Journal of Photochemistry and Photobiology B: Biology 95 (2009) 162–169

Contents lists available at ScienceDirect

Journal of Photochemistry and Photobiology B: Biology

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

Specific interaction of 4′-*O*-(a-L-Cladinosyl) daunorubicin with human serum albumin: The binding site II on HSA molecular using spectroscopy and modeling

Fengling Cui [a,*], Xiaoduo Kong [c], Lixia Qin [a], Guisheng Zhang [a,*], Qingfeng Liu [a], Beilei Lei [b], Xiaojun Yao [b]

[a] College of Chemistry and Environmental Science, Key Laboratory for Environmental Pollution Control Technology of Henan Province, Henan Normal University, Xinxiang 453007, People's Republic of China
[b] Department of Chemistry, Lanzhou University, Lanzhou 730000, People's Republic of China
[c] Vocational and Technical College of Medicine in Hebi, Hebi 458030, People's Republic of China

ARTICLE INFO

Article history:
Received 30 October 2008
Received in revised form 25 February 2009
Accepted 2 March 2009
Available online 12 March 2009

Keywords:
4′-*O*-(a-L-Cladinosyl) daunorubicin
Human serum albumin (HSA)
Fluorescence quenching
Molecular modeling
Synchronous fluorescence

ABSTRACT

This study was designed to examine the interaction of 4′-*O*-(a-L-Cladinosyl) daunorubicin (CDNR), a disaccharide anthracycline with potent antitumor activity against leukemia cell line K562 cells and colon cancer cell line SW620 cells, with human serum albumin (HSA) for the first time by fluorescence spectroscopy in combination with UV-absorption and molecular modeling under simulative physiological conditions. The quenching mechanism was suggested to be static quenching according to the fluorescence measurement. The thermodynamic parameters, enthalpy change (ΔH) and entropy change (ΔS) were calculated to be −19.89 KJ/mol and 26.23 J/mol/K, according to the Van't Hoff equation. These data suggested that hydrophobic interaction was the predominant intermolecular forces stabilizing the complex, which was in good agreement with the results of molecular modeling study. The effect of CDNR on the conformation of HSA was analyzed using synchronous fluorescence spectroscopy and UV/vis absorption spectroscopy. In addition, the effects of common ions on the binding constant of CDNR–HSA complex were also discussed at room temperature.

1. Introduction

The anthracycline quinone daunorubicin (DNR) and doxorubicin are potent antitumor agents against a variety of solid tumors [1]. So far doxorubicin is still one of the most effective drugs against a variety of human solid tumors. The major problems associated with anthracycline drugs are cardiotoxicity and drug resistance mediated by multidrug resistance gene. Many researchers have explored to modify the structure of anthracycline to generate more analogues to reduce the side effects and reverse multidrug resistance; however, these efforts only have limited success. Recently, Zhang et al. reported a novel class of disaccharide analogues of daunorubicin against drug-resistant leukemia [2,3]. In these disaccharide analogues the first (inner) sugar in the carbohydrate chain is a daunosamine and the second sugars that linked to he first sugar are a series of uncommon sugars. Of all these disaccharide anthracyclines, 4′-*O*-(a-L-Cladinosyl) daunorubicin (CDNR, Fig. 1) emerged as the most active compound, showing similar antitumor activity against leukemia cell line K562 cells and colon cancer cell line SW620 cells to the parent compound DNR [2] and at least 5-fold higher activity against drug-resistant cells (K562/Dox) than DNR [3]. The compound ADNR-3 is worthy of further evaluation as a new drug candidate.

Serum albumins are the most abundant proteins in the circulatory system of a wide variety of organisms. Being the major macromolecule contributing to the osmotic blood pressure [4], they can play a dominant role in drug disposition and efficacy [5]. Many drugs and other bioactive small molecules bind reversibly to albumin and other serum components, which then function as carriers. Serum albumin often increases the apparent solubility of hydrophobic drugs in plasma and modulates their delivery to cells in vivo and in vitro. Consequently, it is important to study the interactions of drugs with this protein. The effectiveness of drugs depends on their binding ability.

It is well known that HSA is a monomeric protein comprising 585 amino acids. And its secondary structure is mainly α-helix and 17 disulfide bridges. The initial crystal structure analyses have revealed that the principal regions of ligand binding sites of albumin are located in hydrophobic cavities in subdomains IIA and IIIA, and the sole tryptophan residue (Trp-214) is in subdomain IIA [6]. Numerous studies confirmed that the binding of small molecule substances to HSA could induce the conformational change of HSA, HSA to maintain the secondary structure which results in the conformational change of protein [7–18]. For macromolecules, fluorescence measurements can give some information of the

* Corresponding authors. Tel./fax: +86 373 3326336 (F. Cui), tel./fax: +86 373 3325250 (G. Zhang).
E-mail addresses: fenglingcui@hotmail.com (F. Cui), zgs6668@yahoo.com (G. Zhang).

