Journal of Photochemistry and Photobiology B: Biology 140 (2014) 381–389

Contents lists available at ScienceDirect

Journal of Photochemistry and Photobiology B: Biology

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

Biophysical study on the interaction of an anesthetic, vecuronium bromide with human serum albumin using spectroscopic and calorimetric methods

Yulin Zhu [a,1], Renxia Zhang [b,1], Yuxia Wang [b], Jiahai Ma [b], Kezhong Li [b], Zhi Li [b,*]

[a] Department of Anesthesiology, Yantaishan Hospital, 91# Jiefang Road, Yantai 264000, China
[b] Department of Anesthesiology, Yantai Yuhuangding Hospital, Affiliated Hospital of Medical College, Qingdao University, 20# East Yuhuangding Road, Yantai 264000, China

ARTICLE INFO

Article history:
Received 27 April 2014
Received in revised form 21 August 2014
Accepted 26 August 2014
Available online 10 September 2014

Keywords:
Vecuronium bromide
Human serum albumin
Spectroscopy
Calorimetry
Physiology

ABSTRACT

The interactions between an anesthetic, vecuronium bromide (VB) and human serum albumin (HSA) have been investigated systematically by steady-state/time-resolved fluorescence, circular dichroism (CD), UV–vis absorption, Fourier transform infrared spectroscopy (FTIR), mass spectroscopy and differential scanning calorimetry (DSC) methods under physiological conditions. The fluorescence quenching observed is attributed to the formation of a complex between HSA and VB, and the reverse temperature effect of the fluorescence quenching has been found and discussed. Fluorescence analysis has proved that there is one classical binding site on HSA for VB with a relative weak binding constant of $1.07 \times 10^4\ M^{-1}$ at 298 K. The primary binding pattern is determined by hydrogen bonding or van der Waals forces occurring in site I of HSA with $\Delta G^\circ = -2.30 \times 10^4\ J\ mol^{-1}$, $\Delta S^\circ = -233\ J\ mol^{-1}\ K^{-1}$ and $\Delta H^\circ = -9.23 \times 10^4\ J\ mol^{-1}$ at 298 K. VB could slightly change the secondary structure and induce unfolding of the polypeptides of protein. The DSC results provide quantitative information on the effect of VB on the stability of serum albumin. It is shown that VB can efficiently bind with HSA and be transported to the focuses needed.

1. Introduction

HSA is the most abundant protein in plasma, present at a concentration of about 0.6 mM. The functions of HSA include the maintenance of colloid osmotic pressure and transportation and metabolism of many endogenous and exogenous ligands (fatty acids, metal ions, or hormones). HSA, a globular protein molecular weight 66 kDa, consists of 585 amino acids. Its amino acid sequence contains 18 tyrosines, 6 methionines, 1 tryptophan (Trp 214), 17 disulfide bridges, and only one free thiol (Cys 34) [1]. The disulfides are positioned in a repeating series of nine loop-link-loop structures centered on eight sequential Cys–Cys pairs. HSA comprises three homologous domains (I, II, and III) that assemble to form a heart-shaped molecule. Each domain contains two subdomains (A and B) that possess common structural motifs. The binding of some ligands with active sites of the proteins can change their structure and function and cause toxic effects [2,3]. The single tryptophan of HSA at residue 214 has been used extensively as a fluorescent reporter group for ligand binding and conformational studies [4–6].

