Journal of Chromatography B, 879 (2011) 1934–1938

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Investigation of competitive binding of ibuprofen and salicylic acid with serum albumin by affinity capillary electrophoresis

Zhu-Mei Li[a,1], Chuan-Wan Wei[a,1], Yintang Zhang[b], Di-Shan Wang[a], You-Nian Liu[a,*]

[a] College of Chemistry and Chemical Engineering, Central South University, Changsha, Hunan 410083, PR China
[b] Department of Chemistry, Shangqiu Normal University, Shangqiu, Henan 476000, PR China

ARTICLE INFO

Article history:
Received 31 January 2011
Accepted 17 May 2011
Available online 23 May 2011

Keywords:
Affinity capillary electrophoresis
Competitive binding
Ibuprofen
Salicylic acid
Serum albumin
Binding constant

ABSTRACT

Ibuprofen and salicylic acid, two typical non-steroidal anti-inflammatory drugs, are used commonly as analgesic drug in clinical medicine and sometimes are co-administered. When the drugs are co-administered, the drug–drug interactions may occur, and can lead to alter the safety and efficacy of drugs, resulting in variations in drug response of the co-administered drugs. Affinity capillary electrophoresis (ACE) was employed to investigate the competitive binding of ibuprofen and salicylic acid on serum albumin. Mobility ratio, derivatives from mobility shift method, was used to deduce the binding constant (K_b). The binding constants of ibuprofen with HSA are $2.97 \times 10^6\ M^{-1}$ and $7.07 \times 10^4\ M^{-1}$, respectively; while for salicylic acid, the binding constant is $5.99 \times 10^4\ M^{-1}$. The competitive binding of the two drugs was investigated by addition of excessive ibuprofen into the solutions containing constant concentrations of salicylic acid and serum albumin. The results confirmed that ibuprofen and salicylic acid have different high-affinity binding site, but share some low-affinity binding sites on the serum albumin; and ibuprofen is able to partially replace salicylic acid from the preformed binary complexes of serum albumin and salicylic acid.

1. Introduction

Serum albumin, the most abundant protein in blood plasma, accounts for about 60% of the plasma's total protein contents, and contains three homologous helical domains (I–III), each divided into A and B subdomains. The binding of drugs with serum albumin plays an important role in the study of the bioavailability, efficacy, transport and toxicity of the drugs. Many drugs bind to serum albumin at one of two primary sites (I and II) located in subdomains IIA and IIIA, respectively [1,2]. The investigation of the interaction of drugs and serum albumin appeals lots of interests. Apart from interaction with serum albumin, drug co-administration in therapy has a significant influence on binding to serum albumin. The bound drugs could be displaced by another one which has higher affinity to serum albumin, or the structure of serum albumin may be altered, the change of affinity binding of drugs to serum albumin will result in the variation of the level of a drug, which is closely related to the drug's therapeutic effect [3].

Non-steroidal anti-inflammatory drugs have been widely used in the pharmaceutical field as the performances of treating inflammation, pain and fever, especially in the treatment and prevention of Parkinson's disease (PD) [4] and Alzheimer's disease (AD) [5]. Ibuprofen (Ibu), salicylic acid (Sal) and their derivatives are used commonly as analgesic and anti-inflammatory drugs in clinical medicine. When interaction with serum albumin, ibuprofen primarily binds to site II of serum albumin, while salicylic acid binds to site I mainly [6–8]. Some researchers studied the competitive binding where two ligands (the drug and the competitor) share the same binding site on the protein [9,10]. In this work, we focus on the competitive binding of drugs which have different high-affinity binding sites on protein.

