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Interaction of characteristic structural elements of persimmon tannin with Chinese cobra PLA_2

Ying Zhang [a], Li Zhong [a], Bin Zhou [c], Jin-yu Chen [a], Chun-mei Li [a,b,*]

[a] College of Food Science and Technology, Huazhong Agricultural University, Wuhan 430070, China
[b] Key Laboratory of Environment Correlative Food Science, Huazhong Agricultural University, Ministry of Education, China
[c] College of Life Science and Technology, Huazhong Agricultural university, Wuhan 430070, China

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ABSTRACT

To more fully understand the mechanism by which persimmon tannin (PT) inhibited phospholipase A_2 (PLA_2) and the structural requirements of PT for the inhibition, the interactions between PLA_2 and seven characteristic structural elements of PT including epigallocatechin-3-gallate (EGCG), myricetin, epicatechin-3-gallate (ECG), epicatechin-3-gallate-(4β → 8, 2β → O → 7)-epicatechin-3-gallate (A-type ECG dimer), epigallocatechin-3-gallate-(4β → 8, 2β → O → 7)-epigallocatechin-3-gallate (A-type EGCG dimer), epicatechin-(4β → 8, 2β → O → 7)-epicatechin (A-type EC dimer) and epicatechin-(4β → 8)-epicatechin (B-type EC dimer) were studied by enzymatic and spectroscopic methods. Molecular docking was also used to explore the possible residues involved in the interactions. The results revealed that A-type EGCG dimer and A-type ECG dimer showed higher inhibitory effects on the catalytic activity of PLA_2 than monomers and B-type dimer. They induced greater conformational changes in PLA_2 than other structural elements. In addition, molecular docking studies revealed that expect for lysine residues, other residues such as Trp18, Try27, Gly29, His47 and Tyr63 were involved in the interactions. We propose that A-type EGCG and ECG dimer units may be structural requirements for the interaction between PT and PLA_2. Our data provide an additional structural basis for anti-PLA_2 activity of persimmon tannin.

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1. Introduction

Phospholipase A_2 (PLA_2, EC 3.1.1.4), which is a main constituent of snake venom, specifically catalyzes the hydrolysis of the sn-2-acyl group in 1,2-diacyl-sn-glycero-3-phospholipids (Kishimura and Hayashi, 1999). Besides its catalytic activity, snake venom PLA_2 possesses wide varieties of pharmacological activities including cardiotoxicity, myotoxicity, neurotoxicity, edema, hemolysis, and anticoagulation (Gutierrez and Lomonte, 1995; Ownby, 1998). Agents including antisera, chemical antidotes, and anti-snake venom proteins are known effective inhibitors of snake venoms (Melo and Ownby, 1999; Faure, 2000; Trento et al., 2001; Biondo et al., 2003; Soares et al., 2003). In addition, plant extracts, especially plant polyphenols, have been reported to have promising protective effect against snake venoms. Melanin (Hung et al., 2004), catechins (Pithayanukul et al., 2010), rosmarinic acid (Ticli et al., 2005) and aristolochic acid (Chandra et al., 2002) were reported to display significant inhibitory effects on PLA_2. Phenols from seed kernels of Thai mango exhibited potent inhibitory effects on the caseinolytic and fibrinogenolytic activities of Malayan pit viper and Thai cobra venoms (Pithayanukul et al., 2009).

Persimmon (*Diospyros kaki* L.) is widespread in China, Japan and Korea. In China, its fruits and leaves were

* Corresponding author. College of Food Science and Technology, Huazhong Agricultural University, Wuhan 430070, China. Tel.: +86 27 87283201; fax: +86 27 87282966.
E-mail addresses: lichmyl@126.com, lichmyl@mail.hzau.edu.cn (C.-m. Li).

