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Investigation on the interaction between endocrine disruptor triphenyltin with human serum albumin

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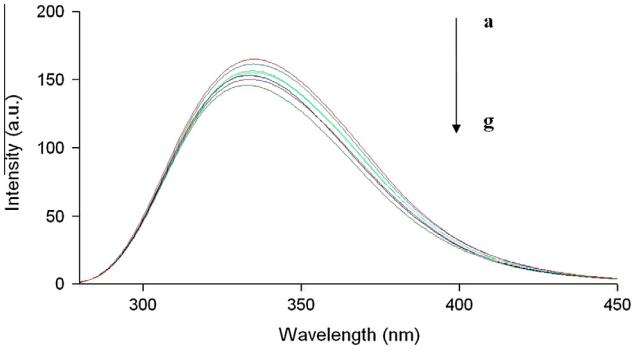
HIGHLIGHTS

- The fluorescence quenching of human serum albumin (HSA) by triphenyltin (TPT) is static.
- The binding of TPT and HSA is a result of the formation of TPT–HSA complex.
- The energy transfer of HSA to TPT occurs.
- Hydrophobic force is the main binding forces.
- Addition of TPT changes the conformation of HSA.

GRAPHICAL ABSTRACT

The changes of FL spectra of TPT-HSA intensity with TPT concentration. a: $R = 0$; b: $R = 0.5$; c: $R = 1$; d: $R = 1.5$; e: $R = 2$; f: $R = 2.5$; g: $R = 3$ (R = [TPT]/[HSA]).



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ABSTRACT

The interaction between triphenyltin (TPT) and human serum albumin (HSA) in physiological buffer (pH = 7.4) was investigated by the fluorescence quenching technique. The results of fluorescence titration revealed that TPT could strongly quench the intrinsic fluorescence of HSA through a static quenching procedure. The apparent binding constants K and number of binding sites n of TPT with HSA were 2.51×10^3 and 0.96 at 298 K which were obtained by the fluorescence quenching method. The thermodynamic parameters enthalpy change (ΔH), entropy change (ΔS) were positive, which indicated that the interaction of TPT with HSA was driven mainly by hydrophobic forces. The process of binding was a spontaneous process in which Gibbs free energy change was negative. The distance r between donor (HSA) and acceptor (TPT) was calculated to be 3.13 nm based on Forster's non-radiative energy transfer theory. The results of synchronous fluorescence, three-dimensional fluorescence and circular dichroism (CD) spectra showed that the triphenyltin induced conformational changes of HSA.

Introduction

Organotin compounds have been widely used as biocides in antifouling paints and agriculture since the 1960s [1]. With increased usage of organotin compounds, considerable attention has focused on potential toxicity. Numerous studies have assessed exposure and toxicological effects in humans. Triorganotin groups are usually more toxic than di-or mono-compounds of the same chain length. Tributyltin and triphenyltin compounds have embryotoxic, myotoxic, genotoxic and immunotoxic effects in mammals [2,3] and have been associated with occupational poisoning, hepatic injury, acute nephropathy, and mucous membrane irritation [4]. Derivatives of these compounds are known to induce imposex (the

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superimposition of male sex organs, penis and vas deferens, in females) in marine neogastropods and to cause developmental and reproductive toxicity in mammals by disrupting endocrine systems [5,6]. These toxic effects have recently become a matter of substantial concern [7]. However, the toxicity produced by their direct interaction with biological macromolecules in vitro has not been explored previously. Consequently, a thorough study on the mechanisms of moleculartoxicity of the TPT is needed.

Human serum albumin (HSA) is the most abundant protein in plasma, which functions in the maintenance of colloid osmotic blood pressure and in the binding and transportation of various ligands such as fatty acids, hormones, and drugs, then transports them between tissues and organs [8]. It has been shown that the distribution, free concentration, and metabolism of various ligands can be significantly altered as a result of their binding to HSA [9,10]. Ligand interactions at the protein binding level will in most cases significantly affect the apparent distribution volume of the ligands and also affect the elimination rate of ligands. Therefore, investigating the interactions of TPT and HSA are significant for knowing their transports and distributions in the body and clarifying it action mechanisms and pharmaceutical dynamics. As of yet, however, no work has been reported for the mechanism of it interactions and the detailed physicochemical characterizations of TPT binding to HSA.

Fluorescence spectroscopy is an appropriate method to determine the binding parameters between small molecules and proteins [11–13]. In this work, the interaction between triphenyltin and HSA was investigated by means of fluorescence spectroscopy to identify the quenched mechanism of TPT in HSA, the binding constant, the binding site, thermodynamic parameters and the changes of conformation. In addition, circular dichroism (CD) spectroscopy was performed to confirm the results.

