学位論文の要旨

Abstract of Thesis

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学位論文題目 Title of Thesis（学位論文題目が英語の場合は和訳を付記）

Tea catechins as potent non-competitive inhibitors of angiotensin converting enzyme
（アンジオテンシン変換酵素の強力な非競合阻害剤としての茶カテキン）

学位論文の要旨 Abstract of Thesis

Renin-angiotensin system (RAS) is one of the major control systems for blood pressure, fluid and electrolyte balance. Angiotensin converting enzyme (ACE) plays a crucial role in the RAS by converting angiotensin I to angiotensin II, a potent vasoconstrictor. The inhibition of ACE is one of the most promising strategies for treatment of hypertension. Synthetic ACE inhibitors, such as captopril and enalapril, are also used as the first-line drugs for the therapy of cardiovascular diseases. However, these artificial ACE inhibitors are believed to have certain side effects such as cough, skin rash, and angioneurotic edema. Thus, there is an urgent requirement for the exploration and development of a new type of ACE inhibitors without adverse effects. Accumulating epidemiological and intervention studies indicated that green tea consumption is inversely associated with the risk of cardiovascular disease, which might be largely attributed to the presence of polyphenols. Tea catechins, including (-)-epicatechin (EC), (-)-epigallocatechin (EGC), (-)-epicatechin gallate (ECg) and (-)-epigallocatechin gallate (EGCg), are the primary class of polyphenols in green tea. Although green tea and tea catechins have been shown to be promising as potential ACE inhibitors, the precise mechanisms underlying ACE inhibition by catechins remain to be clarified. In this thesis, the molecular mechanisms involved in the ACE inhibition by tea catechins are investigated.

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In Chapter 2, the inhibitory effects of four tea catechins, including (-)-epicatechin (EC), (-)-epigallocatechin (EGC), (-)-epicatechin gallate (ECg) and (-)-epigallocatechin gallate (EGCg), on the enzymatic activity of ACE (purified from rabbit lung) were compared. Each catechin treatment significantly reduced the ACE activity with the order of potency being EGCg > ECg > EGC = EC. The addition of 1 mM borate significantly recovered the reduced ACE activities by tea catechins, suggesting that hydroxyl groups at B-ring or at a galloyl moiety play an important role in the ACE-inhibitory mechanism. The covalent modification of ACE by tea catechins was also observed by a redox-cycling staining experiment. A Lineweaver-Burk plot indicated that EGC and ECg were non-competitive inhibitors. The galloylated catechins might more potently inhibit ACE activity in an allosteric manner through the hydrophobic and hydrogen-bonding interaction of the galloyl moiety with the non-catalytic site of ACE.

In Chapter 3, the molecular mechanisms involved in the ACE inhibition by EGCg, a major tea catechin, were further investigated. The enzyme activity of recombinant human ACE was inhibited by EGCg in a dose-dependent manner. Co-incubation with Zn^{2+} ion showed no influence on the ACE inhibition by EGCg, whereas it completely counteracted the inhibitory effect of EDTA, a representative ACE inhibitor chelating Zn^{2+} at the active site of ACE. Although a considerable amount of hydrogen peroxide was produced during the incubation of EGCg, the treatment of ACE with hydrogen peroxide showed little effect on its enzymatic activity. On the other hand, the co-incubation of EGCg with inhibitors of catechol oxidation, such as borate or ascorbic acid, significantly diminished the EGCg inhibition. A redox-cycling staining experiment revealed that ACE was covalently modified by EGCg. Furthermore, a Lineweaver-Burk plot analysis indicated that EGCg also inhibited the ACE activity in a non-competitive manner. These results strongly suggested that EGCg allosterically inhibits the ACE activity through the oxidative conversion into an electrophilic quinone and subsequent binding to the ACE.

3,4-Dihydoxyphenylacetic acid (DOPAC), having a catechol group, is one of the colonic microflora-produced catabolites of quercetin 4′-glucoside. A previous study indicated that DOPAC is oxidized to form *o*-quinone, then covalently binds to sulfhydryls in GSH or proteins due to its catechol structure. In Chapter 4, the modulating effect of DOPAC on ACE activity was

also studied. The co-incubation of DOPAC significantly inhibited the enzymatic activity of ACE in a dose-dependent manner. Among the four tea catechins, DOPAC has the similar potency to inhibit ACE to that of EC. In addition, the covalent binding of DOPAC with ACE was detected by a tag-free DOPAC probe with the azide labeled biotin and a horseradish peroxidase (HRP)-streptavidin complex. These findings suggested that (1) the catechol structure and its oxidative conversion into electrophilic species are involved in the potential ACE inhibitory activity; (2) ACE is one of the potential cellular targets of DOPAC.

In conclusion, this study indicates that: (1) tea catechins and DOPAC, both of which are derived from food stuffs, are potential ACE inhibitors; (2) Zn^{2+}-chelation as well as hydrogen peroxide-dependent mechanism are ruled out in the mechanisms of the ACE inhibition by tea catechins; (3) the galloylated catechins (ECg and EGCg) allosterically inhibit the ACE activity through the oxidative conversion into an electrophilic quinone and subsequent binding to the ACE; (4) the presence of galloyl moiety might play an important role in the hydrophobic and hydrogen-bonding interaction with the non-catalytic site of ACE, associated with more potent ACE inhibitory activity. The present findings encourage further study using not only the plausible structural model based on the docking simulation but also proteomic and reverse genetic approaches to identify amino acid residues of ACE involved in the catechin-protein interaction. It is likely that basic knowledge of the ACE-binding site of tea catechins will provide a platform for the rational design of new domain-selective ACE inhibitors with improved efficacy and pharmacological profiles. Taken together, this study suggests that the galloylated catechins could be potential candidates to develop nutraceuticals against hypertension and its related disease.