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Interaction of 3′-azido-3′-deamino daunorubicin with human serum albumin: Investigation by fluorescence spectroscopy and molecular modeling methods

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ABSTRACT

In this Letter, the binding of 3′-azido-3′-deamino daunorubicin (ADNR) to human serum albumin (HSA) was investigated at different temperatures by fluorescence spectroscopy at pH 7.4. The binding constant was determined according to Stern–Volmer equation based on the fluorescence quenching of HSA in the presence of ADNR. The thermodynamic parameters, ΔH and ΔS, were calculated according to the dependence of enthalpy change on the temperature to be -21.01 kJ mol^{-1} and 24.71 J K^{-1} mol^{-1}, respectively. The results revealed that ADNR had a strong ability to quench the intrinsic fluorescence of HSA through a static quenching procedure. The hydrophobic force played a major role in the interaction of ADNR with HSA, which was in good agreement with the results of molecular modeling study. The effect of various metal ions on the binding constants of ADNR with HSA was also investigated. All the experimental results and theoretical data indicated that ADNR could bind to HSA and be effectively transported and eliminated in body, which might be a useful guideline for further drug design.

Anthracyclines, such as daunorubicin and doxorubicin, are considered to be some of the most effective anticancer drugs for cancer therapy. However, drug resistance and cardiotoxicity of anthracyclines limit their clinical application. In our previous research[1], a daunorubicin analogue, 3′-azido-3′-deamino daunorubicin (ADNR, Fig. 1), were synthesized by directly transforming the amino group of daunorubicin to an azido group. ADNR exhibited potent anticancer activity in both drug-sensitive (K562) and drug-resistant leukemia cells (K562/Dox), with a 25-fold lower drug resistance index than parent compound daunorubicin. An in vivo xenograft model demonstrated that ADNR showed more than 2.5-fold higher maximum growth inhibition rate against drug-resistant cancers and significant improvement for animal survival rate versus daunorubicin. No significant body weight reduction in mice was observed for ADNR at the maximum tolerable dose, as compared to more than 70% body weight reduction for daunorubicin.[1] The compound ADNR is worthy of further evaluation as a new drug candidate.

It has been shown that the distribution, free concentration and the metabolism of various drugs may be strongly affected by drug–protein interactions in the blood stream. This type of interaction can also influence the drug stability and toxicity during the chemotherapeutic process. Serum albumins have many physiological functions and play a dominant role in drug disposition and efficacy.[2,3] Many drugs and other small bioactivity molecules bind reversibly to albumin and other serum components that then function as carriers. Consequently, it is important to know the affinity of a drug to serum albumin, even if it is not the only factor to predict serum concentrations of the free drug.

Serum albumins have been the most studied proteins for many years. They are the most abundant protein in blood plasma, accounting for about 60% of the total protein as a concentration of 42 g L^{-1} [4,5] and provide about 80% of the osmotic pressure of blood.[4] Recently, the three-dimensional structure of human serum

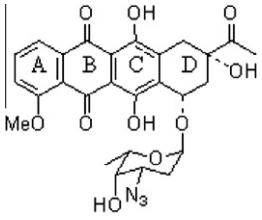

Fig. 1. The structure of ADNR.

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albumin (HSA) has been determined through X-ray crystallographic measurements.[2] The globular protein consists of a single polypeptide chain of 585 amino acid residues and has many important physiological functions.[6,7] There are typical sites of coordination for several substances such as amino acids, fatty acids, hormones, and drugs.[5] The multiple binding sites underlie the exceptional ability of HSA to interact with many substances and make this protein an important regulator of intercellular fluxes and pharmacokinetic behavior of many drugs.

In the present work, the binding of ADNR to HSA was studied by using fluorescence, UV–vis absorption spectrum and molecular modeling methods. The nature of drug binding to protein was described. The effect of the energy transfer was studied according to the Förster theory of non-radiation energy transfer.

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. 3.67×10^{-4} M ADNR was dissolved in *N*,*N*-dimethylformamide (DMF), 0.5 M NaCl working solution, 0.1 M Tris–HCl buffer solution of pH 7.4 and other ionic solutions were prepared. All chemicals were of analytical reagent grade and were used without further purification. Double distilled water was used throughout.

All fluorescence spectra were recorded on a FP-6200 spectrofluorimeter (JASCO, Japan) and a RF-540 spectrofluorimeter (Shimadzu, Japan) equipped with a thermostat bath, using 5/5 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 in the molecular model study.

