Available online at www.sciencedirect.com
ScienceDirect
International Journal of Pharmaceutics 351 (2008) 55–60
INTERNATIONAL JOURNAL OF PHARMACEUTICS
www.elsevier.com/locate/ijpharm

Spectroscopic studies on the interaction of azelnidipine with bovine serum albumin

Nan Wang, Ling Ye*, Fangfei Yan, Ren Xu

School of Chemical Biology and Pharmaceutical Sciences, Capital Medical University, Beijing 100069, PR China

Received 2 May 2007; received in revised form 28 July 2007; accepted 17 September 2007
Available online 21 September 2007

Abstract

Interaction between azelnidipine and bovine serum albumin (BSA) was investigated by fluorescence spectroscopy and circular dichroism (CD). Azelnidipine effectively quenched the intrinsic fluorescence of BSA via a combination of static and dynamic quenching, forming azelnidipine–BSA complex with binding constant (K_a) of the order of 10^5. The thermodynamic parameters obtained from van't Hoff equation revealed that both ΔH° and ΔS° were negative, that is, $-49.77\,\mathrm{kJ\,mol^{-1}}$ and $-64.47\,\mathrm{J\,mol^{-1}\,K^{-1}}$, respectively, suggesting that the binding is mainly driven by the enthalpy and hydrogen bonding plays major role in stabilizing azelnidipine–BSA complex. The binding of azelnidipine to BSA leads to changes in the conformation of BSA according to synchronous fluorescence spectra and CD data. The presence of metal ion decreases the binding constant of azelnidipine–BSA complex.

Keywords: Azelnidipine; Bovine serum albumin (BSA); Fluorescence quenching; Synchronous fluorescence; Circular dichroism (CD)

1. Introduction

Azelnidipine, (±)-(3)-(1-diphenylmethylazetidin-3-yl)-5-isopropyl-2-amino-1,4-dihydro-6-methyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylate, a 1,4-dihydropyridine derivative as shown in Fig. 1, is a newly developed long-acting calcium channel blocker with unique pharmacological features, e.g. cardiac slowing action and high affinity to vascular tissues, which distinguishes itself from other calcium channel blockers (Reed et al., 2001; Kuramoto et al., 2003; Ding et al., 2007). Azelnidipine, thus, became a new generation of calcium channel blocker that can be used for the treatment of hypertensive patients with or without potential ischemic heart diseases. So far, most researches were focusing on its mechanisms as a calcium channel blocker (Kawabata and Urasaki, 2006; Naito et al., 2006), little has been done on the interaction of azelnidipine with serum albumin.

Drug–albumin interactions are important since most of the administered drugs are extensively and reversibly bound to serum albumin and drug is transported mainly as a complex with protein. The nature and magnitude of drug–albumin interaction significantly influences the pharmacokinetics of the drug (Tian et al., 2005) and the binding parameters are useful in studying protein–drug binding as they greatly influence absorption, distribution, metabolism, and excretion properties of typical drugs (Zsila et al., 2003).

Our study focused on the azelnidipine–albumin interaction at the molecular level with the purpose to provide basic information for optimizing the biological utilization and pharmaceutical applications of azelnidipine. Bovine serum albumin (BSA) was selected because of its medically important, low cost, ready availability, unusual ligand-binding properties (Flarakos et al., 2005). Bovine and human albumin tertiary structures are similar in 76% and the results of all the studies are consistent with the fact that human and bovine serum albumin is homologous protein (Carter et al., 1994; Olson and Christ, 1996; Dockal et al., 2000).

Application of the spectral methods can reveal the reactivity of chemical and biological systems in low concentration under physiological conditions. There have been several studies on fluorescence quenching of albumin by drugs or other bioactive small molecules (Caballero-Quintero et al., 2001; Diaz et al., 2003; Cui et al., 2006). In this paper, the quenching of the intrinsic tryptophan fluorescence of BSA has been used as a

* Corresponding author. Tel.: +86 10 83911525.
E-mail address: lingye@ccmu.edu.cn (L. Ye).

0378-5173/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2007.09.016

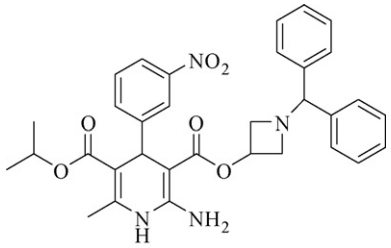

Fig. 1. Structure of azelnidipine.

tool to study the interaction of azelnidipine with this transport protein as well as the impacts of common metal ions on azelnidipine–BSA interaction. Additionally, effect of azelnidipine on conformational changes of BSA was investigated by synchronous fluorescence spectroscopy and circular dichroism (CD).

