Journal of Molecular Structure 1107 (2016) 91–98

Contents lists available at ScienceDirect

Journal of Molecular Structure

journal homepage: http://www.elsevier.com/locate/molstruc

Mechanism of curcumin-induced trypsin inhibition: Computational and experimental studies

Yan-Qing Wang [a, *], Hong-Mei Zhang [a], Yi-Jun Kang [b], Yun-Lan Gu [a], Jian Cao [a]

[a] Institute of Applied Chemistry and Environmental Engineering, Yancheng Teachers University, Yancheng, Jiangsu Province 224002, People's Republic of China

[b] College of Marine and Bio-engineering, Yancheng Teachers University, Yancheng, Jiangsu Province 224002, People's Republic of China

ARTICLE INFO

Article history:
Received 15 August 2015
Received in revised form
5 November 2015
Accepted 11 November 2015
Available online 29 November 2015

Keywords:
Curcumin
Trypsin
Fluorescence quenching
Molecular docking
Activity

ABSTRACT

In the present study, the experimental and theoretical methods were used to analyze the binding interaction of food dye, curcumin with trypsin. The results of fluorescence spectroscopic measurements indicated that curcumin binding resulted in the obviously intrinsic fluorescence quenching with the increase concentration of curcumin. This binding interaction is a spontaneous process with the estimated enthalpy and entropy changes being −15.70 kJ mol^{-1} and 40.25 J mol^{-1} K^{-1}, respectively. Hydrogen bonds and hydrophobic forces played an important role in the complex formation between curcumin and trypsin. Moreover, curcumin could enter into the primary substrate-binding pocket and makes the activity of trypsin decrease remarkably with the increasing concentration of curcumin.

1. Introduction

Curcumin (shown in Fig. 1, A), a major polyphenolic compound in the dried roots of turmeric (*Curcumin longa* L.), is one of the most widely used natural colorants in various branches of industry [1,2]. Many biological and pharmacological activities of curcumin have been reported. For example, curcumin possesses anti-inflammatory, antioxidant, antiproliferative and antiangiogenic activities, which make it a potential compound for treatment and prevention of a wide variety of human diseases [3]. Because of its efficacy and safety, curcumin is widely applied in various food products and its related food products have dramatically increased in the market [1]. When curcumin enters in the body, it has the ability to interact with proteins. Many recent works have been carried out and been focused on the associations of curcumin-human serum and bovine serum albumins to realize the importance of understanding the binding interaction of curcumin with proteins because serum albumin is the most abundant transport protein in circulatory system in vivo [4–8]. M. Macia zek-Jurczyk et al. have studied the binding of curcumin to human serum albumin. The result showed that the binding of curcumin to protein was accompanied by an alteration of the hydrophobicity of the microenvironment [4]. The data from Jean-Sébastien et al. showed that the human serum albumin conformation was altered by curcumin with reduction of α-helix and increase of random coil and turn structures suggesting a partial protein unfolding [5]. According to the paper of Liu et al., curcumin mainly binding on site I (Subdomain IIA) of the protein [6]. However, little is known about the binding interactions of curcumin with digestive proteases in human digestive system. When curcumin enters into digestive system from diet, digestive proteases may be the indirect binding targets.

As one of digestive proteases, trypsin (EC 3.4.21.4, shown in Fig. 1(B)) occurs in the mammalian intestine and belongs to the group of the serine proteases [9]. In trypsin, His-57, Asp-102, and Ser-195 are the mainly catalytic residues [9]. During the process of digestion, trypsin is one of the enzymatic proteins that can selectively degrade peptide at the carboxylic side of amino acids such as lysine and arginine, leading to the break down dietary proteins into their components [10,11]. Therefore, the structural changes and activity inhibition of trypsin induced by compounds in diet may not only contribute to a reduced digestion of protein and other nutrients, but also is considered relevant in medicinal chemistry for the treatment of diseases [9]. In addition, the binding interactions of small molecules that are found in food with trypsin have been studied in the past [12–16]. However, little is known about the

* Corresponding author.
E-mail address: wyqing76@126.com (Y.-Q. Wang).

http://dx.doi.org/10.1016/j.molstruc.2015.11.026
0022-2860/

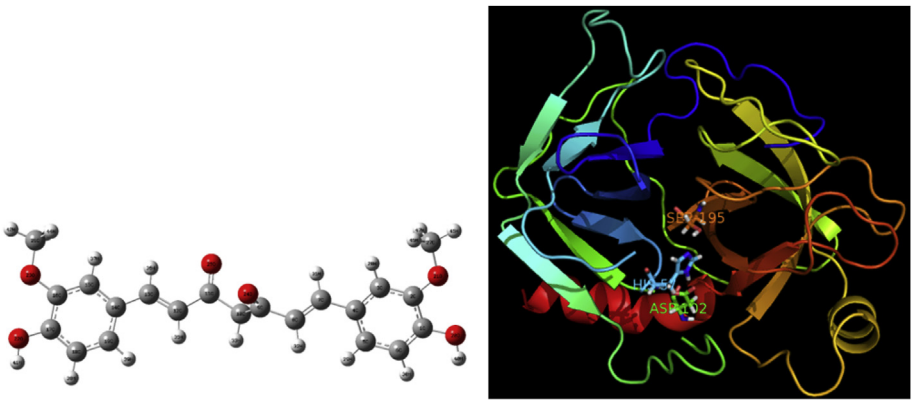

Fig. 1. Structures of curcumin (A) and trypsin (B).

effects of curcumin on the activity and conformation structure of trypsin.

