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Inhibition of tyrosinase by cherimoya pericarp proanthocyanidins: Structural characterization, inhibitory activity and mechanism

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ABSTRACT

In this study, the structure of proanthocyanidins purified from cherimoya (*Annona squamosa*) pericarp was analyzed by ESI-QTOF-MS and HPLC analyses. The result indicated that these compounds were procyanidin-type proanthocyanidins, consisting mainly of (epi)catechin units linked b y B-type interflavan bonds. The analyses of enzymology showed that the activities of monophenolase and diphenolase of tyrosinase could be powerfully inhibited by the proanthocyanidins. Further researches on the inhibition mechanism demonstrated that they were reversible and competitive inhibitors with the K_I value of 27.1 ± 3.1 μg/mL. These inhibitors quenched the fluorescence of tyrosinase through a static quenching mechanism and spontaneously formed proanthocyanidins-enzyme complex. Fluorescence changes of proanthocyanidins in the presence of copper ion suggested that the interactions could reduce the fluorescence intensity of these polymers and the molecular docking analysis revealed that copper irons of the enzyme could be chelated by adjacent hydroxyl groups on the B ring of proanthocyanidins. Moreover, proanthocyanidins were proved to be efficient quencher of substrates. These results would lay scientific foundation for their farther application in food and medicine industry.

1. Introduction

Tyrosinase, also called as polyphenol oxidase, is a multifunctional copper-containing oxidase which exists widely in a variety of organisms, catalyzing both the monophenolase reaction cycle and diphenolase reaction cycle of melanin synthesis (Fig. 1A) (Gowda & Paul, 2002). It plays a critical role in the browning of fruits and vegetables (Lin et al., 2013), development in insects (Pan et al., 2011) and melanin biosynthesis of human skin (Hearing & Tsukamoto, 1991). However, the browning reactions of fruits and vegetables generally lead to the losses of nutritional quality and economic values. Moreover, the melanin accumulation may cause serious skin diseases such as melasma, freckles, senile lentigines, age spots, and sites of actinic damage in human beings. Therefore, the inhibitors of tyrosinase are of great importance in the fields of food, agriculture, and cosmetic. Many tyrosinase inhibitors, such as cardol triene (Zhuang et al., 2010), proanthocyanidins (Chai, Lin, Feng, Zou, & Wang, 2017), thymol (Satooka & Kubo, 2011), polyphenols and organic acids (Zocca, Lomolino, & Lante, 2011) are structurally analogous to phenolic substrate.

Proanthocyanidins are a sort of polyphenols widely existing in cereals, legume seeds, and particularly abundant in some fruits. They have aroused sufficiently interests in the areas of food, agriculture and nutrition owing to their biological activities. Acting as antiviral, anticarcinogen, antibacterial agent, cardiopreventive, and antioxidant, these compounds represent many physiological functions (Aron & Kennedy, 2008; Kruger, Davies, Myburgh, & Lecour, 2014; Zhou, Lin, Li, Li, Wei, & Chai, 2011). Nevertheless, the bioactive capacity of plant proanthocyanidins depends mainly on their structure (e.g., degree of polymerization) (Chai et al., 2014; Zhou, Lin, Wei & Tam, 2011). Therefore, it is quite essential to study the structure of proanthocyanidins. Proanthocyanidins are polymers of flavan-3-ols which present a wide variety of chemical structures (Fig. 1B) (Chai et al., 2012). The diversity of the monomer units, interflavan linkage, degree of polymerization, and substitute of the 3-hydroxyl group caused the varieties of proanthocyanidins' structure (Dixon, Xie, & Sharma, 2005). On account of their structures' complexity and diversity, it still remains difficult for characterization. In this study, ESI-QTOF-MS and HPLC methods were employed to characterize the structure of proanthocyanidins.

Cherimoya (*Annona squamosa*) is an kind of important commercial fruit growing in most tropical and subtropical areas currently, which can be regarded as source of vitamins and other bioactive compounds,

