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Photo-catalytic polymerization of catechin molecules in alkaline aqueous

Ji-Yuan Liang [a], Jun-Yun Wu [a], Ming-Yeh Yang [b], Anren Hu [b], Liang-Yü Chen [a,*]

[a] Department of Biotechnology, Ming-Chuan University, Gui-Shan 33343, Taiwan
[b] Institute of Medical Biotechnology, Tzu-Chi University, Hualien 97004, Taiwan

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ABSTRACT

Polyphenols are associated with a wide range of physiological properties. Catechin is a flavan-3-ol with five phenolic hydroxyl groups. After blue light illumination, the transparent solution of catechin became yellowish. The effects of visible light illumination (400–800 nm) were investigated on molecular structures and antioxidant capacities of catechin. Under the neutral or alkaline aqueous with the illumination of blue light, the photolysis and polymerization of catechin were observed in this study. A chromogenic catechin dimer was separated and identified as a proanthocyanidin by the chromatographic technique and mass spectrometry. For quantitative evaluation, the signal intensities of the catechin and the photochemical product show a negative correlation in the liquid chromatograms. The oligomer of flavan-3-ols (catechin dimer) is suggested as a dimeric B type proanthocyanidin, which has the molecular formula $C_{30}H_{26}O_{12}$ and 578.14 g/mol in exact mass. The mass spectrum of catechin dimer had characteristic ion signals in *m/z* 577, 560, 439 Da. However, the total phenolic contents and scavenging $O_2^{\bullet -}$ activity of catechin treated by blue light illumination are not changed significantly at the neutral or alkaline aqueous. Our results of photocatalytic oligomers of catechin provide a novel way to explain the sensory changes of green tea and a biochemical mechanism under the irradiation environments.

1. Introduction

Polyphenols are secondary metabolites of phytochemicals that are associated with a wide range of physiological properties, including protection from oxidative damage [1], anti-radiation [2,3] and anti-microbial [4]. Phenolic compounds are regarded as the most important antioxidants in plants and plant-based foods [5]. These compounds are polyhydroxylated phytochemicals possess an aromatic ring bearing one or more hydroxyl groups, and their structures may range from that of a simple phenolic molecule to that of a complex high-molecular weight polymer [6].

Catechins are a class polyphenolic flavonoid main include epi-catechin, catechin, epicatechin gallate (ECG), epigallocate catechin (EGC) and epigallocatechin gallate (EGCG) [7]. Catechins are easily oxidized and unstable in solution [8], involving the loss of hydrogen atoms, the semiquinone radical intermediate and formation of quinone oxidized products are generated [9,10].

As shown in Fig. 1, the flavonoid catechin possesses two benzene rings, the catechol group in B ring and the resorcinol group in A ring and it has also the hydroxyl group at position 3 in C ring. Catechins are unstable under the radiation of ultraviolet light (UV), which ranges from 100 to 400 nm in wavelength. Forest et al. [11] observed UV induced epimerization of epicatechin and reported that the photochemistry of catechin was found to undergo a reversible photo-isomerization reaction to give epicatechin [11]. Catechin derivatives were produced by the cross-linkage with bicarboxylic acid under the alkaline and high temperature scenario [12]. Wilhelm-Mouton et al. [13] has been used the catechin and epicatechin were irradiated at 250 nm for 20 h in MeOH. The structural change of catechin molecule has been carried to open the heterocyclic ring (C ring) of flavan-3-ols *via* UV-photolytic cleavage of the ether bond [13].

Proanthocyanidins are oligomeric catechins, and often composed with multiple bonds or mono-linked between the catechin units and classified as A type and B type proanthocyanidins [14]. The proanthocyanidin (catechin dimer) has been identified in various foods including apple, blueberry, cranberry and chocolate [15–17], and been shown to act as cancer chemopreventive and anti-inflammatory agents and by reducing risk of cardiovascular mortality [18].

