Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 132 (2014) 786–794

Contents lists available at ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

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

Study on the interaction between bovine serum albumin and 4′-azido-2′-deoxyfluoroarabinocytidine or analogs by spectroscopy and molecular modeling

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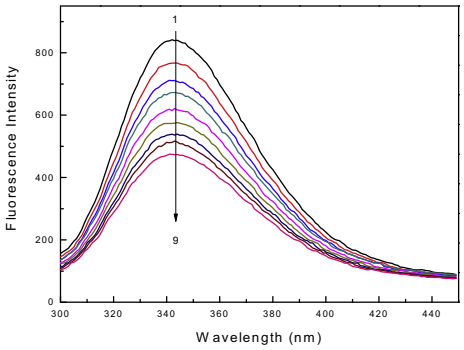
HIGHLIGHTS

- The interactions between BSA and FNC or analogs have been investigated.
- Hydrophobic interactions play major role in the binding process.
- The influence of molecular structure on the binding aspects has been investigated.
- Molecular docking was also applied in the binding study.

GRAPHICAL ABSTRACT

The fluorescence spectra of BSA in the absence and presence of FNC.



ARTICLE INFO

Article history:
Received 17 April 2014
Received in revised form 12 May 2014
Accepted 25 May 2014
Available online 11 June 2014

Keywords:
Bovine serum albumin
FNC
Analogs
Spectroscopy

ABSTRACT

The binding of 4′-azido-2′-deoxyfluoroarabinocytidine (FNC) or analogs (cytidine and 5′-cytidylate monophosphate) to bovine serum albumin (BSA) was investigated by fluorescence, UV–vis absorption spectroscopy and molecular modeling. The three compounds quenched the intrinsic fluorescence of BSA and the results revealed the presence of static quenching mechanism. The positive ΔH and positive ΔS for the systems suggested that the hydrophobic forces stabilized the interaction between the compounds and protein. Results also showed that FNC was the weakest quencher.

Introduction

Proteins are the most abundant macromolecules in cells and are crucial to maintaining normal cell functions. The serum albumin, one of the most abundant proteins, plays an important role in the transport and deposition of a variety of endogenous and exogenous ligands in blood [1]. In this paper, bovine serum albumin (BSA) was selected as the protein model because of its low cost, ready availability, and unusual ligand-binding properties. BSA is constituted by 582 amino acid residues. On the basis of the distribution of the disulfide bridges and of the amino acid sequence it seems possible to regard BSA as composed of three linearly arranged, structurally distinct, and evolutionarily related domains I, II and III. Further, each domain is subdivided into two

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http://dx.doi.org/10.1016/j.saa.2014.05.090
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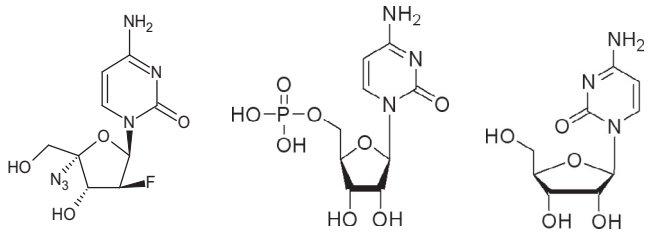

Fig. 1. The structure of 4′-azido-2′-deoxyfluoroarabinocytidine or 2′-deoxy-2′-β-fluoro-4′-azidocytidine (FNC), Cytidine (CDPC) and 5′-cytidylate monophosphate (5′-CMP).

subdomains A and B. Meanwhile, BSA has two tryptophan residues that possess intrinsic fluorescence. Trp-212 is located within a hydrophobic binding pocket of the protein and Trp-134 is located on the surface of the molecule [2].

Human cancer remains a major public health problem worldwide, and right now, tremendous effort has been directed to the discovery and development of novel agents for the treatment of human cancer and great successes have been achieved. So far, several nucleoside analogues have been successfully used as anticancer drugs. 4′-azido-2′-deoxyfluoroarabinocytidine or 2′-deoxy-2′-β-fluoro-4′-azidocytidine (FNC, Fig. 1) is a novel cytidine analogue [3]. Recent studies have demonstrated that FNC is a highly potent and selective deoxycytidine inhibitor, which has anticancer activity and been found to suppress the secretion of the HBV antigens in a dose-dependent manner and hepatitis C virus (HCV) [4,5]. Cytidine (CDPC, Fig. 1) is one of the pyrimidine nucleoside constitute a nucleic acid, which is mainly used for acute craniocerebral trauma and disturbance of consciousness after brain surgery. Cytidine monophosphate (5′-CMP, Fig. 1) is a structural portion of RNA, which can be used as production of nucleic acid drugs intermediates, health food, biochemical reagents and biochemical drugs.

