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Molecular simulation study of the specific combination between four kinds of phthalic acid esters and human serum albumin

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ABSTRACT

The interaction between endocrine disruptor phthalic acid esters (PAEs) and human serum albumin (HSA) was studied by fluorescence spectroscopy and molecular modeling methods. The efficiency of energy transfer and the distance between HSA and PAEs were calculated. The results showed that all of the four kinds of PAEs could quench the intrinsic fluorescence of the HSA, with the mechanisms of static quenching and non-radiative energy transfer. Molecular docking study and thermodynamic analysis revealed that the binding behavior was mainly governed by hydrophobic force. And the results of site marker competitive experiments and modeling method suggested that the four PAEs would mainly bind to the HSA in sub-domain IIIA, which demonstrated that the experimental results could coordinate with the theoretical results. Molecular dynamic simulation (MD) revealed that HSA did have a slight conformational change when it bound with PAEs. It also verified the greater stability of HSA–PAEs complex compared to free protein.

1. Introduction

Human serum albumin (HSA) contains 585 amino acids, which serves as a transporting agent for various endogenous and exogenous compounds, like fatty acids, nutrients, steroids, certain metal ions, hormones and drugs (Carter et al., 1994; Yamasaki et al., 1996; Pu et al., 2014; Ibrahim et al., 2010). The whole structure is composed of three homologous domains (I, II and III), each could divided into two sub-domains (A and B), and only one tryptophan residue (Trp-214) in the sub-domain IIA (Deeb et al., 2010). Ligands generally bind with the two principal regions of HSA that are located in the hydrophobic cavities of sub-domain IIA and IIIA, that is, Sudlow's site I and site II, respectively. Site I is located in the hydrophobic cavity of sub-domain IIA which is capable of binding most neutral or heterocyclic compounds by strong hydrophobic interactions, whereas site II in the sub-domain IIIA could bind many aromatic carboxylic acids by Van der Waals force or hydrogen bonding interaction, respectively.

Phthalic acid esters (PAEs) are widely used in toys and personal care products, etc. and they can migrate from the plastic into the external environment, plants (Blair et al., 2009; Mo et al., 2009; Liu et al., 2013), which scatter the potential risk for human exposure (Romani et al., 2014). Moreover, PAEs are considered to be a class of environmental endocrine disruptors which could cause testicular effect, influence the hormone synthesis and increase miscarriages or other gestational complications (Qu et al., 2009; Hansen and Grafton, 1994; Suzuki et al., 2001). To investigate the interaction between HSA and PAEs in detail has significant meaning for further understanding the pharma-cokinetics and pharmacodynamic properties of PAEs as it strongly affect the distribution of PAEs and the free fraction that is available for subsequent interactions with targeted receptors. However, few experiment studies of the pollution of phthalate esters in the HSA were reported due to the complexity of the sample substrate, and a detailed mechanism of interaction is still elusive (Xie et al., 2011; Zhou et al., 2012).

In this study, we emphasizes particularly on the mechanism and analysis of the interaction of HSA with four test PAEs (structure shown in Fig. 1) such as dimethyl phthalate (DMP), diethyl phthalate (DEP), dibutyl phthalate (DBP) and dioctyl phthalate (DOP) at physiological buffer (pH 7.4) by fluorescence technique and dynamics approaches. Compared the binding mode site I and site II of HSA and its parameters, the nature of interacting forces were discussed. The molecular level interactions, conformational changes of the protein HSA after binding to the probe, and the flexibility at the binding sites were also explored by molecular docking and MD simulations.

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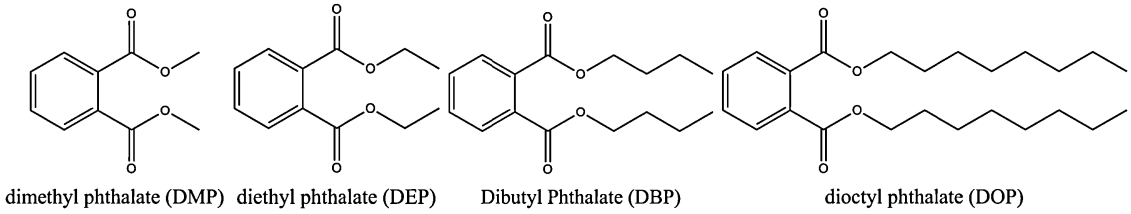

Fig. 1. Structure of DMP, DEP, DBP and DOP.

2. Materials and methods

2.1. Reagents and apparatus

DMP, DEP, DBP and DOP were obtained from Sino-Pharm Co. Ltd, and the 1.0×10^{-3} M PAEs solution was prepared by dissolving them in ethanol solution, respectively; HSA (98%) was purchased from Sigma Chemical Company; 1.0×10^{-5} M HSA stock solution and 1.0×10^{-3} M warfarin (ibuprofen) stock solution was prepared by dissolving them in pH 7.4 Tris–HCl buffer solution (0.2 M, containing 0.15 M of NaCl). The stock solution was stored at −4 °C before assay. All of the reagents were analytical grade and the water used was doubly distilled water.

