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# Interaction of tetramethylpyrazine with two serum albumins by a hybrid spectroscopic method

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ABSTRACT

The interactions of tetramethylpyrazine (TMPZ) with bovine serum albumin (BSA) and human serum albumin (HSA) have been investigated by various spectroscopic techniques. Fluorescence tests showed that TMPZ could bind to BSA/HSA to form complexes. The binding constants of TMPZ–BSA and TMPZ–HSA complexes were observed to be $1.442 \times 10^4$ and $3.302 \times 10^4\ M^{-1}$ at 298 K, respectively. The thermodynamic parameters ($\Delta G$, $\Delta H$ and $\Delta S$) calculated on the basis of different temperatures revealed that the binding of TMPZ–HSA was mainly depended on hydrophobic interaction, and yet the binding of TMPZ–BSA might involve hydrophobic interaction strongly and electrostatic interaction. The results of synchronous fluorescence, three-dimensional fluorescence, UV–vis absorption, FT-IR and CD spectra showed that the conformations of both BSA and HSA altered with the addition of TMPZ. The binding average distance between TMPZ and BSA/HSA was evaluated according to Föster non-radioactive energy transfer theory. In addition, with the aid of site markers (such as, phenylbutazone, ibuprofen and digitoxin), TMPZ primarily bound to tryptophan residues of BSA/HSA within site I (sub-domain II A).



## 1. Introduction

Tetramethylpyrazine (TMPZ) (Fig. 1), an extract from a Chinese herbal medicine, *Ligusticum wallichi* Franchat (Chuan Xiong in Chinese), has been commonly used in the clinic for the treatment of cardiovascular diseases in China [1], and its synthetic compound is known by the patent name ligustrazine, is now being used in China to treat thrombotic disorders, including myocardial infarction and cerebral infarction [2]. For many years, TMPZ has been widely used in China as one of the traditional Chinese medicines for its preventive effect on oxidative stress, renal toxicity and hepatocellular injury [3]. Tsai et al. [4] reported that TMPZ (0.01–10 mM) reduced the vascular tone of isolated aortic rings raised. Later, Shao et al. [5] proved that TMPZ (0.1–1.0 mM) ameliorated cerebral vasospasm and L-NAME (a NOS inhibitor), and induced a dose-dependent increase of intra-endothelium $Ca^{2+}$. Recent studies [6,7] showed that TMPZ protected C2C12 myotubes and retinal cells, and it might serve as a starting point in the search of novel antioxidants for the treatment of diabetes and associated complications, and it could also be applied to prevent and treat cataracts. Although the biological activities of TMPZ have been investigated intensively, the mechanism of action of TMPZ still remains unknown. However, knowledge of interaction mechanisms between TMPZ and proteins is of crucial importance for us to understand the pharmacodynamics and pharmacokinetics of TMPZ. So the studies of interaction between TMPZ and BSA/HSA are very significant.

Serum albumins (SAs) are major soluble protein constituents of the circulatory system, and constitute 50–60% of total amount of plasma proteins [8]. They play important roles in the transport and deposition of various endogenous and exogenous compounds, such as fatty acids, amino acids, hormones, and drugs [9]. However, in recent years, bovine serum albumin (BSA) and human serum albumin (HSA) have been widely used as models in evaluating the interactions between ligands and proteins owing to their similar folding and well-known primary structure [10].

In the paper, the binding interactions between TMPZ and BSA/HSA have been investigated by various spectroscopic techniques under imitated physiological conditions. The quenching mechanism, nature of the binding force and the effect of TMPZ on the conformation of BSA/HSA were analyzed. The binding constants, the number of binding sites and the energy transfer distance of TMPZ–BSA/HSA complexes were estimated. In addition, the effect of some common ions on the binding constant of TMPZ to BSA/HSA was also discussed. We hope this work can provide useful information for the pharmacology of TMPZ.

