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Interaction of erucic acid with bovine serum albumin using a multi-spectroscopic method and molecular docking technique

Yang Shu [a], Weiwei Xue [b], Xiaoying Xu [a], Zhimin Jia [a], Xiaojun Yao [b], Shuwen Liu [a], Lihong Liu [a,*]

[a] School of Pharmaceutical Sciences, Southern Medical University, Guangzhou 510515, China
[b] Department of Chemistry, Lanzhou University, Lanzhou 730000, China

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ABSTRACT

Overconsumption of erucic acid has been shown to cause heart damage in animals. The aim of this study is to evaluate the binding behaviour between erucic acid and bovine serum albumin using multi-spectroscopic methods and a molecular docking technique under physiological conditions. We find that erucic acid can quench the intrinsic fluorescence of BSA by dynamic quenching and there is a single class of binding site on BSA. In addition, the thermodynamic functions ΔH and ΔS are 119.14 kJ mol^{-1} and 488.89 J mol^{-1} K^{-1}, indicating that the hydrophobic force is a main acting force. Furthermore, the protein secondary structure changes with an increase in the content of α-helix, measured using synchronous fluorescence, circular dichroism and Fourier transform infrared spectroscopies. The molecular docking results illustrate that erucic acid can bind with the subdomain IIA of the BSA, and hydrogen bonding is also an acting force.

1. Introduction

Erucic acid (C22:1, n-9), also known as *cis*-13-docosenoic acid, is an unbranched fatty acid with a 22-carbon chain length and a single double bond in the omega 9 position (Bozcali, Süzer, Gürsoy, Atukeren, & Gümüstas, 2009). Erucic acid is one of the major fatty acids commonly found in mustard oil, canola oil and rapeseed oil. Feeding experiments in rats, chicks, pigs and non-human primates indicate that consumption of erucic acid can cause myocardial lipidosis and cardiac steatosis (Roine, Uksila, Teir, & Rapola, 1960; Schiefer et al., 1978). Additionally, excessive ingestion also indicates possible cardiotoxicity in humans (Imamura et al., 2013), because the heart utilises fatty acids as its fuel (Goldberg, Trent, & Schulze, 2012; Lopaschuk, Ussher, Folmes, Jaswal, & Stanley, 2010). Hence, it is very important to study the conformational changes of erucic acid under simulative physiological conditions.

Bovine serum albumin (BSA), the most abundant protein in plasma, is a commonly used reagent in biological study, which has high homologous with human serum albumins. Serum albumins play important roles in many physiological functions, and serve as transporters in the transportation and distribution of endogenous and exogenous substances (Kratz, 2008). Substances are always combined with serum albumins before arriving at the target destinations. The binding of substances to serum albumins changes not only the effectiveness of substances and their conformation but also the activity of serum albumins. Substance-protein interaction experiments have great significance in gaining fundamental binding characteristic of the complex. The objective of this study is to investigate the effect of erucic acid on BSA.

Structurally, BSA is a single chain of 582 amino acids, globular, non-glycoprotein, cross-linked with 17 cysteine residues (Carter & Ho, 1994). BSA has three types of intrinsic fluorophores: tryptophan (Trp), tyrosine (Tyr) and phenylalanine (Phe) (Peters, 1985). There are two tryptophan residues in BSA: Trp-212 is located in a hydrophobic binding pocket, and Trp-134 on the surface of molecule (Kragh-Hansen, 1981). The BSA molecule is made up of three homologous domains (I, II, III) which are divided into nine loops (L1–L9) by 17 disulphide bonds. Each domain in turn is the product of two subdomains (IA, IB, etc.) (Peters, 1995). Trp-134 is in the

* Corresponding author at: School of Pharmaceutical Sciences, Southern Medical University, 1838 North Guangzhou Avenue, Guangzhou 510515, China. Tel.: +86 20 61648595.
E-mail address: lhliu02@126.com (L. Liu).

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first domain, and Trp-212 is in the second domain (He & Carter, 1992).

To evaluate the binding parameters between erucic acid and BSA, we used multi-spectrophotometric methods and molecular docking methods to investigate the interactions of erucic acid and protein. In this paper, the binding mechanism and properties of BSA-erucic acid were investigated by UV–vis absorption spectroscopy and fluorescence quenching technology, while the secondary structure was detected by synchronous fluorescence, circular dichroism (CD) spectroscopy and Fourier transform infrared (FT-IR) spectroscopy (Bi, Pang, Wang, Zhao, & Yu, 2014). The molecular docking studies between erucic acid and BSA (PDB ID: 3V03) were performed by Glide tools.

Up to now, various studies have reported the effect of erucic acid. However, little research has been conducted on the interaction of erucic acid with BSA using a multi-spectroscopic method and molecular docking technique. In this paper, accurate and full data was obtained, which could make a significant contribution to the understanding of erucic acid. It may help us in the search for more particular information regarding its transport and distribution in the human body. In addition, it may aid in the understanding of results from related animal experiments. We believe it could provide valuable information for future clinical pharmacology.