RF-5301PC fluorescence spectrometer with supporting equipments such as thermostatic water bath, heated pool and 1.0 cm quartz cuvette (Shimadzu, Japan); Cary-50 UV–visible absorption spectra spectrophotometer (Varian, USA); EL204 electronic analytical balancer (Mettler-Toledo Instruments Co., Ltd); PT-10 portable pH meter (Sartorius, Germany).

2.2. Spectral measurements

In a typical fluorescence measurement, 1.0 mL HSA stock solution and 2.00 mL pH 7.4 Tris–HCl buffer solution were added to a 10.0 mL test tube. The PAEs solution was then gradually added to the tube using a trace syringe. The fluorescence emission spectra were measured at 291 K, 298 K and 310 K, respectively by a thermostatic bath to maintain the temperatures. The width of the excitation and emission slits was set at 3.0 and 5.0 nm, respectively. 280 nm was chosen as the excitation wavelength and the emission wavelength was recorded from 300 to 500 nm.

Synchronous fluorescence spectra of HSA in the absence and presence of the gradually increasing amount of PAEs was examined with constant differences of $\Delta\lambda = 60$ nm and 15 nm between the excitation and emission fluorescence monochromators, respectively. Site marker competitive experiments were carried out using Warfarin and Ibuprofen as site markers with fluorescence titration methods. The concentrations of HSA and Warfarin/Ibuprofen were all stabilized at 1.0×10^{-6} M. PAEs were then gradually added to the HSA–Warfarin and HSA–Ibuprofen solutions. An excitation wavelength of 280 nm was selected and the fluorescence spectra were recorded in the range of 300–500 nm.

2.3. Molecular docking

The following molecular docking study was conducted to explore possible binding positions between PAEs and HSA. The initial structures of all the molecules were generated by molecular modeling software SYBYL-X 1.1. Surflex-Dock program is used for molecular docking. Water molecules were removed and hydrogen atoms were added to the proteins, and Kollman Uni charges were also assigned to HSA. All other parameters were maintained at their default settings. The known crystal structure of HSA (PDB ID:1N5U, Wardell et al., 2002) was obtained from the Brookhaven Protein Data Bank (http://www.rcsb.org/pdb). The structure geometry was optimized using the Tripos force field with a steepest descent method, followed by conjugate gradient in 10^4 steps, and a convergence criterion for the energy gradient of 0.05 KJ/(mol Å). Finally, the highest-scored conformation was selected as the final bioactive conformation.

2.4. Molecular dynamics simulations

A 10 ns molecular dynamics simulation of the complex was carried out with GROMACS 4.5.5 package by using the GROMOS96 43A1 force field. The topology parameters of PAEs were built by using PRODRG (http://davapc1.bioch.dundee.ac.uk/cgi-bin/prodrg/). Then the complex was immersed in a cubic box of extended single point charge (SPC) water model. The solvated system was neutralized by adding 14 Na^+ counter ions. To release conflicting contacts, energy minimization was performed using the steepest descent method with 2000 steps. The periodic boundary condition was used and the motion equations were integrated by applying the leaf-frog algorithm with a time step of 2 fs. The atomic coordinates were recorded at every 2 ps during the simulation for latter analysis. Finally, the full system was subjected to 10 ns MD at 300 K temperature and 1 bar pressure.

3. Results and discussion

3.1. Characteristics of molecular dynamics simulation

The structural behaviors and energetic features of HSA–PAEs interaction were further analyzed based on the snapshots taken from the molecular trajectory of MD simulation. Root Mean Square Deviation (RMSD) of HSA and PAEs was examined to investigate the stability of the system (protein, ligand, ions, water, etc.). RMSD values for free HSA and HSA–PAEs complexes during 10 ns MD simulations are shown in Fig. 2(a). It is observed that the total energy for all molecular systems remains stable with some fluctuations after 4 ns of simulation, so all of the following analysis was carried out based on the stability of the simulation after the 4 ns of equilibration. The stability of the whole protein structure makes it possible to obtain reliable analysis on ligand binding and its effect on HSA–PAEs interaction through MD simulation. The relative decrease in RMSD value of the complex with respect to free HSA also indicates the conformational change, increased rigidity and stability of the protein HSA upon binding of PAEs (Ko et al., 2008). By comparing the RMSD of PAEs, it can be stated that site II is more favorable for PAEs binding to HSA.

Protein mobility was also analyzed by calculating the time-averaged root mean square fluctuation (RMSF) (Jana et al., 2012) of free HSA and HSA–PAEs complexes based on the 10 ns trajectory data (Fig. 2(b)). The general profiles of atomic fluctuations were found to be very similar to each other. The comparison of RMSF for HSA–PAEs in sub-domains IIIA, where PAEs is binding, shows that RMSF value of HSA–PAEs in sub-domains IIIA was much more than free HSA, with very little fluctuation.

The radius of gyration (*Rg*) values of free HSA and HSA–PAEs complexes are shown in Fig. 2(c). In both systems, *Rg* values were