## 2. Experimental

### 2.1. Reagents and chemicals

Human serum albumin (HSA, 96% purity) acquired from Sigma chemical company (St. Louis, USA), and bovine serum albumin

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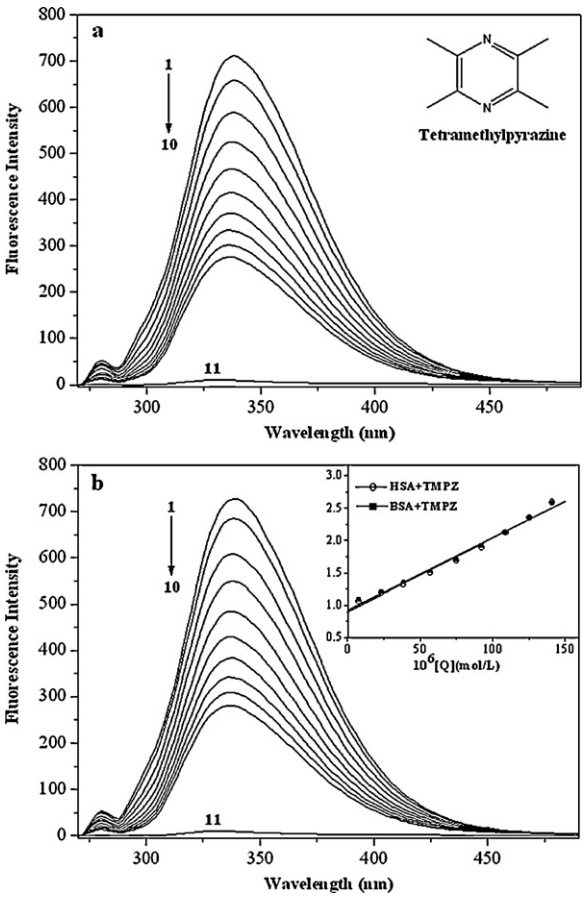


**Fig. 1.** Fluorescence spectra of (a) BSA (5.0 μM) and (b) HSA (5.0 μM) in the presence of various TMPZ concentrations following the excitation wavelength at 280 nm for two serum albumins, (1–10) indicate the emission spectra of proteins in the presence of 0.0, 7.95, 23.53, 38.71, 57.14, 75.00, 92.31, 109.09, 125.37 and 141.18 μM TMPZ. A concentration of 125.37 μM (11) was used for TMPZ only. The inserts correspond to the structure of TMPZ and the Stern–Volmer plots, respectively. $T$ = 298 K.

(BSA, FractionV, 98% purity) purchased from Roche Company, were used without further purification, and dissolved in the Tris–HCl buffer solution of pH 7.4 and then kept in the dark at 4 °C. Tetramethylpyrazine (98% purity) (TMPZ) was purchased from DELTA information centre for natural organic compounds (Anhui, China). The buffer solution of pH 7.40 consisted of Tris (0.1 mol $L^{-1}$) and HCl (0.1 mol $L^{-1}$), which was adjusted to 7.40 by adding 0.1 mol $L^{-1}$ NaOH when the experiment temperature was higher than 298 K. Phenylbutazone (99+% purity) was purchased from ACROS ORGANICS (NJ, USA); ibuprofen (99% purity) was purchased from Shanghai Civi Chemical Technology Co., Ltd. (Shanghai, China); digitoxin (99% purity) was purchased from Hubei Huabei Biomedicine Co., Ltd. (Wuhan, China). Other reagents were of analytical reagent grade and also used without further purification. Doubly distilled water was used throughout the experiment. Sample masses were accurately weighted on an electronic analytical balance ESJ180-4 (Shenyang Longteng Electronic Co., Ltd, China) with a resolution of 0.1 mg.

