Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 115 (2013) 1–11

Contents lists available at SciVerse ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

journal homepage: www.elsevier.com/locate/saa

Comparative study of the binding of Trypsin with bifendate and analogs by spectrofluorimetry

Hua Li, Juanjuan Pu, Yi Wang, Chuang Liu, Jie Yu, Tao Li, Ruiqiang Wang *

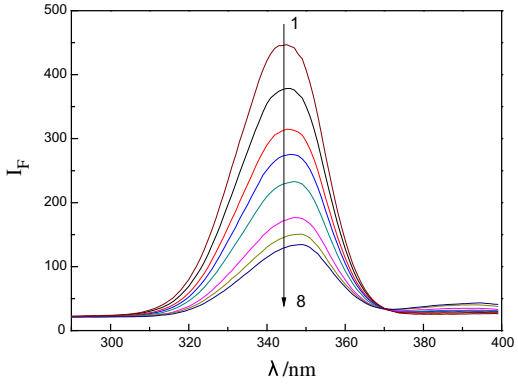
The First Affiliated Hospital of Zhengzhou University, Zhengzhou 450052, China

HIGHLIGHTS

- The interactions between a serious of analogs and Trypsin have been investigated.
- Results reveal that DDB has the strongest affinity for Trypsin among four compounds.
- Hydrophobic interaction plays major role in the binding process.
- The influence of molecular structure on the binding aspects has been investigated.

GRAPHICAL ABSTRACT

The synchronous fluorescence spectra of Trypsin in the absence and presence of DDB.



ARTICLE INFO

Article history:
Received 18 April 2013
Received in revised form 27 May 2013
Accepted 5 June 2013
Available online 19 June 2013

Keywords:
Trypsin
DDB
Analogs
Fluorescence
Interaction

ABSTRACT

The interactions between Trypsin and bifendate (DDB) or analogs (I, II and III) were investigated by fluorescence, UV–visible absorption, resonance light scattering, synchronous fluorescence and 3D spectroscopy under mimic physiological conditions. The results revealed that DDB and analogs caused the fluorescence quenching of Trypsin by the formation of DDB/I/II/III–Trypsin complex. The quenching and energy transfer mechanisms were discussed. The binding constants and thermodynamic parameters at three different temperatures were obtained. The hydrophobic interaction was the predominant intermolecular forces to stabilize the complex. Results showed that DDB was the stronger quencher and bound to Trypsin with higher affinity than other three analogs.

© 2013 Elsevier B.V. All rights reserved.

Introduction

Proteinases–drugs interactions have been extensively studied in recent years [1–3]. Trypsin (EC3.4.21.4), which is detected in the pyloric ceca and intestine of fish, is a medium-sized globular protein [4,5]. Trypsin (Try) is a serin protease of 23,300 Da with an isoelectric point between 11.0 and 11.4 [6]. It is secreted by pancreas, which is converted from the inactive trypsinogen and plays an important role in digestion deconstruction of food proteins and other biological process in vertebrates [7]. Trypsin could cleave the peptide bonds on the carboxyl side of lysine, arginine and ornithine working optimally at pH 7.5–8.5 [8]. Trypsin has four tryptophans (Trp 51, Trp 141, Trp 215, and Trp 237) that can be used as intrinsic fluorophores [9,10].

* Corresponding author. Tel./fax: +86 371 66295193.
E-mail address: wangruiqiang@zzu.edu.cn (R. Wang).

