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Investigation of the interaction between five alkaloids and human hemoglobin by fluorescence spectroscopy and molecular modeling

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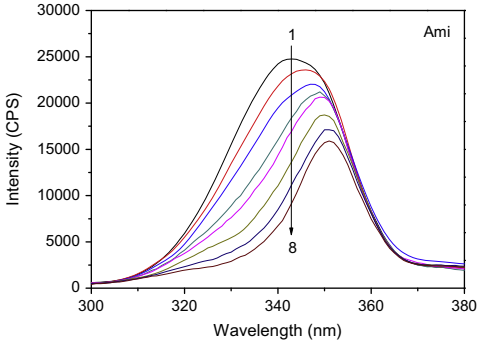
HIGHLIGHTS

- The interactions between HHb and five alkaloids have been investigated.
- Hydrophobic and electrostatic interactions play major role in the binding process.
- The influence of molecular structure on the binding aspects has been investigated.
- Molecular docking was also applied in the binding study.

GRAPHICAL ABSTRACT

The synchronous fluorescence spectra of HHb in the absence and presence of Ami.



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ABSTRACT

This work studied the interaction of human hemoglobin (HHb) with aminophylline, acefylline, caffeine, theophylline and diprophylline systematically by UV–vis absorption spectroscopy and fluorescence spectroscopy in combination with molecular modeling. Five alkaloids caused the fluorescence quenching of HHb by the formation of alkaloids-HHb complex. The binding constants and thermodynamic parameters were obtained. The hydrophobic and electrostatic interactions were the predominant intermolecular forces to stabilize these complexes. Results of thermodynamic analysis and molecular modeling showed that aminophylline was the strongest quencher and diprophylline was the weakest quencher.

Introduction

Hemoglobin (Hb), the major protein component in erythrocytes, exists as a tetramer of globins chains that is composed of two α and two β subunits [1]. Each subunit has one redox iron heme as its prosthetics group, and the heme is located in the crevices at the exterior of the subunit [2]. Hb is well known for its function in the vascular system of animals, being a carrier of oxygen. It also aids, both directly and indirectly, the transport of carbon dioxide and regulates the pH of blood [3]. Human hemoglobin (HHb) has six Trp and twelve Tyr residues in its tetramer, three Trp and six Tyr residues in each αβ dimmer. The β-37 Trp residue is located at the $\alpha_1\beta_2$ interface, which has been assigned as the primary source of fluorescence emission [4]. The fluorescence intensity and the location of fluorescence peak reflect the microenvironment of the chromophore group of protein [5].

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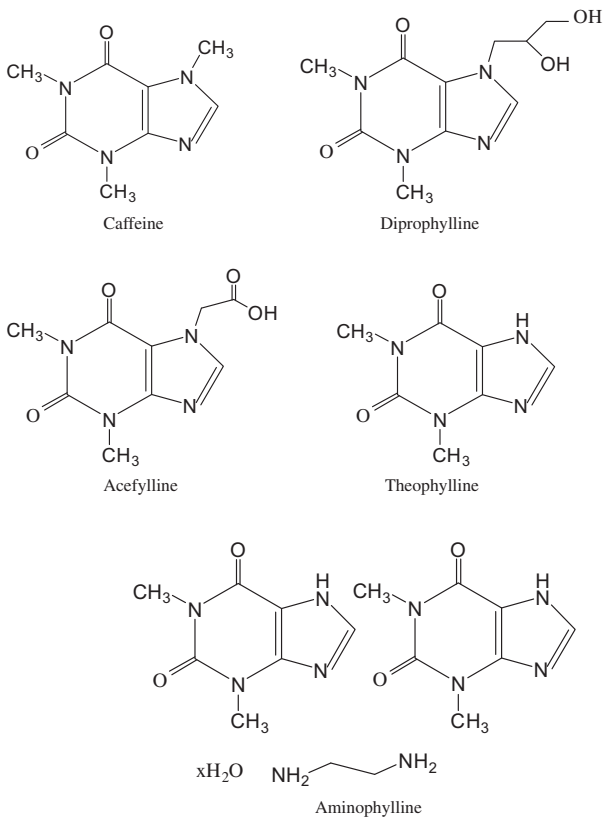

Fig. 1. The molecule structure of five alkaloids.

Alkaloids, natural products of plant origin, are gaining increasing importance for their potential therapeutic utility with high potency and low systemic toxicity [6,7]. Because of their abundance in the human diet and drugs, their bioavailability and biological properties have raised a great interest [8]. The structure of aminophylline (Ami), acefylline (Ace), caffeine (Caf), theophylline (Tph) and diprophylline (Dip) are listed in Fig. 1. A similar main structure is found in these five alkaloids.