ADNR was synthesized starting from daunorubicin hydrochloride according to the known method[1] as a red solid in 70% yield: HRMS $(M+Na)^+$ (ESI) calcd for $C_{27}H_{27}N_3O_{10}Na^+$ 576.1589, found 576.1612; ^{1}H NMR (500 MHz, $CDCl_3$) 13.95 (1H, s, HO-6), 13.19 (1H, s, HO-11), 7.98 (1H, d, *J* = 7.4 Hz, H-1), 7.74 (1H, t, *J* = 8.2 Hz, H-2), 7.36 (1H, d, *J* = 8.4 Hz, H-3), 5.54 (1H, d, *J* = 3.6 Hz, H-1′), 5.23 (1H, d, *J* = 1.9 Hz, H-7), 4.37 (1H, s, HO-9), 4.10 (1H, m, H-5′), 4.05 (3H, s, MeO-4), 3.69 (1H, br, H-4′), 3.60 (1H, m, H-3′), 3.15 (1H, dd, *J* = 1.6 Hz, *J* = 18.8 Hz, Ha-10), 2.87 (1H, d, *J* = 18.8 Hz, Hb-10), 2.38 (3H, s, H-14), 2.28 (1H, m, Ha-8), 2.09 (2H, m, Hb-8, Ha-2′), 1.91 (1H, m, Hb-2′), 1.30 (3H, d, *J* = 6.6 Hz, H-6′); ^{13}C NMR (125 MHz, $CDCl_3$) 211.5, 186.9, 186.7, 161.1, 156.3, 155.7, 135.7, 135.5, 134.2, 133.9, 120.8, 119.8, 118.5, 111.5, 111.3, 100.6, 76.7, 70.1, 69.5, 67.1, 56.8, 56.7, 34.9, 33.3, 28.5, 24.7, 16.8.

For the fluorescence measurement, 2.0 mL Tris–HCl buffer solution, 2.0 mL NaCl solution, appropriate amounts of HSA and ADNR 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). The UV absorption and synchronous fluorescence spectra of the above system were also recorded. In addition, fluorescence spectra in the presence of other ions were measured at the same conditions.

The synchronous fluorescence spectra were obtained by simultaneously scanning the excitation and emission monochromators.[8] When the wavelength interval ($\Delta\lambda$) was fixed at 60 nm, 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.[9–11] Thus, the synchronous fluorescence measurements can be applied to calculate association constants similar to the emission fluorescence measurements and deduce the binding mechanism. In this study, the synchronous fluorescence spectra of tyrosine residues and tryptophan residues were measured at λ_{em} = 280 nm ($\Delta\lambda$ = 15 and 60 nm) in the absence and in the presence of various amounts of ADNR.

The potential of the 3D structures of HSA was assigned according to the Amber 4.0 force field with Kollman-all-atom charges. The initial structures of all the molecules were generated by molecular modeling software SYBYL 6.9.1. The geometries of this drug were subsequently optimized using the Tripos force field with Gasteiger–Marsili charges. The AutoDock3.05 program was used to calculate the interaction modes between the drug and HSA. Lamarckian genetic algorithm (LGA) implemented in Autodock was applied to calculate the possible conformation of the drug that binds to the protein. During docking process, a maximum of 10 conformers was considered for the drug. The conformer with the lowest binding free energy was used for further analysis. All calculations were performed on SGI FUEL workstation.

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.[12] Numerous studies confirmed that the binding of small molecule substances to HSA could induce the conformational change of HSA, because the intramolecular forces involved to maintain the secondary structure could be altered, which results in the conformational change of protein.[13–24] Actually, the intrinsic fluorescence of HSA is almost contributed by tryptophan alone.[25] The change of intrinsic fluorescence intensity of HSA is that of fluorescence intensity of tryptophan residue when small molecule substances are added to HSA. The fluorescence quenching spectra of HSA at various concentrations of ADNR are shown in Figure 2. Obviously, HSA had a strong fluorescence emission band at 342 nm by fixing the excitation wavelength at 280 nm, while ADNR had no intrinsic fluorescence. The fluorescence intensity of HSA decreased regularly, and slight blue shift was observed for the emission wavelength with increasing ADNR concentration. Therefore, we inferred that the binding took place near Trp-214 and led to a conformational change with a local perturbation of the IIA binding site in HSA.

The synchronous fluorescence spectra present the information about the molecular microenvironment in the vicinity of the fluorophore functional groups. In the synchronous fluorescence of HSA, the shift in position of maximum emission wavelength corresponds to the changes of polarity around the fluorophore of amino acid residues. The $\Delta\lambda$ values (scanning interval, $\Delta\lambda = \lambda_{em} - \lambda_{ex}$)

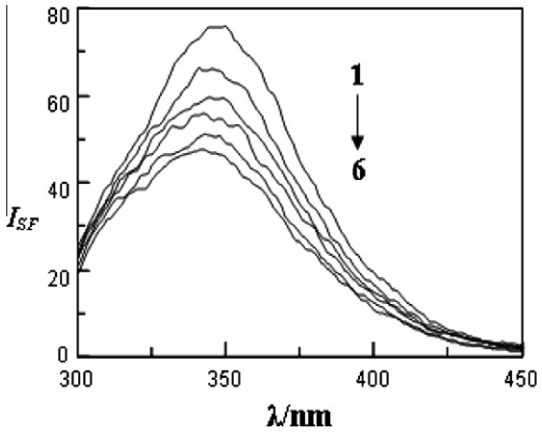

Fig. 2. The fluorescence spectra of ADNR–HSA system. From 1 to 6: $C_{HSA} = 8.0 \times 10^{-7}$ M; C_{ADNR} = 0, 1.8, 3.6, 5.4, 7.2, 9.0 $\times 10^{-6}$ M.