On-line preconcentration of sodium dodecyl sulfate-protein complexes using electrokinetic supercharging method with a prefilled water plug in capillary sieving electrophoresis

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Abstract An electrokinetic supercharging (EKS) method with a prefilled water plug at the head column of capillary was developed for on-line preconcentration of sodium dodecyl sulfate-SDS-protein complexes in capillary sieving electrophoresis (CSE). Conventional EKS is a combination of electrokinetic injection with transient isotachophoresis (tr-ITP). The capillary is first filled with background electrolyte and then an appropriate amount of a leading electrolyte is filled and electro-injection is carried out for a certain duration. After that terminating electrolyte is filled and tr-ITP is subsequently initiated followed by capillary electrophoresis (CE) separation. In this work, the performance of EKS was evaluated by integrating multiple sub-methods step by step and a water plug containing polymer was introduced before electrokinetic injection in order to further improve the concentration effect. The positive effects of the sub-methods were verified including molecular sieving effect of polymer field enhanced sample injection (FESI) with and without a water plug and transient isotachophoretic electrophoresis-based FESI. It was observed that analyte discrimination usually encountered in conventional electrokinetic injection was eliminated due to the similar charge to mass ratios of SDS-protein complexes. Based on these results, a hybrid on-line preconcentration method (EKS with injecting a water plug containing polymer before sample electrokinetic injection) was proposed and used to indiscriminately preconcentrate SDS-protein complexes which provided a sensitivity enhancement factor of more than 1000. It was very suitable for the analysis of low-abundance proteins providing the information of their molecular mass.

Key words field enhanced sample injection, electrokinetic supercharging, capillary sieving electrophoresis, on-line preconcentration, protein

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Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) has been widely used for protein separation according to the relative molecular mass. However, conventional slab SDS-PAGE is associated with apparent drawbacks such as time-consuming, difficult to quantitate and automate. Capillary electrophoresis (CE), which provides the advantages of high speed, high separation efficiency, automation and low consumption of sample, has been a powerful tool for protein analysis. The capillary version of SDS-PAGE has been developed to overcome the drawbacks of the slab-gel format. While cross-linked polyacrylamide gel filled in capillary brings some problems, such as short lifetime of the gel bubble formation and high background absorbance, entangled polymers such as polyethylene glycol and dextran are good alternatives to cross-linked gel. Solutions of these polymers are easy to prepare and are replaceable after each analysis, which are favorable for providing high resolution and repeatability. However, like other CE modes, capillary sieving electrophoresis (CSE) also suffers from insufficient sensitivity with UV absorbance detection due to the short optical path and small sample volume. This hampers the application of CSE for the analysis of low concentration protein samples.

Many efforts have been made to increase the detection sensitivity in CE. Compared with new instrumental designs and sensitive detectors, on-line preconcentration is a simple, effective and inexpensive strategy which can improve the sensitivity by a factor of 1–6 orders of magnitude. Based on different principles, a variety of preconcentration techniques have been established, including sample stacking, pH junction, transient isotachophoresis (tr-ITP), sweeping, and so on. However, only a limited number of on-line preconcentration methods were developed for the analysis of low-abundance protein samples. Kim et al. demonstrated a large volume sample stacking with an electroosmotic flow EOF pump with 100-fold sensitivity enhancement for proteins. Li and co-workers used field-enhanced sample injection (FESI) with a water plug to separate basic proteins in bubble cell capillary. FESI is one of the most effective methods providing enhanced concentration sensitivity of several hundreds to thousands fold without degradation of resolution. In FESI, analytes dissolved in low-conductivity buffer are electrokinetically injected into the capillary which is prefilled with high-conductivity running buffer. The charged analytes move rapidly and then stack at the boundary between low-conductivity zone and high-conductivity running buffer due to the amplified electric field strength across the boundary. The main drawback of FESI is the analyte discrimination effect which favors the preconcentration of analytes with high mobility. tr-ITP is another effective method for on-line preconcentration of proteins without the limitation of low-conductivity sample matrix. The analyte ions are concentrated between the leading and terminating ions which migrate faster and slower than the analyte ions respectively. Stutz et al. used cationic tr-ITP to preconcentrate metal-binding proteins which increased the separation efficiency to 230,000 plates/m. It was demonstrated that ITP was an effective approach to press the sample zone into a narrow band. The ITP-zone electrophoresis combination was employed to concentrate proteins on a plastic chip by Ōlivečká et al. Hirokawa and co-workers proposed a novel hybrid on-line preconcentration method electrokinetic supercharging (EKS) which was the combination of tr-ITP and electrokinetic sample injection. EKS has been applied to a variety of
analytes including metal ions, pharmaceuticals, peptides, DNA fragments, and SDS-protein complexes in microchip gel electrophoresis. So far, few investigations have focused on the preconcentration of proteins in CSE. Foote et al. employed a porous silica membrane between adjacent microchannels to preconcentrate proteins and achieved 600-fold signal enhancement in CSE.

In the present study, EKS with a water plug containing polymer was used to improve the detection sensitivity of SDS-protein complexes in CSE. The sensitive enhancement of EKS was proved by integrating multiple preconcentration methods one by one including polymer sieving, FESI and transient ITP. The indiscrimination of electrokinetic injection was avoided due to the similar mobility of SDS-protein complexes. Sensitivity enhancement by more than 1000-fold was obtained.

