Environmental Toxicology and Pharmacology 30 (2010) 45–51

Contents lists available at ScienceDirect

Environmental Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/etap

Probing the molecular mechanism of C.I. Acid red 73 binding to human serum albumin

Yumei Guo[a,b,1], Qinyan Yue[a,2], Baoyu Gao[a,*]

[a] School of Environmental Science and Engineering, Shandong University, No. 27 Shanda South Road, Jinan 250100, Shandong, People's Republic of China
[b] School of Chemistry and Chemical Engineering, Shandong University, Jinan 250100, People's Republic of China

ARTICLE INFO

Article history:
Received 29 December 2009
Received in revised form 13 March 2010
Accepted 16 March 2010
Available online 25 March 2010

Keywords:
C.I. Acid red 73
Human serum albumin (HSA)
Molecular mechanism
Spectroscopy
Molecular docking

ABSTRACT

The molecular mechanism of C.I. Acid red 73 binding to human serum albumin (HSA) was investigated by spectroscopy and molecular docking procedures. The molecular docking results indicated that subdomain IB of HSA was the main active binding site for C.I. Acid red 73. The spectroscopic experiment results showed that the mechanism of the interaction between C.I. Acid red 73 and HSA was dominantly initiated by complex formation and the number of binding site (n) was 1.71 at 298 K. The molecular docking study and thermodynamic analysis suggested that the forces acting was predominantly hydrophobic and hydrogen bond interactions. Far-UV circular dichroism (CD) spectroscopy also revealed that the conformation of the HSA changed slightly after C.I. Acid red 73 bound to the HSA. The mean distance between the bound dye and the Trp residue is 3.28 nm as calculated from Förster energy transfer.

1. Introduction

Azo dyes are the largest and most versatile group whose share in industrial application amounts to some 70% of all dye stuffs consumed (Gholamreza and Maryam, 2009). Recent studies indicate that approximately 12% of synthetic dyes are lost during manufacturing and processing operations and that 20% of the resultant color discharged into the environment through effluents from industrial wastewater treatment plants (Ollgaard et al., 1998). So, azo dyes might be present in relatively high concentrations in wastewaters (Saquib and Muneer, 2002). The dye-containing wastewaters pose a great threat to the environment. This consequently adversely affects the health of living beings and fertility of the soil, thereby resulting in being a source of many of man's illnesses. Moreover, some azo colorants have been related to bladder cancer in humans, to splenic sarcomas, hepatocarcinomas and nuclear anomalies in experimental animals and to chromosomal aberrations in mammalian cells (Santosa et al., 2005). Due to their potential carcinogenicity, the German government has recently banned the imports of textiles, leathers and other items dyed with azo dyes (Ding et al., 2009).

To ensure safety of hair dye products for consumers, the European Commission has banned 22 hair dye substances that could potentially cause bladder cancer if used for a long time. The ban took effect on December 1, 2006. The azo dye C.I. Acid red 73 is one of the 22 hair dye chemicals banned. Bioassay tests indicated that the LC_{50} values for Acid red 73 dye effluent are 54.76% (48 h), 48.71% (72 h) and 39.8% (96 h), respectively, and also indicated that the LC_{50} value for Acid red 73 shown higher toxicity compared to the other tested dyes (Acid red 88, Acid red 18, Acid orange 7 and Acid orange 10) (Muthukumar et al., 2005). However, to date, very little has been reported about the molecular mechanism of interactions of C.I. Acid red 73 with its target proteins. This point is important for further toxicology investigation and environmental risk assessment of C.I. Acid red 73. The molecular structure of C.I. Acid red 73 is shown in Fig. 1.

Transportation, distribution, physiological and toxicological actions of dyes in vivo is closely related to their binding with proteins. The binding of the dyes with active sites of the proteins can change their structure and function and cause toxic effects. Human serum albumin (HSA) is the principal extracellular protein as it is responsible for transporting many smaller exogenous and endogenous organic molecules, such as drugs, dyes and other physiological substances (Ding et al., 2009). The binding of dyes on the protein will change the molecular conformation and thus affect the physiological function of the protein (Ma et al., 2009). So it has great significance in studying the interaction between C.I. Acid red 73 and HSA to better understand the disposition, transportation and metabolism of C.I. Acid red 73 in vivo at the molecular level.

