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Spectroscopic studies on H_2O_2 damaging BSA induced by 1,2-dihydroxy-9, 10-anthraquinone-3-aminomethyl-*N*, *N*-diacetate-Ferrous(III)

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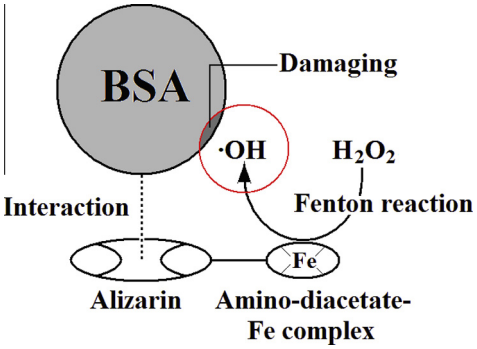
HIGHLIGHTS

- Iron amino acid complexes were used to study the interaction and damage to BSA.
- Oxidative damage of the Fe(III)/H_2O_2 system to BSA were studied at first.
- Some quenchers were used to determine the kind of the generated ROS.

GRAPHICAL ABSTRACT

The interaction between bovine serum albumin (BSA) and Alizarin–DA–Fe (1,2-dihydroxy-9, 10-anthraquinone-3-aminomethyl-*N*, *N*-diacetate-Ferrous(III)) as well as the induced H_2O_2 damage to BSA by Alizarin–DA–Fe were studied by using UV–vis and fluorescence spectra. The results showed that the fluorescence quenching process of BSA caused by Alizarin–DA–Fe belongs to the static quenching. Otherwise, in the presence of H_2O_2 in aqueous solution the BSA molecules were obviously damaged by Alizarin–DA–Fe. The experimental results demonstrate that the damage degree increase with the increase of standing time, Alizarin–DA–Fe concentration and H_2O_2 concentration. Finally, the generation of reactive oxygen species (ROS) induced by Alizarin–DA–Fe as Fenton-like reagent was estimated by some quenchers.



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ABSTRACT

The interaction between 1,2-dihydroxy-9, 10-anthraquinone-3-aminomethyl-*N*, *N*-diacetate-Ferrous(III) (Alizarin–DA–Fe(III)) and bovine serum albumin (BSA) was studied by using UV–vis and fluorescence spectra. And then, the H_2O_2 damage of BSA induced by Alizarin–DA–Fe(III) was examined. The results show that due to the interaction the fluorescence of BSA solution can be obviously quenched by Alizarin–DA–Fe(III) and that the quenching process belongs to the static quenching. In addition, in the presence of Alizarin–DA–Fe(III) the BSA molecules were markedly damaged by H_2O_2. Meanwhile, the effects of the standing time, Alizarin–DA–Fe(III) concentration and H_2O_2 concentration on the damage of BSA molecules were also researched. The experimental results demonstrate that the damage degree increase with the increase of standing time, Alizarin–DA–Fe(III) concentration and H_2O_2 concentration. Finally, the generation of reactive oxygen species (ROS) from H_2O_2 induced by Alizarin–DA–Fe(III) as Fenton-like reagent was estimated by some quenchers. Because the Imino-diacetic–Ferrous(III) (IDA–Fe(III)) and Nitrilotriacetic–Ferrous(III) (NTA–Fe(III)) can be thought of as the active part of Alizarin–DA–Fe(III), they were used to compare the catalytic activity with Alizarin–DA–Fe(III). Owing to the special plane structure, the experiment results showed that the Alizarin–DA–Fe(III) exhibited higher damage ability than IDA–Fe(III) and NTA–Fe(III). Perhaps, the Alizarin–DA–Fe(III) may be used as a new antitumor compound to induce peroxides in body to kill cancer cells.

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Introduction

1,2-Dihydroxy-9, 10-anthraquinone (Alizarin) is a kind of anthraquinone fluorescent dye [1,2]. Because of the plane structural features, Alizarin displays a natural antitumor activity [3–6]. Moreover, as an Anthraquinoid compound, 1,2-dihydroxy-9, 10-anthraquinone-3-aminomethyl-*N*, *N*-diacetic acid (Alizarin–DA) may display a better antitumor activity due to the targeted selectivity and biological compatibility of amino acid groups.

Serum albumin is the most abundant protein in human or animal plasma, and albumin concentration in plasma is an indicator of health and disease. In addition, serum albumin is one of targets for many drugs [7], especially for anticancer drugs, so the research of the interaction or combination of drug with serum albumin is the important content in pharmacokinetics and clinical pharmacology. Considering the high homology degree of Bovine serum albumin (BSA) and human serum albumin (HSA), as well as the low prices and rich source of BSA, it is often used as a kind of model protein to be instead of HSA in the drug development and protein study system [8,9].

It has been well known that, in the process of tumor growth, the abnormal metabolism generally tends to generate some peroxides (for example: hydrogen peroxide (H_2O_2)). If these peroxides are not removed or utilized in time, they may damage the other normal tissues and cause lesions. And if they are used properly, it may also have the effect of killing tumor cells. H_2O_2 is a medium strong oxidant (standard electrode potential: 1.80 and 0.87 V at pH 0 and 14, respectively, Degussa Corporation) and its application in the treatment of various inorganic and organic pollutants is well established [10]. Therefore, numerous applications of H_2O_2 in the removal of pollutants from wastewater (such as sulphites, hypochlorites, nitrites, cyanides and chlorine) are known [11]. In addition, H_2O_2 is also one of major components of Fenton reagent. Although Fenton reagent was discovered over 100 years, its application in an oxidizing process for destroying toxic organics was not applied until late 1960s [11,12]. In recent years, some researches have been focused on the damage of biomolecules (such as BSA) by using Fenton like reaction process. As we known, the valence of iron is much easy to change to form different complexes with oxygen or nitrogen ligands. For example, most of the iron which is surrounded by the protein form the chelate compounds tightly. And such trivalent iron performs specific functions in human plasma which usually alters the valence state. Of which the Fenton like reaction generating from the reaction between the iron complex and H_2O_2 in body is essential for normal metabolism. We can use this mechanism to make further research on the treatment of tumors by the new iron complexes in the presence of H_2O_2. Alizarin–DA has the special construction and composition, so the iron complex with Alizarin–DA as a ligand, 1,2-dihydroxy-9, 10-anthraquinone-3-aminomethyl-*N*, *N*-diacetate-Ferrous(III) (Alizarin–DA–Fe), was prepared and used as a new Fenton like reagent, inducing H_2O_2 generating reactive oxygen species (ROS) to damage some biomolecules.

