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Arsenic trioxide binding to serum proteins

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ABSTRACT

Arsenic trioxide (ATO) also known as Trisenox, is an anticancer chemotherapeutic drug which has been used in treating diagnosed and relapsed patients with acute promyelocytic leukemia (APL). Serum albumin is the most abundant of the proteins in blood plasma and is the major transporter for delivering several drugs in vivo. The current study was designed to evaluate the potential ability of human and bovine serum albumin for delivering arsenic trioxide. Therefore, interaction of arsenic trioxide with HSA and BSA was investigated in aqueous solution at physiological conditions using a constant protein concentration and various drug contents. FTIR and UV–Vis spectroscopic methods were used to analyze arsenic trioxide and protein binding modes, the binding constants and the effect of drug complexation on HSA and BSA stability and conformation. Results of this study showed that drug complexation altered protein conformation by major reduction of α-helix and increase of turn structure which is indicative of a partial protein destabilization. Structural analysis revealed that arsenic trioxide bind HSA and BSA with overall binding constants of $K_{ATO\text{-}HSA} = 1.07\ (\pm 0.01) \times 10^4\ M^{-1}$ and $K_{ATO\text{-}BSA} = 1.27(\pm 0.02) \times 10^4\ M^{-1}$. It could be concluded that serum albumins can be considered as good carriers for delivering arsenic trioxide to target tissue.

1. Introduction

Arsenic trioxide; ATO (As_2O_3) is an antineoplastic (anti-cancer) drug which was approved by FDA for treatment of refractory acute promyelocytic leukemia (APL). It has shown preliminary activity in patients with relapsed/refractory multiple myeloma and substantial efficacy in treating both newly diagnosed and relapsed patients [1–4]. As a single agent, it induces complete remissions, causing few adverse effects and only minimal myelosuppression. ATO affects numerous intracellular signal transduction pathways and causes alterations in cellular function [5]. This action is related to induction of apoptosis, inhibition of growth and angiogenesis, and promotion of differentiation [6,7]. Such effects have been observed in cultured cell lines and animal models, as well as clinical studies. Preclinical studies of As_2O_3 and arsenical-based cancer drugs have shown antitumor activity in murine solid tumor models, including breast, cervix, brain, liver, gastric, prostate, renal, and bladder cancer [8–10].

Although the mechanism of arsenic trioxide action is not completely understood, substantial data show that arsenic trioxide produces remissions in patients with APL at least in part through a mechanism that results in degradation of the aberrant PML-arsenic trioxide receptor-fusion protein [11]. It also appears to correct the gene responsible for making flawed protein (called PML–RAR fusion protein) that causes acute promyelocytic leukemia (APL) [12].

Serum albumins are constituents of circulatory system and have many physiological functions [13]. The most important property of this group of proteins is that they serve as transporters for a variety of compounds. BSA has been one of the most extensively studied of this group of proteins. BSA and HSA molecules are made up of three homologous domains (I, II, III) which are divided into nine loops (L_1–L_9) by 17 disulfide bonds [14–18]. The loops in each domain are made up of a sequence of large–small–large loops forming a triplet. Each domain in turn is the product of two subdomains (IA, IB, etc.) [15,18]. X-ray crystallographic data show that albumin structure is predominantly α-helical with the remaining polypeptide occurring in turns and in extended or flexible regions between subdomains with no β-sheets. BSA has two tryptophan residues that possess intrinsic fluorescence [19,20], Trp-134 in the first domain and Trp-212 in the second domain. Trp-212 is located within a hydrophobic binding pocket

Abbreviations: ATO, Arsenic trioxide; FTIR, Fourier transform infrared spectroscopy.

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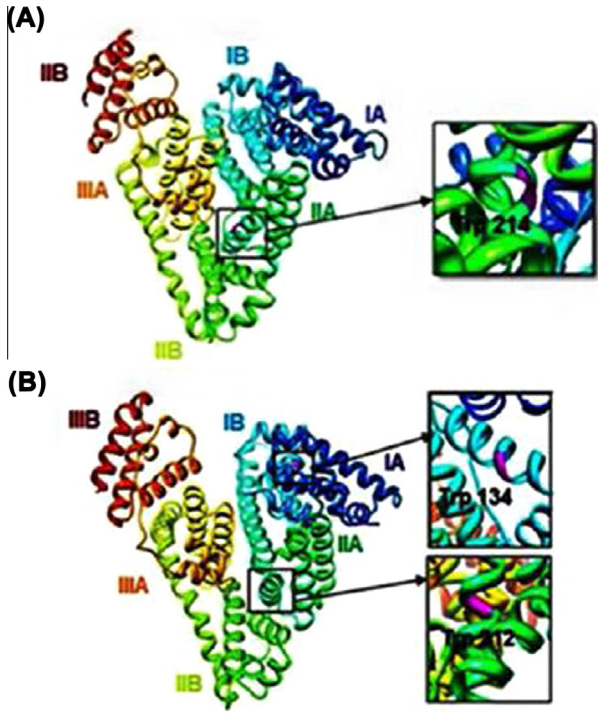

Scheme 1. Three-dimensional structures of serum albumins, (A) HSA and (B) BSA.

of the protein and Trp-134 is located on the surface of the molecule. While there are marked similarities between HSA and BSA in their compositions, HSA has only one tryptophan residue; Trp-214. Drug-protein interaction may result in the formation of a stable drug-protein complex [21]. Resulting complex can exert important effects on distribution, biological activity and metabolism of the drug in blood stream [22,23] (Scheme 1).

