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Comparative study of the binding of trypsin to caffeine and theophylline by spectrofluorimetry

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ABSTRACT

The interactions between trypsin and caffeine/theophylline were investigated by fluorescence spectroscopy, UV–visible absorption spectroscopy, resonance light scattering and synchronous fluorescence spectroscopy under mimic physiological conditions. The results revealed that the fluorescence quenching of trypsin by caffeine and theophylline was the result of the formed complex of caffeine–trypsin and theophylline–trypsin. 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 caffeine was the stronger quencher and bound to trypsin with higher affinity than theophylline.

1. Introduction

In recent years, much attention has been paid to the interaction between small molecules and proteins [1–3]. The water-soluble globular protein, Trypsin (EC 3.4.21.4), which is secreted by pancreas, is converted from the inactive trypsinogen and plays an important role in digestion deconstruction of food proteins and other biological process in vertebrates [4]. Trypsin is a medium-sized globular protein. It has a molecular mass of 23,300 Da and consists of 223 amino acid residues [5]. A trypsin molecule contains two domains of nearly equal size, the major constituent of each domain being a set of six anti-parallel strands of polypeptide chain laced together into a β-sheet unit by a network of H–bonds [6]. Details have been reported earlier [7].

Caffeine (Caf) is widely found in coffee, tea, citrus fruits, olive oil and cola nuts. Theophylline (Tph) is found in tea. There is a similar main structure between Caf and Tph as shown in Fig. 1. Caf has been generally used as a central nervous system, cardiac, and respiratory stimulant. Tph has been widely used as smooth muscle relaxants. However, no adequate attention has been paid to the interaction of Caf/Tph target to trypsin by spectroscopy.

Fluorescence quenching is an important method to study the interaction of substances with protein because it is sensitive and relatively easy to use. Fluorescence spectroscopy is a probe technique sensing changes in the local environment of the fluorophore. Also, various possibilities of structural rearrangements in the environment of the fluorophore may lead to a similar fluorescence signal; they can complicate interpretation of the experimental result and be exploited to obtain unique structural and dynamic information [8,9].

In this paper, the interaction of Caf/Tph on trypsin was evaluated by spectroscopic methods including fluorescence spectroscopy, UV–visible absorption spectroscopy, resonance light scattering (RLS) and synchronous fluorescence spectroscopy. This research provided investigations on the effect of Caf/Tph on the spectral properties of trypsin, the thermodynamic aspects in the binding process, and characterization of the binding sites.

2. Materials and methods

2.1. Apparatus

All fluorescence spectra were recorded on 970CRT fluorescence spectrophotometer (SANCO, Shanghai, China) equipped with 1.0 cm quartz cell. 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 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.

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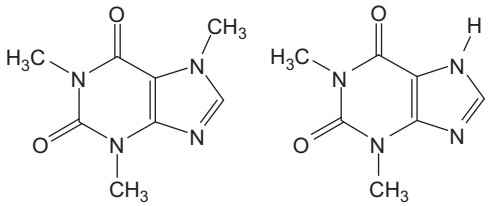

Fig. 1. Molecular structures of caffeine and theophylline.

2.2. Reagents

Caf, Tph, trypsin and BAEE (N_α-benzoyl-L-arginine ethyl ester) were purchased from Aladdin chemistry Co. Ltd. A phosphate buffer (0.2 mol L^{-1}) was used to keep the pH value at about 7.4 of all the solutions, which is to provide physiological conditions to the chemical reactions. The deionized water was used throughout the whole experiment.

Stock solutions of Caf (1.0×10^{-3} mol L^{-1}), Tph (1.0×10^{-3} mol L^{-1}) and trypsin (3.0×10^{-4} mol L^{-1}) were prepared in 50 mL volumetric flasks, respectively. All solutions were stored in refrigerator at 4 °C in dark.

2.3. Experimental procedures and methods

Trypsin (0.5 mL, 3.0×10^{-4} mol L^{-1}) solution, 2 mL phosphate buffer and a certain volume of Caf/Tph 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 300–450 nm) with a 970CRT fluorescence spectrophotometer at three temperatures (296 K, 306 K and 316 K). Synchronous fluorescence spectra were recorded with λ_{ex}=287 nm. The *D*-value ($\Delta\lambda$) was set at 15 nm or 60 nm.

The activity of trypsin was measured by the absorbance changes of substrate, viz. BAEE. BAEE was catalysed to BA (N_α-benzoyl-L-arginine) by trypsin [10]. The absorbance of the reaction system was measured at 253 nm in the absence and presence of drugs, respectively. BAEE (3.0 mL, 1.0×10^{-3} mol L^{-1}), trypsin (0.2 mL, 3.0×10^{-5} mol L^{-1}) and different concentration of drugs were added into 1 cm uartz cuvette and mixed. Then the absorbance was measured at 253 nm.

3. Results and discussion

3.1. UV/vis absorption spectra

UV/vis absorption measurement is a simple method and applicable to explore the structural change and to know the complex formation [11]. The absorption spectral change of trypsin in the presence of drugs was shown in Fig. 2. There are two absorption peaks at 203 nm and 273 nm on the trypsin absorption spectrum, which correspond to peptide bond absorption and the aromatic amino acid absorption, respectively [12]. The absorption peak at 203 nm increased upon the addition of Caf/Tph. The results indicated that Caf/Tph interacted with trypsin.

