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Absorption and fluorescence study of the interaction between (2-hydroxy-benzimido)ethyl-n-hexylselenide and bovine serum albumin

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Abstract

The binding of Schiff base selenide, (2-hydroxy-benzimido)ethyl-n-hexylselenide, to bovine serum albumin (BSA) was studied using fluorescence spectroscopy. The measurement was performed in Tris-HCl buffer aqueous medium at pH 7.4. Stern–Volmer graphs were plotted and quenching constants were estimated. The quenching constant at 303 K was $(1.639 \pm 0.046) \times 10^{13}\,L\,mol^{-1}\,s^{-1}$. Decreased quenching was observed as temperature increased, but at the temperature range of 303–313 K, the association of Schiff base selenide to BSA was not significantly different. The static quenching presented in the system of Schiff base selenide and BSA. A complex was possibly formed between Schiff base selenide and BSA, which was responsible for the quenching of the fluorescence of BSA. This fact was also confirmed by differences in the absorption spectra of BSA before and after Schiff base selenide addition. The hydrophobic interaction was found to play a main role in the binding according to the thermodynamic parameters, enthalpy change (ΔH) and entropy change (ΔS) of reaction. Schiff base selenide most likely binds to the hydrophobic pockets within sub domain IIA of BSA, which can be proved by competition experiments for sodium dodecyl sulfate. By constant-wavelength synchronous fluorescence spectra, the influence of (2-hydroxy-benzimido)-ethyl-n-hexylselenide on the surrounding environment of tyrosine and tryptophan residues in BSA was also investigated. The red shift of the fluorescence peak of tryptophan residues indicated that the hydrophobic amino acid structure surrounding tryptophan residues in BSA collapsed slightly after the addition of (2-hydroxy-benzimido)ethyl-n-hexylselenide.

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Introduction

Schwarz & Foltz (1957) demonstrated that trace elements of selenium protected against liver necrosis in vitamin E-deficient rats and established their nutritional essentiality. Human diseases in China that have been associated with a particularly low level of selenium include cardiomyopathy, Keshan disease and Kaschin-Beck disease, involving osteoarthropathy (Li et al 1990, 1992). Meanwhile, selenium status has been implicated in a wide range of disorders, including heart disease, cancer and AIDS (Shamberger 1983; Clement & Howard 1990).

To compare organoselenium compounds, many organoselenium compounds were synthesized and their bioactivity was studied (Xu & Huang 1994). Our previous studies (Li et al 2002) showed that the antimicrobial activity of organoselenium compounds is many times higher than that of sulfur and oxygen analogues having isosteric elements. Schiff base compounds have been used in several areas (Fioravanti et al 1995; Metri et al 1995) for their antiviral, anti-inflammatory and antioxidative activity. (2-Hydroxy-benzimido) ethyl-n-hexylselenide (Figure 1) is a kind of selenium-containing Schiff base compound or a Schiff-base-group-containing selenide (Schiff base selenide). For the Schiff base group contained, a Schiff base selenide would hopefully exhibit a better drug effect than other organoselenium compounds. In a previous study (Li 2001), Schiff-base selenide had clearly demonstrated high antimicrobial activity.

It has been shown that the distribution, free concentration and metabolism of various drugs may be strongly affected by drug–protein interactions in the blood-stream. This type of interaction can also influence drug stability and toxicity during the

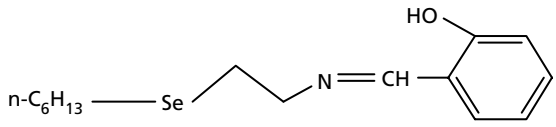

Figure 1 Structure of (2-hydroxy-benzimido)ethyl-n-hexylselenide.

chemotherapeutic process. Serum albumin is the major transport protein for unesterified fatty acids, but it is also capable of binding an extraordinarily diverse range of metabolites, drugs and organic compounds. Since the efficacy of many drugs in the body are correlated with their affinity towards serum albumin (Wesley 1996), the investigation of pharmaceuticals with respect to albumin–drug binding is imperative and of fundamental importance. These studies may provide information about the structural features that determine the therapeutic effectiveness of drugs, and become an important research field in life sciences, chemistry and clinical medicine.

Bovine serum albumin (BSA) has a wide range of physiological functions involving the binding, transport and delivery of fatty acids, porphyrins, bilirubin, tryptophan, thyroxin and steroids. There is evidence of conformational changes in BSA induced by its interaction with low-molecular-weight drugs. These changes appear to affect the secondary and tertiary structure of albumin (Hushcha et al 2000). The molecular interactions are often monitored by optical techniques. These 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 measurement of albumin fluorescence is an important method used to study interactions of several substances with protein (Lakowicz 1999; Klajnert & Bryszewska 2002; Sulkowska et al 2003). It can reveal the accessibility of quenchers to albumin's fluorophore groups, help us understand albumin's binding mechanisms to drugs and provide clues to the nature of the binding phenomenon.