The electrochemical evidences also revealed that the B ring hydroxyl configuration dominates the scavenging activity of the superoxide anion radicals ($O_2^{\bullet -}$), in contrast to the other rings [9,19–21]. The antioxidant activity could be increased by polymerization of flavan-3-ol monomers, the proanthocyanidins are excellent antioxidants due to the high polymeric units and the various types of reactive oxygen species [22].

Many natural molecules also are sensitive to the irradiation of visible lights in 400 to 800 nm, which is relatively harmless to organism. Our previous study reported that the effectiveness of riboflavin photolysis is mainly determined by blue light illumination [23]. The effects of

* Corresponding author.
E-mail address: loknath@mail.mcu.edu.tw (L.-Y. Chen).

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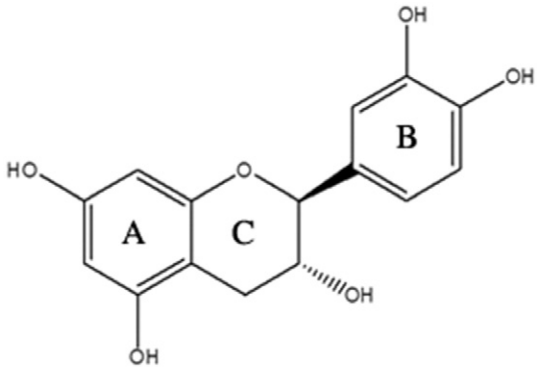

Fig. 1. The molecular structure of (+)-catechin.

light qualities, such as wavelength and irradiation dosage, on the photolysis of catechin may affect the interactions of catechin and matrix molecules. The structural changes of catechin might modify their functional properties and the apparent phenolic contents in solution [20].

There were 2.4 million bottles of tea beverages were discarded by Food and Drug Administration in Taiwan [24]. Catechins are considered as major integrates in tea beverages and argued on relative contents of these constituents which may result in natural degradation [24]. Otherwise, the healthy claims are usually appraised through the antioxidant capacity of catechins. Determination of total polyphenol content and scavenging $O_2^{\bullet-}$ activity, either *in vivo* or *in vitro*, is an important topic in the fields of biochemistry and nutritional sciences [25,26].

Various processing and storage techniques would obviously determine the sensory quality, including the appearance, flavor, and the nutritional value of tea products. Thus, the shortage and quality controls of catechins is receiving increasing attention in food processing and commercial marketing. Our aim was to investigate the effects of light and pH on the molecular changes of catechin by the chromatographic separation and mass spectrometry techniques. The effects of photo-catalytic process on total phenolic contents and scavenging $O_2^{\bullet-}$ activity of catechin were also inspected in this study.

2. Materials and Methods

2.1. Setup of Illumination Units

As used in previous study, the photo-induced reactions were performed in a plastic box (104 cm × 74 cm × 55 cm) with a light source. The box was made of white cardboard, and its outer surface was covered with a black shade cloth [27]. The light-emitting diode (LED) tube lights (VITALUX T8HO, 58 cm length, Vita LED Technologies Co., Tainan, Taiwan) in red, green and blue, were used as light sources. The wavelengths of the emitted maxima (λ_{max}) of the blue, green, and red lights were 463, 529 and 632 nm, and the spectral widths at half height ($W_{1/2}$) were 23, 31 and 14 nm, respectively. The temperature of photoreaction system was maintained at 25 ± 2 °C by an air conditioner. Irradiance was measured by the power of the electromagnetic radiation per unit area (mW/cm^2) with radiometry and validated by a solar power meter (TM-207, Tenmars Electronics Co., Taipei, Taiwan).

2.2. Chemicals

Folin–Ciocalteu reagent, (+)-catechin ($C_{15}H_{14}O_6$, 2*R*,3*S*), riboflavin, gallic acid, methanol, acetonitrile, mono-potassium phosphate, potassium dihydrogen phosphate and sodium carbonate were purchased from Sigma-Aldrich (St. Louis, USA). Nitro blue tetrazolium (NBT) was purchased from Bio Basic, Inc. (Markham, Ontario, Canada). The phosphate buffer was prepared from mono-potassium phosphate and potassium dihydrogen phosphate, and the pH was adjusted to 5, 6, 7 and 8, respectively. Ultra-pure deionized water from a Milli-Q system was used as a solvent in this study.