Many strategies were adopted to investigate the interactions between drugs and protein [11–17]. For example, Hage and coworkers investigated the interaction of lidocaine with serum protein using high-performance affinity chromatography [18]. Liu et al. studied the competitive reaction between bovine serum albumin (BSA) and neomycin (NM) with ponceau S (PS) as a fluorescence probe [19]. NMR method was also utilized in competitive binding. Liu's group investigated competitive binding of ligands, such as ibuprofen and salicylic acid, tolmetin and salicylic acid with HSA [20]. Capillary electrophoresis (CE) is a rapid, accurate assay to measure the binding constant and study the affinity interaction [21–25]. Yang et al. studied the detection of prion protein using a capillary electrophoresis-based competitive immunoassay with laser-induced fluorescence detection and cyclodextrin-aided separation [26]. Zou and coworkers studied the competitive binding of enantiomers (D-, L-tryptophan) to protein by affinity capillary

* Corresponding author.
E-mail address: liuyounian@mail.csu.edu.cn (Y.-N. Liu).
[1] Authors contributed equally to this work.

1570-0232/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2011.05.020

electrophoresis [27]. In our previous work, ACE was employed to determine the binding properties of diclofenac sodium with BSA [28].

The aim of this work is to determine the binding constants of ibuprofen and salicylic acid with proteins, and the competitive binding properties of these two drugs on serum albumin by ACE.

2. Materials and methods

2.1. Reagents and chemicals

BSA was purchased from AMRESCO (Solon, OH, USA); HSA, salicylic acid and ibuprofen were purchased from Sigma (St. Louis, MO, USA). Boric acid, borax, and other reagents were of analytical grade and used as received. All solutions were prepared with deionized water treated with a water purification system (18 MΩ cm, Simplicity Model, Billerica, MA, USA).

2.2. Apparatus

CE experiments were performed with a Beckman-Coulter P/ACE MDQ system (Fullerton, CA, USA). Data were collected and analyzed with the Beckman 32 Karat Software. The uncoated fused-silica capillary was 50 cm in length with I.D. of 75 μm and O.D. of 375 μm, purchased from Ruipu Chromatography Equipment Co. (Hebei, China). The effective length of the capillary from the inlet to the detector is 40 cm. Operational voltage of 20 kV was performed in normal polarity and the detection wavelength was set to 214 nm. The temperature of the capillary was kept at 25 °C.

2.3. Sample and solution preparations

The borate buffer containing 15 mM borax was adjusted to pH 8.5 with 60 mM boric acid. Samples were prepared by diluting a 10 mM stock solution to 0.05 mM using borate buffer. All the solutions were filtered through 0.22 μm membrane filters (Xingya Purification Materials Inc., Shanghai, China) before use.

2.4. Procedures

The capillary was rinsed with NaOH (1 M) for 2 min, and deionized water for 2 min, then buffer for 2 min at 20 psi successively after each run. Different amounts of serum albumin were then added into the borate buffers, and the 0–20 μM serum albumin solutions which used as the ACE running buffers were obtained. When the samples were injected into the capillary by applying a pressure of 0.5 psi at the anode for 5 s, the volume and the length of the resultant sample plug were ca. 18 nL and 4 mm (calculated using the Poiseulle equation), respectively. A 1% (v/v) dimethyl sulfoxide (DMSO) was used as the neutral EOF marker in all CE experiments.

3. Results and discussion

3.1. Optimization of ACE experimental conditions

For drug/protein interaction studies, either the drug or the protein can be added into the ACE running buffer [29,30]. According to our previous work [28], ibuprofen and salicylic acid were injected into running buffer with or without serum albumin, respectively. The mobility shifts of drugs in the presence of serum albumin are obviously different from that in the absence of serum albumin. However, the mobility of serum albumin is close to that of serum albumin/drug complex. Therefore, serum albumin was used to be an additive for the experiment.

The adsorption of serum albumin onto the capillary inner wall will result in width and tailing of peak, even low resolution and efficiency [31–33]. To eliminate the adsorption of proteins the serum albumin concentration was controlled lower than 20 μM and a borate buffer (60 mM, pH 8.5) was employed according to our previous works [21,28]. In order to investigate the effects of pH on the peak shape and separation efficiency in CE system, we studied drug–protein interactions in different pH buffer solutions over the pH range from 6 to 9. The experimental results revealed that the peaks of serum albumin were broad and tailed due to the protein adsorption onto the capillary inner wall at acidic or neutral buffer solutions. Also, we found that the high separation efficiency was obtained when in the borate buffer solution (pH 8.5). Thus, we chose pH 8.5 as the optimal condition for all experiments. The SA solution was injected at 1 h intervals and it was found that the mobility time of SA is essentially unchanged for 4 h and peak areas maintain almost constant. Hereby, in the case that the adsorption of serum albumin onto the capillary inner wall was neglectable, the concentrations of serum albumin were controlled in the range of 0–20 μM.