In this work, we demonstrated the binding of Schiff base selenide to BSA by using fluorescence spectroscopy. The nature of the binding of drug to protein was described. The effect of the energy transfer was studied according to the Förster mechanism of non-radiation energy transfer. By constant-wavelength synchronous fluorescence spectra of BSA at different wavelength intervals, the effect of Schiff base selenide on the surrounding environment of tyrosine and tryptophan residues in BSA was also investigated.

Materials and Methods

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.

Reagents

Fatty-acid-free BSA purified from bovine serum (fraction V) was obtained from Bo'ao Biotechnology Company (Shanghai, China) and used without further purification; its molecular weight was assumed to be 67 000. (2-Hydroxy-benzimido)ethyl-n-hexylselenide was synthesized and characterized by the Department of Chemistry, Wuhan University (P. R. China) (Yang 2001). Tris-HCl buffer medium (0.02 M), pH 7.4 (containing 0.2 M NaCl, except for ionic strength experiment), was used for preparing BSA solution. All reagents were of analytical reagent grade and double-distilled water was used throughout.

Procedures

The mixture solution containing 1.0×10^{-5} M BSA and the varying amounts of Schiff base selenide was prepared. After 10 min, fluorescence spectra (excitation at 282 nm and emission wavelengths of 300–500 nm), absorption spectra and synchronous fluorescence spectra ($\Delta\lambda = 60$ nm and $\Delta\lambda = 15$ nm, respectively) were obtained.

Theoretical background

The fluorescence intensity values at 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 did not undergo any structural degradation.

The quenching constants were calculated according to the Stern–Volmer equation (Lakowicz & Weber 1973):

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

where F and F_0 are current and initial fluorescence, respectively, [Q] is ligand concentration, K_q is the quenching rate constant of the biomolecule, K_{SV} is the Stern–Volmer quenching constant and τ_0 (10^{-8} s^{-1}) is the average lifetime of the biomolecule without quencher (Lakowicz & Weber 1973).

If the static quenching interaction is noted, the binding Lineweaver–Burk equation is presented as (Ma et al 1995):

$$(F_0 - F)^{-1} = F_0{}^{-1} + K_{LB}{}^{-1}F_0{}^{-1}[Q]^{-1} \quad (2)$$

where K_{LB} is the binding constant of drug with the biological macromolecule, which can be determined by the slope of the curve $(1/(F_0 - F)$ vs $1/[Q])$. The thermodynamic parameters, which are important for confirming binding modes, were determined from the plot lnK vs 1/T according to the van't Hoff equation.

If binding reaction in the BSA molecule happens for the static quenching interaction, there are similar and independent binding sites in the BSA.

$$nQ + B \rightleftharpoons BQn \quad (3)$$

The relationship between the fluorescence intensity and the quenching medium can be deduced as (Xi & Zhang 1998):

$$\mathrm{Log}[(F_0 - F)/F] = \log K + n\log[Q] \quad (4)$$

where K is the apparent binding constant for the protein–ligand complex, and n is the number of binding sites.

We assume the binding reaction in the BSA molecule happens in a sequential manner and 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 (Wu & Brand 1994).

The efficiency of energy transfer, E, is given by:

$$E = 1 - (F/F_0) = R_0^6/(R_0^6 + r^6) \quad (5)$$

where r is the distance between donor and acceptor and R_0 is the distance at 50% transfer efficiency.

$$R_0^6 = 8.8 \times 10^{-25} K^2 n^{-4} \Phi J \quad (6)$$

where K^2 is the orientation factor related to the geometry of the donor–acceptor of dipole and $K^2 = 2/3$ for random orientation as in fluid solution, n (= 1.4) is the refractive index of medium, Φ (= 0.10) is the fluorescence quantum yield of the donor (Liu et al 2003) and J is the spectra overlap of the donor emission and the acceptor absorption. J is given by:

$$J = \Sigma F(\lambda)\varepsilon(\lambda)\lambda^4 \Delta\lambda / \Sigma F(\lambda)\Delta\lambda \quad (7)$$

where $F(\lambda)$ is the fluorescence intensity of fluorescence reagent when the wavelength is λ and $\varepsilon(\lambda)$ is the molar absorbance coefficient at the wavelength of λ. From these relationships, J, E and R_0 can be calculated; so the value of r can also be calculated. A distance between the acceptor and the donor of less than 7 nm after interaction will indicate again the static quenching interaction between the acceptor and the donor.