2.3. Effects of Illumination and pH on the Catechin Solutions

The effects of the pH and color lights on the changes of catechin were determined by a UV/Vis spectrometer. In brief, (A) 1 mM catechin in 0.1 M phosphate buffer solution (pH 5, 6, 7 and 8) was as a standard. (B) 1 mM catechin in 0.1 M phosphate buffer solution (pH 5, 6, 7 and 8) was illuminated by blue, green and red lights at 2.0 mW/cm^2 for 4 h. The absorbance of catechin solution was detected at 250–650 nm by a UV/Vis spectrometer (Lambda35, Perkin-Elmer).

2.4. HPLC Analysis of the Catechin Solution After Blue Light Illumination

The HPLC was performed on a Hitachi L system equipped with a quaternary pump (Hitachi-L2130) with vacuum degasser, a thermostatted column compartment, a manual injector, a photodiode array detector (DAD, Hitachi-L2450), and an EZChrom Elite workstation. The Mightysil RP-18 GP Aqua column (5 μm, 4.6 mm id × 250 mm, Kanto Chemical Co., Tokyo, Japan) eluted at a rate of 1.0 mL/min. The mobile phase consisted of 0.5% phosphate in water (A) and methanol (B) using a gradient program of 15% (B) in 0–1 min, 15–50% (B) in 1–5 min, and held for 5 min. The 2.0 mM catechin solutions were prepared as described in Section 2.3 and the sample solutions was filtered through a 0.45 μm filter (Millipore) before use. DAD detector was set at 280 nm for acquiring chromatograms, and the UV spectra were monitored and recorded between 220 and 580 nm in the chromatographic analysis.

2.5. Total Phenolic Assay

The catechin solutions were 0.1 mM and prepared as described in Section 2.3. The method of total phenolic contents was measured by a modified Folin-Ciocalteu method [12]. The 1 N Folin-Ciocalteu reagent (250 μL) was mixed with 250 μL catechin solution, and incubated for 5 min. The solution was mixed with 0.5 mL of 20% Na_2CO_3 and 4 mL water, and incubated at room temperature for 25 min. The mixture was centrifuged at 4 °C and 5000 rpm for 10 min. The supernatant was collected and quantitated to 10 mL and then measured at 730 nm by a UV/Vis spectrometer (Lambda35, Perkin-Elmer). The amount of total phenols was expressed as a gallic acid equivalent in mg per gram of dry sample extract.

2.6. Determination of Scavenging $O_2^{\bullet-}$ Activity

Superoxide anion radicals ($O_2^{\bullet-}$) are intermediate products generated during oxidation or reduction. The scavenging $O_2^{\bullet-}$ activity was determined by the method developed by Cheng et al. [27]. The photo-induced reactions were performed in a plastic box with a blue LED tube light (VITALUX T8HO LED tube lights, Vita LED Technologies Co., Tainan, Taiwan) that contained white cardboard and an outer surface covered with black cloth. The distance between the reactant and the lamp was fixed and the reactant was illuminated by blue light illumination at 0.12 mW/cm^2 for 30 min. For the control treatment, the reactant was kept in the dark [27].

The catechin solutions were prepared as described in Section 2.3. All solutions were 50 mM in phosphate buffer (pH 7.8). The total volume of reactant was 3 mL and the concentrations of riboflavin, methionine and nitro blue tetrazolium (NBT) were 2.4×10^{-6} M, 0.01 M and 1.6×10^{-4} M, respectively. The catechin solution (50 μL) was added to 3 mL reactant to final concentrations of 0, 100, 200, 300, 400 and 500 μg/mL. The photochemical-reduced riboflavins generated $O_2^{\bullet-}$ which reduced NBT to form blue formazan that can be detected at 560 nm (Lambda35, Perkin-Elmer).