It is well-known that the concentration of sample has a significant impact in ACE [34,35]. Generally, the higher concentration of analyte tends to lower the separation efficiency; whereas, the data precision could be improved greatly. Fig. 1 shows that the mobility shift of drugs decreases greatly with the increases of the concentration of drugs initially while the migration times of DMSO remain essentially unchanged (from 2.16 to 2.17 min for Sal-BSA and from 2.51 to 2.53 min for Ibu-BSA). When the concentrations of the drug reached 1 mM, the trend of mobility shifts turned to gradual decrease, suggesting that the estimation of K_b value can be achieved with drug concentrations between 0.1 and 1 mM. Thus, the solution of 1 mM ibuprofen and salicylic acid were adopted since the drugs can be readily detected in this concentration.

3.2. Estimation of the binding constants for the binary system

In ACE, the binding constant K_b can be determined from the variation of mobility shifts of a sample as a role of additive concentrations in the running buffer. The electrophoretic mobility of a drug is given by

$$\mu = \frac{l_c l_d}{Vt} \quad (1)$$

In this work, the mobility ratio M was inducted to eliminate the effects of the variation of electroosmotic flow (EOF) [29,36], as defined in the following equation:

$$M = \frac{\left\lfloor l_c l_d/(Vt)\right\rfloor}{\left\lfloor l_c l_d/(Vt_{EOF})\right\rfloor} + 1 = \frac{t_{EOF}}{t} + 1 \quad (2)$$

where l_c (cm) is the total length of the capillary, l_d (cm) is the effective length of the capillary, t (s) is the measured analyte migration time, t_{EOF} (s) is the migration time of the neutral marker, and V is the operating voltage. The K_b value was estimated by typical Scatchard analysis (Fig. 2). The binding constants of ibuprofen with BSA are $1.27 \times 10^6\ M^{-1}$ and $8.02 \times 10^4\ M^{-1}$ with correlation coefficients $R^2 = 0.99$ and 0.97, respectively. The binding constant of salicylic acid with BSA is $3.92 \times 10^4\ M^{-1}$ with correlation coefficients $R^2 = 0.99$. The yield binding constants of ibuprofen with HSA are $2.97 \times 10^6\ M^{-1}$ and $7.07 \times 10^4\ M^{-1}$ with correlation coefficients $R^2 = 0.99$ and 0.99, respectively. The binding constants of salicylic acid with HSA is $5.99 \times 10^4\ M^{-1}$ with correlation coefficients $R^2 = 0.98$. The results demonstrated that the binding constant of ibuprofen with serum albumin is much bigger than that of salicylic acid, i.e., ibuprofen has a significantly larger affinity to serum

Table 1
Binding constants of ibuprofen and salicylic acid with BSA and HSA.

Serum albumin	Complex	K_1 (M^{-1})	K_2 (M^{-1})	Coef	Method	Conditions	Ref.
BSA	Ibu-BSA	1.27×10^6	8.02×10^4	0.990.97	ACE	0.060 M borate (pH 8.50) 25 °C	This work
		1.31×10^6	1.76×10^4		ED	0.033 M phosphate (pH 7.4), 37 °C	[38]
	Sal-BSA	3.92×10^4		0.99	ACE	0.060 M borate (pH 8.50), 25 °C	This work
		7.38×10^4		0.98	Fluorescence	0.05 M Tris (pH 7.0), 0.01 M NaCl, 30 °C	[39]
HSA	Ibu-HSA	2.97×10^6	7.07×10^4	0.990.99	ACE	0.060 M borate (pH 8.50), 25 °C	This work
		2.47×10^6	1.47×10^4		ED	0.033 M phosphate (pH 7.4), 37 °C	[38]
	Sal-HSA	5.99×10^4		0.98	ACE	0.060 M borate (pH 8.50), 25 °C	This work
		1.89×10^4			Capacitive sensing technique	0.01 M phosphate (pH 7.0)	[40]
	$(Sal)_nSA\,(Ibu)_m$	4.65×10^5 **(Ibu)**	2.24×10^4 **(Ibu)**	0.990.99	ACE	0.060 M borate (pH 8.50), 25 °C	This work
		3.47×10^4 **(Sal)**		0.99	ACE	0.060 M borate (pH 8.50), 25 °C	This work

albumin than that of salicylic acid, the results are consistent with the previous studies (see Table 1).

