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Use of capillary electrophoresis-based competitive immunoassay for a large molecule

J.P. Ou[a], Q.G. Wang[b], T.M. Cheung[a], S.T.H. Chan[c], W.S.B. Yeung[a,*]

[a]*Department of Obstetrics and Gynaecology, The University of Hong Kong, Queen Mary Hospital, Pokfulam Road, Hong Kong, Hong Kong*

[b]*Department of Chemistry, Tsinghua University, Beijing, China*

[c]*Department of Zoology, The University of Hong Kong, Queen Mary Hospital, Hong Kong, Hong Kong*

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Abstract

A systematic study on the optimization of capillary electrophoresis-based immunoassay (CEIA) was performed using bovine serum albumin (BSA) and monoclonal anti-BSA. The immunocomplex could not be resolved from free BSA or anti-BSA with UV detection. When fluorescein isothiocyanate-labeled BSA (FITC-BSA) was used as tracer, the free and bound FITC-BSA were well separated giving definite peaks with laser induced fluorescence detection. The factors affecting the separation of the free and bound FITC-BSA, including voltage, pH and ionic strength of the running buffer, were systematically analyzed. Competitive CEIAs were demonstrated in uncoated and coated capillaries with whole or Fab fragment of the antibody. The coefficient of variation for the quantification of BSA in coated capillary was less than that in uncoated capillary. This study demonstrated that competitive CEIA could be applied to quantify high-molecular-mass protein in biological fluids.

Keywords: Bovine serum albumin

1. Introduction

Immunoassays are commonly used in clinical, pharmaceutical and chemical analyses for detection and quantification of trace analytes in biological fluids and other complex matrices. These assays offer high selectivity on the analytes determined due to specific antibody/antigen binding. The sensitivity of the assays can be enhanced by various labeling techniques such as fluorescent, radioactive, chemiluminescent and enzyme labeling. Conventional immunoassays are usually performed using solid-phase techniques and quantification is achieved by measuring either the radioactivity as in radioimmunoassay (RIA) or enzyme activity as in enzyme-linked immunoasorbent assay (ELSIA). Most conventional immunoassays are carried out manually with multiple incubation, washing and rinsing steps that may take hours to complete.

Capillary electrophoresis (CE) is a powerful technique for the separation of macromolecules, such as peptides, proteins and immunocomplex [1]. Recently researchers utilize the selectivity of immunoaffinity interactions in combination with the sensitivity and efficiency of CE to improve the performance of

*Corresponding author. Fax: +852-2855-0947.
E-mail address: wsbyeung@hkucc.hku.hk (W.S.B. Yeung)

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immunoassays [2]. These capillary electrophoresis-based immunoassays (CEIAs) offer several advantages over conventional immunoassays. In conjunction with laser induced fluorescence (LIF) detection, CEIA can performed rapid separations with high detection sensitivity, and is capable of determining multiple analytes simultaneously [3,4]. In addition, only minute amount of sample ranging from 10–20 μl is required for these assay. Nielsen et al. [5] first reported the use of CE with UV detection to separate human growth hormone (hGH) from its immunocomplex at μ*M* levels [5]. Shimura and Karger [6] subsequently used a fluorescent labeled antibody fragment (Fab′) to detect methionyl recombinant hGH at p*M* concentration. In 1993, Schultz and Kennedy [2] first demonstrated the concept of performing competitive and non-competitive immunoassays using CE with LIF detection. In that study labeled insulin was used as a tracer and the Fab fragment of monoclonal anti-insulin was used as the immunoreagent. Separation was completed within 3 min and the detection limit for insulin was about 3 n*M* or 420 zmol injected. A modification of this assay has been used to determine the insulin content in a single islet of Langerhans [7] and to quantify insulin secretion from single islet with or without glucose stimulation [7,8].

CEIA has also been applied to quantify other antigens including morphine [3,9], angiotensin II [10,11], cortisol [12,13], theophylline [14–16], hGH [6], immunoglobulin G (IgG) [17], thyroxine (T4) [18], digoxine [19–21], and phencyclidin (PCP) [3]. In addition, Chen and co-workers [3,4] demonstrated the determination of multiple analytes simultaneously in CEIA. Most reported CEIAs used low-molecular-mass antigens ranging from 180 to 6000 dalton. These antigens can usually be separated easily from their high-molecular-mass antibodies or complexes. However, the immunocomplex may not be easily separated from the free antibody or antigen for many large proteins such as IgG [17,22] and IgA [23]. This is partly because the heterogeneity of the labeled antigen will generate multiple peaks especially when the antibody concentration is high. The problem is further amplified with the use of polyclonal antibodies that lead to the formation of multiple immunocomplex peaks between the antigen and different antibody species in the reaction mixture [24].

In this study, the feasibility of CEIA with a large molecule is investigated. The bovine serum albumin (BSA) (M_r=67 000) and its antibodies are used as a model system. The objectives of this study are (1) to systematically optimize the separation conditions for this model system, and (2) to demonstrate that CEIA is possible for the quantification of this large molecule.