* Corresponding author. Tel.: +86 0531 88365258; fax: +86 0531 88365258.
E-mail addresses: gym@sdu.edu.cn (Y. Guo), qyyue@sdu.edu.cn (Q. Yue), bygao@sdu.edu.cn (B. Gao).
[1] Tel.: +86 0531 88361595; fax: +86 0531 86185990.
[2] Tel.: +86 0531 88365258; fax: +86 0531 88365258.

1382-6689/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.etap.2010.03.013

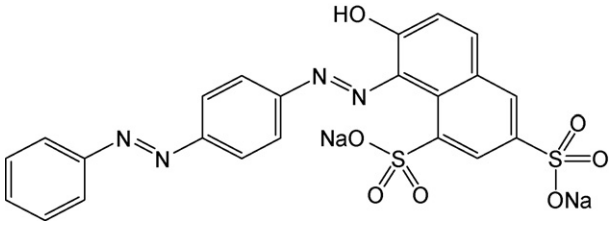

Fig. 1. Molecular structure of C.I. Acid red 73.

Fluorescence spectroscopy is an appropriate method to determine the interaction between the small molecules and biomacromolecules. By means of analysis of the fluorescence parameters, much information concerning the structural changes in biomacromolecule can be obtained (Farzaneh and Hamideh, 2009; Zhang et al., 2007).

Although the distance between the donor and acceptor can be obtained according to the Förster energy transfer theory, it is difficult to confirm the binding site of the small molecule binding to biomacromolecule. Molecular docking procedures are also suitable tools to analyze, investigate and predict the possible binding sites and to assess the microenvironment around ligand–protein binding sites to acquire a deeper insight on ligand–protein interaction mechanism from atomic and molecular levels (Yue et al., 2008; An et al., 2009; Iribarne et al., 2007).

The aim of the work was to probe molecular mechanism of C.I. Acid red 73 binding to human serum albumin by fluorescence, UV–vis, far-UV CD spectroscopy and molecular docking procedures. The mechanism of the interaction between C.I. Acid red 73 and HSA regarding thermodynamic functions, the efficiency of energy transfer between the donor and acceptor, binding distances and the effect of C.I. Acid red 73 on the protein conformation were investigated in detail. We also tried to locate the active binding sites and to construct the binding modes according to the molecular docking results. The results reported are expected to provide some useful information for further toxicology investigation and environmental risk assessment of C.I. Acid red 73 dye.

2. Materials and methods

2.1. Materials

Human serum albumin (HSA) was purchased from Shanghai Boao Biochemical Technology (Shanghai, China) and used without further purification. All HSA solutions were prepared based on its molecular weight of 66,500 and kept in the dark at 4 °C. C.I. Acid red 73 was supplied by Bin Zhou Dye Printing Co. (China) and was used without further purification. The buffer used was 0.2 mol L^{-1} sodium phosphate buffer at pH 7.40. All other materials were of analytical reagent grade and double distilled water was used throughout.

2.2. Apparatus

Fluorescence measurements were performed on a Hitachi spectrofluorimeter Model F-4500 (Japan) equipped with a 150 W Xenon lamp and a slit width of 10 nm. A 1 cm quartz cell was used for measurements. The CD measurements were made on JASCO J-810S circular dichroism spectropolarimeter (Japan) using a 0.1 cm cell at 0.2 nm intervals, with two scans averaged for each CD spectrum in the range of 200–240 nm. The absorption spectra were recorded on a double-beam UV-4100 spectrophotometer (Hitachi, Japan) equipped with a 150 W Xenon lamp and a slit width of 1 nm. Two 1-cm quartz cells were used for measurements. All pH measurements were made on a Delta 320-S pH meter (Mettler Toledo, China).

2.3. Procedures

Fluorescence spectra of HSA were recorded in the absence and presence of C.I. Acid red 73 at 288, 298 and 308 K in the range of 300–450 nm upon excitation at 280 nm. Very dilute solutions were used in the experiment (HSA 1.0×10^{-6} mol L^{-1} and C.I. Acid red 73 in the range of 0.40–1.6×10^{-6} mol L^{-1}) to avoid the inner filter effect. The CD spectra of HSA (1.0×10^{-6} mol L^{-1}) in the presence of C.I. Acid red 73 (0 and 1.6×10^{-6} mol L^{-1}) were made at 298 K in the range of 200–240 nm. Far-UV–vis spectra of HSA (1.0×10^{-6} mol L^{-1}) in the presence of C.I. Acid red 73 (0 and 1.0×10^{-6} mol L^{-1}) were recorded at 298 K in the range of 295–450 nm.

