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Journal of Pharmaceutical and Biomedical Analysis 39 (2005) 263–267
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
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Short communication

Some properties of the interaction between 2,2′-diselenadibenzoic acid and serum albumins

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Received 18 September 2004; received in revised form 13 January 2005; accepted 13 February 2005
Available online 27 April 2005

Abstract

The binding of 2,2′-diselenadibenzoic acid to bovine serum albumin (BSA) and human serum albumin (HSA) was studied by using fluorescence spectroscopy. The measurement was performed in Tris–HCl buffer aqueous medium at pH = 7.40. The quenching constant at 303 K was $(3.277 \pm 0.046) \times 10^{13}$ L mol^{-1} s^{-1} for BSA, and $(3.946 \pm 0.002) \times 10^{12}$ L mol^{-1} s^{-1} for HSA. Decreased quenching was observed in association with increased temperature. Our findings show that the observed binding constant is dependent on the ionic strength of the medium. It is said that electrostatic interactions play a role in the binding of 2,2′-diselenadibenzoic acid to serum albumin, in addition to the hydrophobic association. The decrease of the linearity of S–V plot demonstrates reduced binding of ligand to the protein in the presence of anionic surfactants such as sodium dodecyl sulfate (SDS), which indicates that 2,2′-diselenadibenzoic acid most likely binds to the hydrophobic pockets within sub-domain IIA of serum albumin, the same site as SDS.

Keywords: 2,2′-Diselenadibenzoic acid; BSA; HSA; Binding; Fluorescence; SDS

1. Introduction

Many organoselenium compounds have been synthesized and their bioactivity studied [1]. Our previous studies [2] showed that the anti-microbial activity of organselenium compound is many times higher than that of sulfur and oxygen analogs having isosteric elements. Ebselen is a lipid-soluble selenoorganic compound that has an array of pharmacological effects including anti-inflammatory and antioxidant activities. Ebselen exhibits glutathione (GSH) peroxide-like activity, reducing hydrogen peroxide with thiol cosubstrates such as GSH and *N*-acetylcysteine [3–5]. 2,2′-Diselenadibenzoic acid, as shown in Fig. 1, is the intermediate of Ebselen synthesis. It has much stronger oxidation–reduction activity than sulfur and oxygen analogs and has good anti-inflammatory and anti-cataracta activities [1].

Serum albumin is the major transport protein for unesterified fatty acids, but is also capable of binding an extraordinarily diverse range of metabolites, drugs and organic compounds. Since the overall distribution, metabolism and efficacy of many drugs in the body are correlated with their affinities towards serum albumin [5], the investigation of pharmaceuticals with respect to albumin–drug binding is important. These studies may provide information of the structural features that determine the therapeutic effectiveness of drugs, and have become an important research field in the life sciences, chemistry and clinical medicine. There is evidence of conformational changes in bovine serum albumin induced by its interaction with low molecular weight drugs. These changes appear to affect the secondary and tertiary structure of albumin [6]. These molecular interactions are often monitored by optical techniques. Such methods are sensitive and relatively easy to use whereas fluorescence spectroscopy is a valuable technique for study of the binding of ligands to proteins. Quenching measurements of albumin fluorescence are an important method to study the

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doi:10.1016/j.jpba.2005.02.028

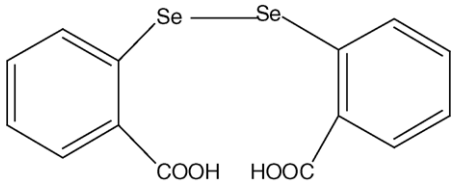

Fig. 1. Structure of 2,2′-diselenadibenzoic acid.

interactions of substances with protein [7–9]. It can reveal the accessibility of quenchers to albumin's fluorophore groups, help understand albumin's binding mechanisms to drugs, and provide clues to the nature of the binding phenomenon.

In the present work, we demonstrate the binding of 2,2′-diselenadibenzoic acid to serum albumin by using fluorescence spectroscopy and have investigated the nature of the binding of drug to protein. Based on the binding difference between bovine and human serum albumin, and study of the effect of ionic strength, surfactant SDS on the binding, the association mechanism was characterized.

2. Materials and methods

2.1. Apparatus

All fluorescence measurements were carried out on an F-2500 FL Spectrophotometer (Hitachi, Tokyo, Japan) equipped with a Xenon lamp source, a thermostat system and 1.0 cm quartz cells, using 2.5 nm slit width. A TU-1901 UV–VIS recording spectrophotometer (Puxi Analytic Instrument Ltd., Beijing, China) equipped with 1.0 cm quartz cells was used for scanning the UV spectrum.