2. Experimental

2.1. Chemicals

BSA, monoclonal anti-BSA (mouse $IgG_{2\alpha}$ in mouse ascites fluid), boric acid and Fluoro Tag FITC conjugation kit were purchased from Sigma (St. Louis, MO, USA). The dissociation constant of the anti-BSA with BSA was 3.1 n*M* (Wang and Yeung, unpublished data). An ImmunoPure Fab preparation kit (44885G) was obtained from Pierce (Rockford, IL, USA). One hundred m*M* borate buffer, pH 8.35, 0.1 *M* NaOH regeneration buffer and eCAP 20 m*M* Tricine buffer (pH 8.0) were purchased from Beckman Instruments (Fullerton, CA, USA). Sodium tetraborate was obtained from BDH (Poole, UK). All buffers were made with deionized water, filtered through 0.45-μm syringe filters and sonicated for 10 min before use. BSA and anti-BSA solution were diluted in 10 m*M* phosphate-buffered saline (PBS), pH 7.4. All reagents used were of analytical grade.

2.2. Apparatus

A P/ACE 5010 system (Beckman) equipped with an UV detector and a 5 mW air-cooled argon ion laser detector (excitation 488 nm, emission 520 nm) was used. An IBM PS/2 Model 350-P100 personal computer with Beckman P/ACE system software controlled the CE system. An uncoated fused-silica capillary (47 cm×75 μm I.D.) in an UV cartridge, an uncoated capillary (57 cm×75 μm I.D.) in a LIF cartridge, and a neutral capillary (part No. 477441, 47 cm×50 μm I.D.) which has been permanently bonded with a neutral hydrophilic polymer layer in a LIF cartridge were used. On-line UV absorbance detection was done at 214 nm. A DU650 spectrophotometer (Beckman) was used to measure ab-

sorbance of gel filtration fractions after fluorescent labeling. Microcentrifuge tubes (LW2072, Alpha Labs., Hampshire, UK) were used as reaction vials.

2.3. Labeling of BSA

BSA was labeled with FITC according to the manufacturer's instruction. FITC-BSA was purified by Sephadex-25M gel column. The labeling efficiency was determined with the DU650 spectrophotometer. The fluorescein/protein ratio (F/P) and concentration of the stock FITC-BSA was 1.15:1 and 2.49 mg/ml, respectively. The conjugate was protected from light and stored at 4°C until use.

2.4. Preparation of Fab of monoclonal anti-BSA

Fab fragments of anti-BSA were prepared according to the instructions of the ImmunoPure Fab preparation kit. Briefly, monoclonal anti-BSA was digested with immobilized papain. The Fab fragments were collected from the void fraction of a protein A affinity column in Tris buffer, concentrated and resuspended in 10 m*M* PBS, pH 7.4, using Centricon 30 microconcentrators (Amicon, Beverly, MA, USA) which has a molecular mass cut-off of 30 000. The concentration of the Fab fragment as estimated by absorbance at 280 nm in a DU650 spectrophotometer was 7.54 mg/ml (conversion factor 1.42 absorbance unit/mg per ml in a 1-cm cuvette [2,7]).

2.5. Immunoassay protocols

BSA and anti-BSA solutions were diluted to the appropriate concentrations with 10 m*M* PBS, pH 7.4. Fifteen microliters each of BSA and anti-BSA solution were mixed in a 0.5-ml microcentrifuge tube and incubated at room temperature (23°C) for 10 min before injection for non-competitive CE–UV assay of BSA. To perform competitive assay, 15 μl of 0.02 mg/ml (300 n*M*) FITC-BSA was mixed with 15 μl of 0.0002 to 0.2 mg/ml (3–3000 n*M*) BSA. Fifteen microliters of anti-BSA with concentrations ranging from 0.01–0.05 mg/ml (67–333 n*M*) or 1 mg/ml of partially purified Fab was then added to the mixture and incubated for 10 min before analysis with CE–LIF. Intra-assay variation was defined as the variation in the concentration of BSA determined in three consecutive runs performed on the same day. Inter-assay variation was determined by the variation in the BSA concentration determined in three runs performed on separate days.

2.6. Capillary zone electrophoresis

Uncoated fused-silica capillary was rinsed successively with 0.1 *M* NaOH for 5 min, deionized water for 2 min and running buffer for 3 min under 20 p.s.i. pressure prior to injection or between runs (1 p.s.i.=6894.76 Pa). Coated capillary was regenerated by 1-min rinse with 0.1 *M* HCl, followed by 4-min rinse with 20 m*M* Tricine buffer. The volume of sample used was 20 μl. Injection was done by applying 0.5 p.s.i. pressure for 3 s to the sample vials. The injection volume was approximately 15 nl. Separation voltage was 25–30 kV for uncoated capillary and 23.5 kV for coated capillary. The polarity of the system was reversed when coated capillary was used. The temperature of the capillary was maintained at 23°C throughout the separation.