The sole theoretical absorption spectrum of trypsin in the presence of drug was obtained by subtracting the spectrum of drug from that of trypsin–drug complex. Fig. 2 c and d showed that the spectra of trypsin, trypsin–drug and drug were different. Spectra 1 and 4 should be identical in Fig. 2c and d if no interaction occurred between trypsin and drugs. The UV/vis absorption spectrum of trypsin shows a strong band in the near-UV region with a maximum at 203 nm, which appears due to peptide bond absorption of tryptophan [11]. The peak at 273 nm corresponds to the aromatic amino acid absorption of trypsin. It was found that absorbance at 203 nm decreased

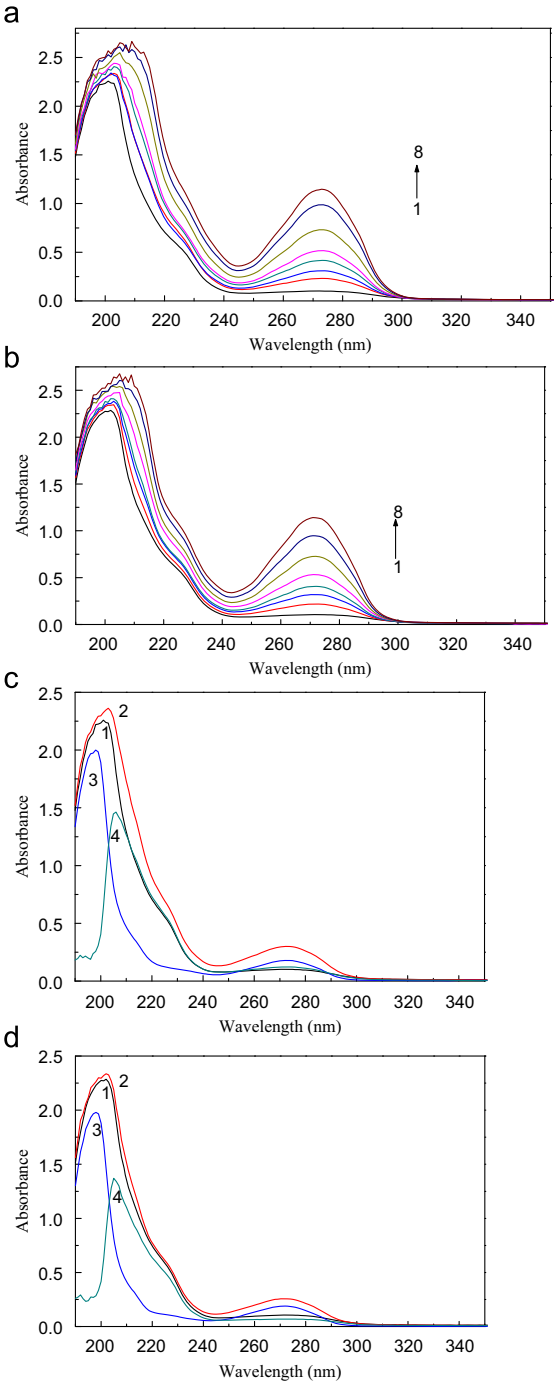

Fig. 2. (a) Influence of Caf on the absorbance spectrum of trypsin. Conditions: pH 7.4, T=291 K. $C_{Trypsin}=1.5\times10^{-5}$ mol L^{-1}, C_{Caf}=0, 1.0, 2.0, 3.0, 4.0, 6.0, 8.0, and 10.0×10^{-5} mol L^{-1}, respectively (From spectra 1 to 8). (b) Influence of Tph on the absorbance spectrum of trypsin. C_{Tph}=0, 1.0, 2.0, 3.0, 4.0, 6.0, 8.0, and 10.0×10^{-5} mol L^{-1}, respectively (From spectra 1 to 8). Other experimental conditions were the same as described in (a). (c) The UV absorption spectra of trypsin–Caf system. (1) The absorption spectrum of trypsin only, 1.5×10^{-5} mol L^{-1} (2) Caf (1.5×10^{-5} mol L^{-1})+trypsin (1.5×10^{-5} mol L^{-1}); (3) Caf, 1.5×10^{-5} mol L^{-1} (4) [Caf (1.5×10^{-5} mol L^{-1})+trypsin (1.5×10^{-5} mol L^{-1})]−[Caf, 1.5×10^{-5} mol L^{-1}]. (d) The UV absorption spectra of trypsin–Tph system. (1) The absorption spectrum of trypsin, 1.5×10^{-5} mol L^{-1}; (2) Tph (1.5×10^{-5} mol L^{-1})+trypsin (1.5×10^{-5} mol L^{-1}); (3) Tph, 1.5×10^{-5} mol L^{-1}; (4) [Tph (1.5×10^{-5} mol L^{-1})+trypsin (1.5×10^{-5} mol L^{-1})]−[Tph].