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doi:10.1016/j.jphotobiol.2009.03.001

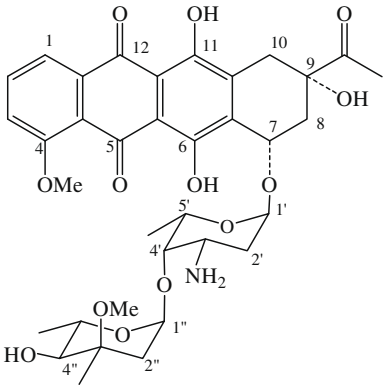

Fig. 1. The structural of CDNR.

binding of small molecule substances to protein, such as the binding mechanism, binding mode, binding constants, binding sites, intermolecular distances, etc. For HSA, there are only three intrinsic fluorophore – tryptophan, tyrosine and phenylalanine. Actually, the intrinsic fluorescence of HSA is almost contributed by tryptophan alone, because phenylalanine has a very low quantum yield and the fluorescence of tyrosine is almost totally quenched if it is ionized, or near an amino group, a carboxyl group, or a tryptophan. This viewpoint was well supported by the experimental observation coming from Sułkowska [19]. That is, the change of intrinsic fluorescence intensity of HSA is that of fluorescence intensity of tryptophan residue when small molecule substances are added to HSA. It has been shown that the distribution, free concentration and the metabolism of various drugs can be significantly altered as a result of their binding to HSA [20]. Drug interactions at protein binding level will in most cases significantly affect the apparent distribution volume of the drugs and also affect the elimination rate of drugs; therefore the studies on this aspect can provide information of the structural features that determine the therapeutic effectivity of drugs, and have been an interesting research field in life sciences, chemistry and clinical medicine.

Fluorescence spectroscopy has been widely used to investigate the interaction of drug and protein. After the protein is treated by quenchers of different concentrations, quenching of the protein intrinsic fluorescence can be used to infer the binding mechanism and to calculate the number of binding site, binding constant and binding distance from the tryptophan residues [21–23]. The manner in which the fluorescence emission spectra of the bound drug-protein affected by simultaneous presence of ligands which bind specifically on the protein, leads to use the drug as a fluorescent probe to determine the environment at the drug binding site [24,25]. However, at routine experiment conditions, only tryptophan and tyrosine amino acid residues in protein can emit fluorescence, so the information relating to protein conformational changes got from fluorescence spectra must be correlative to these two residues [26–28].

In the present paper, the mechanism of interaction between CDNR and HSA has been studied using fluorescence spectroscopic technique and molecular modeling method under physiological condition. Spectroscopic data were used to quantify the binding constants of CDNR to HSA and the action distance which was based on the Förster's energy transference (FET). Synchronous spectroscopy revealed that the change of protein structure resulted from the CDNR binding to several amino acids on the hydrophobic pocket of HSA. What is more, the interaction of the mainly acting forces and the binding site of the location were characterized by optical spectroscopy.

2. Materials and methods

2.1. Materials

Appropriate amounts of human serum albumin (Hualan Biological Engineering Limited Company) was directly dissolved in water to prepare stock solution at final concentration of 2.0×10^{-5} M and stored in the dark at 0–4 °C. 2.84×10^{-4} M CDNR, 0.5 M NaCl working solution, 0.1 M Tris–HCl buffer solution of pH 7.4 and other ionic solutions were prepared. The CDNR sample was synthesized according to the known method as a red solid and its spectra data were identical with those reported in literature [2]. All chemicals were of analytical reagent grade and were used without further purification. Double distilled water was used throughout.

2.2. Apparatus

All fluorescence spectra were recorded on an FP-6200 spectrofluorimeter (JASCO, Japan) and a RF-540 spectrofluorimeter (Shimadzu, Japan) equipped with a thermostat bath, using 5 nm/5 nm slit widths. The UV-absorption spectra were performed on a Tu-1810 ultraviolet–visible spectrophotometer (Beijing General Instrument, China). The pH values were measured on a pH-3 digital pH-meter (Shanghai Lei Ci Device Works, Shanghai, China) with a combined glass electrode. All calculations were performed on SGI workstation while studying the molecular model.

2.3. Measurements of spectrum

Under the optimum physiological conditions described above, 2.0 mL Tris–HCl buffer solution, 2.0 mL NaCl solution, appropriate amounts of HSA and CDNR were added to 10.0 mL standard flask and diluted to 10.0 mL with double distilled water. Fluorescence-quenching spectra of HSA were obtained at excitation wavelength (280 nm) and emission wavelength (300–450 nm). Fluorescence spectra in the presence of other ions were also measured at the same conditions. In addition, molecular modeling and synchronous fluorescence spectra of system were recorded.

2.4. Characteristics of synchronous fluorescence method

The synchronous fluorescence spectra were obtained by simultaneously scanning the excitation and emission monochromators. Thus, the synchronous fluorescence applied to the equation of synchronous luminescence [29]:

$$F = kcdE_{ex}(\lambda_{em} - \Delta\lambda)E_{em}(\lambda_{em}) \quad (1)$$

where F is the relative intensity of synchronous fluorescence, $\Delta\lambda = \Delta\lambda_{em} - \lambda_{ex}$ is a constant, E_{ex} the excitation function at the given excitation wavelength, E_{em} the normal emission function at the corresponding emission wavelength, c the analytical concentration, d the thickness of the sample cell, and k is the characteristic constant comprising the "instrumental geometry factor" and related parameters. Since the relationship of the synchronous fluorescence intensity (F) and the concentration of CDNR should follow the F equation, F should be in direct proportion to the concentration of CDNR.

The optimal values of the wavelength intervals ($\Delta\lambda$) are important for the correct analysis and interpretation of the binding mechanism. When the wavelength interval ($\Delta\lambda$) was fixed at 60 nm of protein, the synchronous fluorescence had the same intensity as the emission fluorescence following excitation at 280 nm, just the emission maximum wavelength and shape of the peaks were changed [30–32]. Thus, the synchronous fluorescence measurements can be applied to calculate association con-