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Spectroscopic study on the interaction of Trypsin with Bicyclol and analogs

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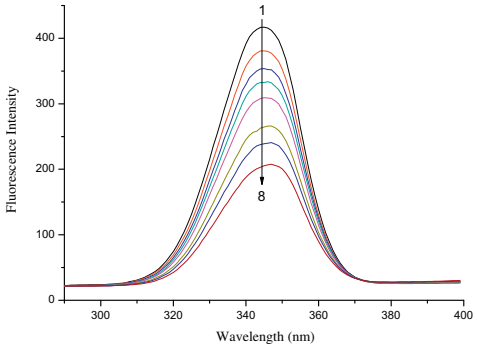
HIGHLIGHTS

- The interactions between Trypsin and Bicyclol or analogs have been investigated.
- Results reveal that Bifendate has the strongest affinity for Trypsin among five compounds.
- Hydrophobic and electrostatic interactions play 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 Bicyclol.



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ABSTRACT

The interactions between Trypsin and Bicyclol or analogs (Bifendate, I, II and III) were investigated by spectrophotometric methods. It was found that Bicyclol or analogs had strong ability to quench the intrinsic fluorescence of Trypsin by a static quenching procedure. The binding constants were obtained at three temperatures. The thermodynamics parameters reveal that the hydrophobic and electrostatic interactions play an important role in the interaction. Results showed that the microenvironments of tryptophan residue of Trypsin were disturbed by the analogs. Results indicated that Bifendate was the strongest quencher among five compounds.

1. Introduction

Trypsin (Try), a class of serine protease [1], is widely existed in pancreas and can catalyze a variety of protein. It is an important protein in digestive system which was activated by enterokinase or Trypsin after secrete to duodenum [2] and also activate other zymogen (chymotrypsin) [3]. Trypsin owns about 223 amino acid residues and each consists of two size close tubbiness structural domains which were connected by six disulfide bonds. Every domain have six antiparallel of β-foldings and between two of domains are His, Asp and Ser which are catalytic activity center of Try, the three of structures above form catalysis triplet [4,5].

Bicyclol and Bifendate are synthetic anti-hepatitis drugs, which have extraordinary hepatoprotective abilities against chemical liver injury induced by tetrachloride, acetaminophen or ethanol [6]. Bifendate is a synthetic hepatoprotective agent derived from Schizandrin C, a component of Fructus. Experimental studies have proved that Bifendate protect the liver cells by reducing the abnormally elevated aspartate amino transferase and alanine amino transferase level [7]. Besides, it has also been reported that

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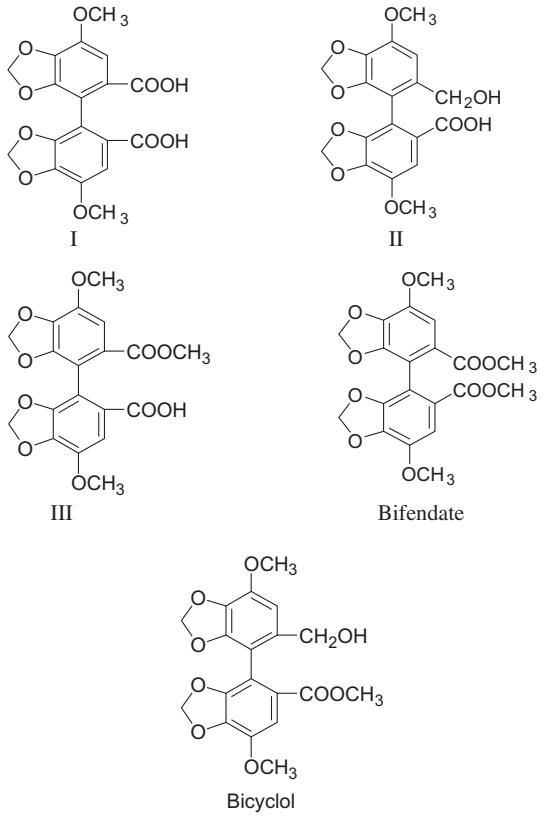

Fig. 1. The molecular structures of Bicyclol and analogs (Bifendate, I, II and III).