The reproducibility of mobility ratio M from ibuprofen and salicylic acid was determined in the presence of various concentrations of serum albumin (0–20 μM). The relative standard deviations (RSD) for five replicate injections ranged from 3.5% to 8%. And the RSD for day-to-day M_D values tested over a period of 5 days ranged from 4.0% to 9.6%.

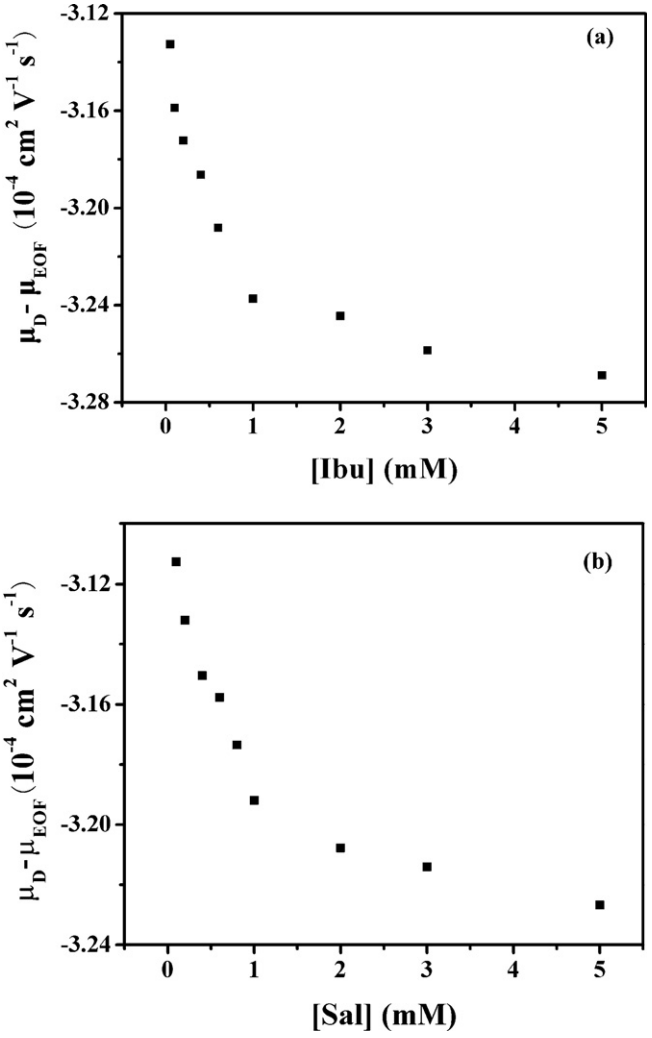

Fig. 1. Mobility shifts of ibuprofen and salicylic acid as the function of ibuprofen and salicylic acid concentrations, respectively. (a) The concentration of BSA in the running buffer was kept constant at 10 μM and the ibuprofen concentrations used were 0.05, 0.1 0.2, 0.4, 0.6, 1.0, 2.0, 3.0 and 5.0 mM. (b) The concentration of BSA in the running buffer was kept constant at 20 μM and the salicylic acid concentrations used were 0.1, 0.2, 0.4, 0.6, 0.8, 1.0, 1.5, 2.0, 3.0 and 5.0 mM. ACE conditions: 20 kV, 25 °C, injection at 0.5 psi for 5 s, and detection at 214 nm.