3. Results and discussion

3.1. CZE–UV detection

The electropherograms of BSA, monoclonal anti-BSA and their mixture are shown in Fig. 1A–E. The limit of detection (LOD), the concentration of BSA used in the standard curve that gave a peak height 3-times the standard deviation above the baseline noise (S/N=3:1), was 0.02 mg/ml (300 n*M*). The electropherogram of BSA alone showed two major overlapping peaks, confirming that BSA was heterogeneous (Fig. 1A). Addition of increasing concentrations of anti-BSA led to the continuous decrease in the BSA peaks (Fig. 1C–D). Simultaneously, the height of an asymmetrical peak that corresponded to a mixture of the anti-BSA and its immunocomplexes increased. This latter peak could not be completely resolved from the BSA peak especially when the amount of anti-BSA added was high. The inability of the system to resolve anti-

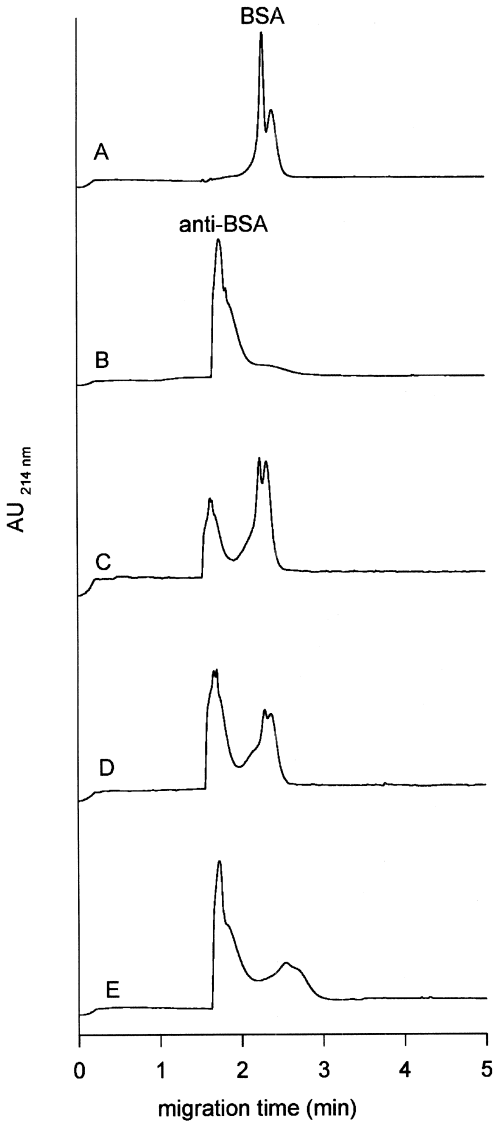

Fig. 1. CE–UV electropherograms of (A) BSA 1 mg/ml; (B) monoclonal anti-BSA 1 mg/ml; and mixtures of 1 mg/ml BSA with different concentrations of anti-BSA: (C) 0.25 mg/ml anti-BSA; (D) 0.5 mg/ml anti-BSA; (E) 1 mg/ml anti-BSA. Conditions: uncoated fused-silica capillary, 47 cm×75 μm I.D.; applied potential, 30 kV/30 μA; injection 3 s; buffer, 100 m*M* borate, pH 8.35; UV detection, 5 Hz, at 214 nm; temperature, 23°C.

BSA, BSA and the immunocomplexes indicates that the charge-to-mass ratios of these molecules were similar. The poor electrophoretic discrimination can be attributed to the binding of an antigen to its antibody does not alter the electrophoretic mobility of the resulting immunocomplex significantly [25]. This is probably because the charge to mass ratios in these proteins are relatively similar because of their large molecular sizes, and any change in charge due to antibody and antigen interaction is not sufficient to alter the ratios of the immunocomplexes considerably. Thus non-competitive CEIA of BSA cannot be performed in CZE with UV detection. Attempts to optimize the separation using other buffers (e.g., PBS) did not improve the resolution (data not shown). Chen [23] modified a fluorescein-labeled antibody with a succinyl group that resulted in a net gain of two charges at neutral pH for every lysine residual group (ϵ-NH_2) on the antibody. Although the separation of the immunocomplex from this antibody was improved, baseline separation was not achieved.

3.2. CE–LIF detection

To reduce the requirement of resolving BSA, anti-BSA and their immunocomplexes with similar charge-to-mass ratios in CZE, CEIA was performed in a competitive manner using FITC-BSA. Fifteen microliters of 300 n*M* FITC-BSA were added to 167 n*M* of anti-BSA and incubated at room temperature (23°C) for 10 min before injection. CE was performed with 100 m*M* borate buffer (pH 8.35) in an uncoated capillary at a voltage of 25 kV. With LIF detection, only the free FITC-BSA and immunocomplex containing FITC-BSA were revealed in the electropherogram (Fig. 2). The multiple peaks of FITC-BSA in Fig. 2A suggested that the BSA was heterogeneous and/or BSA had been tagged with different amount of FITC [2]. Free FITC was partially separated from FITC-BSA in the electropherogram.