### 2.2. Apparatus

All fluorescence measurements were carried out on a Cary Eclipse fluorescence spectrofluorimeter (Varian, USA) equipped with a 1.0 cm quartz cell and a thermostat bath. UV–vis absorption spectra were recorded at room temperature on a UV-3600 spectrophotometer (Shimadzu, Japan) equipped with 1.0 cm quartz cells and an s-1700 thermoelectric single cell holder. FT-IR spectra were measured on a Nicolet-6700 FTIR spectrometer via the attenuated total reflection (ATR) at a resolution of 4 $cm^{-1}$ and 64 scans. Circular dichroism (CD) measurements were performed on a Jasco-810 automatic recording spectropolarimeter (Japan).

### 2.3. Methods

Fluorometric titration experiments as follows: 3.0 mL of BSA/HSA solution (pH 7.4) was added accurately to the quartz cell and then titrated by successive additions of TMPZ solution with the concentration of $1.2 \times 10^{-3}$ mol $L^{-1}$ using a 50 μL microsyringe to attain a series of final concentrations. Titrations operated manually and mixed moderately. Fluorescence emission spectra were measured at 293, 298, 304 and 310 K with the width of the excitation and emission slit both adjusted at 5 nm. The excitation wavelength was 280 nm, and fluorescence emission spectra were recorded from 250 to 500 nm. Synchronous fluorescence spectra were recorded from 220 to 350 nm at $\Delta\lambda$ = 15 and $\Delta\lambda$ = 60 nm, respectively. Also appropriate blanks corresponding to the buffer were subtracted to correct the fluorescence background.

Binding location studies between TMPZ and BSA/HSA in the presence of three site markers (ibuprofen, phenylbutazone and digitoxin) were performed by the fluorescence titration methods. The concentration of BSA/HSA and site markers was all stabilized at $5.0 \times 10^{-6}$ mol $L^{-1}$ (pH 7.4). TMPZ was then gradually added to the BSA–ibuprofen, BSA–phenylbutazone, BSA–digitoxin, HSA–ibuprofen, HSA–phenylbutazone, or HSA–digitoxin mixtures. The fluorescence quenching data for six systems were recorded over the range of 250–500 nm using an excitation wavelength of 280 nm.

FT-IR spectra of free BSA/HSA and TMPZ–BSA/HSA complex were mensurated on a Nicolet-6700 FT-IR spectrometer via the attenuated total reflection (ATR) at a resolution of 4 $cm^{-1}$ and 64 scans. Their FT-IR spectra were recorded from 400 to 4000 $cm^{-1}$ at 298 K. The corresponding absorbance contributions of buffer and free TMPZ solutions were recorded and digitally subtracted with the same instrumental parameters.

CD spectra of BSA/HSA in the absence and presence of TMPZ were performed on a Jasco-810 automatic recording spectropolarimeter (Japan) using a 0.1 cm cell at room temperature. The CD spectra were recorded in the range of 200–260 nm at a scanning rate 100 nm/min.

Three-dimensional fluorescence spectra of free BSA/HSA and TMPZ–BSA/HSA complex were performed under the following conditions: the initial excitation wavelength was set at 200 nm with increment of 2 nm, the number of scanning curves was 76; the emission wavelength was recorded between 200 and 500 nm at a scanning rate of 24,000 nm/min, and other scanning parameters were the same as those of fluorescence quenching spectra.

## 3. Results and discussion

### 3.1. Fluorescence quenching spectra

The intrinsic fluorescence of BSA/HSA appears at 338 nm using an excitation wavelength of 280 nm, which is owing to tryptophan (Trp), tyrosine (Tyr) and phenylalanine (Phe) residues. Intrinsic fluorescence characteristics are very sensitive to its microenvironment. The fluorescence spectra of both BSA and HSA upon the addition of TMPZ are illustrated in Fig. 1. As shown in Fig. 1, the fluorescence intensities of both BSA and HSA reduced gradually with increasing TMPZ concentrations, and a slightly blue shift (from 338 to 336 nm) was also observed for the emission wavelengths of both SAs, indicating that the microenvironment around HSA/BSA changed after the addition of TMPZ and the formation of