Statistical analysis

The protein and the drugs were measured separately by measuring the fluorescence of the protein quenched by the drugs. For the proteins, the concentration of Schiff base selenide was kept unchanged. The fluorescence intensity was measured before and after the addition of Schiff base selenide with the concentration of the protein changed. The linear relationship between the difference in fluorescence intensity and concentration of the protein was applied in the determination of the protein. For the drugs, the concentration of the protein was kept unchanged with different concentrations of Schiff base selenide. The linear relationship between fluorescence intensity and the concentration of Schiff base selenide was used in the determination of the drugs.

Each group of experiments was replicated 3 times, and the present method had good reproducibility. The statistical analysis was performed using the SPSS 10.0 software system (SPSS Inc., Chicago IL). Data were statistically analysed using Kruskal–Wallis test. A one-way analysis of variance test was used for multiple comparisons between results from different temperature experiments. This was followed by a post-hoc test.

Results and Discussion

Binding properties of BSA and Schiff base selenide

The change in the relative fluorescence of serum albumin can be correlated with changes in its structure as the ligand concentration increased. The addition of Schiff base selenide quenched the fluorescence of BSA remarkably (Figure 2), and the drug-concentration-dependent quenching of the intrinsic fluorescence of protein indicated that there is an interaction between Schiff base selenide and BSA. The quantitative analysis of the binding of Schiff base selenide to BSA was carried out using the fluorescence quenching at 340 nm at various temperatures (Figure 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. Linearity may reveal the occurrence of a single type of quenching, either static or dynamic. Decreasing of quenching observed in association with temperature increase and the values of K_q being larger than 10^{13} suggest the occurrence of static quenching between Schiff base selenide and BSA.

Statistical comparisons between various temperatures were made by Kruskal–Wallis test. The results failed to show significant differences between temperatures ($\chi^2 = 0.751$, $P = 0.687$). It can be said that over the temperature range 303–313 K, the association of Schiff base selenide to BSA is not significantly different. Individual differences between the temperatures was then identified using post-hoc test ($P < 0.05$). In all the cases, the mean difference between both measurement conditions did not exceed 0.40 and was not more significant.

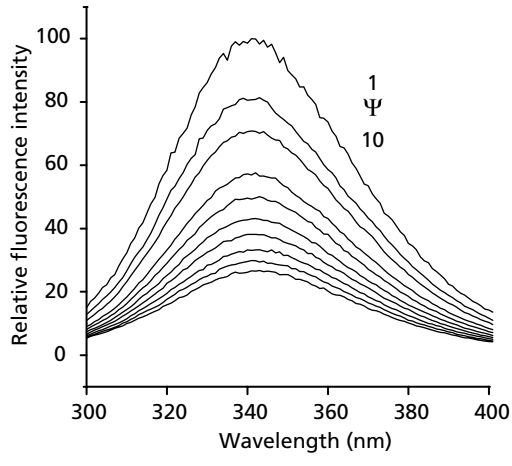

Figure 2 The fluorescence emission spectra of Schiff base selenide–BSA system. The concn of BSA was 1.0×10^{-5} mol L^{-1}. The molar ratio of selenide to BSA corresponded to 1–10:0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8; temperature = 303 K; pH = 7.4.

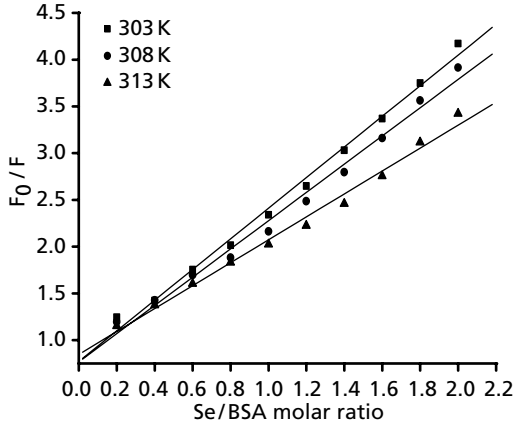

Figure 3 Stern–Volmer curves for Schiff base selenide–BSA at different temperatures. F and F_0 are current and initial fluorescence, respectively; $\lambda_{ex} = 282$ nm, $\lambda_{em} = 340$ nm; BSA concn: 1.0×10^{-5} mol L^{-1}; pH = 7.4.