2.2. Reagents

Fatty acid-free BSA and HSA were obtained from Bo'ao Biotechnology Company (Shanghai, China) and used without further purification. 2,2′-Diselenadibenzoic acid was synthesized and characterized by the Group of Organic Synthesis, Department of Chemistry, Wuhan University, PR China [10]. 0.02 mol L^{-1} Tris–HCl buffer of pH 7.40 (0.2 mol L^{-1} analytical grade NaCl, except for the ionic strength experiment) was used for preparing BSA solutions. All reagents were of analytical reagent grade and double-distilled water was used throughout.

2.3. Procedures

A 2.0 mL solution, containing appropriate concentrations of BSA or HSA, was titrated by successive additions of a 2.0×10^{-3} mol L^{-1} DMF stock solution of 2,2′-diselenadibenzoic acid (to give a final concentration of 2.5×10^{-5} mol L^{-1}). Titrations were done manually by using trace syringes, and the fluorescence spectra (excitation at 282 nm and emission wavelengths of 300–450 nm) and absorption spectra were recorded.

The fluorescence intensity values at the emission maximum (340 nm) were used for calculating the relative fluorescence, considering the fluorescence intensity of control untreated BSA as 100. The temperatures chosen were 303, 308, and 313 K, so that BSA does not undergo any structural degradation. The quenching constants were calculated according to Stern–Volmer equation [11]:

$$\frac{F_0}{F} = 1 + K_q \tau_0 [Q] = 1 + K_{SV}[Q] \quad (1)$$

where F and F_0 are current and initial fluorescence, respectively, $[Q]$ the ligand concentration, K_q the quenching rate constant of the biomolecule, K_{sv} the Stern–Volmer quenching constant and τ_0 (10^{-8} s^{-1}) the average lifetime of the fluorescent substance without quencher [11].

If the binding reaction in the BSA molecule happens for the static quenching interaction, there are similar and independent binding sites in the BSA. The apparent binding constant K and binding sites n can be found from equation [12]:

$$\log\left(\frac{F_0 - F}{F}\right) = \log K + n \log[Q] \quad (2)$$

All experiments were replicated three times, and the present method has good reproducibility.

3. Results and discussion

3.1. Binding of 2,2′-diselenadibenzoic acid to serum albumins

The effect of 2,2′-diselenadibenzoic acid on the fluorescence intensity of albumin is shown in Fig. 2; it can be seen that the addition of 2,2′-diselenadibenzoic acid quenched the fluorescence of serum albumins remarkably, which indicates that there are strong interactions between them and 2,2′-diselenadibenzoic acid. It can be observed that the fluorescence intensity of BSA reaching the steady state 10 s after the mixing, indicated the interaction between 2,2′-diselenadibenzoic acid and BSA attained equilibrium fast. For the molar ratio [ligand]:[SA] = 1:1, the decrease of protein fluorescence due to the presence of 2,2′-diselenadibenzoic acid as a quencher attains about 50% for BSA and 33% for HSA, respectively. The quantitative analysis of the binding of 2,2′-diselenadibenzoic acid to these albumins were carried out using the fluorescence quenching at 340 nm at various temperatures as shown in Fig. 3 and Table 1. The Stern–Volmer plots are linear and increasing temperature does not change the linearity of the serum albumin Stern–Volmer plot. The linearity may reveal the occurrence of a single type of quenching, either static or dynamic. Decreased quenching observed in association with increased temperature and the values of K_q are larger than 10^{13} suggesting the occurrence of static quenching between 2,2′-diselenadibenzoic acid and serum albumins.

