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Mechanism of cinnamic acid-induced trypsin inhibition: A multi-technique approach

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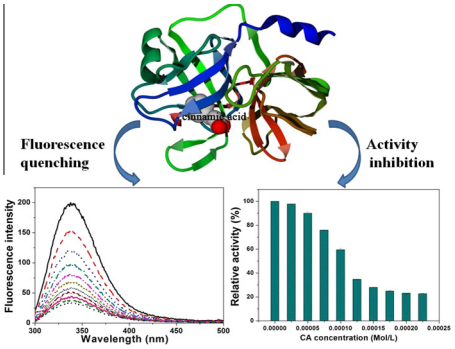
HIGHLIGHTS

- Binding interactions of trypsin with cinnamic acid were investigated.
- Noncovalent interactions stabilize the trypsin–cinnamic acid complex.
- Cinnamic acid induces the inhibition of trypsin activity.

GRAPHICAL ABSTRACT

The fluorescence intensity of trypsin decreased obviously and trypsin molecule could bind with cinnamic acid by noncovalent interactions. Cinnamic acid can bind into the primary substrate-binding pocket and leads to the enzyme inhibition. These results constitute sound work for establishing a new strategy to probe the inhibition of digestive enzymes induced by other organic acids.



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ABSTRACT

In order to investigate the association of the protease trypsin with cinnamic acid, the interaction was characterized by using fluorescence, UV–vis absorption spectroscopy, molecular modeling and an enzymatic inhibition assay. The binding process may be outlined as follows: cinnamic acid can interact with trypsin with one binding site to form cinnamic acid–trypsin complex, resulting in inhibition of trypsin activity; the spectroscopic data show that the interaction is a spontaneous process with the estimated enthalpy and entropy changes being $-8.95\ kJ\ mol^{-1}$ and $50.70\ J\ mol^{-1}\ K^{-1}$, respectively. Noncovalent interactions make the main contribution to stabilize the trypsin–cinnamic acid complex; cinnamic acid can enter into the primary substrate-binding pocket and alter the environment around Trp and Tyr residues.

Introduction

Knowledge of the interactions between proteinase inhibitors and proteinases play an important role in understanding fields such as medicine, toxicology, chemistry, biology, food science, and agriculture [1]. Trypsin (EC 3.4.21.4, Fig. 1A) is the most abundant proteases in nature and plays essential roles in many physiological processes such as hemostasis, apoptosis, signal transduction, reproduction, and immune response [2]. Therefore, studies on the interactions between small molecules and trypsin have an important meaning on realizing the inhibition mechanisms of the small molecules. Several reports were published in order to analyze the interactions of trypsin with some proteinase

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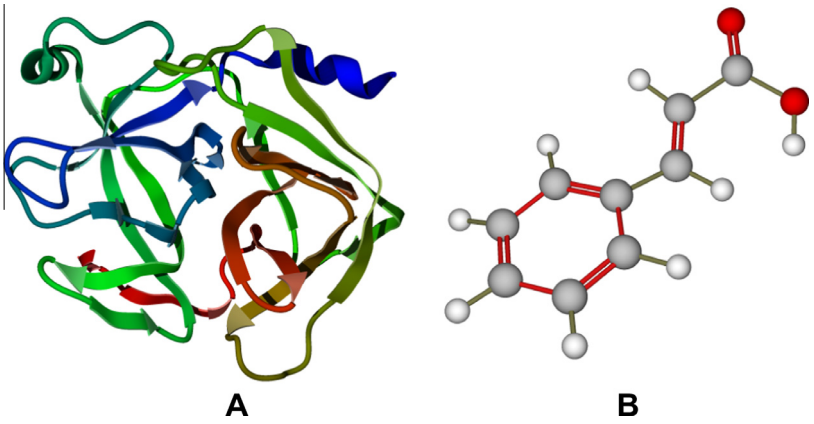

Fig. 1. The molecular structure of trypsin (A) and CA (B).

inhibitors, such as oxytetracycline [3], tannins [4], procyanidins [1], flavonoids [5], and other small molecules [6,7]. Many proteinase inhibitors often enter the human diet mainly through fruits and vegetable-based beverages and affect the activity of trypsin in vivo.