2. Materials and methods

2.1. Chemicals

Essentially fatty-acid-free bovine serum albumin (BSA, >99%, lyophilized power) was purchased from Sigma (USA). Azelnidipine was obtained from Garlin Pharmaceutical Co. (Beijing, China). All other chemicals were of analytical grade and used as received.

2.2. Apparatus

Fluorescence emission spectra and synchronous spectra were collected on a RF-5301PC spectrofluorophotometer (Shimadzu, Japan) equipped with a SB-11 water bath (Eyela) and 1.0 cm quartz cells. The absorption spectrum was recorded on a UV-2250 UV–vis spectrophotometer (Shimadzu, Japan). Circular dichroism spectra were measured with a Jasco J-810 Spectropolarimeter (Jasco, Tokyo, Japan) using a 0.1 cm quartz cell at room temperature over a wavelength range of 280–200 nm and under nitrogen flush.

2.3. Procedures

Ten micromolar BSA solution, based on the molecular weight of 68,000, was prepared by dissolving BSA in 0.1 M pH 7.4 phosphate buffer solution (PBS) containing 0.15 M NaCl. Azelnidipine solution was prepared by dissolving the drug in methanol with final concentration of 1.25×10^{-3} M. Various metal ion solutions of 2.5×10^{-3} M were prepared in ultra pure water.

For the fluorescence measurement, 2.5 mL 10 μM BSA and various amount of azelnidipine was added to a 1.0 cm quartz cell in the presence and absence of metal ions. The concentrations of azelnidipine were ranged from 5 to 50 μM and the total accumulated volume of azelnidipine was no greater than 100 μL. Corresponding fluorescence emission spectra were then recorded from 300 to 500 nm upon excitation wavelength at 295 nm using 3/3 nm slit widths. The wavelength 295 nm leads to the excitation of two tryptophanyl, i.e. Trp135 and Trp214 residues in BSA. All experiments were conducted at three temperatures, 298, 304, and 310 K, respectively.

Synchronous fluorescence spectra of BSA with various concentrations of azelnidipine were obtained from 300 to 400 nm ($\Delta\lambda = 60$ nm) and from 280 to 350 nm ($\Delta\lambda = 15$ nm) with the excitation and emission slit widths of 3/1.5 nm, respectively. The absorbance spectra of 10 μM azelnidipine were recorded at 298 K. CD spectra were collected from 200 to 280 nm at 0.2 nm intervals with five scans averaged for each CD spectrum. The molar ratio of azelnidipine to BSA was varied as 0:1, 1:1, 3:1 and 5:1.

3. Results and discussion

3.1. Fluorescence studies of BSA quenched by azelnidipine

Fluorescence spectra of BSA were recorded in the presence of various amounts of azelnidipine at 298 K. It is found that addition of azelnidipine results in a concentration dependent quenching of the intrinsic fluorescence of BSA without notable change of the emission maximum (Fig. 2). Fluorescence quenching is the decrease of quantum yield of fluorescence from a fluorophore induced by a variety of molecular interactions with quencher molecule. Therefore, the quenching of BSA fluorescence indicates that the formation of complex between azelnidipine and BSA occurs.

Fluorescence intensity data was then analyzed according to Stern–Volmer quenching equation:

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

where F and F_0 are the fluorescence intensity with and without the quencher (azelnidipine), respectively. K_{sv} and $[Q]$ are dynamic quenching constant and the concentration of quencher. The fluorescence quenching curve of BSA was plotted in Fig. 3.