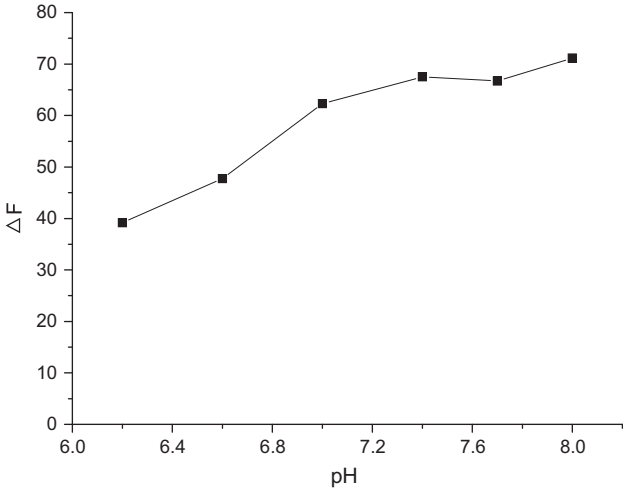

Fig. 2. The ΔF–pH curves at an excitation wavelength of 280 nm in 0.05 mol L^{-1} phosphate butter solution at 298 K, [NaCl] = 0.10 mol L^{-1}, [Try] = 1.5×10^{-5} mol L^{-1}.

Bifendate can be effective in improving the detoxification function of chronic hepatitis patients without noticeable toxicity and side effect [8].

Bicyclol and Bifendate are developed to cure hepatitis with evident liver protection and aminotransferase decrease. In our lab, Bifendate, Bicyclol and the structural analogs (I, II, III) are synthesized to investigate the effect of substituent on the binding affinity [9]. The structures of the five compounds are shown in Fig. 1. Four analogs investigated in this study were different from those in Ref. [5].

In this paper, the effect of Bicyclol or analogs on the conformation and optical properties of Trypsin was investigated by synchronous fluorescence, UV–vis absorption and three-dimensional fluorescence spectra under physiological conditions. The binding constants, binding distance, binging force and quenching mechanism of the interaction were also obtained.

Materials and methods

Reagents

Trypsin (from Aladdin) was directly dissolved in double-distilled water to prepare stock solutions (3.0×10^{-4} mol L^{-1}) and then stored at 0–4 °C. Stock solutions of Bicyclol and analogs (1.0×10^{-3} mol L^{-1}) were dissolving in ethanol. 0.20 mol L^{-1} pH 7.4 Na_2HPO_4–NaH_2PO_4 buffer solution. 0.50 mol L^{-1} NaCl working solution. All other reagents were analytical grade and double-distilled water was used throughout.

Apparatus

Fluorescence spectra were obtained with a 970-CRT Spectrofluorimeter (Sanke, Shanghai, China) equipped with 1.0 cm quartz cells. The absorption spectra were recorded on a UV-1800 spectrophotometer (Meipuda, Shanghai). The pH measurements were made with a pH-3 digital pH-meter (Lei Ci, Shanghai) with a combined glass electrode.

Procedures

In 10 mL colorimetric tubes, 2.0 mL 0.50 mol L^{-1} NaCl solution, 2.5 mL pH 7.4 Na_2HPO_4–NaH_2PO_4 buffer solution (0.20 mol L^{-1}), 0.5 mL 3.0×10^{-4} mol L^{-1} Trypsin, and Bicyclol (or analogs) solution were added and mixed to the mark with double distilled water. An excitation wavelength of 280 nm and 5 nm/5 nm slit widths were chosen in fluorescence measurement. Synchronous fluorescence spectrometry were recorded by setting $\Delta\lambda$ = 15 nm and $\Delta\lambda$ = 60 nm, respectively. The three-dimensional fluorescence spectra were obtained under the following conditions: the photomultiplier tube voltage 700 v, the excitation and emission wavelengths at 200–300 nm, 290–470 nm, and scanning number 15. The UV–vis absorption spectra of Trypsin, Bicyclol (or analogs) and their mixture were obtained at room temperature.

Optimization of experimental conditions

In order to select an ideal experimental system, various experimental parameters were investigated with the concentration of Trypsin being 1.5×10^{-5} mol L^{-1} throughout the experiments.

The effect of pH in the range of 6.20–8.00 was shown in Fig. 2. The result displayed that fluorescence intensity of Trypsin gradually increased with the increase of pH value. So pH 7.4 was chosen for the experiment to simulate physiological conditions. Several buffer solutions (Tris–HCl, NaOH–KH_2PO_4, $Na_2B_4O_7{\cdot}10H_2O$–$C_6H_8O_7$ and Na_2HPO_4–NaH_2PO_4) were investigated at pH 7.4 in the experiment, and the result showed that the sensitivity was highest in Na_2HPO_4–NaH_2PO_4 buffer solution. Moreover, the volume of phosphate buffer solution in the range of 0.5–5.0 mL was tested and it was found that 2.0–3.0 mL buffer solution was optimal. For these reasons, 2.5 mL pH 7.4 Na_2HPO_4–NaH_2PO_4 (0.20 mol L^{-1}) buffer solution was used in our work.

In addition, the concentration effect of NaCl was studied in the range of 0.025–0.25 mol L^{-1}. The result showed that the fluorescence intensity of Trypsin was highest in 0.10 mol L^{-1} NaCl. The effect of ethyl alcohol amount was also investigated. However the