Cinnamic acid (CA, Fig. 1B) is a naturally occurring organic acid that is found in some fruits and a number of spices. CA has shown a variety of pharmacologic properties including hepatoprotective, anti-oxidant, and anti-diabetic activities [8]. In order to understand the modes of CA action, human serum albumin [9], bovine serum albumin [10] and lysozyme [11] etc. have been used as possible biological targets. However, the mechanism of CA-induced trypsin inhibition and the binding mode of CA with trypsin at the molecular level have seldom been reported. In this work, the effect of CA on the activity and conformation of trypsin and the binding interaction mechanism of CA with trypsin were studied by UV–vis absorption, fluorescence spectroscopy, and molecular modeling methods. The data analysis of the intrinsic fluorescence of trypsin in the presence of CA allows evaluating the binding constant, the number of binding sites, and thermodynamic parameters between CA and trypsin.

Material and methods

Reagents

Trypsin (from porcine pancreas), CA and *N*-benzoyl-D,L-arginine-*p*-nitroanilide (BApNA) were all obtained from Sigma Aldrich and stored at −20 °C. The Tris, NaCl, HCl, etc. were all of analytical purity. The trypsin solution (3.0×10^{-5} mol L^{-1}) was prepared in pH 7.40 Tris–HCl buffer. CA solution (2.5×10^{-2} mol L^{-1}) was prepared in Tris–HCl buffer (pH 7.40, 0.05 mol L^{-1} Tris, 0.1 mol L^{-1} NaCl)/methanol 20%. BApNA was dissolved in dimethylsulfoxide to prepare a stock solution (3.0×10^{-3} mol L^{-1}) that was stored at 0–4 °C. Ultrapure water was used throughout.

Apparatus and methods

UV–vis absorbance measurements

The UV–vis absorbance spectra of trypsin, CA, trypsin–CA system were recorded at 294 K equipped on a SPECORD S600 (Jena, Germany) with 1.0 cm quartz cells. A fixed concentration of trypsin (5.0×10^{-6} mol L^{-1}, 2.5 mL) in absence and presence of CA (1.0×10^{-5} mol L^{-1}) was added to the sample cell. Because trypsin can catalyze BApNA into p-nitroanilide, detectable by spectrophotometer at 410 nm, the activity of trypsin was measured by using BApNA as the substrate according to Ref. [4].

Fluorescence measurements

A 2.5 mL solution, 1.0×10^{-5} mol L^{-1} trypsin, was titrated by successively adding CA solution (5.0×10^{-3} mol L^{-1}) using trace syringe to give a final volume of 50 μL. The overall dilution induced by CA solution could be ignored. The CA–trypsin solution was kept in dark place before measurement. The fluorescence spectra were then measured (excitation at 280 nm and emission wavelengths of 300–500 nm) at three temperatures (294 K, 302 K, 310 K) on LS-50B spectrofluorimeter (Perkin–Elmer USA). The fluorescence spectra of Tris–HCl buffer was measured (excitation at 280 nm and emission wavelengths of 300–500 nm) to eliminate background influences on the trypsin fluorescence quenching values. In fluorescence quenching experiments, tryptophan fluorescence from trypsin has also been corrected for inner filter effect according to Ref. [12]. Synchronous fluorescence spectra of trypsin in the absence and presence of CA were recorded at 15 nm and 60 nm, respectively. For three-dimensional fluorescence spectra, the emission wavelengths range was selected from 290 to 500 nm, the initial excitation wavelength was set to 200 nm with increment of 10 nm, and the scanning number was 15 with other parameters just the same as that of the fluorescence quenching spectra.

Molecular modeling study

Molecular modeling docking calculations were carried out by using of Autodock 4.2.3. The three-dimensional structure of CA was optimized at DFT/B3LYP/6–311G by Gaussian 03 [13]. The

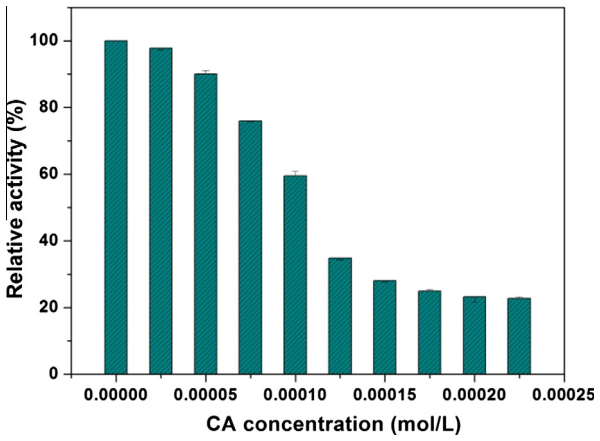

Fig. 2. Trypsin activity in the absence and presence of CA at different concentrations (pH = 7.4, T = 310 K), c (trypsin) = 5.0×10^{-6} mol L^{-1}.