Study of drug–protein interaction has great significance in discovering pharmacokinetic and pharmaco-dynamics implications, which is an essential step for a new drug design. Investigation of the interaction is helpful for revealing the transportation and distribution of the drugs in vivo, explaining the toxicity at the molecular level [6]. Fluorescence assay has been widely applied to study the interaction because of some advantages such as high sensitivity, high selectivity and easy operation. In this work, for the first time the interaction between BSA and FNC (analogs) has been studied by fluorescence spectroscopy, UV–vis absorption spectroscopy and molecular modeling method.

Experimental section

Materials

BSA (Fraction V, heatshock isolation, >99%) was obtained from Sigma–Aldrich. FNC was synthesized in our lab. Both CDPC (99%) and 5′-CMP (98%) were obtained from Aladdin Chemistry Co., Ltd. BSA was directly dissolved in double distilled water to prepare stock solutions (1.0×10^{-4} mol L^{-1}) which were then stored at 0–4 °C. Stock solutions (0.02 mol L^{-1}) of FNC or analogs (CDPC and 5′-CMP) were dissolved in double distilled water. 0.5 mol L^{-1} NaCl solution was used to keep the ion strength of solutions. 0.1 mol L^{-1} Tris–HCl buffer was selected to keep the pH of the solution at 7.40. All chemicals were of analytical grade and were used without further purification. Double-distilled water was used throughout.

Apparatus

Fluorescence spectra were carried out on a 970-CRT Spectrofluorimeter (San Ke, Shanghai, China) equipped with a 1 cm quartz cell and a thermostat bath. The excitation and emission slit widths were set at 5.0 nm. Absorption spectra were acquired in an Agilent 8453 UV–visible spectrophotometer. The pH values were measured by a pH-3 digital pH-meter (Lei Ci, Shanghai) with a combined glass electrode.

Experimental

Fluorescence measurement

In titration experiments, the concentration of protein was kept fixed at 8.00×10^{-6} mol L^{-1} while that of FNC or analogs (CDPC and 5′-CMP) were varied from 0 to 3.2×10^{-5} mol L^{-1} in a total volume of 2.0 mL. Fluorescence spectra were recorded in the range of 300–450 nm for BSA (λ_{ex} = 290 nm). Quenching experiments were carried out at 302 and 317 K, respectively. Synchronous fluorescence spectra were recorded with $\Delta\lambda$ = 15 nm and 60 nm ($\Delta\lambda = \lambda_{em} - \lambda_{ex}$) in the absence and presence of compounds and the spectra were recorded in the range of 280–400 nm. The three-dimensional fluorescence spectra of BSA were recorded by scanning excitation wavelength in the range of 230–305 nm, and emission wavelength in the range of 280–460 nm at an interval of 5 nm, respectively.

Absorbance measurements

The UV–vis absorption spectra of BSA, FNC (or analogs) and their mixture were obtained in the range of 190–350 nm at room temperature, respectively.

Molecular modeling study

The PDB entry of the BSA crystal structure employed in docking study was 1H9Z. The structure of FNC, CDPC and 5′-CMP was optimized using Gaussian 09 program [7]. Molecular docking simulations were performed with the software package AutoDock4.2 [8], in which the Lamarckian Genetic Algorithm was applied. The program was used to calculate the interaction modes between FNC (CDPC or 5-CMP) and BSA. Geometry optimizations were carried out using the hybrid B3LYP [9] functional together with the 6–31 g (d, p) basis set for C, H, O, F and N atoms. A grid map of 126 × 78 × 126 grid points in size with a grid-points pacing of 0.708 Å was created for the two proteins. According to the binding energy and the geometry matching after 250 runs, the most favorable docking model was selected for further analysis.

Results and discussion

Fluorescence quenching spectra

Fluorescence quenching is the decrease of the quantum yield of fluorescence from a fluorophore induced by a variety of molecular interaction which including excited state reactions, molecular rearrangements, energy transfer, ground state complex formation, and collisional quenching. Such decrease in intensity of fluorescence is called fluorescence quenching. For macromolecules, the fluorescence measurements can give some information of the binding of small molecule substances to proteins, such as the binding mechanism, binding mode, binding constants, binding sites, and intermolecular distances [10].

The fluorescence emission spectra of BSA in the presence of FNC or analogs at 302 K were shown in Fig. 2. BSA had a strong fluorescence emission band with a peak at 342 nm by fixing the excitation