Addition of anti-BSA to FITC-BSA resulted in the formation of two immunocomplex peaks (Fig. 2B) at 3.8 and 4.2 min, and a decrease in the FITC-BSA peaks. The two immunocomplex peaks might be due to the binding of the antibody to one or two BSA molecule [2]. One of these peaks overlapped slightly with the free FITC-BSA under the present CE–LIF conditions.

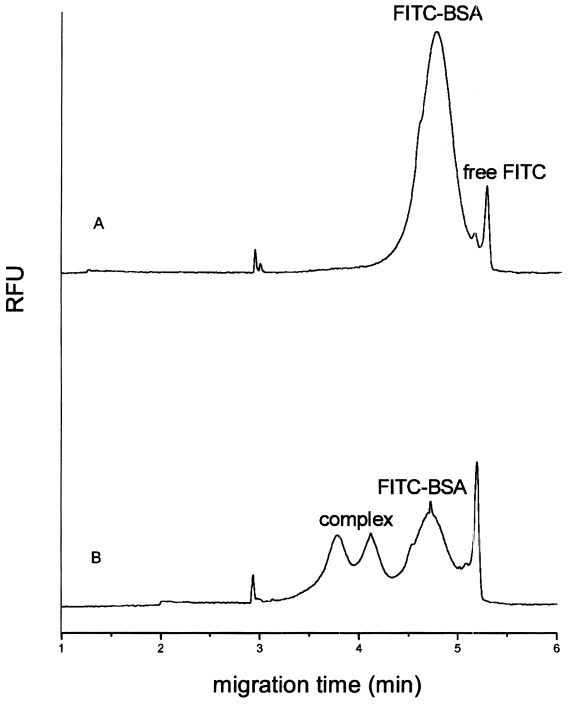

Fig. 2. CE–LIF electropherograms of (A) 300 n*M* FITC-BSA; (B) mixture of 300 n*M* FITC-BSA and 167 n*M* monoclonal anti-BSA. Conditions: uncoated capillary, 57 cm×75 μm I.D.; applied voltage, 25 kV; 100 m*M* borate, pH 8.35; 3 s injection; LIF detection.

3.3. Optimization of CE–LIF separation conditions

3.3.1. Effect of pH on separation

Borate buffers (100 m*M*) with different pH were used. The applied voltage was 25 kV. When the pH of the running buffer was 7.0, the immunocomplexes were partially separated from free FITC-BSA (Fig. 3A). The separation improved as the pH increased to pH 8.5 (Fig. 3B–C). At pH 9.0, peak broadening was obvious, and the separation time was much prolonged (Fig. 3D). Therefore, the pH of the borate buffer in the subsequent experiments was chosen to be 8.5. The increase in pH also increased the current during capillary electrophoresis. Similar experiments was performed using phosphate buffer (data not shown) but the peaks were not as well resolved as those in the borate buffer. Our unpublished data also demonstrated that the borate buffer gave the best resolution and a reasonable current in the separation

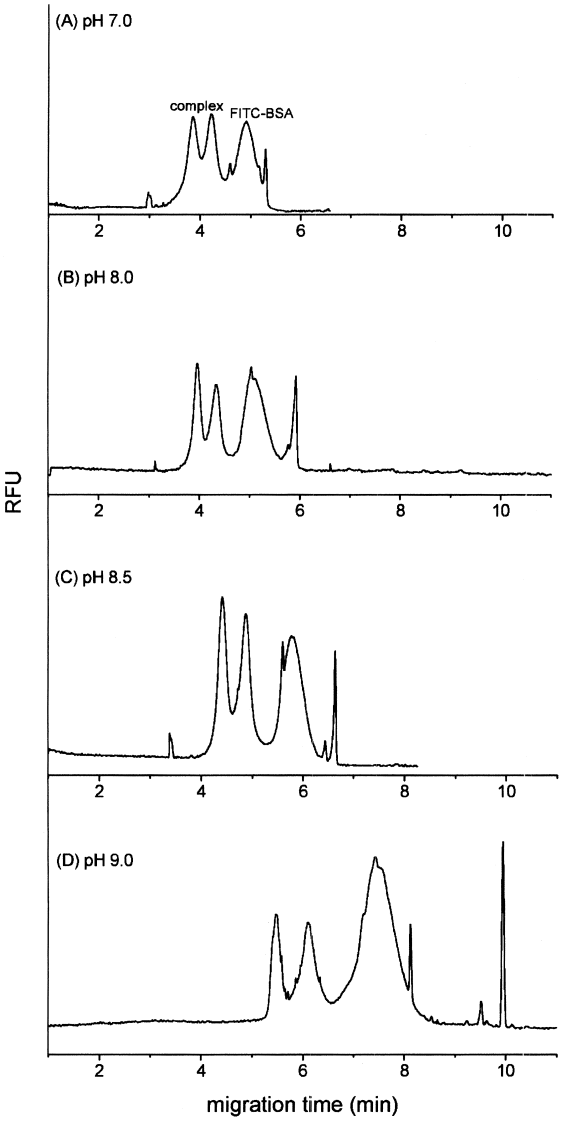

Fig. 3. Comparison of electropherograms in 100 m*M* borate with different pH. Sample and conditions as in Fig. 2.

of FITC-BSA from its immunocomplexes among other buffers such as Tricine and 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid (HEPES).

