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Study on the Interaction between Strychnine and Bovine Serum Albumin by Capillary Electrophoretic Frontal Analysis

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Abstract: The protein binding constant, binding sites of the *Strychnos* alkaloid-strychnine and bovine serum albumin (BSA) was determined by capillary electrophoretic frontal analysis (CE-FA) for the first time. The experiment was carried out in a polyacrylamide-coated fused silica capillary (48.4 cm×50 μm i.d., 38.1 cm effective length) with 20 mmol/L citrate/MES buffer (pH 6.0, ionic strength 0.17). The applied voltage was 12 kV and detection wavelength was set at 257 nm. The plateau height of the peak was employed to determine the unbound concentration of drug in BSA equilibrated sample solution based on the external drug standard in the absence of protein. The present method provides a convenient, accurate technique for the early stage of drug screening.

Keywords: Strychnine, binding constant, capillary electrophoretic frontal analysis, bovine serum albumin.

Most drugs are usually bound to proteins such as serum albumin, α1-acid glycoprotein, lipoprotein, and other blood constituents. The plasma concentration of an unbound drug shows better correlation to the pharmaceutical activity[1]. It is therefore necessary to know the extent of drug-protein binding in order to adjust the optimal therapeutic dose of the drug.

Strychnos nux-vomica L. (Loganiaceae) is an evergreen tree native to southeast Asia. Its dried seeds are used for treatment of nervous diseases, arthritis, and vomiting as herbal remedies in traditional Chinese medicine[2-3]. Strychnine and brucine are major pharmacologically active components from *Strychnos nux-vomica* L., which are central nervous stimulant, but they can also lead to lethal poisoning at high dosage[3]. Strychnine and brucine have similar pharmacological action. The curative effect of brucine is amount to 1/40 of that of strychnine and its toxicity is 1/70 of that of strychnine[4]. So, in the development of *Strychnos nux-vomica* L. and strychnine as secure, responsible curatives, it is important to study the interaction of strychnine and albumin.

Many methods, chiefly chromatographic and electrophoretic methods, have been developed for evaluation of the interaction between the drug and protein[5-6]. Recently, CE-FA has attracted much attention and may possess potential in interaction studies[7-8].

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J. Østergaard *et al*. showed the advantages and limitations of CE-FA and manifested its latest developments in their reviews[9-10], which proved that CE-FA could be used as a general method to study and quantify drug-albumin interactions. In this study, CE-FA was employed to study the interaction between strychnine and BSA through several quantitative models from molecular-based viewpoint.

Experimental

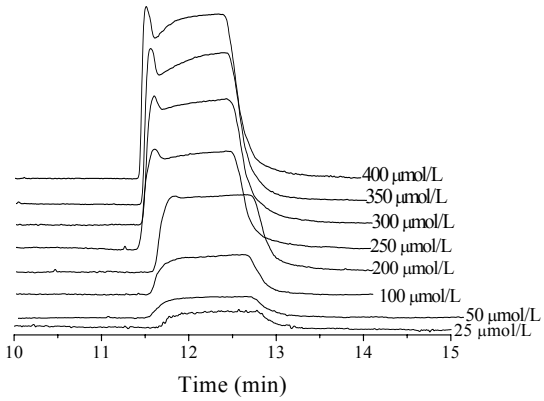
BSA (Fraction V) was purchased from Amresco (Ohio, USA). Strychnine was separated from *Strychnos nux-vomica* L. by preparative chromatography and identified by IR, UV, NMR and MS in our laboratory. 2-(N-Morpholine)ethane sulfonic acid (MES) (biological reagent) was purchased from Shanghai Chemical Reagent Company (Shanghai, China). Capillary electrophoresis was carried out on a Beckman P/ACE MDQ Capillary Electrophoresis System (Beckman Coulter, USA) equipped with photodiode array detector. The polyacrylamide-coated fused-silica capillary (Beckman Coulter, USA) of 48.4 cm total length (38.1 cm to the detector) × 50 μm i.d. was used throughout the experiments. Temperature of the capillary tube during electrophoresis was maintained at 25 ˚C by liquid coolant in the capillary cartridge. The applied voltage of the electrophoretic separation was set at 20 kV and the samples were injected by pressure mode with 0.5 psi for 30 s (1 psi = 6894.76 Pa). The detection wavelength was 257 nm.