Materials and methods

Materials

HSA was obtained from Sigma–Aldrich Company and used without purification. TPT was purchased from Acros Co. The stock solution of HSA was prepared in the Tris–HCl buffer solution (0.05 mol L^{-1} Tris, 0.15 mol L^{-1} NaCl, pH 7.4) with a final concentration of 1.0×10^{-4} mol L^{-1} and was kept in the dark. All other materials were of analytical reagent grade and double distilled water was used throughout all the experiment.

Apparatus

Fluorescence measurements were measured by a spectrofluorimeter Model F-7000 spectrophotometer (Hitachi, Japan) equipped with a 150 W Xenon lamp and a slit width of 2.5 nm. A quartz cell of 1.00 cm was used for measurements. The absorption spectra were performed on a double beam U-3900 pectrophotometer (Hitachi High Technologies, Japan) equipped with a 150 W Xenon lamp and a slit width of 10 nm. A quartz cell of 1.00 cm was used for measurements. CD spectra were recorded on a MOS-450 (Bio-Logic, France)

Procedures

Fluorescence measurements

Fluorescence quenching spectra were recorded at room temperature from 300 to 500 nm at an excitation wavelength of 280 nm at 298 and 310 K. The excitation and emission band widths were both 2.5 nm. Synchronous fluorescence spectra of HSA ($\Delta\lambda = 60$ and 15 nm) in the presence of TPT were measured at pH 7.4 buffer. Concentration of HSA for each run was fixed at 1.0×10^{-5} mol L^{-1} and the TPT concentrations were 0, 0.5, 1, 1.5, 2, 2.5 and 3×10^{-5} mol L^{-1}. The three-dimensional fluorescence spectra were measured under the following conditions: the emission wavelength was recorded between 200 and 600 nm, the initial excitation wavelength was set at 200 nm with an increment of 10 nm, and the other scanning parameters were just the same as those of the fluorescence emission spectra.

UV–vis absorption spectra measurements

The absorption spectrum of TPT (1.0×10^{-5} mol L^{-1}) was recorded in the range of 300–500 nm. Then, the overlap of the UV absorption spectrum of TPT with the fluorescence emission spectrum of HSA was used to calculate the energy transfer.

CD measurements

Circular dichroism (CD) measurements were recorded on a MOS-450 spectropolarimeter (200–250 nm and cell length path was 1 cm) by keeping the concentration of HSA constant (1.0×10^{-7} mol L^{-1}) while varying the TPT concentration from 0 to 1.0×10^{-5} mol L^{-1}.

Results and discussion

The quenching mechanism

Generally, the fluorescence of protein is caused by three intrinsic fluorophore present in the protein, i.e. tryptophan, tyrosine and phenylalanine residues. Actually, the intrinsic fluorescence of many proteins is mainly contributed by tryptophan and tyrosine residues, because the quantum yield of the phenylalanine residue is very low [14].

Fluorescence quenching refers to any process that is a decrease of the fluorescence intensity from a fluorophore due to a variety of molecular interactions. These include excited-state reactions, molecular rearrangements, energy transfer, ground-state complex formation, and collisional quenching.

Fig. 1 shows that with the addition of TPT, the fluorescence intensity of HSA decreased regularly. These phenomena indicated that TPT could quench HSA intrinsic fluorescence.

Quenching can occur by different mechanisms, which are usually classified as dynamic quenching and static quenching. For dynamic quenching, the decrease in intensity is usually described by the well-known Stern–Volmer equation [15–17].

$$F_0/F = 1 + K_q\tau_0[Q] = 1 + \mathrm{Ksv}[Q] \quad (1)$$

where F_0 and F are the fluorescence intensity in the absence and presence of quencher (triphenyltin), respectively, K_q the bimolecular

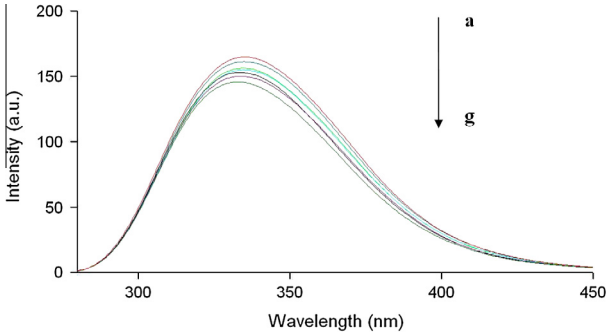

Fig. 1. The changes of FL spectra of TPT–HSA intensity with TPT concentration. (a) $R = 0$; (b) $R = 0.5$; (c) $R = 1$; (d) $R = 1.5$; (e) $R = 2$; (f) $R = 2.5$ and (g) $R = 3$ ($R = [\mathrm{TPT}]/[\mathrm{HSA}]$).