3.3.2. Effect of ionic strength on separation

Borate buffers (pH 8.5) with different ionic strength ranging from 25–200 m*M* were used. Results showed that increasing the ionic strength of the buffer improved the resolution and increased the

migration time (Fig. 4). The best resolution was achieved when the separation was carried out in 200 m*M* borate buffer.

3.3.3. Effect of voltage on separation

The BSA and anti-BSA mixture was separated in 200 m*M* borate buffer, pH 8.5 at different voltages, namely 15, 20, 25 and 30 kV. As expected, the higher the applied voltages, the higher the electroosmotic flow, and hence the shorter the migration times. The best separation was achieved when the applied voltage was 25 kV or 30 kV. However, the current (>90 μA) was high at 30 kV, making the separation less stable. As the voltage and buffer strength were increased, the current passing through the capillary also increased. In the present CE system, the temperature of the capillary is kept constant at 23°C by a liquid coolant. An increase in capillary temperature would decrease the analysis time. However, this may not be associated with an increase in resolution [29]. At a voltage of 25 kV, the LOD of FITC-BSA in 200 m*M* borate buffer (pH 8.5) was 2 n*M*.

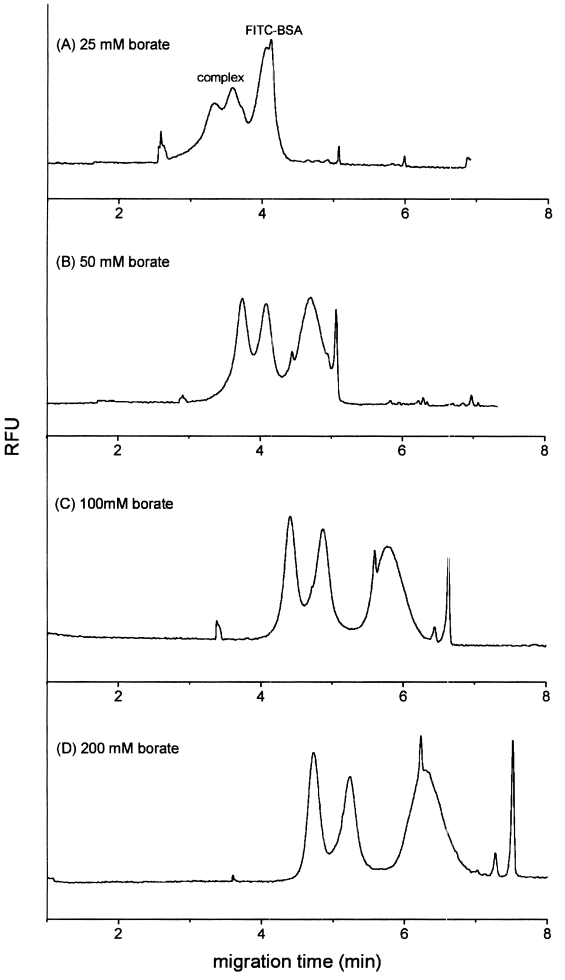

Fig. 4. Comparison of electropherograms in borate buffer of different ionic strength (pH 8.5). Conditions as in Fig. 2.

3.3.4. Effect of incubation time on complex formation

BSA and anti-BSA were incubated for 10 min in the above experiments. In order to determine the effect of incubation time on CEIA in this system, BSA and anti-BSA solution were incubated at room temperature for 10, 20, 30, 60 and 120 min before injection into the capillary. There was no difference in the bound/free FITC-BSA ratio (B/F) at all the time points tested. The present result and that of others [2,12,26] suggested that equilibrium of B/F ratio was established after 10 min. There is also report showing that reliable result can be achieved with less than 1 min incubation, and suggesting that prolonged incubation time is not necessary [23].

3.4. Competitive CEIA in uncoated capillary

Competitive immunoassay of BSA was performed using FITC-BSA as tracer in uncoated capillary. The separation conditions for CE was 200 m*M* borate buffer (pH 8.5) and 25 kV applied voltage. The concentrations of FITC-BSA and anti-BSA were 300 n*M* and 333 n*M*, respectively. As the concentration of unlabeled BSA increased, the peak height and area percentage of the immunocomplex in the electropherogram decreased while that of the free FITC-BSA increased (Fig. 5). The peak height and area percentage of the free FITC remained the same throughout the experiment. A typical sigmoidal calibration curve using area percentage of complex against logarithm of the concentration of unlabeled BSA is shown in Fig. 6. Each point represented the average of three consecutive runs. The intra-assay and inter-assay variation for area percentage of complex for this experiment were 0.98–6.69% and 0.62–9.69%, respectively (Table 1). The coefficients of variation (CVs) of the migration time for the two immunocomplex peaks were less than 1%. The LOD

