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Molecular interactions of flavonoids to pepsin: Insights from spectroscopic and molecular docking studies

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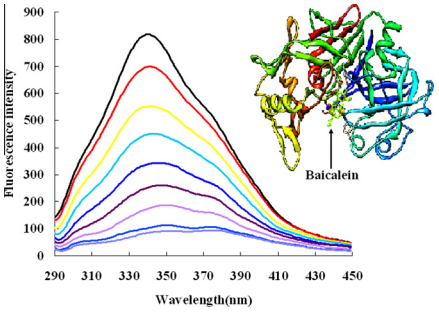
HIGHLIGHTS

- The inhibitory effects of 10 flavonoids on pepsin were measured in vitro.
- Binding mechanisms were investigated by spectroscopic and docking methods.
- Pepsin fluorescence was quenched via static quenching with *r* less than 7 nm.
- The interaction of Pepsin with flavonoids occurred in the hydrophobic cavity.
- The common residues lining the flavonoids in the catalytic site were investigated.

GRAPHICAL ABSTRACT

The complex was formed by non-covalent reactions between flavonoids (baicalein here) and pepsin, which resulted in the significant decrease in the fluorescence intensity of pepsin. The molecular docking study shows that flavonoids are located in the hydrophobic cavity of pepsin. Since the binding of flavonoids affected the microenvironment of the pepsin activity site, flavonoids caused the inhibition of pepsin activity.



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ABSTRACT

In the work described on this paper, the inhibitory effect of 10 flavonoids on pepsin and the interactions between them were investigated by a combination of spectroscopic and molecular docking methods. The results indicated that all flavonoids could bind with pepsin to form flavonoid–pepsin complexes. The binding parameters obtained from the data at different temperatures revealed that flavonoids could spontaneously interact with pepsin mainly through electrostatic forces and hydrophobic interactions with one binding site. According to synchronous and three-dimensional fluorescence spectra and molecular docking results, all flavonoids bound directly into the enzyme cavity site and the binding influenced the microenvironment and conformation of the pepsin activity site which resulted in the reduced enzyme activity. The present study provides direct evidence at a molecular level to understand the mechanism of digestion caused by flavonoids.

1. Introduction

Pepsin, an enzyme expressed as a prototype of zymogen and pepsinogen and was released by the chief cells in the stomach to degrade food proteins into peptides, was the first animal enzyme

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to be discovered by Theodor Schwann in 1836 [1]. As an important digestive protease in the stomach, pepsin is responsible for the most of the digestive activities [2]. When the food enters the stomach, pepsin will not only digest the protein of the food, but also interact with ingredients of the food at the same time, and then its activity may be affected by these compounds. And what is worse, some adverse effects, such as hiccup singultation, nausea and vomiting, will be caused in this process. Therefore, in order to evaluate the toxicity and binding mechanism of these small molecules that enter the stomach through food and drug, recently several reports were investigated on the interactions between pepsin and some molecules [3–7].

Flavonoids are the important phytonutrient components that occur in edible plants, vegetables, fruits and plant-originated foodstuffs [8]. Therefore, flavonoids may be a class of natural compounds that people take daily through the consumption of plant food. Generally, the trace mount of flavonoids in food do not cause obviously indigestive symptoms. However, due to exhibiting broad pharmaceutical activities, several flavonoids were extracted from plants as the main component of drugs in clinic, such as Lpriflavone Tablets and Silybin Capsules. According to published results and the data on file with the manufacture of these drugs, some adverse effects on digestion would occur in some patients even if they took the normal dosage [9]. The reason for this might be due to indigestion caused by flavonoids. Therefore, in order to improve the safety of drug usage in clinical, it is very significant to investigate the inhibitory effect of flavonoids on pepsin and learn about the knowledge that whether the drug could interact with the pepsin, what the mechanism of this action was in this process. Recently, several public and scientific interests have been focused on the interactions of flavonoids with some proteins, such as lysozyme [10], human serum albumin [11], bovine serum albumin [12] and tyrosinase [13]. However, to the best of our knowledge, little concern was placed on the inhibition of flavonoids on the activity of pepsin and the bindings of them to pepsin.