The running buffer in the CE experiments was 20 mmol/L citrate-20 mmol/L MES-10 mmol/L NaCl, pH=6.00. The background electrolyte solution-phosphate buffer saline (PBS) was prepared with 5 mmol/L sodium dihydrogen phosphate, 5 mmol/L disodium hydrogen phosphate, and 150 mmol/L sodium chloride (pH 7.40, ionic strength 0.17) as well as the solvent of the sample solution. A series of strychnine solutions of different concentration (25-400 μmol/L) were made to obtain strychnine calibration line. Another series of strychnine-BSA solutions were made up of different concentration (50-400, 100-350, 200-250, 250-200, 300-150, 350-100, 400-50 μmol/L) and the solutions were mixed at 25 ˚C for 2 hour before analysis. All solutions were filtered through 0.45 μm membrane and degassed by ultrasonication *prior to* use.

Results and Discussion

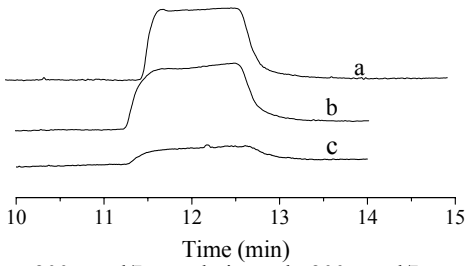
Injection of a large sample was needed for the FA experiment. It was evident that when injection time was longer than 20 s, a slant plateau region appeared slowly, and a complete plateau region appeared at injection time of 30 s. So an injection time of 30 s was used for all the binding studies. Under the above experimental conditions, the calibration line was got by measuring the plateau height of a series of strychnine standard solutions, from 25-400 μmol/L (see **Figure 1**). The linear regression equation was y=-3.604+0.09852×x (r = 0.99995, n = 9), where x represented the height of the plateau and y represented the concentration of the sample. There was a good linear relation in the concentration range of 25-400 μmol/L.

Figure 1 Electropherogram of different concentrations of strychnine (25-400 μmol/L) by CE-FA



CE conditions: polyacrylamide-coated fused silica capillary: 48.4 cm total length (38.1 cm to the detector) × 50 μm i.d.; detection wavelength: 257 nm; temperature: 25 ˚C; running voltage: 20 kV; injection: 0.5 psi×30 s.

Figure 2 Electropherograms of strychnine-BSA equilibrium systems



CE conditions as in **Figure 1**. a. 200 μmol/L strychnine; b. 200 μmol/L strychnine-250 μmol/L BSA; c. 50 μmol/L strychnine-400 μmol/L BSA.

Three strychnine peaks are shown in **Figure 2**, two of strychnine-BSA mixtures and one of pure strychnine solution. Because the capillary inner surface was coated with polyacrylamide, the electroosmotic flow was negligible. So, negatively charged BSA (isoelectric point pI=4.8) migrated backward to the inlet vial and the positively charged drug migrated toward the detection end, which resulted in appearance of the drug peak only.

Seven strychnine-BSA solutions with different amounts of strychnine were subjected to electrophoresis according to CE conditions in **Figure 1** and the concentrations calculated from the peak height are presented in **Table 1**. Data were processed to obtain binding parameters. The estimated binding constant (K) and the binding sites on one protein (n) are shown in **Table 2** according to the Scatchard analysis, the Klotz analysis and non-linear regression analysis. The estimated binding parameters from the three regression methods are in good agreement.

The proposed method has been proven to be a powerful and convenient tool in quantitating molecular interaction and the polyacrylamide coating of the inner wall in the

capillary was favorable for CE-FA of basic drugs. The peak of negatively charged protein did not appear in the electropherogram and a wide analytical window was obtained. The adsorption of the protein onto the capillary wall was successfully avoided, which often caused many troubles in CE with a non-coated fused silica capillary.

Table 1 Concentration concerned data of strychnine-BSA equilibrium solutions determined by CE-FA

C_0 (μmol/L)	C_f (μmol/L)	C_b (μmol/L)	C_p (μmol/L)	$r=C_b/C_p$	r/C_f (L/mol)
50	38.96	11.04	400	0.0276	708.7
100	82.80	17.20	350	0.0491	593.5
200	179.84	20.16	250	0.0806	448.4
250	231.76	18.24	200	0.0912	393.5
300	285.26	14.74	150	0.0983	344.6
350	338.95	11.05	100	0.1105	326.0
400	394.02	5.98	50	0.1196	303.5

Table 2 Binding parameters of strychnine-BSA determined by CE-FA

Sample	Regression method	*K*-binding constant (10^3 L/mol)	*n*-the number of binding sites	*r*-correlation coefficient
Strychnine-BSA	*Scatchard*	4.54±0.25	0.180±0.014	0.9927
	Klotz	4.78±0.17	0.175±0.004	0.9997
	Non-linear regression	4.12±0.42	0.189±0.030	0.9977

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