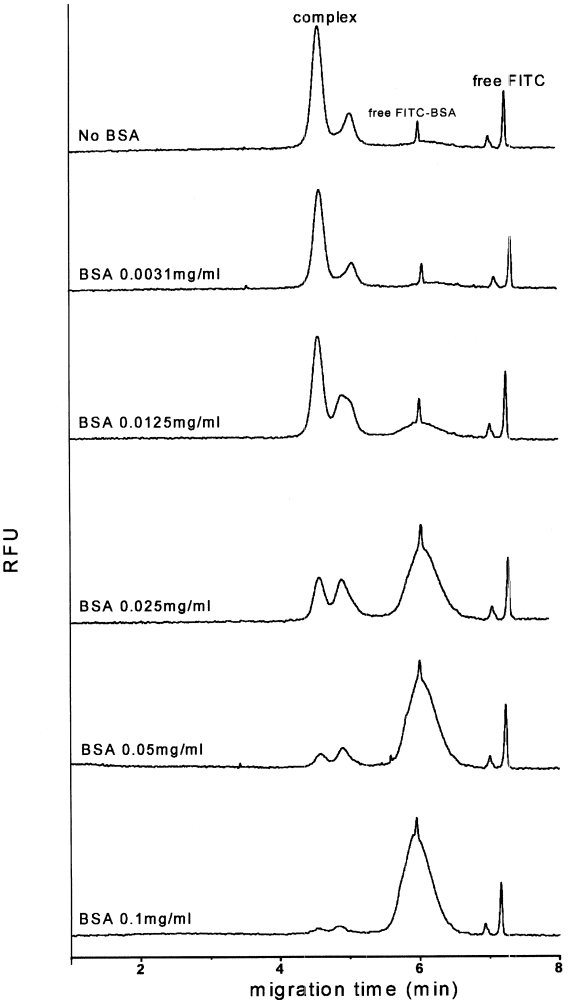

Fig. 5. Comparison of electropherograms of competitive CEIA with whole anti-BSA. The reaction mixtures contained FITC-BSA (300 n*M*), anti-BSA (333 n*M*) and BSA solution in different concentrations. Buffer, 200 m*M* borate, pH 8.5; other conditions as in Fig. 2.

for the assay was 0.0031 mg/ml (47 n*M*) of BSA. Reducing the concentration of anti-BSA by half without changing the amount of FITC-BSA increased the sensitivity of the assay and shifted the calibration curve to the left (Fig. 6). Further reduction of the anti-BSA concentration did not improve the sensitivity, but drastically reduced the linear dynamic range of the assay as a result of decreased formation of labeled immunocomplexes (Fig. 6). Our unpublished data also showed that increasing the

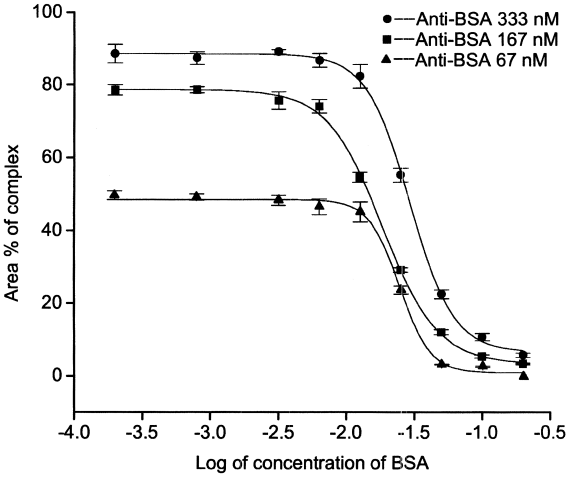

Fig. 6. Calibration curves of competitive immunoassay in CE–LIF with area percentage of complex vs. logarithm of concentration of BSA. The reaction mixture contained 300 n*M* FITC-BSA, 3–3000 n*M* BSA and 67–333 n*M* of anti-BSA.

concentration of FITC-BSA led to peak broadening and overlapping of the peaks, while reducing its concentration decreased the signal-to-noise ratios and resulted in higher LOD.

3.5. CE–LIF in coated capillary

When CZE is applied to the separation of multicharged proteins, adsorption can be a serious problem [27,28]. This absorption lead to zone broadening [27], irreproducible separation, reduced efficiency and in some cases, the inability to detect the analytes due to adsorption of the analyte to the capillary wall

Table 1
Comparison of coefficients of variation in CEIA with uncoated and coated capillary[a]

BSA concentration (mg/ml)	Intra-assay (%)		Inter-assay (%)	
	Uncoated	Coated	Uncoated	Coated
0.0002	0.98	0.39	2.91	2.78
0.0008	1.03	0.85	2.02	5.97
0.0031	2.41	0.43	0.62	2.79
0.0062	2.67	0.16	2.21	0.94
0.0125	1.73	0.84	3.96	4.00
0.025	4.26	0.81	3.49	0.91
0.05	0.15	5.71	5.48	1.55
0.10	6.69	2.23	9.69	2.35

[a] Three consecutive runs, $n=3$.