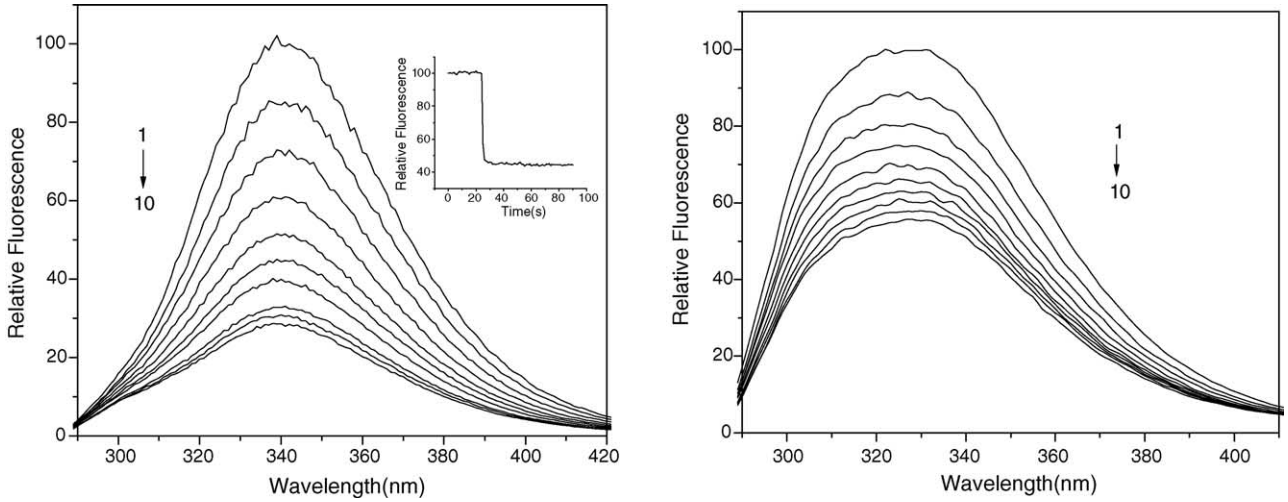

Fig. 2. Spectrofluorometric titration of BSA (left) and HSA (right) with 2,2′-diselenadibenzoic acid. Samples of albumin in buffer (pH 7.40) were titrated with addition of 2,2′-diselenadibenzoic acid from a stock solution in DMF (2.0 mM). Albumin concentration: 1.0×10^{-5} mol L^{-1}; the molar ratio of C_{SA}:C_{drug} corresponding to 1–11:0–1.0, T = 303 K. The inserts correspond to time dependence of the fluorescence emission intensity at 340 nm of BSA after drug addition with stirring.

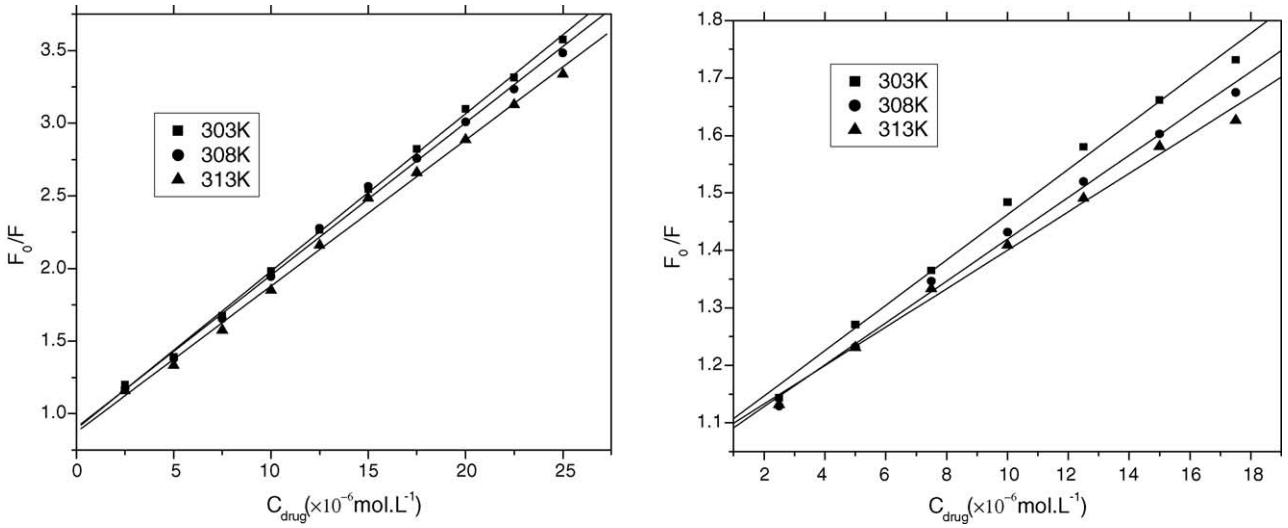

Fig. 3. Stern–Volmer curves for 2,2′-diselenadibenzoic acid–albumins, BSA (left) and HSA (right) at different temperatures. Albumin concentration: 1.0×10^{-5} mol L^{-1}; pH = 7.40; T = 303 K.