VB (Fig. 1B) is a muscle relaxant in the category of non-depolarizing blocking agents. It is indicated as an adjunct to general anesthesia, to facilitate endotracheal intubation and to provide skeletal muscle relaxation during surgery or mechanical ventilation. As it is intravenous, it can be used perioperatively when patients are unable to take oral medications [7]. A drug's efficiency may be affected by its ability to bind the proteins within blood plasma. Thus to study the interaction between drugs and plasma proteins would be of great value to illustrate the pharmacological and toxicological mechanisms. In this paper the study of the interaction between VB and HSA was performed by steady-state fluorescence. CD experiments have enabled us to monitor changes in the secondary structure of the protein. Spectroscopy was used to calculate the binding constants and the number of binding sites in HSA. Moreover, FTIR, mass spectrometry, and DSC measurements were also carried out. The influence of some common ions in the formation of the complexes was also investigated. This study tries to unveil the changes in the structure of the protein due to drug interactions

* Corresponding author at: Department of Anesthesiology, Yantai Yuhuangding Hospital, Affiliated Hospital of Medical College, Qingdao University, 20# East Yuhuangding Road, Yantai 264000, Shandong Province, China. Tel./fax: +86 0535 6691999.
E-mail address: leezhi@163.com (Z. Li).
[1] Both authors contributed equally to this work and share the first authorship.

http://dx.doi.org/10.1016/j.jphotobiol.2014.08.019
1011-1344/

in order to assist in the understanding the behavior and clinical applications of the drug.

2. Materials and methods

2.1. Materials

HSA and VB (purity ⩾ 98%) were purchased from Sigma (St. Louis, MO) and used without further purification. HSA and VB were all prepared with 0.1 M Tris–HCl buffer (pH 7.4). Other chemicals employed are at least of analytical reagent grade and were all bought from Sinopharm (Beijing, China). Ultrapure water of 18.0 MΩ cm^{-1} resistivity from a Milli-Q (Millipore, Bedford, MA) water purification system was used in this study.

2.2. Apparatus and methods

1 mL BSA (1.0×10^{-5} M) and various amounts of drug solution were added to a 10 mL colorimetric tube in sequence, then diluted with ultrapure water to 10 mL. An F-7000 spectrophotometer (Hitachi, Japan) is used for the fluorescence measurement with a slit of 5 nm and a scan speed of 1200 nm min^{-1}. The voltage of photomultiplier was set to 500 V. Three runs of one sample were averaged to obtain one spectrum. For ion effects on the association of drug-protein, all the metal ions sources are chloride salts and the experimental conditions are the same with the above fluorescence test. Inner filter effects (IFE) are corrected for the related calculations using methods from van de Weert [8]:

$$F_{ideal}(\lambda_{ex}, \lambda_{em}) = F_{obs}(\lambda_{ex}, \lambda_{em})CF_p(\lambda_{ex})CF_s(\lambda_{em}).$$
$$\approx F_{obs}(\lambda_{ex}, \lambda_{em})10^{(A_{em}+A_{ex})/2}$$

where CF_p is the correction factor for the absorption of excitation radiation, which depends on the total absorbance of the sample at λ_{ex}, whereas CF_s is the correction factor for the absorption of emission radiation, which depends on the total absorbance of the sample at λ_{em}. A_{ex} and A_{em} represent the absorbance at the fluorescence excitation and emission wavelengths, respectively. F_{obs} is the observed fluorescence. The synchronous fluorescence spectra were obtained by setting the excitation and emission wavelength interval ($\Delta\lambda$) at 15 and 60 nm. The experimental condition is the same with the fluorescence measurement.

For mass spectrometry determination, positive ion mode mass spectra were recorded on a micro-TOF Q (Bruker Daltonics, Bremen, Germany) equipped with an electrospray ionization source. For these measurements, the HSA concentration used was 0.1 μM and the VB concentration was 0.2 μM. Free HSA and HSA-VB were prepared in 5 mM ammonium acetate (pH 7.4) mixed with 20% acetonitrile, and was introduced into the mass spectrometer source with a syringe pump at 3 mL min^{-1}. Scanning was performed over an m/z range of 50–3000, with collision energy of 10 eV. Data were averaged for 2 min and then smoothed using the Gaussian algorithm in the Bruker data analysis 3.4 software program.

