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Binding of erucic acid with human serum albumin using a spectroscopic and molecular docking study

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ABSTRACT

Erucic acid (EA) is one of the key fatty acids usually found in canola oil, mustard oil and rapeseed oil. Consumption of EA in primates was found to cause myocardial lipidosis and cardiac steatosis. To have an insight of the effect of EA in humans, we performed *in vitro* interaction studies of EA with the primary plasma protein, human serum albumin (HSA). Spectroscopic (UV–vis and fluorescence) analysis of the HSA-EA interaction revealed a static mode of quenching with binding constant K_b ~10^4 reflecting high affinity of EA for HSA. The negative value of ΔG° for binding of EA to HSA in the fluorescence studies indicates the process to be spontaneous. Thermodynamic signatures of the HSA-EA interaction in the complex reflect dominance of hydrogen bonds. Despite predominance of hydrogen bonds, hydrophobic interactions in the HSA-EA complex were found acting as a contributing factor in the binding of EA to HSA, observed as structural change in the far-UV CD spectra. Förster's resonance energy transfer of the EA-HSA complex revealed a distance of 3.2 nm between acceptor molecules (EA) and the donor Trp residue of HSA. To have a deeper insight of the structural dependence of the HSA-EA interaction in the complex, thermodynamic study was supplemented with molecular docking. The molecular docking analysis further highlighted the EA binding in the subdomain IIIA (Sudlow site II) of HSA. The information generated in the study reflects greater pharmacological significance of EA and highlights its importance in the clinical medicine.

1. Introduction

Fatty acids (FAs) are the major fuel source of heart and skeletal muscles, but excessive FAs consumption causes, deposition in the heart muscles; thereby enhances cardiotoxic effects in humans [1]. Free FAs become bound to serum albumin (SA) and are carried via blood to other tissues, such as, heart and skeletal muscles, which oxidize them as fuel. The rate of uptake of free FAs at tissue surface is correlated with the concentration of SA bound FAs in blood. Erucic acid (EA) is a long-chain monounsaturated fatty acid (FA) with a single double bond at its omega 9 position (C22:1). It is digested, absorbed and metabolized, for the most part, like other fatty acids. EA is weakly oxidized by the mitochondrial β-oxidation (particularly in myocardial cells) and this results in EA accumulation, leading to myocardial lipidosis that cause decrease in the contractile performance of heart muscles [2].

SA is a major constituent of blood plasma (concentration up to 40 g L^{-1}), responsible for maintaining osmotic pressure of the blood vessels [3]. It serves as a carrier protein for a variety of molecules, including metabolites of both endogenous and exogenous origin, hormones, and drugs [4,5]. Having high affinity for free FAs, it overcomes poor solubility of FAs; thereby acts as a major vehicle for their transport in blood [6,7]. FAs stabilize SA against denaturation. Under normal physiological conditions, 1 mole of HSA carries around 0.1–2 mole of FAs [8]. With at least seven binding sites of varying affinities [9], it not only accommodates FAs but also carry certain divalent cations, bilirubin, xenobiotics, and other entities. SA present as a major protein in the circulatory system, and it

Abbreviations: ASA, accessible surface area; CD, circular dichroism; EA, erucic acid; FAs, fatty acids; ΔH, enthalpy; HSA, human serum albumin; K_m, Michaelis-Menten constant; λ_{max}, wavelength maxima; MRE, mean residue ellipticity; V_{max}, maximum velocity; SA, serum albumin.

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contributes significantly to osmotic blood pressure in wide range of organisms [10]. Therefore, albumin is a key regulator of fluid movement between plasma and interstitial compartments under physiological conditions; although interestingly, the absence of plasma albumin can be compensated by the increased secretion of other proteins from liver to maintain physiological plasma oncotic pressures [11]. Similar to HSA, other plasma protein such as transferrin known for iron transport in blood stream for delivery iron to tissues.

Tryptophan based fluorescence spectroscopic studies have commonly been exploited in the association studies of HSA with wide range of endogenous and exogenous molecules. With only one tryptophan residue (Trp214) in its structure, interaction of molecules with the indole moiety of tryptophan chromophore modifies the fluorescence of HSA [12]. The effect, however depends on the concentration of molecules and the distance between molecule and the tryptophan chromophore. Being highly sensitive to environmental polarity, tryptophan fluorescence shifts the emission spectra to lower wavelength (blue shift) observed commonly as increase in the hydrophobicity. The changes in emission spectra of tryptophan is attributed to factors that affect the local milieu of indole ring, common being the protein conformational transitions, ligand binding, subunit association and denaturation. FAs bind at subdomain IIA (Sudlow site I) in the same orientation with hydrogen bond to carboxyl group (—COOH) group of Arg117 [9]. Though long-chain FAs have greater affinity for SA than medium-chain FAs, multiple binding interactions of SA increases the complexity that makes poor understanding of the SA-FA complexes [13,14].