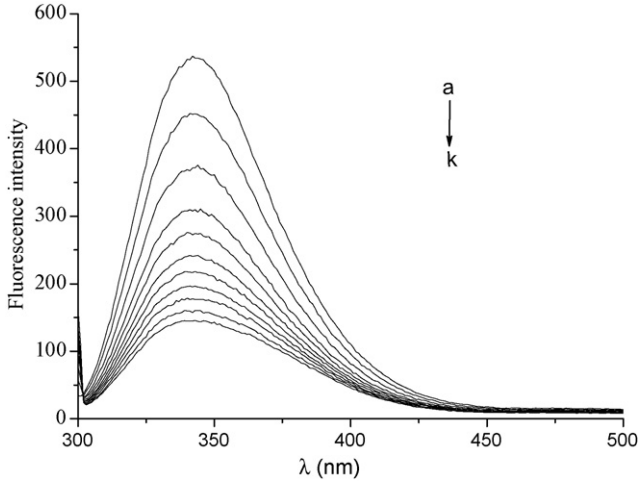

Fig. 2. Fluorescence quenching spectra of BSA in the presence of different concentrations of azelnidipine at 298 K. $C_{BSA} = 10$ μM. $C_{azelnidipine}$ from a to k: 0 μM (a), 5 μM (b), 10 μM (c), 15 μM (d), 20 μM (e), 25 μM (f), 30 μM (g), 35 μM (h), 40 μM (i), 45 μM (j), and 50 μM (k).

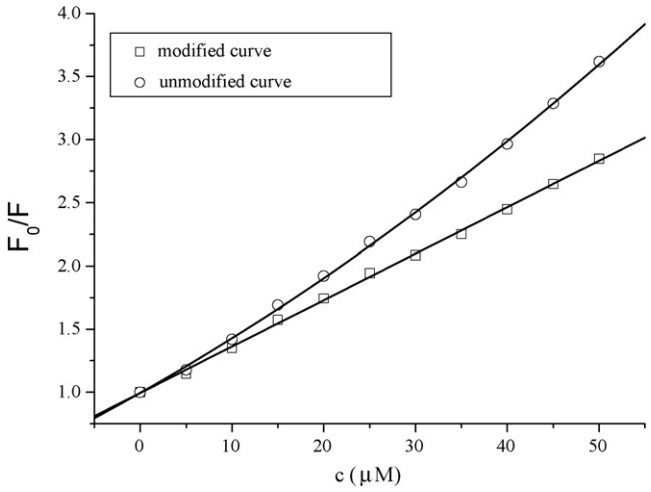

Fig. 3. The modified and unmodified Stern–Volmer plots of azelnidipine interaction with BSA at 298 K. $C_{BSA} = 10\ \mu M$.

The Stern–Volmer plot follows the linear relation at low concentration of azelnidipine but exhibits an upward curvature when the concentration of azelnidipine is greater than 10 μM. According to Eftink's investigations, this indicates that an additional static quenching takes place near the subdomain(s) where tryptophanyl residues are located. Thus, the positive deviation is due to the presence of a quencher molecule in the same solvent cage as the fluorophores at the moment of excitation. Some of the excited states are deactivated almost instantaneously after formation of the complex because azelnidipine molecules appear randomly in BSA fluorophores proximity at the time of their excitation (Boaz and Rollefson, 1950).

Since the positive deviation of the Stern–Volmer polt is caused when both static and dynamic quenching occurs.

To determine the dynamic and static quenching constants, the modified Stern–Volmer equation was applied (Eftink and Ghiron, 1975):

$$\frac{F_0}{F} = 1 + K_{sv}[Q]\ \exp(V[Q]) \tag{2}$$

where V is the static quenching constant and the value of V can be obtained from Eq. (2) by plotting $\{F_0/F \exp(V[Q])\} - 1$ versus $[Q]$ for varying V until a linear plot is acquired. The K_{sv} can be then obtained from slope of $\{F_0/F \exp(V[Q])\} - 1$ versus $[Q]$ plot. Values of V and K_{sv} at different temperatures (298, 304 and 310 K) are consequently presented in Table 1.

A positive deviation observed in Fig. 3 indicates that both static and dynamic quenching occurs in the alzelnidipine quenching of BSA intrinsic fluorescence (Thipperudrappa et al., 2006). However, it is found in Table 1 that the value of K_{sv} is much greater than that of V. This suggests that the overall quenching is dominated by dynamic quenching, while a small static quenching component contributing to the positive deviation of Stern–Volmer plot (Eftink and Ghiron, 1975).

Table 1
Static and dynamic quenching constants for azelnidipine–BSA system at different temperatures

T (K)	V ($\times 10^{-3}$ M^{-1})	K_{sv} ($\times 10^{-5}$ M^{-1})	R^2	S.D.
298	4.80	3.66	0.9988	0.0224
304	1.60	3.95	0.9995	0.0157
310	0.40	4.31	0.9988	0.0261

Table 2
Binding parameters obtained from the interaction of azelnidipine with BSA

T (K)	K_a ($\times 10^{-5}$ M^{-1})	n	R^2	S.D.
298	2.21	1.15	0.9985	0.0149
304	1.05	1.09	0.9988	0.0128
310	1.02	1.08	0.9962	0.0227