3.2. Binding sites

According to the relationship between $\log\left(\frac{F_0 - F}{F}\right)$ versus log[Q], the fit to the fluorescence data using Eq. (2) for the system of 2,2′-diselenadibenzoic acid and serum albumin was found by setting $n = 1.135 \pm 0.010$ and $K = (4.709 \pm 0.020) \times 10^5$ L mol^{-1} for BSA, and $n = 0.843 \pm 0.016$ and $K = (7.713 \pm 0.081) \times 10^3$ L mol^{-1} for HSA, respectively.

3.3. Comparison of quenching effect on BSA and HSA fluorescence

The fluorescence quencher interacts in the protein sub-domain containing tryptophan residues. From the

Table 1
Binding and quenching constants according to Stern–Volmer curves

T (K)	K_{sv} (BSA) (10^5 L mol^{-1})	K_q (BSA) (10^{13} L mol^{-1} s^{-1})	R	K_{sv} (HSA) (10^4 L mol^{-1})	K_q (HSA) (10^{12} L mol^{-1} s^{-1})	R
303	1.084 ± 0.033	1.084	0.9993	3.946 ± 0.020	5.288	0.9962
308	1.049 ± 0.048	1.049	0.9984	3.645 ± 0.016	3.645	0.9974
313	1.005 ± 0.052	1.005	0.9980	3.344 ± 0.017	3.344	0.9962

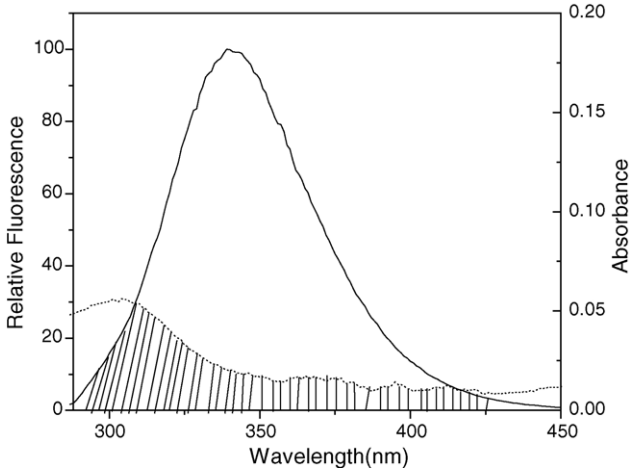

Fig. 4. The overlap of the fluorescence spectra of BSA (real line) and the absorption spectra of 2,2′-diselenadibenzoic acid (dashed line). $C_{BSA} = C_{drug} = 1.0 \times 10^{-5}$ mol L^{-1}; pH = 7.40; T = 303 K.

spectroscopic point of view one of the main differences between the two proteins is that BSA is formed by 582 amino acid residues, and contains a first tryptophan residue in position 135, in sub-domain IB of the albumin molecule, and a second tryptophan residue in position 214, in sub-domain IIA [13], while HSA has only one tryptophan residue in position 214. A large hydrophobic cavity is present in the sub-domain IIA, and a wide variety of arrangements can take place in this sub-domain. So sub-domain IIA is the target of 2,2′-diselenadibenzoic acid binding. At the same time, the tryptophan residues in the vicinity of W135 interact directly with the polar headgroup of 2,2′-diselenadibenzoic acid possibly, which is the reason for the difference between the two proteins.

3.4. The energy transfer between 2,2′-diselenadibenzoic acid and BSA

We assume the binding reaction in the BSA molecule happens in a sequential manner, the distance between the binding site and the fluorophore in the protein can be evaluated according to the Förster mechanism of non-radiation energy transfer [14]. The overlap integral calculated is 2.933×10^{-7} cm^3 L mol^{-1} (Fig. 4). The distance between the acceptor and the donor is 4.56 nm, lower than 7 nm after interaction, indicating the static quenching interaction between the acceptor and the donor. The variation in the environment of BSA fluorophores (sub-domain IIA) in the presence of drug induces changes in relative fluorescence due to an energy transfer from sub-domain IIA to the chromophore in drug.

3.5. The effect of ionic strength

Generally, small molecules are bound to a macromolecule by four binding modes: H-bonding, Van der Waal's forces, electrostatic and hydrophobic interactions. The albumin polypeptide chain contains more than 580 amino acid residues, and the loop region of each sub-domain contains three helical segments, about 30% of the surface being hydrophobic. Several amino acids are found to be important in the binding. Basic amino acids like His, Arg and Lys are involved in the binding process. These groups may interact with the hydrophobic moieties of the 2,2′-diselenadibenzoic acid.

