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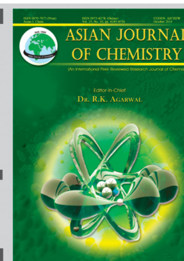

Study on the Conformational Change of the Interaction Between Bovine Serum Albumin and Troxerutin by Fourier Transform Infrared Spectroscopy

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The conformational change of the binding interaction of troxerutin with bovine serum albumin (BSA) was investigated by Fourier transform infrared (FT-IR) spectroscopy under simulative physiological conditions. The results showed that the secondary structure changes of BSA were changed by the addition of troxerutin. Combining the curve-fitting results of amide I bands, the alterations of protein secondary structure after drug complexation were quantitatively determined. The α-helix structure has a decrease of ≈ 13 %, from 59-46 % and the β-sheet increased ≈ 5 %, from 28-33 % and the β-turn increased ≈ 8 %, from 13-21 % at high drug concentration. It is very significance to study the conformational change of interaction between troxerutin and serum albumin for understanding of troxerutin's toxicity and its distribution in the organism.

Key Words: Bovine serum albumin, Troxerutin, FT-IR spectroscopy, Conformational change, Amide I.

INTRODUCTION

Serum albumins are the most extensively studied and applied proteins because of their availability, low cost, stability and unusual ligand binding properties[1]. Albumins are the most abundant protein in blood plasma and serve as a depot protein and transport protein for numerous endogenous and exogenous compounds. Albumins are also the principle factor in contributing to the colloid osmotic pressure of the blood and have been suggested as a possible source of amino acids for various tissues[2,3]. Among the serum albumins, bovine serum albumin (BSA) has a wide range of physiological functions involving the binding, transportation and delivery of fatty acids, porphyrins, bilirubin, steroids, *etc*. It is home to specific binding sites for metals, pharmaceuticals and dyes. Bovine serum albumin (BSA) is a single-chain 582 amino acid globular nonglycoprotein cross-linked with 17 cystine residues (eight disulfide bonds and one free thiol)[4].

Many drugs, including anticoagulants, tranquillizers and general anaesthetics, are transported in the blood while bound to serum albumins[5]. The binding ability of drug-albumin in blood stream may have a significant impact on distribution, free concentration and metabolism of drug. It is then important to study the binding parameters in order to know and try to control the pharmacological response of drugs and design of dosage forms. Troxerutin is a flavonoid present in tea, coffee, cereal grains and a variety of fruits and vegetables. Because of its role in the treatment of Chronic Venous Insufficiency (CVI), varicosity and capillary fragility[6], the chemistry and biochemistry of troxerutin and related compounds have been, for many years, of considerable interest for chemists, biologists and medical scientists.

In the previous study, we reported the spectroscopic studies to characterize the interaction troxerutin and bovine serum albumin by fluorescence spectroscopy[7]. The present paper deals with the conformational changes of the interaction between troxerutin and bovine serum albumin by Fourier transform infrared (FT-IR) spectroscopy and discusses detailedly the conformational changes. This kind of studies may provide salient information for understanding of troxerutin's toxicity and its distribution in the organism and hence become an important research field in chemistry, life science and clinical medicine.

EXPERIMENTAL

Bovine serum albumin was purchased from Boquan Biochemical Technology Co. Ltd. (Nanjing, China) and used without further purification. Troxerutin was obtained from Shanxi Yabao Pharmaceutical Group Co. Ltd. (Shanxi, China). BSA stock solution (7.9×10^{-4} mol L^{-1}) was prepared in pH 7.4 phosphate buffer solution containing 0.1 mol L^{-1} NaCl. The troxerutin solution (6.0×10^{-4} mol L^{-1}) was prepared in pH 7.4 phosphate buffer solution, respectively. All other chemicals were of analytical reagent grade and double distilled water was used throughout.

FT-IR measurements were carried out at room temperature on a Nicolet Nexus 670 FT-IR spectrometer (USA) equipped with a germanium attenuated total reflection (ATR) accessory, a DTGS KBr detector and a KBr beam splitter. The absorption spectrum was recorded with a UV-3600PC spectrophotometer (Shimadzu, Japan) equipped with 1.0 cm quartz cell.