In order to reveal the reason of indigestion caused by flavonoid, in the present study the inhibitory effect of 10 flavonoids (including baicalein, apigenin, luteolin, keampferol, quercetin, morin, liquiritigenin, naringenin, daidzein and genistein, structures shown in Fig. 1) on pepsin was investigated in vitro. Moreover, to further reveal the mechanism of digestion caused by these flavonoids, the interactions between 10 flavonoids and pepsin were studied by multiple spectroscopic techniques and molecular modeling in this study. This study provides basic data for clarifying the binding mechanism of flavonoids with pepsin and is help for understanding the symptoms of indigestion after oral administration of some flavonoid-contained drugs.

2. Experimental

2.1. Reagents

The pepsin was obtained from Sigma–Aldrich Chemical Co. (USA) and was used without further purification. Flavonoids were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (China) and dissolved in methanol to form a 1.0×10^{-3} mol L^{-1} solution, which was used to determine the binding sites of flavonoids on pepsin. 0.2 mol L^{-1} of Citric acid–sodium citrate buffer solutions containing 0.1 mol L^{-1} NaCI were prepared to adjust the acidity of the system pH 2.0, which is the most common pH for pepsin digests. Water was purified with a Milli-Q purification system (USA). All the chemicals were of analytical-reagent grade and used without further purification.

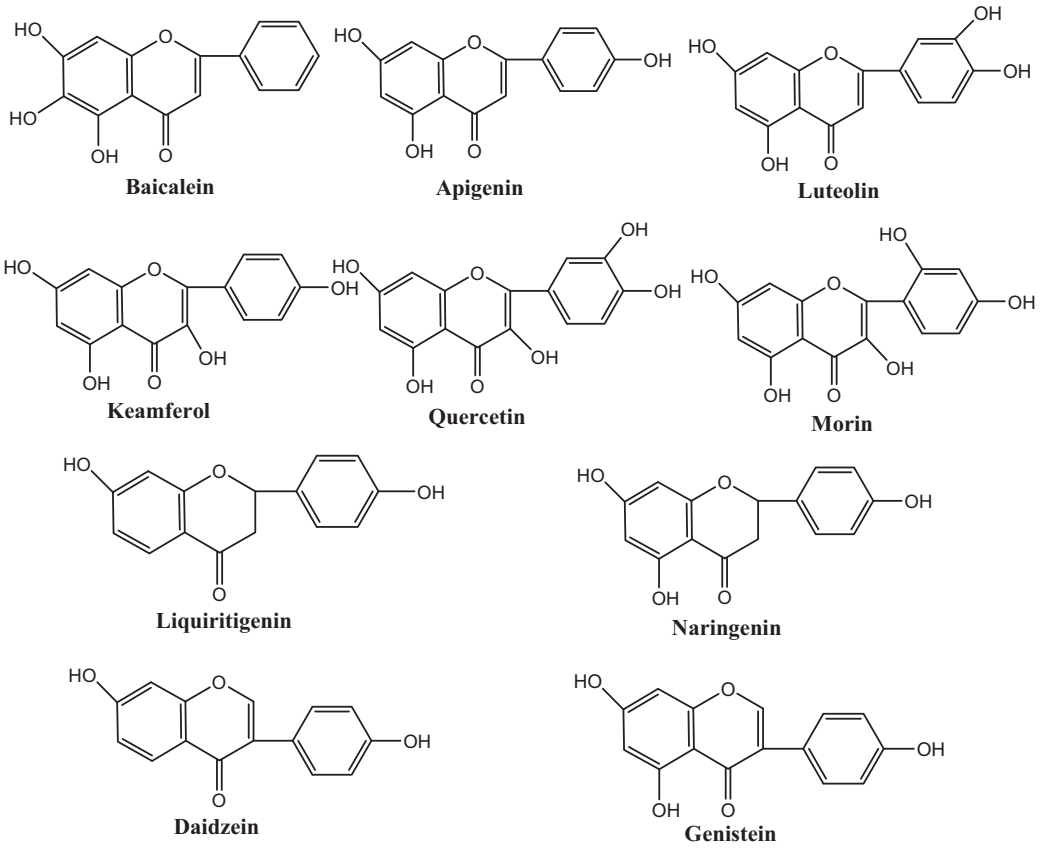

Fig. 1. The molecular structures of the tested flavonoids.