In order to examine the electrostatic contribution to the interaction of 2,2′-diselenadibenzoic acid and serum albumin, experiments were performed in the presence of different concentrations of NaCl. The Stern–Volmer plots are presented in Table 2, we can find that the slope of Stern–Volmer plots are dependent on the ionic strength of the medium. The binding constants increase markedly with the decreasing of ionic strength, when the concentration of NaCl vary from 0.90 to 0.0015 M, the binding constant increases to almost three-fold for BSA. The result supports an electrostatic nature of 2,2′-diselenadibenzoic acid–albumin interaction.

Thus, we suggest that the electrostatic and hydrophobic interactions exist both between 2,2′-diselenadibenzoic acid and serum albumin.

3.6. The effect of SDS

Generally, results of competitive binding experiments can be interpreted either in terms of direct competition for the same binding site or/and in terms of (non)-cooperative interactions of two ligands with the protein [15,16].

The analysis of binding data in the presence of 1 mM SDS revealed an appreciable decrease in the binding affinity of 2,2′-diselenadibenzoic acid–albumin, with a Stern–Volmer binding constant of $(1.014 \pm 0.011) \times 10^4$ L mol^{-1} for BSA, and $(1.301 \pm 0.009) \times 10^4$ L mol^{-1} for HSA, respectively (S–V curves not shown).

The low value of the quenching constant observed in the presence of 1 mM SDS can be explained as due to the

Table 2
Binding constants according to Stern–Volmer curves at different NaCl concentrations in Tris–HCl buffer

C_{NaCl} (mol L^{-1})	K_{sv} (BSA) (10^5 L mol^{-1})	R	K_{sv} (HSA) (10^4 L mol^{-1})	R
0.0015	1.537 ± 0.033	0.9976	4.263 ± 0.019	0.9986
0.045	1.310 ± 0.044	0.9984	4.188 ± 0.019	0.9971
0.15	1.097 ± 0.042	0.9979	3.946 ± 0.020	0.9971
0.45	0.7964 ± 0.037	0.9969	3.240 ± 0.019	0.9949
0.90	0.5836 ± 0.039	0.9952	3.113 ± 0.010	0.9986

formation of the BSA–SDS complex, reducing the accessibility of the tryptophan side chain to 2,2′-diselenadibenzoic acid. It is indicated that 2,2′-diselenadibenzoic acid and SDS are likely bind to the same site within the subdomain of serum albumin. Tryptophan 214 located in domain IIA is more sensitive to denaturation, so surfactant-induced protein denaturation could start in domain IIA [17]. The fact that SDS reduces the chance of binding for 2,2′-diselenadibenzoic acid reveals that Tryptophan 214 located in domain IIA is also the target of 2,2′-diselenadibenzoic acid binding.

4. Conclusions

A fluorescence method for the rapid and simple determination of the interaction between 2,2′-diselenadibenzoic acid and serum albumin is provided. The method is easy to operate and is reliable, practical, and simple. Analysis of the binding of 2,2′-diselenadibenzoic acid to the protein was made in the present work using the data for protein fluorescence changes induced by drug molecules. The results obtained give preliminary information on the binding of 2,2′-diselenadibenzoic acid to serum albumins. The binding constant at 303 K was $(3.277 \pm 0.046) \times 10^{13}\,\mathrm{L\,mol^{-1}\,s^{-1}}$ for BSA, and $(3.946 \pm 0.002) \times 10^{12}\,\mathrm{L\,mol^{-1}\,s^{-1}}$ for HSA, respectively. Both hydrophobic association and electrostatic interactions play roles in the binding of 2,2′-diselenadibenzoic acid to serum albumin. Binding sites $n = 1.135$ for BSA and $n = 0.843$ for HSA indicate that the existence of just a single binding site in albumin for 2,2′-diselenadibenzoic acid. Sub-domain IIA in serum albumin is the main target of 2,2′-diselenadibenzoic acid binding. The decrease of the linearity of S–V plot demonstrates reduced binding of the ligand to the protein in the presence of SDS.

Acknowledgements

We gratefully acknowledge financial support of National Natural Science Foundation of China (Grant no. 20373051, 30170010) and the Teaching and Research Award Program for Outstanding Young Professors of High Education Institutes, Ministry of Education, PR China.

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