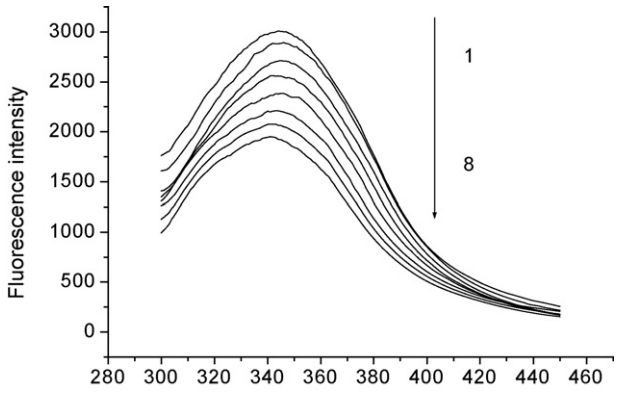

Fig. 2. Quenching effect of C.I. Acid red 73 on fluorescence spectra of HSA. Conditions: HSA: 1.0×10^{-6} mol L^{-1}; phosphate buffer at pH 7.40; C.I. Acid red 73 ($\times 10^{-6}$ mol L^{-1}, 1–8): 0, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6.

2.4. Molecular docking

Molecular docking was accomplished by means of the molecular docking software, AutoDock Version 4.0. The 3D structure of C.I. Acid red 73 was made using Gaussview 3.07. The geometry of C.I. Acid red 73 was optimized using Gaussian 03. The crystal structure of HSA (PDB Id: 1bj5) was downloaded from the Brookhaven Protein Data Bank (http://www.rcsb. org/pdb). The small molecules in the crystal structure of the 1bj5 were removed prior to the docking by the software of UCSF Chimera. The polar hydrogens and the Gasteiger charges were added to the HSA during the preparation of the protein input file. Moreover, rotatable bonds were assigned with Auto Dock Tools. Grid maps were generated with 0.375 Å spacing by the Autogrid4 program using a grid box with npts (number of points in xyz) of 70–70–70 Å, which defines the simulation space. The genetic algorithm (GA) parameters used were: number of GA run: 50, population size: 150, maximum number of energy evaluations retries and generations: 10,000 and 27,000, respectively and others used were default parameters. Genetic algorithm was implemented in SSH Shell software on Red Hat Enterprise VMware player workstation that was applied to calculate the possible conformations of C.I. Acid red 73 that binds to the protein. The docked complexes were selected according to the criteria of interacting energy combined with geometrical matching quality for further analysis. Cluster analysis was performed on the docked results using a root mean square (RMS) tolerance of 2.0 Å.

3. Results and discussion

3.1. Fluorescence quenching

The fluorescence measurements can give some information on binding mechanism of small molecule substances to protein, including binding mode, binding constants, binding sites and intermolecular distances (Liu et al., 2004). Fluorescence intensity of a compound can be decreased by a variety of molecular interactions with quencher molecules, including exciting-state reactions, molecular rearrangements, energy transfer, ground-state complex formation and collisional quenching processes (Ranjan et al., 2007). Quenching can be classified as either dynamic or static quenching by different mechanisms. Dynamic quenching results from collision between fluorophore and quencher, and static quenching is due to the formation of ground-state complex between fluorophore and quencher. In general, dynamic and static quenching can be distinguished by their different dependence on temperature and viscosity (Qi et al., 2008). The quenching rate constants decrease with increased temperature for static quenching, whereas the reverse effect is observed for dynamic quenching. The fluorescence spectra of HSA in the presence of different amounts of C.I. Acid red 73 were recorded in the range of 300–450 nm upon excitation at 280 nm (Fig. 2). It can be seen that the fluorescence intensity of HSA gradually decreased with the increased concentration of C.I. Acid red 73, indicating the binding of C.I. Acid red 73 to HSA.