2. Materials and methods

2.1. Materials

Bovine serum albumin was purchased from Xi'an Biological Engineering Company (Xi'an, China) and its molecular weight was approximately 66,430. Erucic acid (PubChem CID 5281116) was purchased from Aladdin Company (Los Angeles, America) and its molecular weight was 338.57. Tris (Tris(hydroxymethyl) aminomethane) was of biochemical grade and was purchased from Ke'hao Biological Engineering Company (Xi'an, China). BSA was dissolved in a Tris–HCl (0.05 mol l^{-1}, pH = 7.4) to form the BSA stock solution with a concentration of 1.0×10^{-6} mol l^{-1}. A Tris–HCl buffer (0.05 mol l^{-1}, pH = 7.4) containing 0.1 mol l^{-1} NaCl was selected to maintain the ionic strength of the solution. The Tris–HCl buffer was used to imitate physiological conditions throughout all the experiments. All other reagents except erucic acid were prepared using doubly deionised water, and erucic acid stock solution was prepared using ethanol. They all stocked at 4 °C.

The UV spectra were acquired using U-3010 (Hitachi, Japan). Intensity and spectra of synchronous fluorescence were obtained with a RF-5301PC (Shimadzu, Japan). FT-IR measurement is carried out on a Nicolet 6700 (Thermo Fisher, America) equipped with a Germanium attenuated total reflection (ATR) accessory, a DTGS KBr detector and a KBr beam splitter. CD was measured using J-810 automatic recording spectropolarimeter (JASCO, Japan).

2.2. Measurements

2.2.1. UV–vis absorption spectra

The UV–vis absorption spectrum was measured in the wavelength range of 200–400 nm at 298 K. The BSA solution had a fixed concentration of 1.5×10^{-6} mol l^{-1} while the erucic acid concentration varied from 0 to 12.35×10^{-6} mol l^{-1}.

2.2.2. Fluorescence quenching and synchronous fluorescence

3.0 ml solution containing 1.5×10^{-6} mol l^{-1} BSA was titrated manually by successive addition of a 4.63×10^{-5} mol l^{-1} erucic acid, and the final erucic acid concentration was 4.63×10^{-7} mol l^{-1}. The fluorescence intensity was measured with excitation wavelength at 295 nm, the widths of the excitation and emission slit set at 3.0 and 5.0 nm, respectively. All experiments were measured at different temperatures (298, 304 and 310 K). Synchronous fluorescence experiment of $\Delta\lambda = 60$ nm was measured. Its excitation wavelength was set from 230 to 360 nm, and slit width was 5/5 nm.

2.2.3. Circular dichroism (CD) spectroscopy

Solutions containing BSA (1.5×10^{-6} mol l^{-1}), as well as a mixture of BSA (1.5×10^{-6} mol l^{-1}) and erucic acid (3×10^{-6} mol l^{-1}) were measured using CD spectrum. The spectra were recorded in the range of 200–350 nm using a 1 mm cell at 298 K.

2.2.4. Fourier transform infrared spectroscopy (FT-IR)

FT-IR measurement was carried out at room temperature; spectra were taken by the Attenuated Total Reflectance (ATR) method with a resolution of 4 cm^{-1} and 60 scans. The infrared spectra of BSA and the erucic acid complex (the molar ratio of erucic acid to BSA was 1:1) were obtained in the featured region of 4000–400 cm^{-1}.

2.2.5. Molecular modelling

The structure of erucic acid was constructed with the Maestro building tools. The erucic acid was then preprocessed by the LigPrep which used OPLS-2005 force field (Kaminski, Friesner, Tirado-Rives, & Jorgensen, 2001) and gave the corresponding low energy three dimensional (3D) conformers of the compound. The 3D structure of bovine serum albumin was obtained from the Protein Data Bank (PDB ID: 3V03). The Protein Preparation Wizard in Maestro was used to remove crystallographic water molecules, add hydrogen atoms, assign partial charges and protonation states, and minimise the structures. The minimisation was terminated when the root-mean-square deviation (RMSD) reached a maximum value of 0.30 Å. Molecular docking was used to study the binding mode of erucic acid with bovine serum albumin. The prepared erucic acid structure was docked into the binding site of the minimised bovine serum albumin using the Glide with the standard precision (SP) scoring mode. The docking grid box for erucic acid was generated using the binding site identified by the SiteMap. In molecular docking, 5000 poses were generated during the initial phase of the docking calculation, out of which the best 1000 poses were chosen for energy minimisation by 1000 steps of conjugate gradient minimisations. All of the molecular modelling calculation process was carried out with the Schrödinger 2009 software.

3. Results and discussion

3.1. Fluorescence quenching mechanism

An excitation wavelength of 295 nm was applied to selectively excite the tryptophan residues of BSA. Though the Try residue could also contribute to fluorescence, it presented very weak emission when excited at 295 nm. Trp fluorescence was highly sensitive to the environment polarity, and shifted in its emission spectrum towards lower wavelengths (blue shift) that could be seen as hydrophobicity increased. Changes of Trp emission spectra could be seen in response to protein conformational transitions, subunit association, ligand binding, or denaturation, all of which could affect the local environment surrounding the indole ring. Firstly, the effect of various concentrations of erucic acid at different temperatures (298, 303 and 310 K) on the fluorescence spectra of BSA was investigated. The results are shown in Fig. 1. It can be seen from this figure that the fluorescence intensity values of BSA decreased progressively with increasing concentration of erucic acid, which indicated that erucic acid could bind to BSA and