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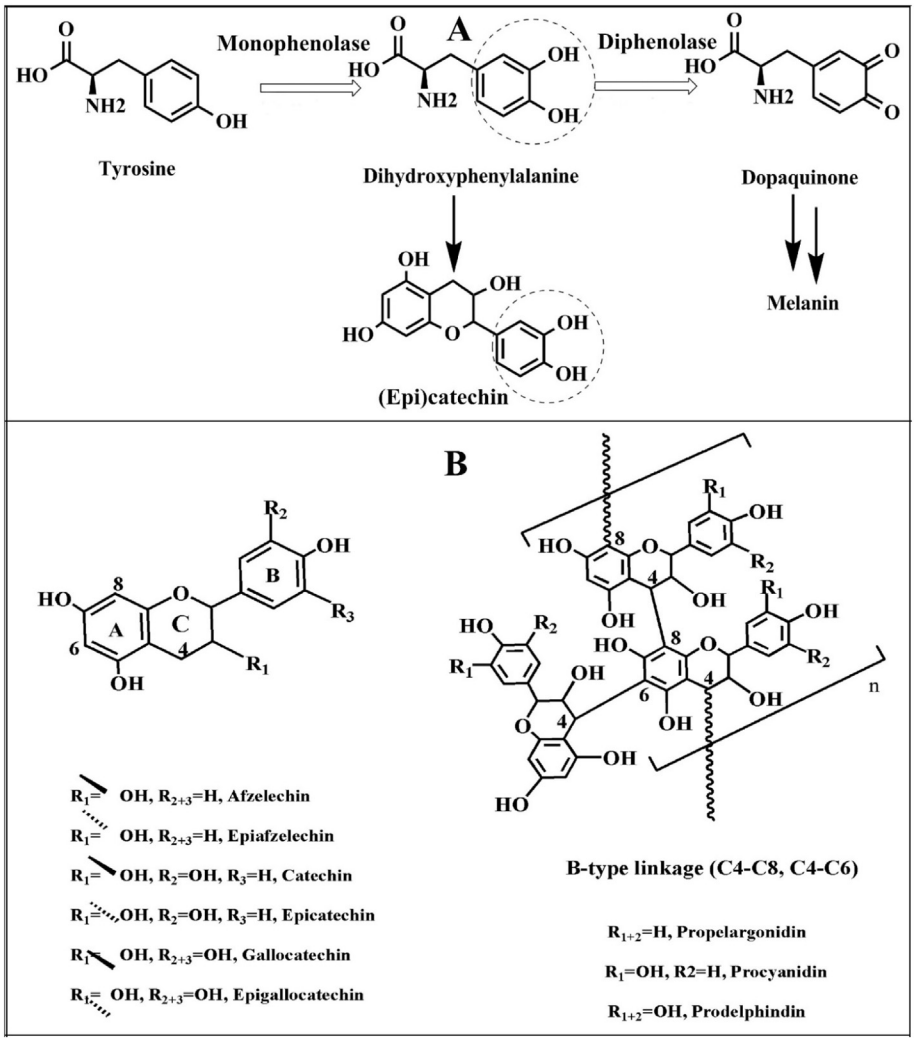

Fig. 1. Chemical structures of proanthocyanidins, flavan-3-ol monomer units, and substrates of tyrosinase. (A) The processes of melanogensis and structure similarity between epicatechin/catechin and L-DOPA. (B) Chemical structures of flavan-3-ol monomer units and proanthocyanidins.

like polyphenols or carotenoids (Albuquerque et al., 2016). Prior researchers have paid much more attentions to antioxidant activity and antifungal activity of extracts obtained from different parts of cherimoya (Ochoa, Chávez, Flores, Camacho, & Ortiz, 2012). However, to the best of our knowledge, it is still unknown about the structure, antityrosinase activity and mechanism of cherimoya proanthocyanidins. New products and added values may be created for the food industry by exploiting the phytochemicals of cherimoya waste. Therefore, the objective of this study is to evaluate the structure, antityrosinase activity and mechanism of proanthocyanidins extracted from cherimoya pericarp, which will be conducive to the development of novel tyrosinase inhibitor.

2. Experimental

2.1. Chemicals and materials

All analytical grade solvents, including acetone, petroleum ether, ethyl acetate and methanol, were purchased from Sinopharm (Sinopharm, Shanghai, China). HPLC grade acetonitrile, MS grade methanol, L-tyrosine, 3,4-dihydroxyphenylalanine (L-DOPA), mushroom tyrosinase, quercetin, Sephadex LH-20, benzyl mercaptan, trifluoroacetic acid, and all HPLC standards were purchased from Sigma-Aldrich (St. Louis, MO, USA). The fresh fruits of cherimoya were picked from the trees growing in Xiamen University (Xiamen, China) in August 2015. The pericarp (waste product of the food industry) of fruit was manually separated. The fresh pericarp was (about 500 g) immediately washed, freeze-dried, ground, and sieved to obtain dry powders, these powders were stored at − 20 °C before further processes.

2.2. Total phenolics and extractable proanthocyanidins assay

Total phenolic content was measured by Folin-Ciocalteu method (Zhou, Lin, Li, et al., 2011; Zhou, Lin, Wei, and Tam, 2011). The absorbance was read at 725 nm using a Beckman UD-800 spectrophotometer (Beckman coulter, Pasadena, California, USA). Gallic acid (Sigma-Aldrich) was used as a standard and the result was expressed in mg gallic acid equivalent per g dry weight. The content total extractable proanthocyanidins was measured by butanol-HCl method (Terrill, Rowan, Douglas, & Barry, 1992). Purified polymeric proanthocyanidins from our purified procedure were used as the standard and the result was expressed as mg purified proanthocyanidins equivalent per g dry weight.

2.3. Extraction and purification of proanthocyanidins

Acetone/water (70:30, v/v, each 200 mL) solution was used as solvent for ultrasonical extraction of 10.0 g cherimoya pericarp powders for three times at 25 °C (Fig. 2). To prevent the oxidation of proanthocyanidins, 0.05% ascorbic acid was added simultaneously. Then petroleum ether and ethyl acetate were selected as extractant to eliminate chlorophyll, lipophilic compounds, as well as low molecular phenolics. The remaining aqueous fraction was chromatographed on a Sephadex LH-20 column (50 × 1.5 cm i.d.) where the ingredients were

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