For fluorescence lifetime, measurements were carried out on a time-correlated single photon counting spectrophotometer (Edinburgh, Livingston, UK). The samples were excited at 278 nm so that all the fluorephores could be excited. The lifetime was obtained using deconvolution technique, which is based on a convolution integral. The software Origin 8.0 was used for curve fitting.

UV–vis absorption spectra were recorded on a T6 UV–vis spectrophotometer (Purkinje General Instruments, Beijing, China) with a 1.0 cm quartz cuvette at room temperature (298 K). The spectra

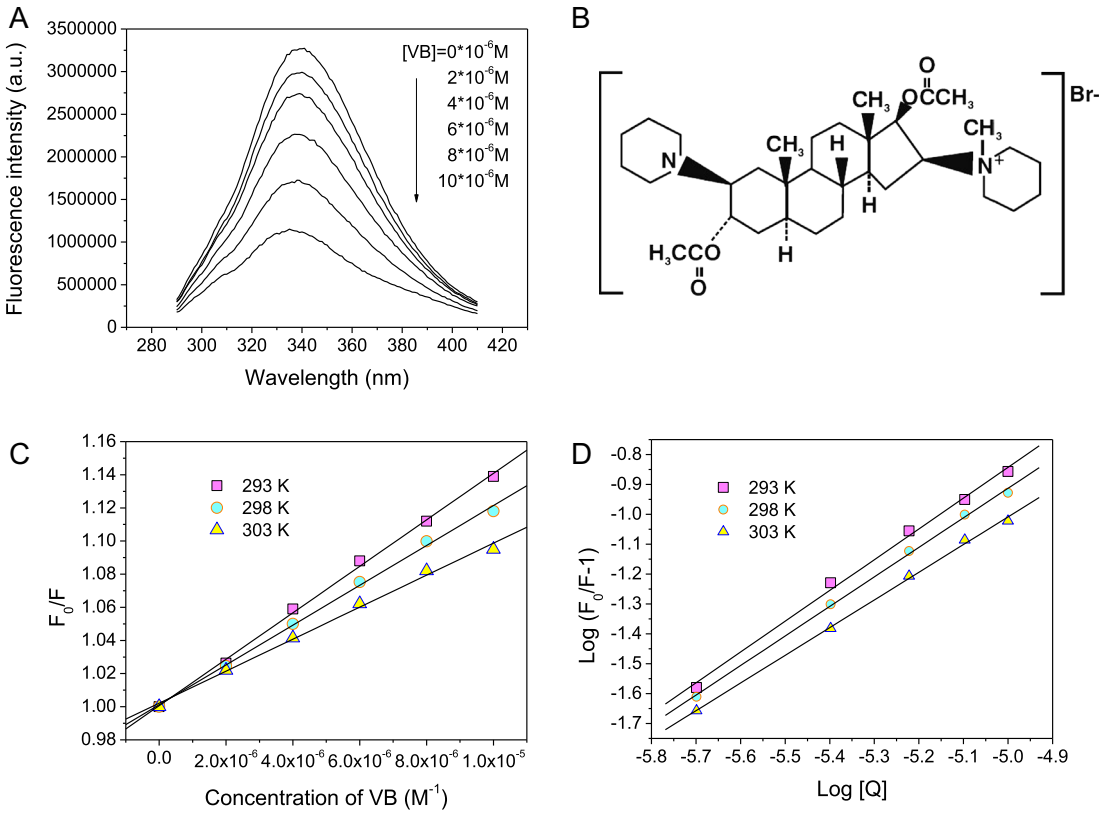

Fig. 1. (A) Fluorescence emission spectra of the HSA-VB system. HSA concentration was kept at [HSA] = 1.0×10^{-6} M, while concentration of [VB] = 0, 2, 4, 6, 8, and 10×10^{-6} M; pH 7.4, T = 298 K. (B) The molecular structure of VB. Stern–Volmer (C) and Hill (D) plots of fluorescence quenching for protein with VB at different temperatures (293, 298 and 303 K) after eliminating inner filter effect. Data in C and D have been linearly fitted.