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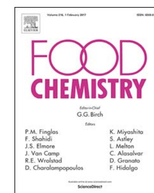

Characterization of binding interactions between selected phenylpropanoid glycosides and trypsin

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A B S T R A C T

Phenylpropanoid glycosides (PPGs) are important bioactive polyphenolic compounds that are widely distributed in plants. In this paper, the inhibitory effects of four selected PPGs against trypsin were investigated. The interactions between these PPGs and trypsin were further investigated by multiple spectroscopic methods and molecular docking studies. The results showed that the binding of each of these PPGs to trypsin induced changes in the natural conformation of trypsin, which inhibited the enzyme in the following order: acteoside > syringalide A 3′-α-L-rhamnopyranoside > lipedoside A-I > osmanthuside B. The binding constant (K_a) values followed the same trend. The hydrogen bond force played an important role in the interaction between each PPG and trypsin. Interestingly, the binding affinity and inhibitory effect increased as the number of phenolic hydroxyl groups increased. In addition, the effect of the phenolic hydroxyl group on the A ring had a greater effect than one on the B ring.

1. Introduction

Trypsin, a type of serine protease, plays an important role in digestion. The inhibition of this digestive enzyme may reduce the absorption of nutrients. On the other hand, reducing the activity of this type of enzyme may be considered for the treatment of diseases such as pancreatitis, rheumatoid arthritis, cystic fibrosis, asthma, platelet aggregation disorders, and pulmonary emphysema (Brandl et al., 2016; Leung, Abbenante, & Fairlie, 2000).

Polyphenols are secondary metabolites in seeds, fruits, and vegetables and are the most abundant antioxidants in the human diet (Cohen & Kennedy, 2010). Polyphenols have been investigated intensively due to their potential health-beneficial effects on humans (Mushtaq & Wanim, 2013). However, polyphenols can also exhibit a negative role by blocking nutrient absorption, which is caused by their interactions with digestive enzymes (Ozdal, Capanoglu, & Altay, 2013). To optimize the biological utilization of polyphenols for human nutrition, knowledge of the interaction between polyphenols and trypsin is desirable.

Phenylpropanoid glycosides (PPGs) are important bioactive polyphenolic compounds that are distributed in a variety of plants as well as foods of plant origin (Pan, Yuan, Lin, Jia, & Zheng, 2004) and are associated with a wide range of biological activities (Chen et al., 2002; Díaz et al., 2004; Wang, Li, Ma, Zhang, & Jia, 2013; Zhang et al., 2008). A previous investigation has shown that PPGs from *Ligustrum robustum* inhibit trypsin activity due to their binding to this enzyme (Wu, Wang, et al., 2013). However, the detailed binding interactions and structure–activity relationships between PPGs and trypsin are not clear.

The different structures of polyphenols significantly affect their binding process with digestive enzymes (Ozdal et al., 2013). In this study, we selected four PPGs (acteoside, syringalide A 3′-α-L-rhamnopyranoside, lipedoside A-I, and osmanthuside B) with the same carbon skeleton and different numbers and positions of the phenolic hydroxyl groups to evaluate their inhibitory activity against trypsin. We used CD, FTIR, and fluorescence spectroscopy as well as molecular docking studies to help characterize the nature of binding between the PPGs and trypsin. Furthermore, structure–activity relationship analyses were conducted to elucidate the binding interactions between these four different PPGs and trypsin. This study may provide useful information for further revealing the mechanisms behind the interactions between PPGs and trypsin.

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2. Materials and methods

2.1. Materials

Bovine trypsin (2500 U/mg), casein, and *N*-α-benzoyl-l-arginine 4-nitroanilide hydrochloride (BAPNA) were from Sigma-Aldrich Co. (St. Louis, MO, USA). Trypsin solution (1.0×10^{-4} M) was prepared in 0.05 M phosphate-buffered saline (PBS, pH 7.5). All other chemicals were of analytical grade.

2.2. Extraction and determination of PPGs

The PPGs were extracted as described (He et al., 2003). In brief, dry leaves (1.2 kg) of *L. purpurascens* were extracted with 10 L hot EtOH 2 times under reflux; the extract was concentrated in a rotary evaporator to produce a residue of 236 g. The EtOH extract was loaded onto a silica column and eluted with varying rations of $CHCl_3$-MeOH solvent, producing fractions 1 to 20. Fraction 3 was subjected to a silica H column with $CHCl_3$-MeOH (9:1, v/v) as an eluent, leading to purification of acteoside (90.1 mg) and lipedoside A-I (112.3 mg). Fraction 8 was loaded onto a silica H column eluted with $CHCl_3$-MeOH (8:1.5, v/v), followed by purification twice to yield syringaride A 3′-α-L-rhamnopyranoside (89 mg) and osmanthuside B (125 mg). Each purified glycoside was subjected to verification of their chemical structures on the basis of the spectral data. HPLC analysis of the PPGs involved the Shimadzu LC-20AD HPLC system (Shimadzu, Japan) equipped with an SIL-20A auto-injector and an Inertsil ODS-3 column (250 × 4.6 mm ID, 5 mm) fitted with a C18 guard column. The SPD-M20A diode array detector was set to 227 nm. The purities of all the PPGs were > 98%.