1386-1425/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.saa.2013.06.025

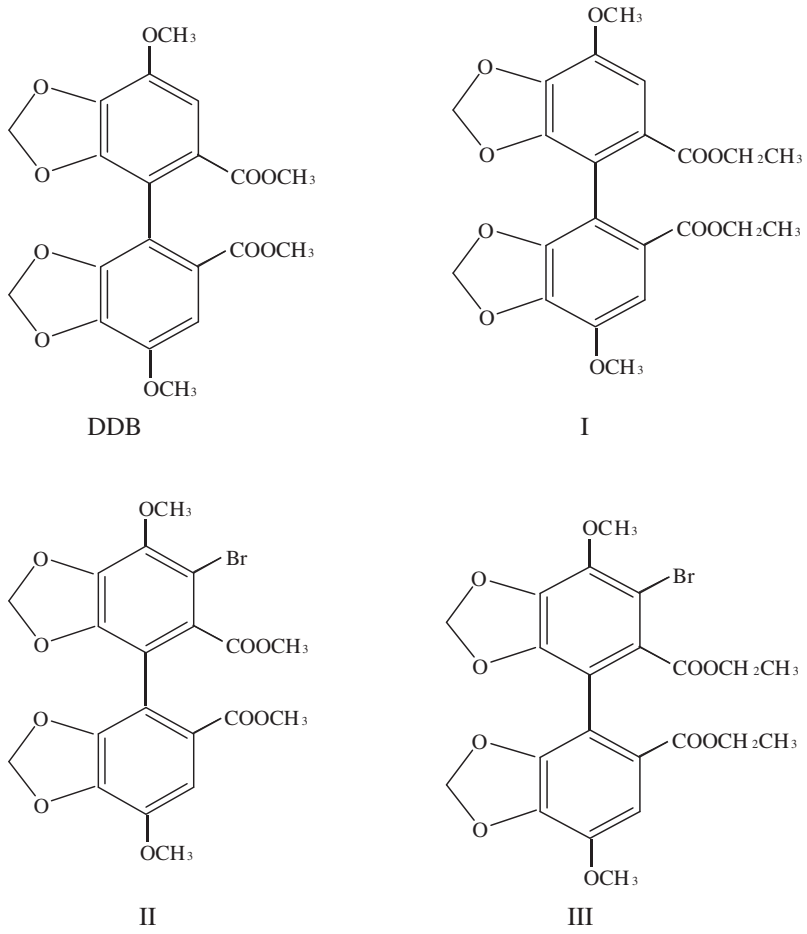


Fig. 1. Molecular structures of DDB, I, II and III.

Bifendate (DDB) is a synthetic intermediate of Schizandrin C, and has still been clinically used for the treatment of hepatitis with minimal observable side effects at the prescribed dosage [11]. It is regarded as a positive control for exploring other hepatoprotective agents [12]. Recently the bioavailability and biological properties of DDB and analogs have raised a great interest [13]. There is a similar main structure between DDB and analogs as shown in Fig. 1. Several reports were published on the interactions of some molecular with Trypsin [10,14]. However, no adequate attention has been paid to the interaction of DDB/I/II/III target to Trypsin by spectroscopy.

This paper is designed to demonstrate the interaction between DDB/I/II/III and Trypsin at three different temperatures under simulating physiological pH conditions using spectroscopic methods including UV–visible absorption, fluorescence, resonance light scattering, synchronous fluorescence and 3D fluorescence spectroscopy. This report provided investigations about the effect of DDB and analogs on the spectral properties of Trypsin, the thermodynamic aspects in the binding process and characterization of the binding sites.

Materials and methods

Apparatus

UV–vis absorption spectra were measured at room temperature with a UV-1800PC spectrophotometer (Mapada, Shanghai, China) equipped with 1.0 cm quartz cell. All fluorescence spectra were recorded on 970CRT fluorescence spectrophotometer (SANCO, Shanghai, China) equipped with 1.0 cm quartz cell. All of pH values were measured with a pHS-3C acidity meter (Leici, Shanghai, China). The widths of the excitation and the emission slits were set to 5.0 nm/5.0 nm for Trypsin, respectively.

Reagents

Trypsin (Aladdin chemistry Co. Ltd.) was directly dissolved in double distilled water to prepare the stock solution (3.0×10^{-4} mol/L). DDB and analogs were synthetized as described in Ref. [15]. Stock solutions of DDB and analogs were dissolving in ethyl alcohol and kept at room temperature. A $Na_2HPO_4{\cdot}H_2O$–NaH_2PO_4 buffer (0.2 mol/L) was used to keep the pH value at about 7.4. All solutions were stored in refrigerator at 4 °C in dark except stock solutions of DDB and analogs. The deionized water was used throughout the whole experiment.

Experimental procedures

Try (0.5 mL, 3.0×10^{-4} mol/L) solution, 2.0 mL $Na_2HPO_4{\cdot}H_2O$–NaH_2PO_4 buffer and 1.0 mL different concentration of DDB/I/II/III were added into a 10 mL volumetric flask. Deionized water was added to dilute the mixture to the scale mark. The fluorescence intensities of all solutions were measured (excitation at 287 nm and emission wavelengths of 300–500 nm) at three temperatures (296 K, 306 K and 316 K). Synchronous fluorescence spectra were recorded with scanning ranges, the *D*-value ($\Delta\lambda$) was set at 15 or 60 nm in the absence and presence of DDB/I/II/III.

Download English Version:

https://daneshyari.com/en/article/7672648

Download Persian Version:

https://daneshyari.com/article/7672648

Daneshyari.com