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A Novel Method for Studying the Interaction of Macromolecule with Small Molecule by Means of Affinity Capillary Electrophoresis

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Abstract: A novel capillary electrophoresis method coupled with on-line microdialysis using an attachable electrode has been developed to study the interaction of macromolecule with small molecule. The binding constants of bovine serum album (BSA) with D,L-tryptophan (Trp), sulfamethoxazole (SMZ) with trypsin and chymotrypsin were determined. These values are 2.3×10^4L/mol for BSA-L-Trp; 1.77×10^3 L/mol for BSA-D-Trp in pH 7.4, 50 mmol/ L phosphate; 1.4×10^4 L/mol for SMZ- trypsin and 6.0×10^3 L/mol for SMZ-chymotrypsin in pH 6.5, 25 mmol/L Tris buffer. The proposed method has merits of speed, low sample consumption and readily available to be performed in desired physiological conditions.

Keywords: Interaction of macromolecule with small molecule, binding constant, affinity capillary electrophoresis, microdialysis membrane.

Introduction

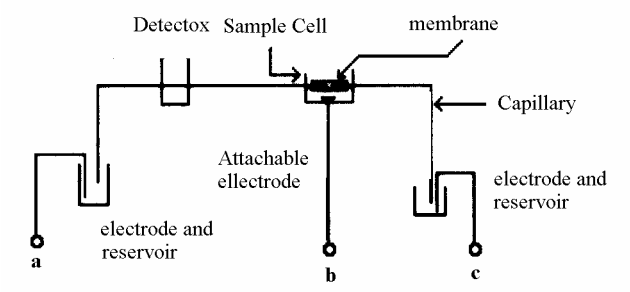
The determination of binding constants plays an important role for the studies of biomolecule recognition. Compared with traditional methods the affinity capillary electrophoresis (ACE) has advantages of small sample consumption, and observing the interaction in free solution. But the application of ACE is limited because macro-molecules are easily adsorbed to capillary wall. This paper developed a novel method for determining the binding constant of macromolecule with small molecule by utilizing capillary electrophoresis coupled with on-line microdialysis using an attachable electrode. The proposed method is simple with on-line detection, it can be used under the desired physiological conditions and has the merits of relatively small volume of sample and faster analysis speed. As examples, the binding constants of BSA-L-tryptophan, BSA-D-tryptophan, SMZ- trypsin and SMZ-chymotrypsin were determined.

Experimental

Equipment

The home-made equipment is shown in **Figure 1**.

Figure 1. .Schematic diagram of experimental set-up



Procedure

Wash the capillary with Tris buffer of pH 8 for 20 min, and water for 5min, respectively. Add Trp solution (dissolved in buffer of pH 7.4, 50 mmol/ L phosphate.) to the sample cell and immediately apply voltage followed by displacing the Trp solution with the running buffer. Perform the electrophoresis, resulting an electropherogram as shown in **Figure 2** and the peak area can be obtained by a chromatographic integrator. For the sample of SMZ the same procedure was carried out except that the sample was dissolved in pH 6.5, 25 mmol/L Tris buffer.

Results and discussion

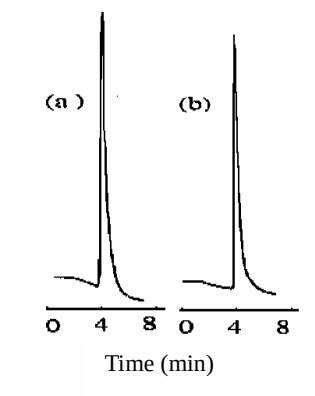
Determination of binding constants of L-tryptophan-BSA and D-tryptophan-BSA

Incubate different concentrations of L-Trp with the fixed concentration of BSA for 15 min, respectively. Add the incubated solution to the sample cell to make the determination as described in the procedure. Then the free concentration of Trp can be obtained from the calibration curve (**Figure.** 3). The experimental data are listed in **Table 1**. Suppose protein (P) interacts with drug (D), forming a complex of PD, Klotz equation exists:

$$\frac{1}{r} = \frac{1}{nk}\frac{1}{[D]} + \frac{1}{n} \quad , \text{r=[PD]/Cp}$$

In which, nk=K represent the total binding constant of PD; [D], [PD] the free concentration of D and PD respectively; Cp the total concentration of P. Fitting the data in **Table 1** to Klotz equation and K was obtained.

Figure 2. Electropherogram of tryptophan



(a.)160μmol/L Trp (b) 160μmol/L Trp +100μmol/L BSA Condition: 50μm I.D capillary; injection E_{bc}= −267v.cm^{-1} for 1 min; temperature 25° C running buffer: pH7.4, 50 mmol/L phosphate, E_{ac}=−242V.cm^{-1},detection: 210nm.

Figure 3. Electrophoresis peak area calibration curve for L-Trp

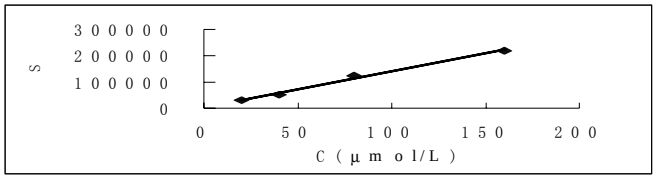


Table 1. The data of determination of binding constant of tryptophan with BSA

C_p:C_{Trp} μM:μM	S	[Trp] μM	r=(C_{Trp}-[Trp])/Cp	Klotz plot regression equation	K (L/mol)	Literature Value[1]
100:160	178241	127.5	0.32	1/r=1/(2.3×10^4[Trp])		
100:50	39433	25.8	0.24	+2.7	2.3×10^4	2.2×10^4
100:40	31865	20.29	0.20	r=0.97		

There is few report on the binding constant of BSA with D-Trp. Using the same method as above the value was determined as K=1.77×10^3 L/mol.

Determination of the binding constants of SMZ-trypsin and SMZ-chymotrypsin

Using the similar method the binding constants of SMZ-trypsin and SMZ-chymotrypsin in the medium of pH 6.5, 25 mmol/L Tris buffer have been determined as $K=1.4\times10^4$ L/mol and $K=6\times10^3$ L/mol. The results are concordant with those by affinity chromatography.

The mechanism of sample introduction

Three electrophoresis experiments were performed using different sample introductions: 1) applying voltage at b and c electrodes (E_{bc}= -267 $V\cdot cm^{-1}$); 2) without applying voltage; 3) applying voltage at a and c electrodes (E_{ac}= -242 $V\cdot cm^{-1}$). The results showed that the sample introduction adopted in this paper is the combination of free diffusion[2] electromigration and electroosmotic flow[3] effect in the capillary.

In this method a dialysis membrane was coupled with the capillary on-line and thus the free concentration of small molecules can be determined apart from macromolecules. The method was carried out without disturbing the equilibrium established because the relative amount of free small molecule introduced from the sample cell was small as the dimensions of the capillary are small, leading to high mass sensitivity.

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