3.2. Analysis of binding equilibria

Fluorescence intensity data can also be used to obtain the binding constant (K_a) and the number of binding sites (n) for the drug–albumin complex. Equilibrium between free and bound azelnidipine is given by Eq. (3) in the case that drugs bind independently to a set of equivalent sites on a macromolecule,

$$\log \frac{F_0 - F}{F} = \log K_a + n\ \log[Q] \tag{3}$$

where n is the number of binding site, and K_a is the binding constant (or the apparent association constant) for drug–BSA interaction. Values of n and K_a (Table 2) can thereby be determined from the intercept and slope by plotting $\log(F_0 - F)/F$ versus $\log[Q]$. The number of binding site n as calculated is approximately equal to 1, indicating that there is one class of binding site for azelnidipine towards BSA. Value of K_a as obtained is of the order of 10^5, indicating that there is a strong interaction between azelnidipine and BSA.

3.3. Determination of the force acting between azelnidipine and BSA

Thermodynamic parameters relying on temperatures were analyzed to characterize the acting forces between drug and BSA. The interaction forces between drugs and biomolecules may include electrostatic interactions, multiple hydrogen bonds, van der Waals interactions, and hydrophobic interaction within the binding site (Cui et al., 2006). In order to elucidate the interaction of azelnidipine with BSA, the thermodynamic parameters, that is, free energy changes (ΔG°), enthalpy changes (ΔH°), and entropy changes (ΔS°) of interactions were calculated. If the enthalpy change (ΔH°) does not vary significantly over the temperature range studied, then its value as well as that of entropy change (ΔS°) can be determined from the van't Hoff equation:

$$\ln K = -\frac{\Delta H^\circ}{RT} + \frac{\Delta S^\circ}{R} \tag{4}$$

where K is the analog to the associative binding constants corresponding to various temperatures, and R is the gas constant. The enthalpy change (ΔH°) can be calculated from the slope of the van't Hoff relationship and the free energy change (ΔG°)

Table 3
Thermodynamic parameters of azelnidipine–BSA interaction

T (K)	K_a ($\times 10^{-5}$ M^{-1})	ΔG° (kJ mol^{-1})	ΔH° (kJ mol^{-1})	ΔS° (J mol^{-1} K^{-1})
298	2.21			
304	1.05	−30.55	−49.77	−64.47
310	1.02			

can be estimated from the following relationship:

$$\Delta G^\circ = \Delta H^\circ - T\,\Delta S^\circ \quad (5)$$

Values of ΔG°, ΔH°, and ΔS° are summarized in Table 3. The negative sign for free energy (ΔG°) means that the binding process is spontaneous. The negative values of ΔH° and ΔS° indicate that the binding is mainly driven by the enthalpy whereas the entropy is unfavorable for it, and hydrogen bonding along with van der Waals interactions play major role in the formation of azelnidipine–BSA complex (Ross and Subramanian, 1981).

3.4. Stability of azelnidipine–BSA complex

The stability of azelnidipine–BSA complex was studied by monitoring the changes of maximum of fluorescence (F_{max}) of BSA at different temperatures and the results are shown in Fig. 4. F_{max} of BSA decreases about 7.5% with increase of temperature from 298 to 310 K. Addition of azelnidipine at azelnidipine:BSA molar ratio 1:1 causes the similar dependence of F_{max} on temperature, i.e. F_{max} decreases 7.4% in the same temperature range. When azelnidipine:BSA is at molar ratio 5:1, F_{max} ceases to decreases with the increase of temperature. It is likely that azelnidipine stabilize the BSA structure regardless of temperature variation. This may be caused by the formation of the complex that may affect the microenvironment of the binding sites. The sensitivity of BSA to temperature thus is changed.

The breaking of the hydrogen bonds can result in temperature denaturation, therefore, it is the hydrogen bonds that probably stabilize this complex between azelnidipine and $-NH_2$ or OH groups in albumin. Moreover, the lack of dependence on temperature at higher azelnidipine/BSA molar ratio may indicate that there are additional stabilization factors, such as van der Waals interactions, between the azelnidipine and BSA.