For ease of comparison studies, as a part of Alizarin–DA–Fe(III) in structure and composition the Iminodiacetic–Ferrous(III) (IDA–Fe(III)) and Nitrilotriacetic–Ferrous(III) (NTA–Fe(III)) were also used to investigate the interaction and damage to BSA molecules due to their antitumor activity reported early in the literature [13,14]. This paper mainly researches the interaction of IDA–Fe(III), NTA–Fe(III) and Alizarin–DA–Fe(III) complexes with BSA molecules. And then, H_2O_2 was added and the damage of BSA molecules induced by these Iron complexes was reviewed. For convenience, the Iron amino acid complexes were used as the full name of them. At the same time, the reactive oxygen radicals (ROS) generated during the Fenton like reaction process was also identified.

Experimental

Reagents and apparatus

Bovine serum albumin (BSA, biochemical reagent, Aoboxing Biotechnological Company, China) was acted as a model protein to study the interaction and damage. Iminodiacetic acid (IDA) was chemical reagent grade and obtained from Huzhou Biological chemical plant (China). Nitrilotriacetic acid (NTA) was analytical reagent grade and obtained from Shenyang New West reagent plant (China). 1,2-dihydroxy-9, 10-anthraquinone-3-aminomethyl-*N*, *N*-diacetic acid (Alizarin–DA) were analytical reagent grade and obtained from Suzhou Sinoera Chem Co., Ltd. (China). Ferric(III) nitrate ($Fe(NO_3)_3 \cdot 9H_2O$, analytical reagent grade) was purchased from Tianjing Tianhe Chemical Reagent Co., Ltd. (China). 1,5-Diphenyl carbazide (DPCI, purity > 99.0%), Dimethylsulfoxide (DMSO, purity > 99.0%), Thiourea (TU, purity > 99.0%), Mannitol (MT, purity > 99.0%) and Hydrogen peroxide (content ≈ 30%) were purchased from Sinopharm Chemical Reagent Co., Ltd. (China). All other chemical reagents were commercial products of analytical grade and used as received. Double distilled water was used throughout experiment.

UV–vis spectrum apparatus (Cary-50, Varian Company, USA) and fluorescence spectrometer (Cary-300, Varian Company, USA) were adopted to track the change of the mixed solutions of BSA and Iron amino acid complexes (IDA–Fe(III), NTA–Fe(III) and Alizarin–DA–Fe(III)). The solution pH value was measured with a pH meter (PHS-3C, Shanghai Leici Instrument Company, Ltd., China).

Measurement of binding parameters and binding sites

BSA storage solution (2.00×10^{-5} mol L^1) was prepared by dissolving BSA with Tris–HCl–NaCl buffer solution and then stored in refrigerator at 0–4 °C. Hydrogen peroxide solution (50×10^{-5} mol L^{-1}) prepared by dissolving hydrogen peroxide (30%) with double distilled water. The Iron amino acid complex solutions were prepared by directly mixing $Fe(NO_3)_3 \cdot 9H_2O$ with IDA, NTA and Alizarin–DA in aqueous solution at the iron-to-ligand molar ratio (1:1) with stirring for 1.0 h. The storage solutions of Iron amino acid complexes with 5.00×10^{-5} mol L^{-1} were obtained by diluting the above mixing solutions with Tris–HCl–NaCl buffer solution. The proposed molecular structures of Iron amino acid complexes (IDA–Fe(III), NTA–Fe(III) and Alizarin–DA–Fe(III)) were shown in Fig. 1.

In a typical fluorescence measurement, six 12.50 mL BSA storage solutions were taken exactly and put into six cleanly 25.00 mL volumetric flasks in order. And then, a series of different volumes of Iron amino acid complex (IDA–Fe(III), NTA–Fe(III) or Alizarin–DA–Fe(III)) solutions were also added to the above flasks in order, and the concentration was varied from 0.00×10^{-5} mol L^{-1} to 2.50×10^{-5} mol L^{-1} at increment of 0.50×10^{-5} mol L^{-1}. The final concentration of BSA solution is 1.00×10^{-5} mol L^{-1}. All mixtures were diluted to the mark with Tris–HCl–NaCl buffer solution. The corresponding fluorescence emission spectra were recorded in the wavelength range 250–500 nm by exciting the BSA at 278 nm using a slit width of 5.0 nm. The determined results were given in Fig. 2. All tested solutions were incubated for 20 min keeping at 37.0 ± 0.2 °C before measurement. The maximal intensities of BSA intrinsic fluorescence were recorded at 348 nm for the calculation of quenching parameters. The calculated quenching rate constants (K_q), static fluorescence quenching association constant (K_{LB}), equlibrium constants (K_A) and binding site number (n) were got according to the fluorescent data. The corresponding curves were shown in Fig. 3. In order to confirm the binding site, the synchronous fluorescence quenching ratios (R_{SFQ}) of BSA were determined along with the increases of Iron