3.3. Competitive binding of ibuprofen and salicylic acid

As mentioned before, salicylic acid and ibuprofen have different high-affinity binding sites on serum albumin. Salicylic acid prefers to bind to site I, while ibuprofen primarily binds to site II on serum albumin. However, they share some low-affinity binding sites on serum albumin [8]. Generally, when the molar ratio of ligand to protein is less than unity, the ligand prefers to bind to high-affinity

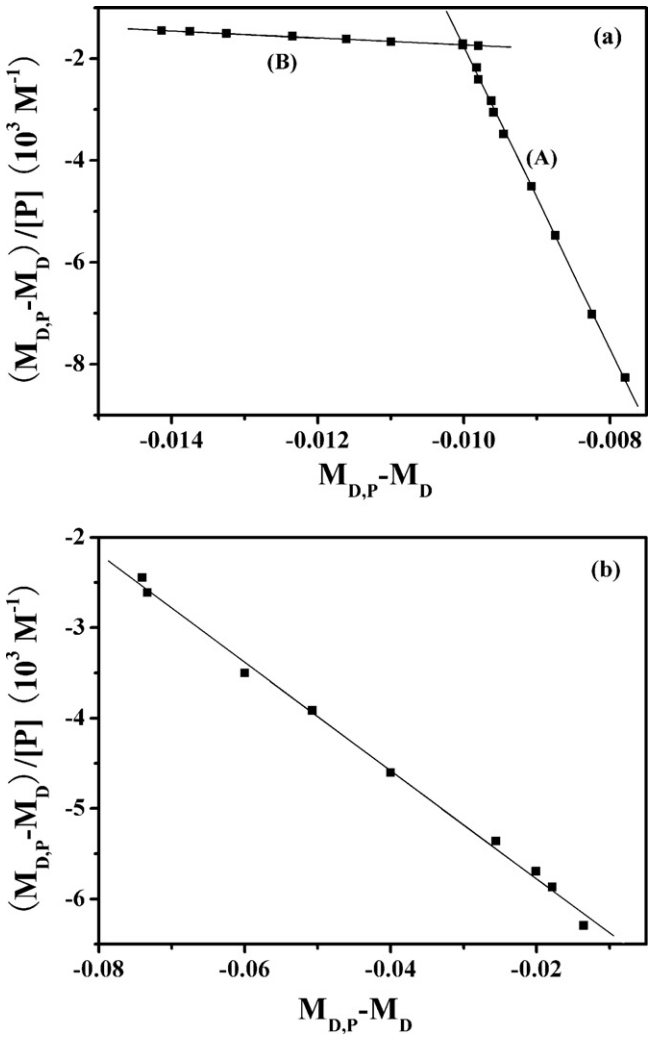

Fig. 2. Estimation of binding constants for ibuprofen and salicylic acid to HSA using Scatchard analysis by ACE. (a) Scatchard plot of the data for ibuprofen and HSA. Equations of the linear fitting: (A) $y = -31.48 - 2.967 \times 10^6 x$, $R^2 = 0.99$; (B) $y = -2.439 - 70.66 \times 10^3 x$, $R^2 = 0.99$. (b) Scatchard plot of the data for salicylic acid and HSA. Equations of the linear fitting: $y = -6.971 - 59.85 \times 10^3 x$, $R^2 = 0.98$.

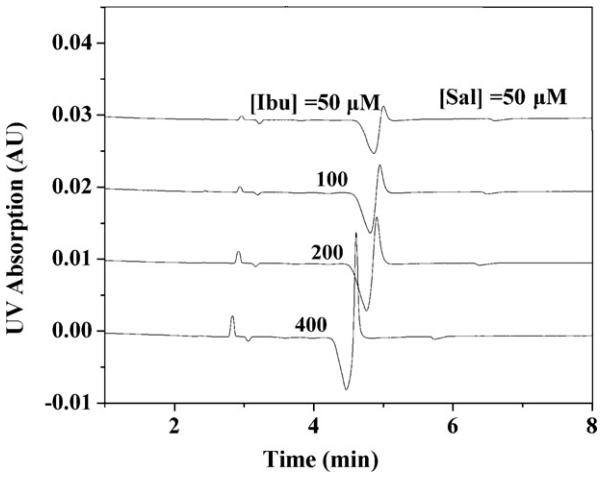

Fig. 3. Representative electropherograms of different concentrations ibuprofen in the presence of constant HSA concentration in the running buffer with constant concentration salicylic acid. The injected ibuprofen concentration was varied from 50, 100, 200, 400 μM. The concentrations of HSA and salicylic acid in the running buffer were 20 μM and 50 μM, respectively. The ACE separation conditions were the same as those described in Fig. 1.