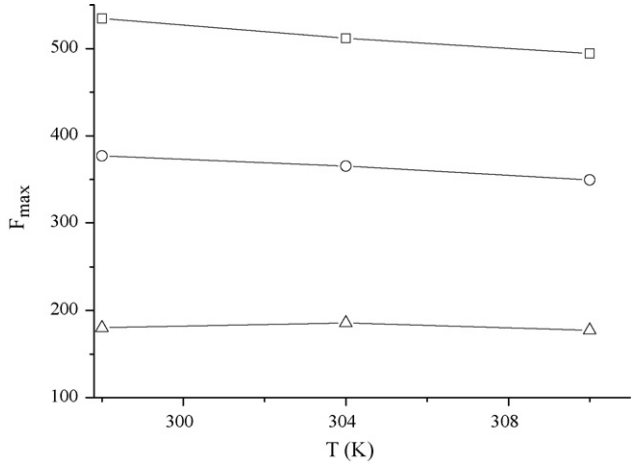

Fig. 4. Dependence of BSA maximum of fluorescence on temperature. The molar ratios of azelnidipine:BSA are (□) 0:1, (◯) 1:1, and (△) 5:1, respectively.

3.5. Conformational investigations

Synchronous fluorescence spectra can be used to analyze the change of the conformation of BSA because the maximum emission wavelength of tryptophan residues is related to the polarity of the environment and the change of emission wavelength is thus used to estimate that of protein conformation. If $\Delta\lambda = 15$ nm, the synchronous fluorescence spectra exhibits the spectral character only of tyrosine residues, and if $\Delta\lambda = 60$ nm, it exhibits that only of tryptophan residues. Synchronous fluorescence spectra of BSA upon addition of azelnidipine gained at $\Delta\lambda = 15$ and 60 nm are shown in Fig. 5.

It can be seen from Fig. 5 that the quenching of the fluorescence intensity of tryptophan residues is stronger than that of tyrosine residue, suggesting that tryptophan residues con-

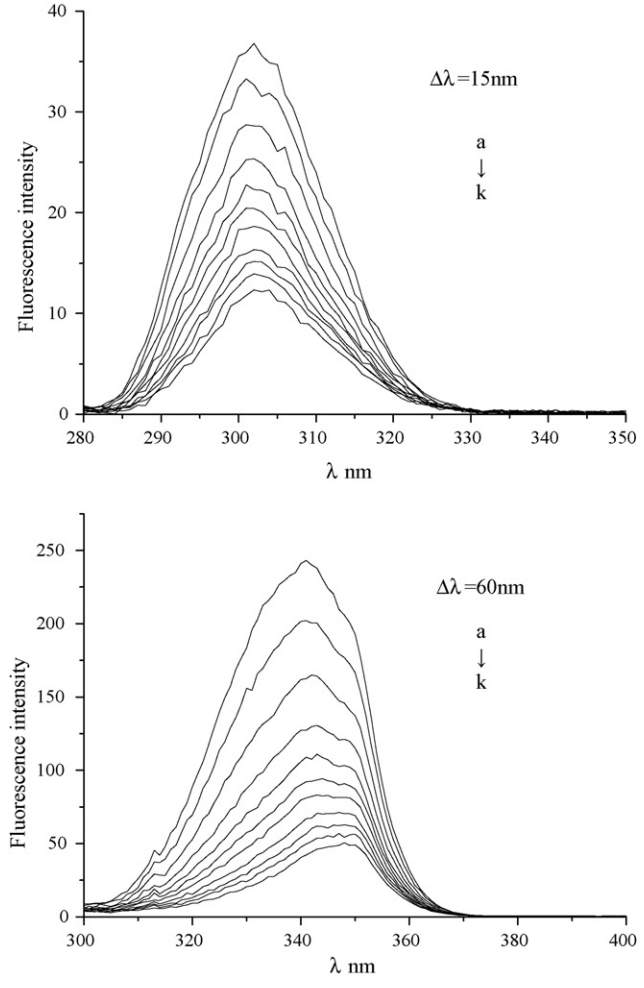

Fig. 5. Synchronous fluorescence spectra of BSA upon addition azelnidipine of at 298 K. C_{BSA} = 10 μM. $C_{azelnidipine}$ from a to k: 0 μM (a), 5 μM (b), 10 μM (c), 15 μM (d), 20 μM (e), 25 μM (f), 30 μM (g), 35 μM (h), 40 μM (i), 45 μM (j), and 50 μM (k).