Herein, the binding interaction of trypsin with curcumin was studied using experimental approach and molecular modeling. Effects of curcumin on the conformational structure and activity of trypsin were also studied to obtain the nature of their binding mechanism.

2. Materials and method

2.1. Materials

Analytical standard of curcumin (≥98%) was obtained from Aladdin Industrial Corporation (Kaplan Ave, City of Industry). Trypsin (from porcine pancreas) and *N*-benzoyl- D, L-arginine-*p*-nitroanilide (BApNA) were purchased from Sigma–Aldrich Company (St. Louis, MO) and were used without further purification. The other reagents were all of analytical purity. Trypsin solution was prepared in a phosphate buffer (0.01 M) of pH 7.40. BApNA (3.0 mM) was dissolved in DMSO and was stored at 0–4 °C. Water was purified with a Millli-Q purification system (Barnstead, Dubuque, IA. USA).

2.2. Methods

2.2.1. UV–vis spectroscopy

The UV–vis spectra of trypsin in the absence and presence of curcumin were measured on a SPECORD S600 (Germany, Jena) equipped with 1.0 cm quartz cells. During the experiment of enzyme activity measurement, *N*-benzoyl- D, L-arginine-*p*-nitroanilide (BApNA) was used as substrate, which is degraded into p-nitroaniline by the presence of trypsin in solution. The enzyme activity was obtained based on the increase in absorbance of p-nitroaniline at 410 nm in Tris–HCl buffer (pH 7.40, 0.05 mol L^{-1} Tris, 0.1 mol L^{-1} NaCl) containing 0.0001 mol L^{-1} in absence and presence of trypsin. The absorbance of curcumin was deducted from trypsin–curcumin system.

2.2.2. Fluorescence measurements

The steady-state fluorescence, synchronous fluorescence, and three-dimension fluorescence spectra of trypsin (2×10^{-5} mol L^{-1}) in the absence and presence of curcumin were measured on a LS–50B Spectrofluorimeter (Waltham, Massachusetts, USA) equipped with 1.0 cm quartz cells and a thermostat bath. During the fluorescence experiments, the scan speed and the slit widths of emission and excitation were set at 500 nm/min and 5.0 nm–5.0 nm, respectively. As for steady-state fluorescence spectral experiments, the excitation wavelength was selected 280 nm and the scan range of emission wavelengths were from 300 to 500 nm. During the synchronous fluorescence spectra experiment, the wavelength interval $\Delta\lambda$ ($\lambda_{em} - \lambda_{ex}$) were set at 15 and 60 nm to obtain the fluorescence spectra of Trp and Tyr resisues in trypsin, respectively. In addition, the three-dimension fluorescence spectra of trypsin in the absence and presence of curcumin were measured by the follow parameters: the scan range of emission wavelengths were selected from 290 to 500 nm, the initial excitation wavelength was set to 200 nm with increment of 10 nm, and the scanning number were 15.

2.2.3. Molecular modeling

The three-dimensional structure of trypsin (PDB ID 2ZQ1) was obtained from RCSB Protein Data Bank [17]. The geometry and UV–vis spectra of curcumin were calculated by Gaussian 09 software at DFT B3LYP/6-31++ G (d, p) and TD-DFT B3LYP/6-31++ G (d, p) levels, respectively [18]. The molecular docking program AutoDock 4.2.3 and AutoDock tools v 1.5.4 were used for molecular docking studies on the binding interaction of trypsin with curcumin [19]. During molecular calculation studies, essential hydrogen atoms, Kollman united atom type charges, and salvation parameters were added. The grid maps of trypsin with curcumin system were set 126 × 126 × 126 Å, with grid spacing of 0.375 Å by using AutoDock tool. The GA population size, the maximum number of energy evaluation, and the number of GA runs were set at 150, 2,500,000, and 100, respectively. And other AutoDock parameters were set to default. In addition, Molegro Molecular Viewer software (Molegro-a CLC bio company, Aarhus, Denmark) was used to generate the final docking figures [20].

3. Results and discussion

3.1. Changes in the fluorescence spectroscopic properties of trypsin in the presence of curcumin

Trypsin is a multi-Trp protein and possesses four Trp residues at locations 34, 121, 193 and 215 [21]. These Trp residues can be used as intrinsic fluorophores. The effect of curcumin on the trypsin fluorescence spectra can provide information about the molecular binding interaction between curcumin and trypsin. Fig. 2(A) shows