FAs are known to alter the structure of SA, and thus, affect drug-SA interactions and drug metabolizing enzyme activity [15]. When FAs are absorbed into the circulatory system, their distribution occurs via red blood cells, leukocytes. FA to HSA binding can direct drug distributions to tissues and control drug free concentrations [16]. These effects of FAs are believed to be associated with the modulation of ligand to albumin binding, which is subject to competitive and allosteric effects [17]. With ability to quench intrinsic fluorescence, interaction of EA with bovine serum albumin (BSA) results in the dynamic quenching of single binding site of BSA. As study of the thermodynamic parameters indicate hydophobic interactions are main driving force between the EA and BSA binding. After addition of EA substantial secondary structural changes observed through circular dichroism and Fourier transform infrared spectroscopy [18].

In the present work, we obtained binding constants and thermodynamic parameters for EA to HSA binding. In addition, Trp quenching mechanism of EA to HSA and binding modes were investigated. Changes in the secondary structure of HSA were examined by circular dichroism (CD) spectroscopy. The effect of EA on HSA functionality was also studied from the perspective of the esterase-like activity of HSA.

2. Materials and methods

2.1. Materials and sample preparation

Fatty acid and globulin free HSA (A1887), erucic acid (E3385; Mw 338.57) and *p*-nitrophenyl acetate (N8130) were purchased from Sigma Aldrich. The stock solution of HSA and EA were prepared by dissolving in sodium phosphate buffer (20 mM, pH 7.4). HSA was dialyzed in 20 mM sodium phosphate buffer at 4 °C before sample preparation. The concentration of HSA was determined by spectroscopic method using extinction coefficient $E^{1\%}_{280nm} = 5.3$. All other solution concentrations were determined on a weight/volume (w/v) basis.

2.2. UV–vis absorption and emission spectroscopy

Perkin-Elmer Lambda 45 double beam UV–vis spectrophotometer attached to a Peltier temperature programmer-1 (PTP-1) was used to scan UV–vis spectra. The UV–vis absorption spectra were acquired in a 1 cm quartz cell.

Fluorescence quenching of HSA were acquired on a Varian Cary Eclipse fluorescence spectrophotometer attached with circulating water bath. The fluorescence emission spectra were performed at three temperatures 25, 30 and 37 °C. Both emission and excitation emission slit width were set to 3.0 nm and an excitation wavelength of 295 nm was used. The concentration of HSA was maintained at 10 μM in UV-vis absorption and 2 μM in fluorescence quenching experiments. Commonly, fluorescence quenching can be described by the Stern-Volmer equation:

$$\frac{I_o}{I} = \mathrm{Ksv[Q]{+}1} = k_q \tau_o [\mathrm{Q}] + 1 \quad (1)$$

where, I_o and I are the fluorescence intensities before and after the addition of quencher respectively. [Q] is the molar concentration of quencher (EA), K_{SV} is the Stern-Volmer quenching constant, k_q is the bimolecular quenching constant and τ_o is the integral fluorescence lifetime of tryptophan which is equal to 5.78×10^{-9} s. Quantitative evaluation of the binding constant (K_b) and of the number of binding stoichiometries (n) for the HSA-EA interaction were determined by analyzing fluorescence quenching data using:

$$\log\left[\frac{I_o - I}{I}\right] = \log K_b + n \log[Q] \quad (2)$$

where, K_b is the binding constant and n is the number of binding stoichiometry. The thermodynamic parameters of the HSA-EA interaction were estimated from binding constant data obtained at three temperatures. We used van't Hoff equation for calculating the change in enthalpy (ΔH°) and change in entropy (ΔS°):

$$\ln K_b = -\frac{\Delta H^\circ}{RT} + \frac{\Delta S^\circ}{R} \quad (3)$$

where, R denotes the universal gas constant (1.987 cal K^{-1} mol^{-1}) and T represents the absolute temperature.

The free energy change (ΔG°) of the process was determined using:

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ \quad (4)$$

2.3. Far-UV circular dichroism (Far-UV CD) spectrometry

The far-UV CD spectra were measured on a JASCO J-815 spectropolarimeter (JASCO, Tokyo, Japan) using a 0.1 cm cylindrical cell. Temperature of cell holder was controlled by Peltier type temperature controller (PTC-4245/15). The buffer solution used as blank was automatically subtracted from the samples during scanning. The measurements were repeated four times and average. Sacn speed of spectra collection was of 50 nm min^{-1}. The obtained CD results were expressed in terms of mean residue ellipticity (MRE) in deg cm^2 $dmol^{-1}$, according to the following equation:

$$\mathrm{MRE} = \frac{\Theta obs(m^\circ)}{10 \times n \times C \times l} \quad (5)$$

where, Θ_{obs} is CD in m°, n is the number of amino acid residues (n = 585-1), l is the path length of the cell in cm, and C is the molar concentration of HSA (2 μM).

2.4. Esterase-like assay of HSA

The effect of EA on the esterase-like activity of HSA was estimated by *p*-nitrophenyl acetate (*p*-NPA) measurements. Increase in the absorbance at 405 nm was measured over 2 min and retained