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Caffeoylquinic acids competitively inhibit pancreatic lipase through binding to the catalytic triad

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ABSTRACT

Caffeoylquinic acid and its isomers inhibited porcine Pancreatic Lipase (PL) activity according to a competitive mode where binding and interaction with the catalytic triad of Ser153, His264 and Asp177 simultaneously occurred. The IC_{50} values under which 3-caffeoylquinic acid (CQA) and its isomers 4-, 5-CQA, 3,4-, 3,5- and 4,5-diCQA inhibited half of the porcine PL activity were 1.10, 1.23, 1.24, 0.252, 0.591 and 0.502 mM, respectively. The binding affinities in the range from −8.4 to −9.5 kCal/mol were well predicted from docking, which showed a high linear correlation coefficient of 0.893 and Spearman correlation of 1.0 with log(IC_{50}) values. Caffeoylquinic acid and its isomers were stabilized by hydrogen bond and hydrophobic interaction in the binding pocket. This finding provided molecular mechanism of coffee and other natural food or drink containing caffeoylquinic acid and its isomers against lipase activity.

1. Introduction

In the past two decades, the prevalence of overweight and obesity has increased dramatically around the world [1]. Obesity is associated with comorbid metabolic and chronic diseases, such as type 2 diabetes, heart diseases, hypertension and several forms of cancer [1,2]. It is considered as a major threat to the public health. Obesity emanates from energy imbalance due to excess caloric intake relative to energy expenditure [3], in which, fat metabolism disorder involves and plays an important role [4].

Inhibiting nutrient digestion and absorption is a major way to treat obesity, where energy intake through gastrointestinal path is weakened while other physiological activities are almost unchanged [5]. Since dietary lipids represent the major source of unwanted calories, specifically inhibiting triglyceride digestion is effective to suppress obesity development [6]. Lipolysis is the key step in lipid digestion and absorption, and it majorly occurs in the duodenum through the action of PL [7]. PL is the key enzyme responsible for dietary triacylglycerol (TG) absorption, hydrolyzing triacylglycerols to monoacylglycerols and fatty acids [8].

Lots of phytochemicals, especially polyphenols, identified from traditional medicinal plants and dietary stuffs exhibited anti-obesity bioactivity, which is, at least partly, attributed to their inhibition effects on digestion enzymes [5,6,9–11]. Polyphenols bind to proteins mainly through a combination of hydrogen bonds and hydrophobic interactions [12]. Their reversible and irreversible interactions depend on the environmental pH, temperature, ionic strength, the concentrations and molecular structure of protein and polyphenols [13]. The nature of their interaction has been investigated by different methods, and the commonly used experimental approaches are fluorescence, UV–vis absorbance, circular dichroism, dynamic laser light scattering, and transmission electron microscopy [14,15]. However, to determine the correlation between polyphenol–protein interaction and the enzyme activity inhibition effect is still challenging [12,16]. In addition, the conformation of the polyphenol–protein complex is difficult to be clarified [14].

Caffeoylquinic acid, i.e., 3-O-caffeoylquinic acid (CQA), and its isomers 4-, 5-CQA, as well as dicaffeoylquinic acids, 3,4-, 3,5- and 4,5-diCQA (Fig. 1) [16] are the major polyphenol compounds present in coffee, which are the ester of caffeic acid and quinic acid. Coffee is considered as one of the richest sources of polyphenols in the western diet and has the bio-functions in prevention of hyperlipidemic disease, type 2 diabetes, liver diseases and obesity [17–20]. These bioactivities are believed to be attributed to the inhibition of lipase activity by caffeoylquinic acid and its isomers [21–24]. However, the corresponding mechanisms require further investigation [21,22].