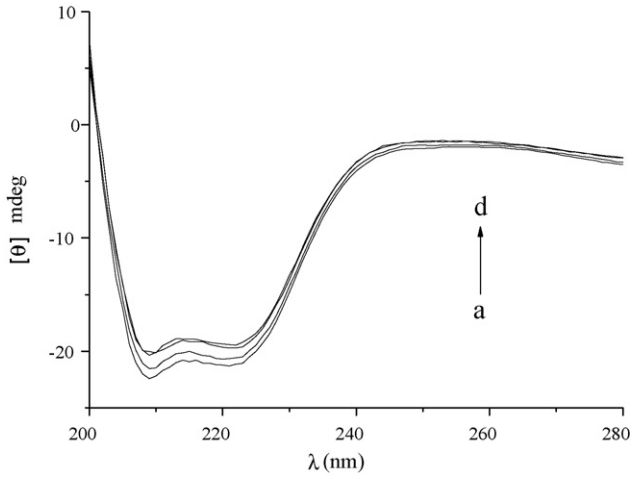

Fig. 6. CD spectra of BSA in the presence of azelnidipine. C_{BSA} = 1 μM. molar ratios of azelnidipine: BSA from a to d are 0:1 (a), 1:1 (b), 3:1 (c) and 5:1 (d), respectively.

tribute greatly to the quenching of intrinsic fluorescence of BSA. Moreover, a notable red shift of maximum emission wavelength of tryptophan residue was observed upon addition of azelnidipine, whereas the tyrosine residue emission maximum kept unchanged. This red shift indicates that the conformation of BSA is changed and tryptophan residues are placed in a less hydrophobic environment, resulting in the increase of polarity of the fluorophore environment. This increased polarity is probably due to the hydrogen bonding between the azelnidipine and BSA, which stabilize the complex.

Typical CD spectra of azelnidipine with BSA are shown in Fig. 6. As expected, the α-helices of BSA show a strong double minimum at 222 and 209 nm, which is the typical α-helix structure of BSA (Gao et al., 2006). The intensities of this double minimum reflect the amount of helicity of BSA. Upon addition of azelnidipine to BSA (1:1, 3:1 and 5:1), the extent of BSA α-helices decreases. The decreased helicity suggests the binding of azelnidipine with BSA induces a slight unfolding of the constitutive polypeptides of protein, which results in a conformational change of the protein that increased the exposure of some hydrophobic regions that were previously buried. This is in agreement with the synchronous fluorescence result.

3.6. Energy transfer from BSA to azelnidipine

Fluorescence resonance energy transfer (FRET) is a non-destructive spectroscopic method that can monitor the proximity and relative angular orientation of fluorophores, the donor and acceptor fluorophores can be entirely separate or attached to the same macromolecule. A transfer of energy could take place through direct electrodynamic interaction between the primarily excited molecule and its neighbors, with an important condition: the distance between the donor and the acceptor is approach in the range of 2–8 nm (Weiss, 1999). Regarding FRET, the energy transfer effect is not only related to the distance between the donor (tryptophan residue) and acceptor (azelnidipine), but also influenced by the critical energy transfer distance R_0. It could be calculated by the following equation:

$$E = 1 - \frac{F}{F_0} = \frac{R_0^6}{R_0^6 + r^6} \tag{6}$$

where r is the distance between the acceptor and the donor, and R_0 is the critical energy transfer distance, at which 50% of the excitation energy is transferred to the acceptor. R_0 depends on the quantum yield of the donor, the extinction coefficient of the acceptor, the overlap of donor emission and acceptor excitation spectra, and the mutual orientation of the chromophores. R_0 is defined by the following equation:

$$R_0^6 = 8.8 \times 10^{-25} k^2 N^{-4} \Phi J \tag{7}$$

In Eq. (7), k^2 is the space factor of orientation, N is the refracted index of medium, and Φ is the fluorescence quantum yield of the donor. J is the effect of the spectral overlap between the emission spectrum of the donor and the absorption spectrum of the acceptor (Fig. 7), which could be calculated by the equation:

$$J = \frac{\sum F(\lambda)\varepsilon(\lambda)\lambda^4 \Delta\lambda}{\sum F(\lambda)\Delta\lambda} \tag{8}$$

where $F(\lambda)$ is the corrected fluorescence intensity of the donor in the wavelength range λ to $\lambda + \Delta\lambda$ and $\varepsilon(\lambda)$ is the extinction coefficient of the acceptor at λ.

In the present case, $k = 2/3$, $N = 1.336$, $\Phi = 0.15$ (Kandagal et al., 2006), according to the Eqs. (6)–(8), we could calculate that $J = 5.33 \times 10^{-15}$ cm^3 L mol^{-1}, $R_0 = 2.29$ nm, $E = 0.295$, and $r = 2.66$ nm. As the distance of donor to acceptor for azelnidipine–BSA binding is in the 2–8 nm scale, the energy transfer from BSA to azelnidipine occurs with high probability.