binding sites of the protein; otherwise, it tends to bind to both high- and low-affinity binding sites [7,37]. Herein, salicylic acid and HSA were mixed with high molar ratio (Sal:HSA = 5:2) to ensure that salicylic acid can bind to both high- and low-affinity binding sites of HSA. Ibuprofen was then injected to the running buffer containing preformed binary complexes of salicylic acid and HSA, primarily it binds to serum albumin on its high affinity sites (site II of serum albumin). As shown in Fig. 3, the mobility time of salicylic acid reduced with the addition of ibuprofen (migration time of DMSO remains almost constant), indicating that the fraction of free Sal increased, i.e., Ibu displaced Sal from its low-affinity binding sites. In addition, the mobility time of ibuprofen reduced as well, further confirming that ibuprofen is able to displace salicylic acid from the complex $SA(Sal)_{n'}$ and form complex $(Sal)_nSA(Ibu)_m$ as described by the following equations:

$$m\text{Ibu} + \text{SA(Sal)}_{n'} \rightleftharpoons (\text{Sal})_n\text{SA(Ibu)}_m + (n' - n)\text{Sal} \quad (3)$$

where $SA(Sal)_{n'}$ and $(Sal)_nSA(Ibu)_m$ represent binary and ternary complexes of the drug(s) and serum albumin.

Moreover, with the further addition of ibuprofen into the above ternary running buffer, the migration time of ibuprofen become closer to that of binary system of serum albumin and ibuprofen (data not shown), suggesting that ibuprofen and salicylic acid have their own specific binding sites besides their mutual ones. That is, the binding sites of ibuprofen and salicylic acid on serum albumin are not fully overlapped, and the competitive binding occurs not only at those mutual binding sites (mainly low-affinity sites on serum albumin), but also at high-affinity sites on serum albumin.

Quantitatively, when ibuprofen was injected into binary system of serum albumin and salicylic acid, the first binding constant of Ibu-HSA decreases from $2.97 \times 10^6\ M^{-1}$ to $4.65 \times 10^5\ M^{-1}$ (see Fig. 4a), and the second binding constant of Ibu-HSA decreases slightly (from $7.07 \times 10^4\ M^{-1}$ to $2.24 \times 10^4\ M^{-1}$), which suggested that the competition of ibuprofen and salicylic acid mainly occur on the high-affinity site of ibuprofen (site II of serum albumin). On the other hand, in the ternary system, the binding constant of salicylic acid with serum albumin was basically unchanged (see Fig. 4b), conforming that salicylic acid does not share high-affinity binding site with ibuprofen. The results revealed that CE has been an alternative method to estimate the binding constants of ligand-receptor in ternary system using Scatchard analysis by ACE.