Table 1 Quenching constants according to Stern–Volmer curves for the binding of Schiff base selenide, (2-hydroxy-benzimido)ethyl-n-hexylselenide, to bovine serum albumin (BSA)

Temperature (K)	K_{sv} (10^5 L mol^{-1})	s.d.	K_q (10^{12} L mol^{-1} s^{-1})	R
303	1.639 ± 0.046	0.084	1.639 ± 0.046	0.997
308	1.511 ± 0.055	0.100	1.511 ± 0.055	0.995
313	1.227 ± 0.043	0.079	1.227 ± 0.043	0.995

K_q is the quenching rate constant of the biomolecule, K_{SV} is the Stern–Volmer quenching constant.

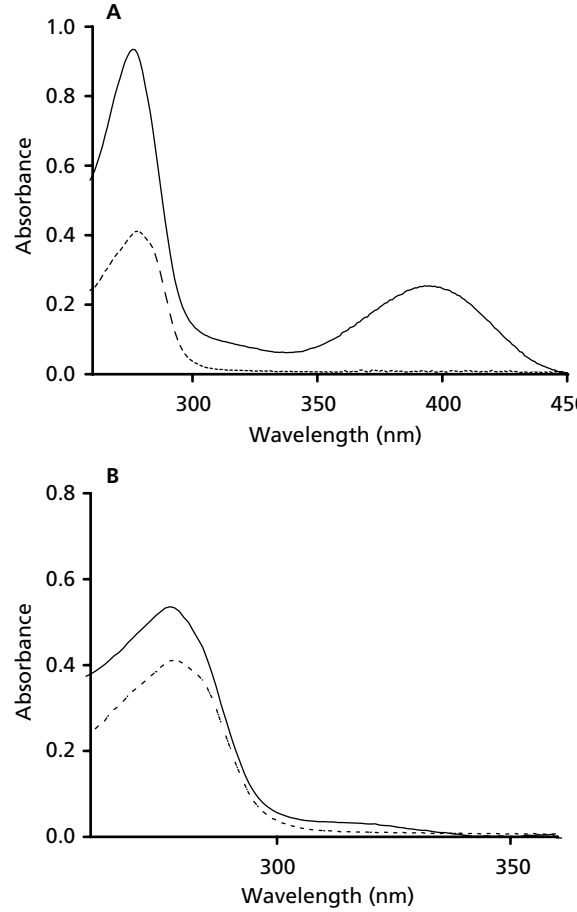

Figure 4 Change in UV absorption spectra of BSA in the presence of Schiff base selenide (A) and differential UV absorption spectrum of complex to Schiff base selenide (solid line) (B). Dashed line represents the spectrum of BSA. $C_{BSA} = C_{selenide} = 1.0 \times 10^{-5}$ mol L^{-1}; pH = 7.4; temperature = 303 K.

It is noted that quenching of intrinsic fluorescence of BSA resulted from the formation of ground-state complex between Schiff base selenide and BSA. To confirm the formation of this complex, the UV spectra of BSA in the presence of Schiff base selenide is shown in Figure 4. The values of absorbance at $\lambda = 278$ of BSA spectra increased in the presence of Schiff base selenide. The spectrum shows distinct difference before and after Schiff base selenide addition. It appears that Schiff base selenide bound to BSA and the complex formed between them.

Binding constants and binding modes

We can obtain binding constants, K_{LB}, of Schiff base selenide with BSA from the intercept and slope of the Lineweaver–Burk curves (Figure 5). The results are given in Table 2. Statistical comparison between various temperatures was also made by Kruskal–Wallis test. The differences between temperatures was not significant ($\chi^2 = 1.840$, $P = 0.399$). Individual differences between the temperatures was then identified using post-hoc test

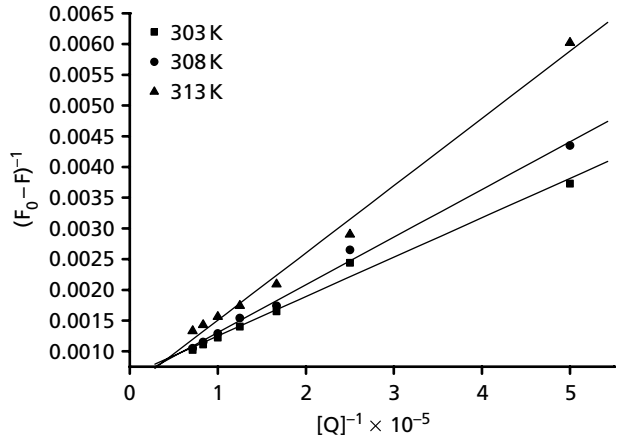

Figure 5 Linewaver–Burk curves of the system of Schiff base selenide–BSA at different temperatures. F and F_0 are current and initial fluorescence, respectively; [Q] is ligand concentration; $\lambda_{ex} = 282$ nm, $\lambda_{em} = 340$ nm; BSA concn: 1.0×10^{-5} mol L^{-1}; pH = 7.4.