3.7. Influences of common metal ions on binding constant

Metal ions are vital to human body and play an essentially structural role in many proteins based coordinate bonds. The presence of metal ions in plasma may affect interaction of drugs with BSA. Therefore, effect of some common metal ions (e.g.

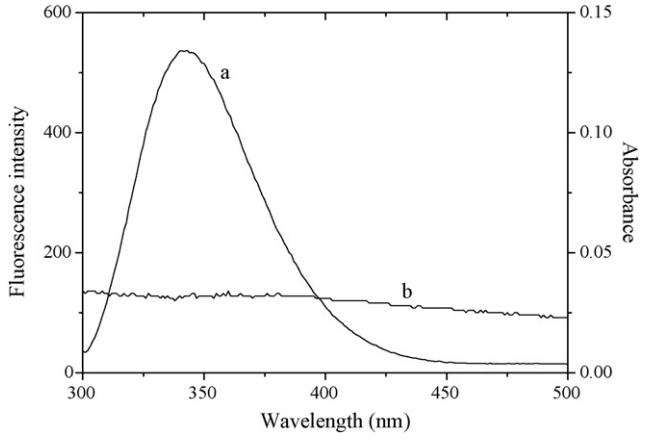

Fig. 7. Spectral overlap between (a) BSA fluorescence spectrum and (b) azelnidipine absorbance spectrum. $C_{BSA} = C_{azelnidipine}$ = 10 μM.

Table 4
Binding constants K_a of azelnidipine–BSA complex in the presence of various metal ions at 298 K

	K_a ($\times 10^{-5}$ M^{-1})	R^2	S.D.
BSA–azelnidipine	2.21	0.9985	0.0224
BSA–azelnidipine–Al^{3+}	1.46	0.9991	0.0234
BSA–azelnidipine–Ca^{2+}	1.31	0.9988	0.0186
BSA–azelnidipine–Cu^{2+}	1.23	0.9992	0.0161
BSA–azelnidipine–K^+	0.86	0.9963	0.0265
BSA–azelnidipine–Mg^{2+}	0.89	0.9995	0.0123
BSA–azelnidipine–Zn^{2+}	1.35	0.9990	0.0137

Cu^{2+}, Zn^{2+}, Mg^{2+}, Al^{3+}, K^+ and Ca^{2+}) on binding constant of azelnidipine–BSA complex was investigated at 298 K. The values of binding constant K_a acquired in the present of various metal ions are listed in Table 4.

As is shown in Table 4, the presence of the tested metal ions, e.g. Cu^{2+}, Zn^{2+}, Mg^{2+}, Al^{3+}, K^+ and Ca^{2+}, decreases the binding constant of azelnidipine–BSA complex. This is likely due to the conformational change in the vicinity of the binding site. Although the binding sites for azelnidipine and metal ion on BSA may not locate in the same domain, the formation of metal ion–BSA complexes is likely to lead to the changes in the conformation of albumin. This may affect azelnidipine binding kinetics and could even inhibit azelnidipine–BSA interaction. From the pharmacokinetic perspective, the decrease of the binding constant in the presence of metal ions may result in the increase in the concentration of free drug in blood plasma, which may enhance its maximum effects.

4. Conclusions

The interaction of azelnidipine with BSA results in the formation of azelnidipine–BSA complex. Azelnidipine quenches BSA intrinsic fluorescence through a combination of dynamic and static modes. The values of thermodynamic parameters suggest that the hydrogen bonding plays a major role in the interaction of azelnidipine with BSA. Synchronous fluorescence spectra and CD data reveal that the conformation of BSA is changed upon addition of azelnidipine. The presence of the metal ions, such as Cu^{2+}, Zn^{2+}, Mg^{2+}, Al^{3+}, K^+ and Ca^{2+}, decreases the binding constants of azelnidipine–BSA complex, which may cause an increase in the concentration of free drug in blood plasma, thus, increase in the effect of free drug.

Acknowledgements

We gratefully acknowledge the financial support provided by Beijing Natural Science Foundation of China (no. 5073043), Scholastic Natural Science Foundation of Capital Medical University (no. 2005ZR06), and Beijing Municipal Project for Developing Advanced Human Resources for Higher Education.