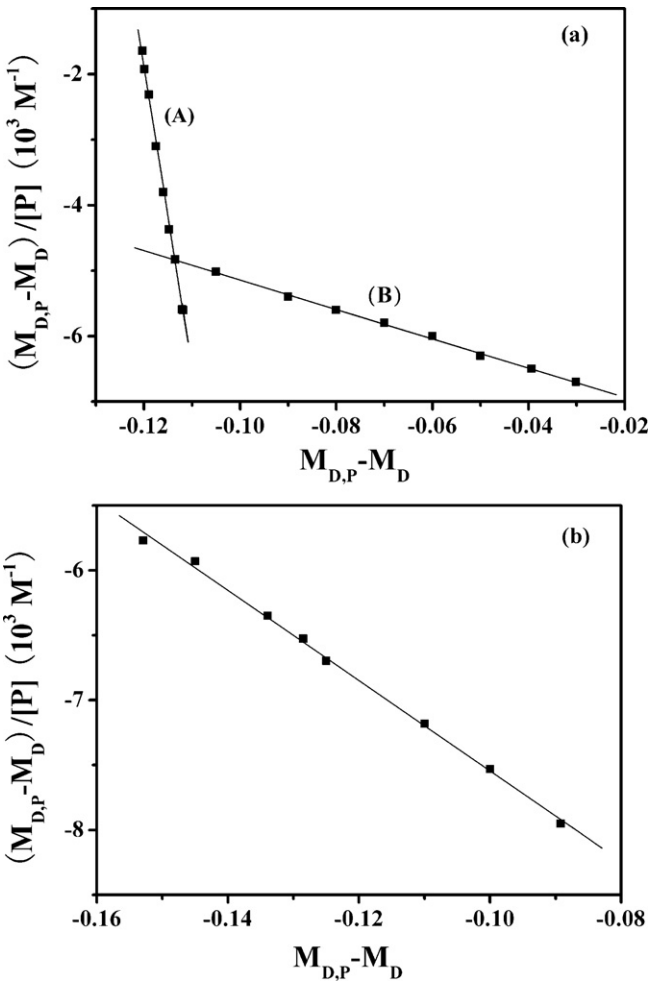

Fig. 4. Estimation of binding constants for ibuprofen and salicylic acid to HSA in the ternary system using Scatchard analysis by ACE. (a) Scatchard plot of the data for ibuprofen and HSA. Equations of the linear fitting: (A) $y = -57.78 - 4.659 \times 10^5 x$, $R^2 = 0.99$; (B) $y = -7.382 - 22.40 \times 10^3 x$, $R^2 = 0.96$. (b) Scatchard plot of the data for salicylic acid and HSA. Equations of the linear fitting: $y = -11.01 - 34.73 \times 10^3 x$, $R^2 = 0.98$.

4. Conclusions

The binding properties of ibuprofen and salicylic acid with serum albumin were studied by ACE. Although ibuprofen and salicylic acid do not share the same high affinity sites in serum albumin, the binding of salicylic acid with serum albumin was affected by ibuprofen when they were simultaneously binding to serum albumin, demonstrating that ibuprofen and salicylic acid share some common binding sites in serum albumin. The competition occurs not only in the low-affinity sites but also in the high-affinity sites.

Compared with other methods, the ACE method is a convenient tool to study the binding properties of drugs with protein. Especially, it can be used as an alternative method to quantitatively analyze the competitive binding of two ligands which simultaneously bind to protein.

Acknowledgements

The authors gratefully thank the financial support of this study by the Natural Science Foundation of China (Nos. 20876179, 20905045 and 21076232).

