kang, Chung H-S., A review: survey and mechanism of skin depigmenting and lightening agents. Phytother Res.2006, 20: 921–934.
[13] Lim JY, Ishiguro K, Kubo I., Tyrosinase inhibitory p-coumaric acid from ginseng leaves. Phytother Res.1999,13: 371–375.
[14] Shin NH, Ryu SY, Choi EJ., Oxyresveratrol as the potent inhibitor on dopa oxidase activity of mushroom tyrosinase. Biochem Biophys Res Commun.1998, 243: 801– 803.