Table 2 The binding constants (K_{LB}) and relative thermodynamics parameters of the system of Schiff base selenide–BSA

Temperature (K)	K_{LB} (10^5 L mol^{-1})	s.d.	R	$\Delta H^{\ominus}$ (kJ mol^{-1})	$\Delta S^{\ominus}$ (J mol K^{-1})	$\Delta G^{\ominus}$ (kJ mol^{-1})
303	1.065	0.102	0.996			−29.12
308	0.868	0.110	0.997	−27.82	177.6	−29.61
313	0.736	0.115	0.994			−29.16

K_{LB} is the binding constant of drug with the biological macromolecule.

($P < 0.05$); the mean difference between both measurement conditions did not exceed 0.25.

Generally, small molecules are bound to macromolecules by four binding modes: hydrogen bonding, Van der Waal's forces, electrostatic interactions and hydrophobic interactions. The thermodynamic parameters enthalpy change (ΔH) and entropy change (ΔS) of reaction are important for confirming binding modes. For this purpose, a plot of lnK vs 1/T gave a straight line according to the van't Hoff equation (not shown). The thermodynamic parameters were determined from the plot and are presented in Table 2. The formation of Schiff base selenide – BSA complex is an exothermic reaction accompanied by positive ΔS value. Consequently, the binding process is entropically controlled and larger positive entropy makes a much greater contribution to the free energy term for BSA. For a drug–protein interaction, positive entropy is frequently taken as being evidence for the role of hydrophobic interaction (Sulkowska & Michnik 1997). Furthermore, specific electrostatic interactions between ionic species in aqueous solution are characterized by a positive value of ΔS and a negative ΔH value. However, ionic interactions cannot be expected for the binding because Schiff base selenide might be considered to be largely un-ionized under the conditions here (pH 7.4), as can be expected from its structure. Furthermore, the fact that the concentration of NaCl in the system almost did not change the binding constant (Figure 6) indicates that ionic strength does not affect the binding. Thus, Schiff base selenide bound to BSA was mainly based on the hydrophobic interaction. The BSA polypeptide chain contains 582 amino-acid residues and the loop region of each subdomain 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 Schiff base selenide.

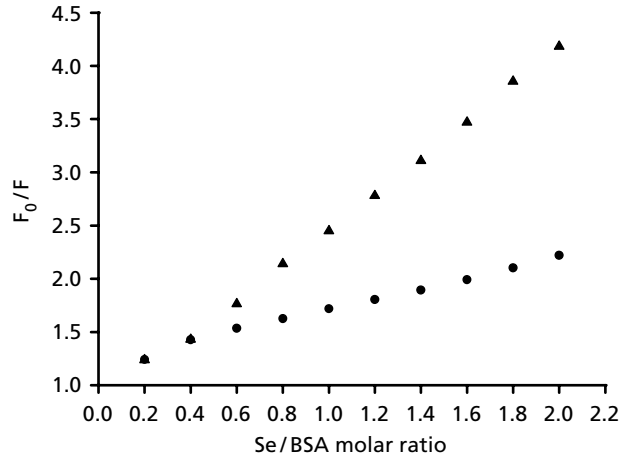

Figure 6 Stern–Volmer curves for Schiff base selenide–BSA at different conditions. Triangles, 0.015 M NaCl contained in buffer; circles, in the presence of 2 mMSDS. F and F_0 are current and initial fluorescence, respectively; $\lambda_{ex} = 282$ nm, $\lambda_{em} = 340$ nm; BSA concn: 1.0×10^{-5} mol L^{-1}; pH = 7.4.

Binding sites

According to the relationship between log[$(F_0 - F)/F$] vs log[Q], the fit to the fluorescence data using equation 4 for the system of Schiff base selenide and BSA was found by setting $n = 1.16 \pm 0.0122$ and $K = (1.183 \pm 0.134) \times 10^5$ L mol^{-1}. It is said that the association of Schiff base selenide and BSA represents a 1:1 binding. Linear Stern–Volmer plots may also indicate the existence of just a binding site for Schiff base selenide in the proximity of the hydrophobic pocket.