References

[1] G. Sudlow, D.J. Birkett, D.N. Wade, Mol. Pharmacol. 11 (1975) 824.
[2] E.L. Gelamo, C.H.T.P. Silva, H. Imasato, M. Tabak, Biochim. Biophys. Acta – Protein Struct. Mol. Enzymol. 1594 (2002) 84.
[3] A. Sułkowska, B. Bojko, J. Równicka, W.W. Sułkowski, Biopolymer 81 (2006) 472.
[4] E. Esposito, V. Di Matteo, A. Benigno, M. Pierucci, G. Crescimanno, G. Di Giovanni, Exp. Neurol. 205 (2007) 295.
[5] S. Weggen, M. Rogers, J. Eriksen, Trends Pharmacol. Sci. 28 (2007) 536.
[6] J. Ghuman, P.A. Zunszain, I. Petitpas, A.A. Bhattacharya, M. Otagiri, S. Curry, J. Mol. Biol. 353 (2005) 38.
[7] T. Kosa, T. Maruyama, M. Otagiri, Pharm. Res. 14 (1997) 1607.
[8] Y. Ni, S. Su, S. Kokot, Anal. Chim. Acta 580 (2006) 206.
[9] Q. Zhang, Y. Huang, R. Zhao, G. Liu, Y. Chen, Biosens. Bioelectron. 24 (2008) 48.
[10] C. Diot, Y. Kibleur, L. Desfrere, Early Hum. Dev. 86 (2010) 315.
[11] I. Vayá, R. Pérez-Ruiz, V. Lhiaubet-Vallet, M.C. Jiménez, M.A. Miranda, Chem. Phys. Lett. 486 (2010) 147.
[12] R. Punith, U. Katrahalli, S.S. Kalanur, S. Jaldappagari, J. Lumin. 130 (2010) 2052.
[13] K. Vuignier, J. Schappler, J.L. Veuthey, P.A. Carrupt, S. Martel, J. Pharm. Biomed. Anal. 53 (2010) 1288.
[14] T. Pan, Z.-D. Xiao, P.-M. Huang, J. Lumin. 129 (2009) 741.
[15] B. Bojko, A. Sułkowska, M. Maciążek-Jurczyk, J. Równicka, W.W. Sułkowski, J. Mol. Struct. 924–926 (2009) 332.
[16] M.J. Yoo, J.E. Schiel, D.S. Hage, J. Chromatogr. B 878 (2010) 1707.
[17] K.S. Joseph, J. Anguizola, A.J. Jackson, D.S. Hage, J. Chromatogr. B 878 (2010) 2775.
[18] S. Soman, M.J. Yoo, Y.J. Jang, D.S. Hage, J. Chromatogr. B 878 (2010) 705.
[19] B.S. Liu, C.L. Xue, J. Wang, C. Yang, F.L. Zhao, Y.K. Lv, J. Lumin. 130 (2010) 1999.
[20] Y.F. Cui, G.Y. Bai, C.G. Li, C.H. Ye, M.L. Liu, J. Pharm. Biomed. Anal. 34 (2004) 247.
[21] Y.T. Zhang, M.T. Xu, M. Du, F.M. Zhou, Electrophoresis 28 (2007) 1839.
[22] D. El-Hady, S. Kühne, N. El-Maali, H. Wätzig, J. Pharm. Biomed. Anal. 52 (2010) 232.
[23] H.M. Serra, D.R. Bronze, A.L. Simplício, J. Chromatogr. B 878 (2010) 1480.
[24] Z. Chen, S.G. Weber, Trends. Anal. Chem. 27 (2008) 738.
[25] W. Chen, W. Li, X. Ling, X. Wang, J. Liu, J. Chromatogr. B 878 (2010) 1714.
[26] W.C. Yang, E.S. Yeung, M.J. Schmerr, Electrophoresis 26 (2005) 1759.
[27] M. Ye, H. Zou, Z. Liu, R. Wu, Z. Lei, J. Ni, J. Pharm. Biomed. Anal. 27 (2002) 651.
[28] D.-S. Wang, Y.T. Zhang, Y.-N. Liu, J.X. Wang, J. Liq. Chromatogr. Related Technol. 31 (2008) 2077.
[29] J. Kawaoka, F.A. Gomez, J. Chromatogr. B 715 (1998) 203.
[30] Y.H. Chu, G.M. Whitesides, J. Org. Chem. 57 (1992) 3525.
[31] X. Huang, W.F. Coleman, R.N. Zare, J. Chromatogr. 480 (1989) 95.
[32] V. Dolník, Electrophoresis 18 (1997) 2361.
[33] S.V. Ermakov, M.Y. Zhukov, L. Capelli, P.G. Righetti, J. Chromatogr. A 699 (1995) 297.
[34] X. Huang, M.J. Gordon, R.N. Zare, J. Chromatogr. 480 (1989) 285.
[35] S. Bose, J. Yang, D.S. Hage, J. Chromatogr. B 697 (1997) 77.
[36] J. Yang, S. Rose, D.S. Hage, J. Chromatogr. A 735 (1–2) (1996) 209.
[37] C. Dufour, O. Dangles, Biochim. Biophys. Acta – Gen. Subjects 1721 (2005) 164.
[38] J.B. Whitlam, M.J. Crooks, K.F. Brown, P. Veng Pedrrsen, Biochem. Pharmcol. 28 (1979) 675.
[39] H.R. Xiao, L.Q. Sheng, C.H. Shi, X.L. Xu, Q.L. Liu, Spectrosc. Spect. Anal. 24 (2004) 78.
[40] T.J. Yin, W.Z. Wei, L. Yang, K. Liu, X.H. Gao, J. Biochem. Biophys. Methods 70 (2007) 587.