References

Boaz, H., Rollefson, G.K., 1950. The quenching of fluorescence. Deviations from the Stern–Volmer law. J. Am. Chem. Soc. 72, 3435–3443.
Caballero-Quintero, A., Pineyro-Lopez, A., Waksman, N., 2001. In vitro binding studies of the peroxisomicine A1–BSA and –HSA interactions. Int. J. Pharm. 229, 23–28.
Carter, D.C., Chang, B., Ho, J.X., Keeling, K., Krishnasami, Z., 1994. Preliminary crystallographic studies of 4 crystal forms of serum–albumin. Eur. J. Biochem. 226, 1049–1052.
Cui, F.L., Wang, J.L., Cui, Y.R., Li, J.P., 2006. Fluorescent investigation of the interactions between *N*-(*p*-chlorophenyl)-*N*′-(1-naphthyl) thiourea and serum albumin: synchronous fluorescence determination of serum albumin. Anal. Chim. Acta 571, 175–183.
Diaz, X., Abuin, E., Lissi, E., 2003. Quenching of BSA intrinsic fluorescence by alkylpyridinium cations—Its relationship to surfactant–protein association. J. Photochem. Photobiol. A Chem. 155, 157–162.
Ding, L., Li, L., Ma, P.C., 2007. Determination of azelnidipine in human plasma by liquid chromatography–electro spray ionization-mass spectrometry. J. Pharm. Biomed. Anal. 43, 575–579.
Dockal, M., Carter, D.C., Ruker, F., 2000. Conformational transitions of the three recombinant domains of human serum albumin depending on pH. J. Biol. Chem. 275, 3042–3050.
Eftink, M.R., Ghiron, C.A., 1975. Fluorescence quenching of indole and model micelle systems. J. Phys. Chem. 80, 486–493.
Flarakos, J., Morand, K.L., Vouros, P., 2005. High-throughput solution-based medicinal library screening against human serum albumin. Anal. Chem. 77, 1345–1353.
Gao, H., Wang, Y.N., Fan, Y.G., Ma, H.B., 2006. Interactions of some modified mono- and bis-beta-cyclodextrins with bovine serum albumin. Bioorg. Med. Chem. 14, 131–137.
Kandagal, P.B., Ashoka, S., Seetharamappa, J., Vani, V., Shaikh, S.M.T., 2006. Study of the interaction between doxepin and human serum albumin by spectroscopic methods. J. Photochem. Photobiol. A 179, 161–166.
Kawabata, K., Urasaki, Y., 2006. Simultaneous determination of azelnidipine and two metabolites in human plasma using liquid chromatography–tandem mass spectrometry. J. Chromatogr. B 844, 45–52.
Kuramoto, K., Ichikawa, S., Hirai, A., Kanada, S., Nakachi, T., Ogihara, T., 2003. Azelnidipine and amlodipine: a comparison of their pharmacokinetics and effects on ambulatory blood pressure. Hypertens. Res. 26, 201–208.
Naito, Y., Shimozawa, M., Manabe, H., Nakabe, N., Katada, K., Kokura, S., Yoshida, N., Ichikawa, H., Kon, T., Yoshikawa, T., 2006. Azelnidipine, a new calcium channel blocker, inhibits endothelial inflammatory response by reducing intracellular levels of reactive oxygen species. Eur. J. Pharmacol. 546, 11–18.
Olson, R.E., Christ, D.D., 1996. Plasma protein binding of drugs. Annu. Rep. Med. Chem. 31, 327–336.
Reed, M.D., Myers, C.M., Blumer, J.L., 2001. Influence of midazolam on the protein binding of ketorolac. Curr. Ther. Res. 62, 558–565.
Ross, P.D., Subramanian, S., 1981. Thermodynamics of protein association reactions: forces contributing to stability. Biochemistry 20, 3906–3912.
Thipperudrappa, J., Biradar, D.S., Lagare, M.T., Hanagodimath, S.M., Inamdar, S.R., Kadadevaramath, J.S., 2006. Fluorescence quenching of BPBD by aniline in benzene–acetonitrile mixtures. J. Photochem. Photobiol. A 177, 89–93.
Tian, J.N., Liu, J.Q., Hu, Z., Chen, X.G., 2005. Interaction of wogonin with bovine serum albumin. Bioorg. Med. Chem. 13, 4124–4129.
Weiss, S., 1999. Fluorescence spectroscopy of single biomolecules. Science 283, 1676–1683.
Zsila, F., Bikadi, Z., Simonyi, M., 2003. Probing the binding of the flavonoid, quercetin to human serum albumin by circular dichroism, electronic absorption spectroscopy and molecular modelling methods. Biochem. Pharmacol. 65, 447–456.