[29]. Several strategies have been developed to overcome this problem. These include changing the pH of the running buffer (<3 or >9), coating the inner surface of the capillary with a hydrophilic polymer, or using additives in the sample solution [28]. In this study, competitive CEIA was also tested in coated capillary permanently bonded with a neutral hydrophilic polymer that minimized the EOF and adsorption of the analyte. Fig. 7A shows the electropherogram of 300 n*M* FITC-BSA using the coated capillary. As the electrode polarity was reversed, the sequence of the eluted peaks in the elctropherogram was reversed when compared to that in the uncoated capillary. The electropherogram of the complex and free FITC-BSA mixture is shown in Fig. 7B. The complexes were separated into two peaks. As EOF in the present coated capillary was reduced to only about 5% that of the untreated fused-silica capillary (supplier's information), migration in this capillary is mainly by electrophoretic flow. This is in contrast to the uncoated capillary in which EOF is the main driving force for the movement of the analyte. The movement of protein driven by EOF was usually more than 10-times faster than that by electrophoretic migration. Therefore, the time for complete separation of the analytes was longer in the coated than in the uncoated capillary. The LOD of BSA in this method was also 0.0031 mg/ml (47 n*M*). The intra-assay and inter-assay variation for area percentage of the immunocomplex in different concentrations of BSA were less than those in the uncoated capillary (Table 1). Although the resolution in the coated capillary is better than that in the uncoated capillary, extreme separation condition is not suitable for the coated capillary used in this study. According to the manufacturer's information, high pH buffer (>9) will damage the coating and shorten the life of the capillary.

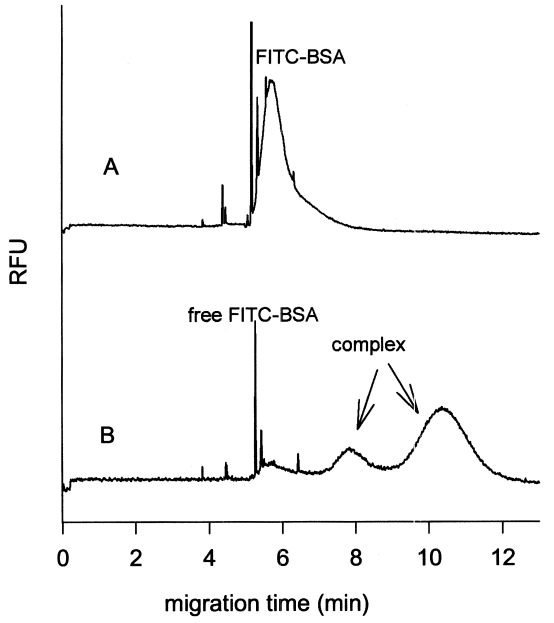

Fig. 7. CE–LIF performed in coated capillary. The electropherogram of (A) FITC-BSA (300 n*M*) and (B) mixture of FITC-BSA, anti-BSA (333 n*M*) and PBS. Conditions: coated capillary 47 cm×50 μm I.D., applied voltage, 23.5 kV; buffer, 20 m*M* Tricine, pH 8.0.

3.6. CEIA with Fab of anti-BSA

Papain digestion of anti-BSA yielded an Fc portion and two Fab fragments. Each Fab fragment has one antigen binding domain. The undigested IgG and Fc fragment was removed by an immobilized Protein A affinity column that bound specifically to the Fc region of the IgG molecule. Using 200 m*M* borate buffer (pH 8.5) as running buffer, the immunocomplexes in the mixture of FITC-BSA and Fab of anti-BSA in uncoated capillary were resolved into two peaks (Fig. 8). The second peak that corresponded to the binding of Fab to one FITC-BSA was the dominant peak. The small first peak was probably undigested anti-BSA. The CV of the area percentage of the immunocomplex peak was comparatively lower than that using whole antibody, and ranged from 0.29% to 3.08%. The LOD of BSA was 47 n*M*.

4. Conclusions

Competitive CEIA is possible for BSA. The immunocomplex of BSA can be resolved from free antigen using LIF detection. The resolution and the coefficient of variation in coated capillary are better than that in uncoated capillary. However, the separation time was longer in the coated capillary. The use of Fab improves the precision of quantification in immunoassay with CE–LIF. We believe that CEIA