Procedures: All the FT-IR spectra were taken *via* the attenuated total reflection (ATR) method with a resolution of 4 cm^{-1} and 60 scans. The spectra processing procedure involved collecting spectra of the buffer solution under the same conditions. Then the absorbance of the buffer solution was subtracted from the spectra of the sample solution to obtain the FT-IR spectra of the proteins. The subtraction criterion was that the original spectrum of the protein solution between 2200 and 1800 cm^{-1} was featureless.

RESULTS AND DISCUSSION

Infrared spectra of proteins can exhibit a number of the amide bands, which represent different vibrations of the peptide moiety. Among these amide bands of the protein, the amide I peak position occurs in the region 1700-1600 cm^{-1} (mainly due to C=O stretch) and amide II band near 1560-1548 cm^{-1} (C-N stretch coupled with N-H bending mode)[8]. They both have a relationship with the secondary structure of the protein. However, the amide I band is more sensitive to the change of protein secondary structure than amide II. The key factors known to govern the conformational sensitivity of the amide bands in proteins are hydrogen bonding and the coupling between transition dipoles. Different spectra also provide information about the conformational changes that arise due to complex formation.

Fig. 1 shows the FT-IR spectra of the free BSA and the difference spectrum after binding with troxerutin. As shown in Fig. 1, BSA in the absence of troxerutin shows the amide I peak at 1656 cm^{-1} and in the presence of troxerutin the amide I peak was shifted to 1654 cm^{-1}. And the amide II also has small shift (from 1548 to 1544 cm^{-1}). From the shift in peak position we confirmed that the conformation of BSA has been affected by the addition of troxerutin.

In order to perform a quantitative analysis of the corresponding secondary structures in amide I, the second derivative of BSA and BSA-troxerutin complexes FT-IR were analyzed. The amide I bands of protein consist of many overlapping component bands that represent different secondary structural elements, such as α-helix, β-sheet, β-turn and random coil[9]. According to the well-established assignment criterion, the spectral ranges from 1637-1615, 1648-1638, 1660-1649, 1680-1660 and 1692-1680 cm^{-1} in the amide I are attributed to β-sheet, random coil, α-helix, β-turn and β-antiparallel structures, respectively.

The quantitative analysis of the protein secondary structure for free BSA and docetaxel-BSA complex was given in Fig. 2 and Table-1. It can be seen that second derivative of free BSA showed a strong peak at 1654 cm^{-1}, which is assigned to α-helix and other peaks at 1624 and 1683 & 1642 corresponding to β-sheet and β-turn, respectively. The curve-fitted results show that the α-helix decreased from 59 to 46 %, β-sheet increased from 28 to 33 % and β-turn increased from 13 to 21 %.

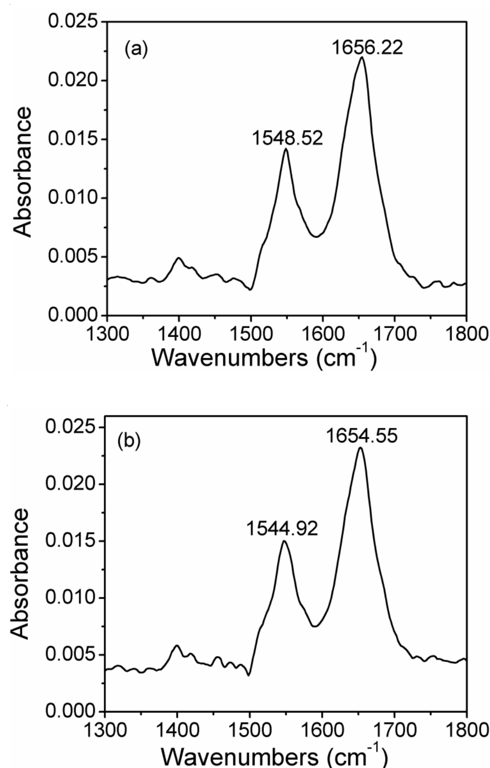

Fig. 1. FT-IR spectra and different spectra [(BSA solution + troxerutin solution)-(troxerutin solution)] of free BSA (a) and troxerutin-BSA complexes (b) in buffer solution in the region of 1800-1300 cm^{-1} $C_{BSA} = 7.9 \times 10^{-4}$ mol/L and $C_{troxerutin} = 6.0 \times 10^{-4}$ mol/L; pH = 7.4