The BSA molecule 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 subdomain IIA (Kragh 1981). Crystal structure analysis has revealed that the drug binding sites are located in subdomains IIA and IIIA (Carter et al 1989; Curry et al 1998). Of both tryptophans in BSA, Trp 135 is more exposed to a hydrophilic environment, whereas Trp 214 is deeply buried in the hydrophobic loop. A large hydrophobic cavity is present in sub-domain IIA and a wide variety of arrangements can take place in this sub-domain. It is suggested that the complex between Schiff base selenide and BSA is formed in sub-domain IIA. Our competition experiment for anionic surfactant sodium dodecyl sulfate (SDS) accorded with this suggestion. The analysis of binding data in the presence of 2 mM SDS revealed appreciable decrease in the binding affinity of BSA for Schiff base selenide, with a Stern–Volmer quenching constant of $(5.069 \pm 0.028) \times 10^4$ L mol^{-1} (Figure 6). The result clearly suggests that Schiff base selenide and SDS likely bind to the

same site within BSA. Tryptophan 214 located in domain IIA is more sensitive to denaturation, so surfactant-induced protein denaturation could start in domain IIA (Gelamo et al 2002). Thus Schiff base selenide most likely binds to the hydrophobic pockets within sub-domain IIA of BSA.

The energy transfer between Schiff base selenide and BSA

From Figure 7, the overlap integral calculated according to Föster's non-radiative energy transfer theory is $19.524 \times 10^{-14}\,cm^3\,L\,mol^{-1}$. So the value of R_0 is 3.79 nm and the value of r is 4.01 nm. The distance between Schiff base selenide and fluorophores in BSA is 4.01 nm, less than 7 nm. Obviously, this result accords with conditions of non-radiative energy transfer theory, indicating again the static quenching interaction between Schiff base selenide and BSA. The variation in the environment of BSA fluorophores (sub-domain IIA) in the presence of drug induces change in relative fluorescence due to an energy transfer from sub-domain IIA to the chromophore in each drug.

The influence of Schiff base selenide on the surrounding of chromophore in BSA

A valuable feature of intrinsic fluorescence of proteins is the high sensitivity of the chromophore to its local environment. The intrinsic fluorescence of proteins can provide considerable information about their structure and dynamics, and is often considered in the study of protein folding and association reactions (Gelamo & Tabak 2000). Changes in emission spectra of chromophores are common in response to protein conformational transitions, subunit association, substrate binding or denaturation (Lakowicz 1999).

Fluorescence spectra of BSA have contributed to tyrosine and tryptophan residues. However, conventional fluorescence spectra of tyrosine and tryptophan residues overlap strongly because of their similar excitation spectra. Thus, it is difficult to identify the two amino acid residues using a conventional fluorescence technique. In synchronous fluorescence spectra, tryptophan and tyrosine residues in BSA can be isolated or separated by selecting suitable wavelength intervals (Yang et al 2002). Thus, synchronous fluorescence spectroscopy can be used to probe the conformational states of proteins. We studied the influence of Schiff base selenide on the relative synchronous fluorescence spectra (Figure 8). When $\Delta\lambda = 60$ nm, the fluorescence peak is attributed to tryptophan residues; when $\Delta\lambda = 15$ nm, the fluorescence peak of tyrosine residues can be observed (Chen et al 1990). There is a 4-nm red shift for the peak position of tryptophan after Schiff base selenide addition (Figure 8A). Because the emission of tryptophan and its derivatives is highly sensitive to the environment,

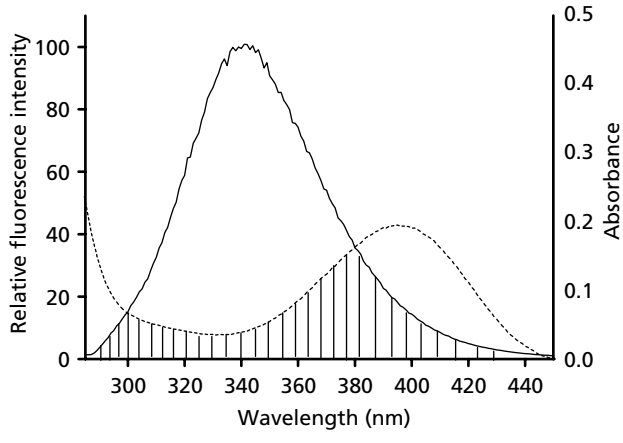

Figure 7 The overlap of the fluorescence spectrum of BSA (real line) and the absorption spectrum of Schiff base selenide (dashed line). $C_{BSA} = C_{selenide} = 1.0 \times 10^{-5}\,mol\,L^{-1}$; pH = 7.4; temperature = 303 K.