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traditionally used for many medicinal purposes such as coughs, hypertension, dyspnea, paralysis, frostbite, burns and bleeding and snakebites (Mowat, 1990). In studying the biological activities of persimmon tannin, we found that a fraction of persimmon tannin (PT40) (Fig. 1) had a unique structure: it is a highly polymerized, highly galloylated condensed tannin with both A and B type linkages and an unusual flavonol terminal unit (Li et al., 2010a). More interestingly, we found that it exerted a very strong inhibitory effect on the catalytic activity of Chinese cobra venom PLA_2, and alleviated the myotoxicity, neurotoxicity and lethality induced by the venom PLA_2 *in vivo* (Xu et al., 2012; Gu et al., 2013). Although the detailed mechanism of polyphenols against venoms is not fully elucidated, the

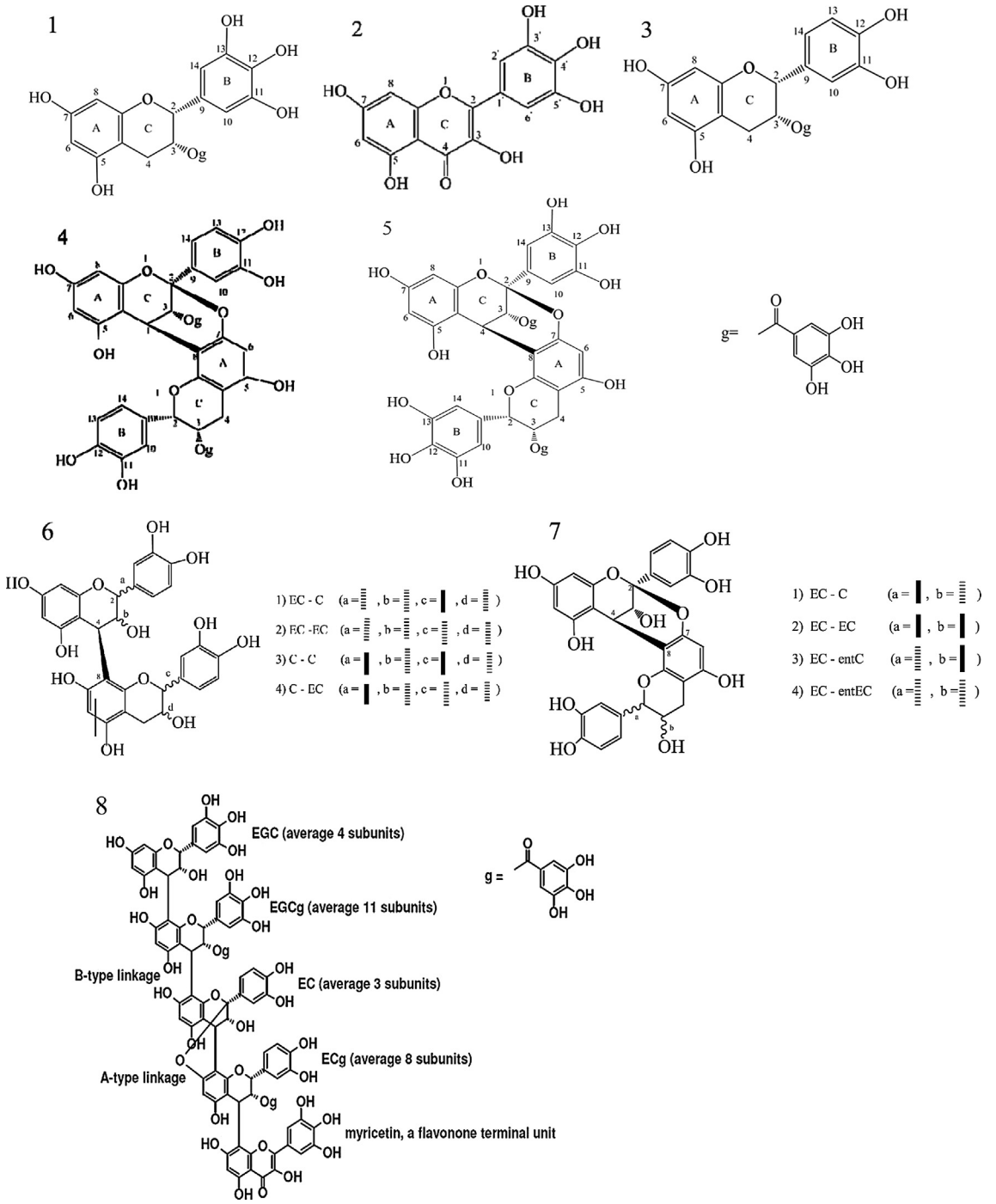

Fig. 1. Structures of the main characteristic subunits of PT and the possible structure of PT40 (1 → 8: EGCG, Myricetin, ECG, A-type ECG dimer, A-type EGCG dimer, B-type EC dimer, A-type EC dimer, PT40).

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