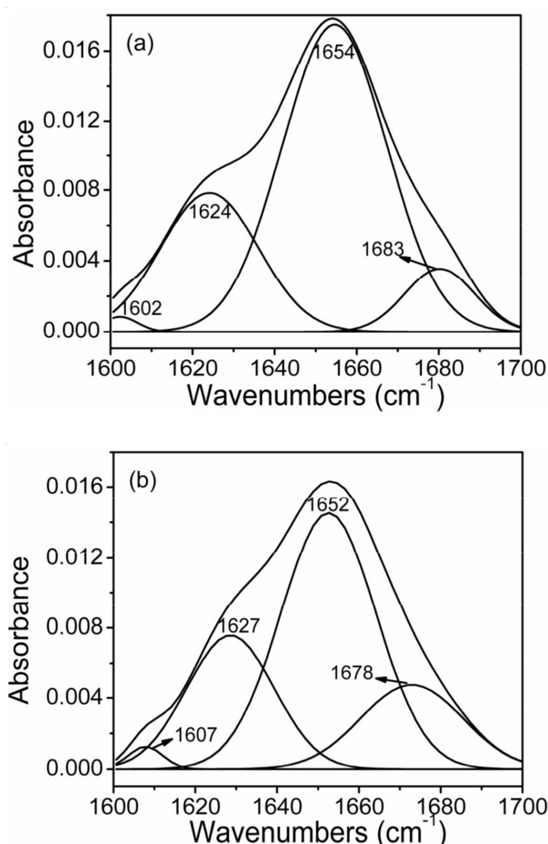

Fig. 2. Curve fitting amide I region with secondary structure determination of the free BSA (a) and troxerutin-BSA complexes (b) in buffer solution in the region of 1600-1700 cm^{-1}. $C_{BSA} = 7.9 \times 10^{-4}$ mol/L and $C_{troxerutin} = 6.0 \times 10^{-4}$ mol/L; pH = 7.4

TABLE-1
CONTENT OF SECONDARY STRUCTURE OF
BSA AND TROXERUTIN-BSA

Amide-I component (cm^{-1})	1637-1615 β-sheet (%)	1660-1649 β-helix (%)	1680-1660 β-turn (%)
Free BSA	28	59	13
Troxerutin + BSA	33	46	21

This significant interaction of troxerutin with BSA was also studied by using UV-VIS spectra (Fig. 3). The UV-VIS spectra of BSA solutions showed the typical peak at 278.5 nm assigned to peptide bond and aromatic amino acid side chains (phenylalanine, tryptophan, histidine and tyrosine), respectively. UV-VIS BSA/troxerutin spectra confirmed FT-IR spectroscopy results showing a significant intensity increment in 278.5 nm band, that could be due to a higher exposure of aromatic amino acid residues probably produced by an enhancement of unfolding in the protein. In addition, it was suggested that strong interactions would be expected at pH 7 between troxerutin and BSA taking into account their association constant[7].

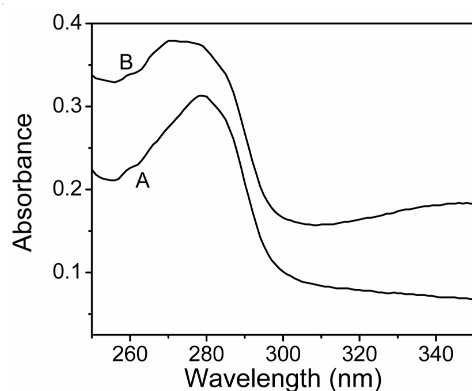

Fig. 3. UV-VIS spectra of (A) free BSA and (B) BSA/troxerutin complex; $C_{BSA} = 1.0 \times 10^{-6}$ mol/L, $C_{troxerutin} = 1.5 \times 10^{-6}$ mol/L

Conclusion

This study clearly shows that the conformational changes of interaction between troxerutin and BSA by Fourier transform infrared (FT-IR) and UV-VIS spectroscopy under simulative physiological conditions. Furthermore, a detailed secondary structural conformation analysis of BSA and BSA/troxerutin complexes could be performed. The result may help to provide more evidences on the mode of action of the troxerutin interacting with the BSA plasma protein.

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