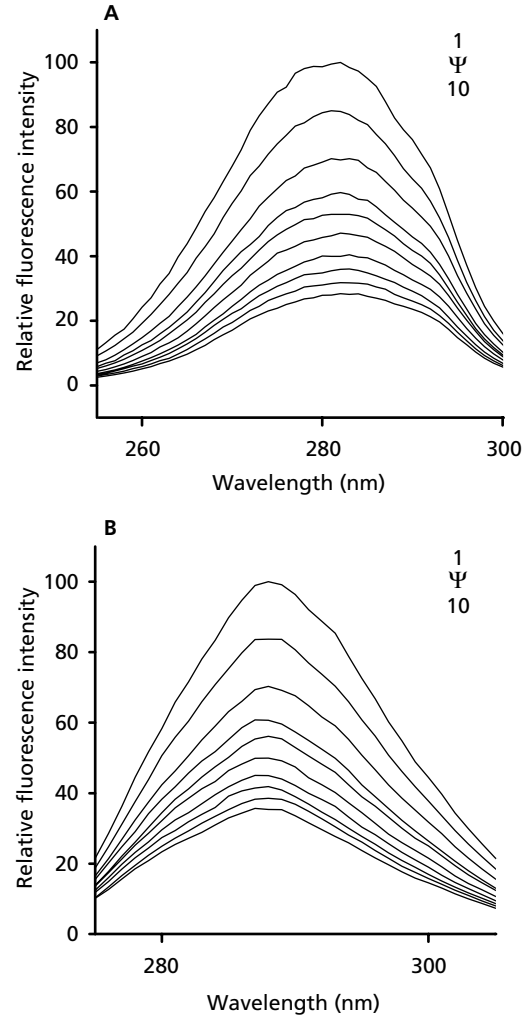

Figure 8 The influence of Schiff base selenide on the synchronous fluorescence spectra of BSA (A: $\Delta\lambda = 60$ nm; B: $\Delta\lambda = 15$ nm). The concn of BSA was $1.0 \times 10^{-5}\,mol\,L^{-1}$. The molar ratio of selenide to BSA corresponded to 1–10:0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8; temperature = 303 K; pH = 7.4.

the red shift is interpreted as the shielding of tryptophan residues from the aqueous solution being decreased. Thus, the hydrophobic amino acid structure surrounding tryptophan residues in BSA tends to collapse slightly and tryptophan residues are exposed more to the aqueous phase after Schiff base selenide addition. But the emission of tyrosine residues is not sensitive to the environment (Figure 8B).

Conclusions

The interaction between (2-hydroxy-benzimido)ethyl-n-hexylselenide and BSA has been studied in buffer solution at pH 7.4 by means of fluorescence and absorption spectra. The results showed that the hydrophobic binding interaction was responsible for the quenching of the fluorescence of BSA, a complex was formed and the binding constants $K_{LB} = 1.065 \times 10^5\,L\,mol^{-1}$ at 303 K, and the association of Schiff base selenide and BSA represents a 1:1 binding. Schiff base selenide most likely binds to the hydrophobic pockets within sub-domain IIA of BSA, which can be proved by competition experiment for SDS. By constant-wavelength synchronous fluorescence spectra, we further investigated the influence of (2-hydroxy-benzimido)ethyl-n-hexylselenide on the surrounding environment of tyrosine and tryptophan residues in BSA. It was found that hydrophobic amino acid structure surrounding tryptophan residues in BSA tended to collapse slightly and tryptophan residues were being exposed more to aqueous phase after addition of (2-hydroxy-benzimido)ethyl-n-hexylselenide. The results obtained give preliminary information on the binding of Schiff base selenide to BSA.

References

Carter, D. C., Há, X. M., Munson, S. H., Twigg, P. D., Gernert, K. M., Broom, M. B., Miller, T. Y. (1989) Three-dimensional structure of human serum albumin. *Science* **244**: 1195–1198

Chen, G. Z., Huang, X. Z., Xue, J. G., Wang, Z. B. (1990) *Methods of fluorescence analysis*. 2nd edition, Science Press, Beijing, pp 115–118

Clement, I. P., Howard, E. G. (1990) Activity of methylated forms of selenium in cancer prevention. *Cancer Res*. **50**: 1206–1211

Curry, S., Mandelkow, H., Brick, P., Franks, N. (1998) Crystal structure of human serum albumin complexed with fatty acid reveals an asymmetric distribution of binding sites. *Nat. Struct. Biol*. **5**: 827–835

Fioravanti, R., Biava, M., Porretta, G. C., Landofi, C., Simonetti, N., Villa, A., Conte, E., Porta, P. A. (1995) Research on antibacterial and antifungal agents. XI. Synthesis and antimicrobial activity of N-heteroaryl benzylamines and their Schiff bases. *Eur. J. Med. Chem*. **30**: 123–132