Fluorescence quenching is an important method to study the interaction of substances with protein. Here we studied the interaction of HHb with five alkaloids by multi spectroscopy. Results showed that Ami was the strongest quencher and Dip was the weakest quencher.

Materials and methods

Apparatus

The UV–vis spectra were recorded on T6-new century UV–vis Spectrophotometer (Persee, Beijing, China) equipped with 1.0 cm quartz cell. Fluorescence quenching was recorded on 970-CRT spectrofluorimeter (San Ke, Shanghai, China) equipped with 1.0 cm quartz cell. The widths of excitation and emission slits were set to 10.0 nm/10.0 nm respectively. Synchronous and three-dimensional fluorescence spectra were performed on Fluoromax-4 Spectrofluorometer (Horiba Scientific, USA) equipped with 1.0 cm quartz cell, using 5/5 nm slit widths. Increment was 2 nm.

Reagents

HHb (Sigma) was dissolved in ultra pure water to form 2.0×10^{-5} mol L^{-1} solution, then preserved at 4 °C and diluted as required. Five alkaloids (Aladdin chemistry Co. Ltd.) were dissolved in ultra pure water to form 1.0×10^{-3} mol L^{-1} solution and were stored in refrigerator at 4 °C in dark.

Procedures

Containing 0.05 mol L^{-1} Tris–HCl buffer (pH 7.4) and 0.10 mol L^{-1} NaCl, 2.0 mL solution protein (6.5×10^{-6} mol L^{-1}) was titrated by successive additions of 1.0×10^{-3} mol L^{-1} alkaloids (concentration from 0 to 1.0×10^{-4} mol L^{-1}). Titrations were done manually by using micro-injector. The fluorescence spectra were then measured (λ_{ex} 280 nm, λ_{em} 300–530 nm) at two temperatures (304 and 320 K). The synchronous fluorescence spectra were obtained through simultaneous scanning of the excitation and emission monochromators while maintaining a constant wavelength interval between them ($\Delta\lambda$, 15 nm and 60 nm). The three-dimensional fluorescence spectrum was performed under the following conditions: the emission wavelengths at 280–470 nm, the excitation at 220 nm with an increment of 2 nm, excitation and emission slit widths was 5/5 nm respectively.

Molecular modeling study

The AutoDock4.2 [9] program was used to calculate the interaction modes between alkaloids and HHb. Lamarckian genetic algorithm (LGA) implemented in Autodock was applied to calculate the possible conformation of alkaloids that binds to HHb. The crystal structure of HHb was taken from the Brookhaven Protein Data Bank (http://www.rcsb.org/pdb) (entry codes: 2D60 for HHb). The structure of five alkaloids was optimized using Gaussian 09 program [10]. Geometry optimizations were carried out using the hybrid B3LYP [11] functional together with the 6–31 g (d, p) basis set for C, H, O and N atoms. A grid map of $80 \times 70 \times 72$ grid points in size with a grid-points pacing of 0.819 Å was created for the protein. The most favorable docking model was selected for further analysis according to the binding energy and the geometry matching after 250 runs.

Results and discussion

UV–vis absorption spectra

UV–vis absorption spectroscopy technique can be used to explore the structural changes of protein and to investigate protein–ligand complex formation [12]. Fig. 2 displayed the absorption spectra of alkaloid (curve a), HHb (curve b), and the mixture of HHb and alkaloid (curve c). Curve d was obtained by deducting the spectrum of alkaloid from the spectrum of the mixed solution. The spectrum of HHb has three absorption peaks. The strong absorption peak at 210 nm not only reflects the framework conformation of protein but also corresponds to the peptide bond [13]. The weak absorption peak at 278 nm appears due to the aromatic amino acids (Trp, Tyr and Phe) [14]. The peak at 405 nm corresponds to the porphyrin Soret band of Hb [15]. According to the curve b and curve d, it can be discovered that the peaks in the far-UV region of HHb shifted and the absorbance decreased with addition of alkaloid. This result indicated that there exists interaction between alkaloids and HHb.

Fluorescence quenching

Hb emits intrinsic fluorescence mainly due to Trp and Tyr residues [16]. Fluorescence measurements can give the information of the binding of small molecule substances to proteins on the molecular level, such as binding mechanisms, binding modes, binding constants and intermolecular distances. The intrinsic fluorescence