The molecular mechanism and specificity mediating the antitumor effects of As_2O_3 is not fully understood so far and little is known about the interaction of As_2O_3 with HSA and BSA. Therefore, it was of interest to study the interaction of As_2O_3 with HSA and BSA in an attempt to characterize the nature of As_2O_3 protein complexation. We report the spectroscopic analysis of HSA and BSA complexes with As_2O_3 in aqueous solution at physiological conditions using Fourier transform infrared (FTIR) and UV methods. The interaction was performed at various drug concentrations (1 μM–1 mM) while keeping the protein concentration constant (0.125 mM). Structural information regarding ATO binding mode and the effects of drug-protein complexation on the stability and conformation of HSA and BSA are also reported here.

2. Material and methods

2.1. Materials

Human and bovine serum albumins fraction V were purchased from sigma chemical Co and used as supplied. Arsenic trioxide was from Merck Chemical Co (Rahway, NJ). Other chemicals were of reagent grade and used without further purification [24].

2.2. Preparation of stock solutions

Human and bovine serum albumins (40 mg/mL or 0.5 mM) were dissolved separately in aqueous solution containing phosphate buffer (pH 7.2). Protein concentration was determined spectrophotometrically using an extinction coefficient of 36,500 M^{-1} cm^{-1} at 280 nm [25]. Arsenic trioxide was dissolved in NaOH (0.1 N) at different concentrations (0.015, 0.062, 0.125 and 0.5 mM). Mixtures of drug with HSA and BSA were prepared by adding arsenic trioxide dropwise to HSA and BSA solutions with constant stirring to give the desired drug-HSA and -BSA molar ratios of 0.015, 0.062, 0.125 and 0.5 mM at a final HSA and BSA concentration of 4% w/v or 0.5 mM. pH of the solution was adjusted between 6 and 7 with NaOH (0.1 N). IR spectra were recorded 1 h after initial mixing of the drug with HSA or BSA solution.

2.3. FTIR spectroscopy measurements

Infrared spectra were recorded on a Jasco FTIR spectrometer (Japan, Tokyo) equipped with a liquid-nitrogen-cooled HgCdTe (MCT) detector and a KBr beam splitter, using AgBr windows. Solution of drug was added dropwise to the protein solution with constant stirring to ensure the formation of a homogenous solution and to reach the drug concentrations of 0.015, 0.062, 0.125 and 0.5 mM with a final protein concentration of 0.5 mM (40 mg/mL). Interferograms were accumulated over the spectral range 4000–400 cm^{-1} with a nominal resolution of 4 cm^{-1} and 100 scans. Spectra were collected and treated using Spectra Manager software supplied by manufacturer of the spectrophotometer. Solution spectra were collected after 2 h incubation of ATO with HSA and BSA solutions, using AgBr windows. Difference spectra [(protein solution + drug solution) – (protein solution)] were generated using the polypeptide antisymmetric and symmetric C—H stretching bands [26], located at 2900–2800 cm^{-1} as internal standard [27]. These bands, which are due to protein C—H stretching vibrations do not undergo any spectral changes (shifting or intensity variation) upon drug interaction and therefore are commonly used as internal standard. When producing difference spectra, these bands were adjusted to the baseline level in order to normalize difference spectra.

2.4. UV–Vis spectroscopy measurements

Absorption spectra were recorded on a double beam Jasco/V-530 spectrophotometer, using a slit of 5 nm and a scan speed of 250 nm min^{-1}. Quartz cuvettes of 1 cm were used and absorption spectra recorded with drug concentrations of 1 μM–1 mM and protein concentration of 12.5 μM in range of 200–400 nm [28].

3. Theory and calculation

3.1. Analysis of protein conformation

Analysis of the secondary structure of HSA and BSA and the complexes of ATO-proteins were carried out based on the procedure previously reported [29]. Protein secondary structure is determined from the shape of the amide I band located around 1660–1650 cm^{-1} which is due almost entirely to C=O stretching vibrations of the peptide linkages. FT-IR spectra were smoothed and their baselines were corrected automatically using Jasco software. Thus, the root-mean square (rms) noise of every spectrum was calculated. By means of the second derivative in the spectral region 1700–1600 cm^{-1}, major peaks for HSA and BSA and the complexes were resolved. The above spectral region was deconvoluted by curve-fitting method, the peaks corresponds to α-helix (1658–1656 cm^{-1}), β-sheet (1640–1610 cm^{-1}), turn (1670–1665 cm^{-1}), random coil (1648–1641 cm^{-1}) and β-antiparallel (1692–1680 cm^{-1}) were adjusted and the area was measured with the Gaussian function. The area of all component