Gelamo, E. L., Tabak, M. (2000) Spectroscopy studies on the interaction of bovine (BSA) and human (HSA) serum albumins with ionic surfactants. *Spectrochim. Acta Part A* **56**: 2255–2271

Gelamo, E. L., Silva, C. H. T. P., Imasato, H., Tabak M. (2002) Interaction of bovine (BSA) and human (HSA) serum albumins with ionic surfactants: spectroscopy and modeling. *Biochim. Biophys. Acta* **1594**: 84–89

Hushcha, T. O., Luik, A. I., Naboka, Y. N. (2000) Conformation changes of albumin in its interaction with physiologically active compounds as studied by quasi-elastic light scattering spectroscopy and ultrasonic method. *Talanta* **53**: 29–34

Klajnert, B., Bryszewska, M. (2002) Fluorescence studies on PAMAM dendrimers interactions with bovine serum albumin. *Bioelectrochemistry* **55**: 33–35

Kragh, H. U. (1981) Molecular aspects of ligand binding to serum albumin. *Pharm. Rev*. **33**: 17–53

Lakowicz, J. R. (1999) *Principles of fluorescence spectroscopy*. 2nd edition, Kluwer Academic Publishers/Plenum Press, New York, pp 237–259

Lakowicz, J. R., Weber, G. (1973) Quenching of fluorescence by oxygen. A probe for structural fluctuations in macromolecule. *Biochemistry* **12**: 4161–4170

Li, X. (2001) *Microcalorimetric study of interaction between selenium compounds and cell or mitochondria*. Ph.D. Thesis, Wuhan University, Wuhan, P R China, pp 54–67

Li, F. S., Yin, Y. J., Yan, S. J. (1990) Presenile changes in tissues of Kaschin-Beck disease and its pathogenic significance. *Mech. Ageing Dev*. **54**: 103–120

Li, F. S., Guan, J. Y., Zhou, L. M. (1992) Three element factors of Kesan Disease and the relationship. *Chin. J. Endemiol*. **11**: 240–243 (Chinese)

Li, X., Liu, Y., Wu, J., Liang, H. G., Qu, S. S. (2002) Microcalorimetric study of *Staphylococcus aureus* growth affected by selenium compounds. *Thermochim. Acta* **387**: 57–61

Liu, J. Q., Tian, J. N., Zhang J. Y., Hu Z. D., Chen, X. G. (2003) Interaction of magnolol with bovine serum albumin: a fluorescence-quenching study. *Anal. Bioanal. Chem*. **376**: 864–867

Ma, G. B., Gao, F., Ren, B. Z., Yang, P. (1995) Study on the interaction of human serum albumin and drugs molecules by fluorescence methods. *Acta Chim. Sin*. **53**: 1193–1197 (Chinese)

Metri, R. M., Kulkarmi, V. H., Patil, S. A. (1995) Synthesis and antihistaminic activity of oxovanadium (IV) complexes of some new schiff bases. *J. Inorganic Biochem*. **59**: 604

Schwarz, K., Foltz, C. M. (1957) Selenium as an integral part of factors against dietary necrotic liver degeneration. *J. Am. Soc*. **79**: 3292–3293

Shamberger, R. J. (1983) *Biochemistry of selenium*. Plenum Press, New York, pp 239–245

Sulkowska, A., Michnik, A. (1997) Interaction of purine bases and nucleosides with serum albumin. *J. Mol. Structure* **410–411**: 27–29

Sulkowska, A., Rownicka, J., Bojko, B., Sulkowski, W. (2003) Interaction of anticancer drugs with human and bovine serum albumin. *J. Mol. Structure* **651–653**: 133–140

Wesley, R. H. (1996) Binding and transport of aluminum by serum proteins. *Coordination Chem. Rev*. **149**: 347–365

Wu, P., Brand, L. (1994) Resonance energy transfer: methods and applications. *Anal. Biochem*. **218**: 1–13

Xi, Z. F., Zhang, L. (1998) Investigation of the interaction between acridine orange and bovine serum albumin. *Talanta* **47**: 1223–1229

Xu, H. B., Huang, K. X. (1994) *Chemistry and biochemistry of selenium and its application in life sciences*. Huazhong University of Science and Technology Press, Wuhan, pp 58–67 (Chinese)

Yang, L. P. (2001) *The synthesis and bioactivity of selenium-containing organic compounds*. Ph.D Thesis, Wuhan University, Wuhan. P R China, pp 34–57

Yang, X. J., Chou, J., Sun, G. Q., Yang, H., Lu T. H. (2002) Synchronous fluorescence spectra of hemoglobin: a study of aggregation states in aqueous solutions. *Anal. Chim. Acta* **466**: 87–92