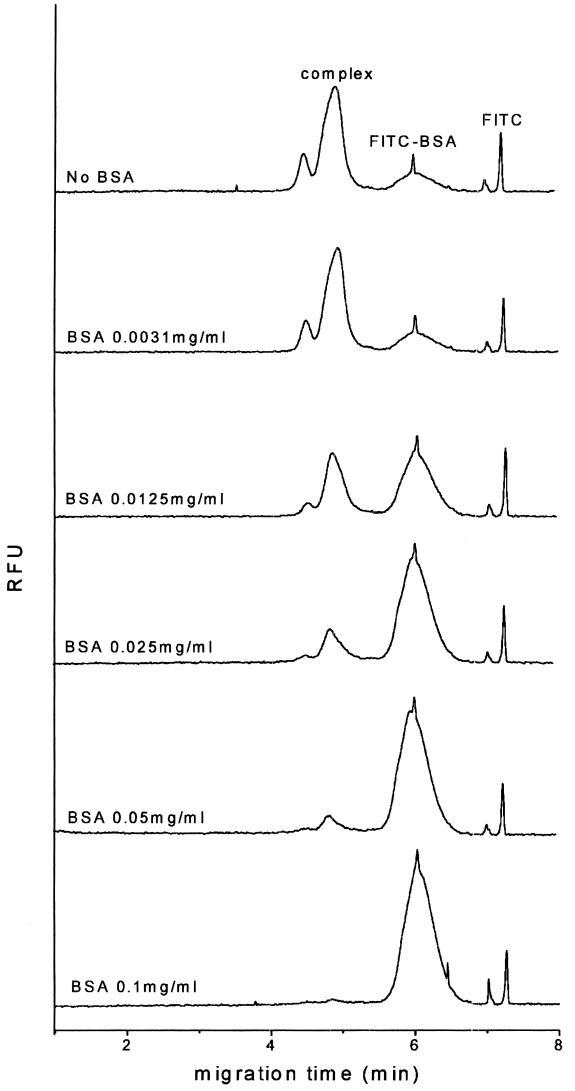

Fig. 8. Comparison of electropherograms of competitive CEIA with Fab of anti-BSA. The reaction mixtures contained FITC-BSA (300 n*M*), Fab of anti-BSA (20 μ*M*) and different concentrations of BSA. Conditions as in Fig. 6.

could be applied to quantify high-molecular-mass protein in biological fluids if proper conditions are used.

References

[1] P.D. Grossman, J.C. Colburn, H.H. Lauer, R.G. Nielsen, R.M. Riggin, G.S. Sittampalam, E.C. Rickard, Anal. Chem. 61 (1989) 1186.
[2] N.M. Schultz, R.T. Kennedy, Anal. Chem. 65 (1993) 3161.
[3] F.T. Chen, R.A. Evangelista, Clin. Chem. 40 (1994) 1819.
[4] R.A. Evangelista, J.M. Michael, F.T. Chen, Am. Clin. Lab., (1995) 27.
[5] R.G. Nielsen, E.C. Rickard, P.F. Santa, D.A. Sharknas, G.S. Sittampalam, J. Chromatogr. 539 (1991) 177.
[6] K. Shimura, B.L. Karger, Anal. Chem. 66 (1994) 9.
[7] N.M. Schultz, L. Huang, R.T. Kennedy, Anal. Chem. 67 (1995) 924.
[8] L. Tao, R.T. Kennedy, Electrophoresis 19 (1998) 403.
[9] R.A. Evangelista, F.T. Chen, J. Chromatogr. A 680 (1994) 587.
[10] T. Pritchett, R.A. Evangelista, F.-T.A. Chen, Beckman Application Information Bulletin A-1778A, 1995.
[11] T. Pritchett, R.A. Evangelista, F.T. Chen, Bio/Technology 13 (1995) 1449.
[12] D. Schmalzing, W. Nashabeh, X.W. Yao, R. Mhatre, F.E. Regnier, N.B. Afeyan, M. Fuchs, Anal. Chem. 67 (1995) 606.
[13] D. Schmalzing, W. Nashabeh, M. Fuchs, Clin. Chem. 41 (1995) 1403.
[14] L. Steinmann, W. Thormann, Electrophoresis 17 (1996) 1348.
[15] N. Chiem, D.J. Harrison, Anal. Chem. 69 (1997) 373.
[16] N.H. Chiem, D.J. Harrison, Clin. Chem. 44 (1998) 591.
[17] R. Lausch, O.W. Reif, P. Riechel, T. Scheper, Electrophoresis 16 (1995) 636.
[18] D. Schmalzing, L.B. Koutny, T.A. Taylor, W. Nashabeh, M. Fuchs, J. Chromatogr. B 697 (1997) 175.
[19] F.T. Chen, J.C. Sternberg, Electrophoresis 15 (1994) 13.
[20] F.T. Chen, S.L. Pentoney Jr., J. Chromatogr. A 680 (1994) 425.
[21] X. Liu, Y. Xu, M.P. Ip, Anal. Chem. 67 (1995) 3211.
[22] O.W. Reif, R. Lausch, T. Scheper, R. Freitag, Anal. Chem. 66 (1994) 4027.
[23] F.T. Chen, J. Chromatogr. A 680 (1994) 419.
[24] J.J. Bao, J. Chromatogr. B 699 (1997) 463.
[25] D. Schmalzing, W. Nashabeh, Electrophoresis 18 (1997) 2184.
[26] L. Tao, R.T. Kennedy, Electrophoresis 18 (1997) 112.
[27] J.W. Jorgonsen, K.D. Lukacs, Science 222 (1983) 266.
[28] K.J. Lee, G.S. Heo, J. Chromatogr. 559 (1991) 317.
[29] R.P. Oda, J.P. Landers, in: J.P. Landers (Ed.), Handbook of Capillary Electrophoresis, CRC Press, Boca Raton, FL, 1997, p. 1.