2.3. Determination of trypsin activity

Trypsin activity was determined at 27 °C with BAPNA as the substrate, as described previously (Zeng, Wang, Yang, You, & Qu, 2016). In brief, trypsin (100 µL, 1.0×10^{-5} M) was incubated with various amounts of each PPG for 30 min at 27 °C before the reaction. Then, BAPNA (200 µL, 20 mM) and PBS (300 µL, 0.05 M, pH 7.5, containing 20 mM $CaCl_2$) were added to the trypsin–PPG mixture, and the volume was adjusted to 1.0 mL with 0.05 M PBS (pH 7.5). The trypsin activity was measured by monitoring the absorbance (Abs) of 4-nitroaniline (the hydrolysis product of BAPNA) at 410 nm every 20 s for 5 min. All the measurements were performed in three replications. To calculate the inhibition (%) of trypsin by each PPG, the following formula was used:

$$\text{Inhibition\%} = (\text{Abs control} - \text{Abs sample})/\text{Abs control} \times 100$$

The IC_{50} value was defined as the concentration of PPG that inhibits 50% of the trypsin activity.

2.4. Determination of CD

CD spectra of trypsin and its PPG complexes were recorded with a Jasco-810 spectrophotometer (JASCO, Tokyo, Japan) according to our previous method (Wu, He, et al., 2013;Wu, Wang, et al., 2013), using cells with a 1.0-mm path length at 37 °C under a nitrogen atmosphere. The trypsin concentration was keep at 1.0×10^{-7} M, while varying each PPG concentration (0.0, 0.5, 1.0, and 2.0×10^{-7} M). The spectra were measured with a scan speed of 50 nm/min from 250 to 190 nm, there times for each spectrum. All the sample spectra were subtracted for PBS (pH 7.5) buffer signal. The CD spectroscopic data were uploaded to DICHROWEB (an online server for protein secondary structure analysis from CD spectroscopic data, http://dichroweb.cryst.bbk.ac.uk) to calculate the secondary structure (Whitmore, 2004).

2.5. Determination of FTIR spectroscopic measurements

The samples for FTIR were prepared according to the method determined by previous reports (Prigent et al., 2003). Samples of 0.1% (w/v) trypsin were incubated with different PPGs (acteoside, lipedoside A-I, syringalide A 3′-α-L-rhamnopyranoside, and osmanthuside B), respectively, at a 50:1 (mol/mol) ratio of PPG to trypsin for 2 h. A control sample without PPG was also incubated under the same conditions. After incubation, free PPG was removed using Slide-A-Lyzer mini dialysis units (with a molecular weight cutoff of 10 kDa, Pierce Chemical Co., Dallas, TX, USA) against distilled water at 4 °C overnight. After dialysis and lyophilization, the PPG–trypsin complex samples were obtained.

All infrared spectra were recorded on a FTIR-8300PCS spectrometer (SHIMADZU Co., Kyoto, Japan) equipped with a KBr beam splitter. Interferograms were accumulated over the spectral range of 1000–4000 cm^{-1}, with a resolution of 5 cm^{-1} and 50 scans at room temperature. KBr spectra were obtained under the same conditions and were subtracted from the spectra of the samples.

Analysis of the secondary structure of trypsin and its PPG complexes was carried out according to a procedure reported previously (Susi & Byler, 1986; Susi, 1986). Baseline correction was carried out to obtain the amide I band located at 1700–1600 cm^{-1}. By performing Fourier self-deconvolution, the width of the component bands, position, and number were estimated. Five major peaks for trypsin and its PPG complexes were resolved. The above spectral region was deconvoluted by a multiple Gaussian curve-fitting process, the peaks corresponding to an α-helix (1650–1658 cm^{-1}), β-sheet (1610–1640 cm^{-1}), β-turn (1660–1700 cm^{-1}), and random coil (1640–1650 cm^{-1}) were adjusted, and the area was measured. The areas under the Gaussian curve assigned to a given conformation were summed up and divided by the total area to calculate the relative percentage of each secondary structure.

2.6. Determination of fluorescence spectroscopy

Fluorescence spectra were measured in a temperature-controlled environment (37 °C) on a Hitachi-850 spectrofluorometer (Hitachi Co., Tokyo, Japan) equipped with 1.0-cm quartz cells. Fluorescence emission spectra were recorded at an excitation of 280 nm over a wavelength range of 300–450 nm, and the excitation and emission bandwidths were 5 nm. A fixed volume (3.0 mL) of 1.0×10^{-6} M trypsin was titrated by successively adding each different PPG (acteoside, lipedoside A-I, syringalide A 3′-α-L-rhamnopyranoside, and osmanthuside B) solution (1×10^{-3} M). All samples were analyzed in triplicate. According to previous reports (Kanakis et al., 2011; Wu, He, et al., 2013; Wu, Raje, et al., 2013), the intensity at 340 nm was used to calculate the number of binding sites per enzyme (n) and the binding constant (K_a).

2.7. Molecular docking studies

For the molecular docking studies, the three-dimensional (3D) structures of acteoside, lipedoside A-I, syringalide A 3′-α-L-rhamnopyranoside, and osmanthuside B were created by use of Chem3D Ultra 6.0; the bovine trypsin crystal structure was downloaded from the Brookhaven Protein Data Bank (accession No. 2ZQ1). The molecular docking study was performed using the Autodock 4.2 software package (Morris et al., 2009). In the initial stage of docking, the polar hydrogen and Gasteiger charges were added, and all of the water molecules were removed. To recognize the binding sites, the grid size was set to 70 Å × 70 Å × 70 Å. The binding site was determined by the FlexX program. In order to obtain the possible binding configurations, the PPG was allowed to move within the whole region of trypsin via 100 runs during the modeling process. The conformer with the lowest binding free energy was chosen for further analysis. The output from

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