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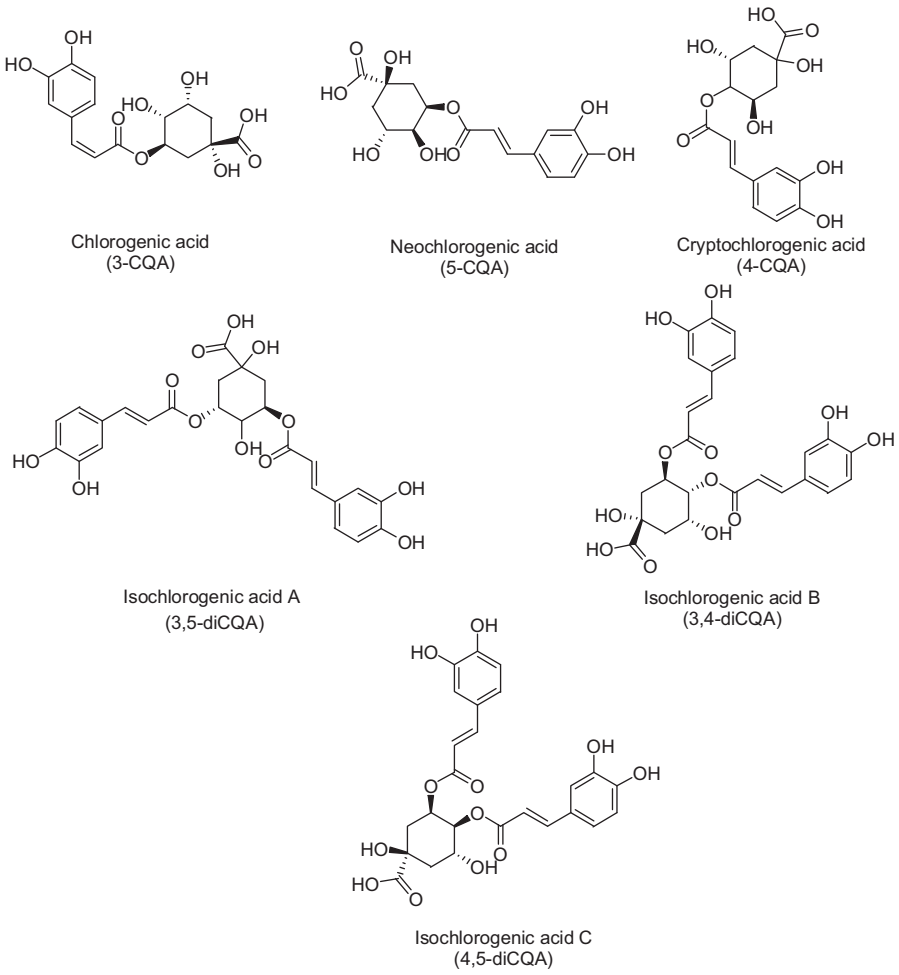

Fig. 1. The chemical structures of the tested caffeoylquinic acid and its isomers.

Therefore, we systematically investigated the inhibition activities of caffeoylquinic acid and its isomers against PL and evaluated the types of their inhibition mode. Like most of the existing studies, porcine PL was used in present study rather than human PL which was very difficult to be obtained. In addition, the detailed interaction nature between caffeoylquinic acid and its isomers with porcine PL was determined by AutoDock Vina program, which has been successfully applied to predict the binding sites, the binding affinity and the structure of various protein–ligand and protein–inhibitor complexes and facilitates structure-based drug design [25–29]. We also applied it to study the binding of tea catechins to trypsin and revealed many details in our recent work [29]. Furthermore, the relevance between the porcine PL activity inhibition effect and the corresponding polyphenol–protein interaction nature was established to explore the mechanism by which caffeoylquinic acid and its isomers inhibit porcine PL activities and the structure–activity relationship associated with different caffeoylquinic acid molecular structures.

2. Methods

2.1. Materials

The standard samples of caffeoylquinic acid and its major isomers (purity >98%), including 3-CQA, 4-CQA, 5-CQA, 3,4-diCQA, 3,5-diCQA, 4,5-diCQA were obtained from Biopurify Phytochemicals Ltd. (Chengdu, China). Porcine pancreatic lipase and Triton X-100 were purchased from Sigma–Aldrich (St. Louis, MO). *p*-Nitrophenyl laurate and *p*-nitrophenol were obtained from Fluka Biochemika. DMSO was purchased from Solarbio Science and Technology Co., Ltd. (Beijing, China). All other chemicals were of analytical grade.

2.2. Pancreatic lipase inhibitory activity

PL inhibitory activity was measured by a chromogenic assay using *p*-nitrophenyl laurate as the substrate, which was modified from previous studies [30]. Briefly, the assay was conducted by mixing 0.5 mL of the PL solution (2 units/mL) in a buffer consisting of 5 mM Tris–HCl (pH 8.0), 50 μL of diluted sample solutions and 0.45 mL of the substrate solution with different *p*-nitrophenyl laurate concentrations to start the enzyme reaction. The substrate stock solutions of 2.25–22.5 mM *p*-nitrophenyl laurate were prepared in CH_2Cl_2. Immediately prior to initiation of the assay, 200 μL of the substrate stock solution was diluted into 20 mL of buffer containing 5 mM Tris–HCl (pH 8.0), 150 mM NaCl, 1.3 mM $CaCl_2$ and 0.01% Triton X-100 while vortexing. The sample stocking solutions of 25 mg/mL caffeoylquinic acid or its isomers were prepared in 3% DMSO solvent. After an incubation period for 2 h at 37 °C, the reaction system was centrifuged at 13,500 rpm for 3 min and the liberation of *p*-nitrophenol in the suspension was measured as the increase in absorbance at 405 nm in an UV–visible Spectrophotometer against a blank without enzyme. All the experiments were