1 Experimental

1.1 Materials
Acrylamide AA, N,N′,N′-tetramethylethylenediamine TEMED, ammonium persulfate APS, tris hydroxymethyl aminomethane Tris, glycine SDS and dithiothreitol DTT were all purchased from Bio-Rad Hercules California USA. Dextran was bought from Fluka Buchs Switzerland. Methanol γ-methacryloxypropyltrimethoxysilane γ-MAPS and SDS-protein standards for CSE were all from Sigma Aldrich Steinheim Germany. Sodium hydroxide and hydrochloride were bought from Nanjing Chemical Reagent Co. Ltd Nanjing China. All chemicals used here were of analytical grade or higher. Ultrapure water produced by a Milli-Q Advantage A10 System Millipore Milford USA was used to prepare all solutions.

1.2 Instrumental
A PACE/MDQ system Beckman Coulter Fullerton USA with a UV detector was employed for SDS-protein complexes analysis. The detection wavelength was set at 214 nm and the temperature was controlled at 25 °C. A bare fused-silica capillary Yongnian Optical Fiber Factory Hebei China of 30 cm, 20 cm to the detector × 75 μm i. d. was used for all separations. The separation voltage was ~12 kV.

1.3 Preparation of linear polyacrylamide LPA coated capillary
LPA coated capillary was prepared and used to eliminate EOF and protein adsorption on the capillary inner wall. The capillaries were coated according to the method described by Hjerten 28. In brief, capillaries were pretreated with 1 mol/L sodium hydroxide 1 mol/L hydrochloric acid water and methanol for 1 h 2 h 10 min and 10 min respectively. After that capillaries were dehydrated at 60 °C overnight under a flow of dry nitrogen followed by silylated with a methanol solution containing 50% w/v γ-MAPS for 12 h in a water bath at 50 °C and then flushed with methanol for 20 min. Finally, the capillaries were quickly dipped into the solution containing 4% w/v AA 0.1% w/v APS and 0.1% w/v TEMED and filled with the mixture under a flow of nitrogen from a nitrogen gas cylinder as soon as possible. After polymerization for 3 h the polymer which was not bonded to the capillary wall was removed by water.

1.4 Preparation of SDS-protein complexes and running electrolytes
Each vial of SDS-protein standards contained 8 purified proteins including α-lactalbumin 14.2 kDa bovine milk trypsin inhibitor 20.1 kDa soybean carbic anhydrase 29.0 kDa bovine erythrocyte ovalbumin 45.0 kDa chicken egg albumin 66.0 kDa bovine serum phosphorylase b 97.4 kDa rabbit muscle β-galactosidase 116.0 kDa Escherichia coli and myosin 205.0 kDa rabbit muscle. Protein mixture 3.5 mg was dissolved in sample matrix which contained 60 mmol/L Tris-HCl 1% w/v SDS and 15 mmol/L DTT at pH 6.8. The solution was heated in boiling water bath for 8 min and cooled to room temperature. Before use it was centrifuged for 3 min at 6 000 r/min. The supernatant was diluted with water by 10 to 1 000-fold for preconcentration experiments. Due to its UV transparency at 214 nm and relatively low viscosity
dextran was chosen as the sieving matrix. The running buffer used for capillary sieving electrophoresis was prepared using 10% w/v dextran powder dissolved in a solution comprised of 100 mmol/L Tris-HCl and 0.1% w/v SDS at pH 8.3 and centrifuged at 13 000 r/min for 30 min. The background electrolyte BGE used for capillary zone electrophoresis CZE was 100 mmol/L Tris-HCl at pH 8.3.

1.5 EKS with a water plug and CSE separation mode

After the capillary was first filled with CSE running buffer a water plug containing polymer or not was hydrodynamically injected for certain length. SDS-protein complexes were electrokinetically injected and then terminating electrolyte was electrokinetically injected. When tr-ITP finished CSE separation was started.

2 Results and discussion

2.1 Molecular mass calibration for conventional CSE

According to the different sizes of protein molecules SDS-protein complexes could be separated based on the differential resistance in polymer network in CSE. The lower the molecular mass is the faster the protein moves. In this work 8 proteins were separated in CSE mode. According to the relationship between the effective mobility and the macromolecular size described by Lumpkin et al. the plot of log $M_i$ $M_i$ relative molecular mass against migration time exhibited a linear relationship $R^2 = 0.99$ as shown in Fig. 1. Therefore the separation mechanism was mainly molecular mass-based.

2.2 Sieving effect of polymer in running electrolyte

When the sample dissolved in free solution traversed the boundary between the free sample matrix and the dextran polymer running buffer stacking occurred because the retardation by polymer matrix led to the decrease of the mobilities of the analytes Fig. 2a. In addition this kind of sieving effect functioned to all the proteins. However if the sample was dissolved in the polymer solution at first there were no differences between the sample matrix and the running buffer. Thereby no sieving effect was observed Fig. 2b.

2.3 Preconcentration and indiscrimination of FESI

The discrimination of electrokinetic injection was first pointed out by Jorgenson and Lukacs. The amount of analytes injected by electrokinetic injection depends on their mobilities. After the interaction between SDS and proteins all saturated SDS-protein complexes take much more negative charges than free proteins and the charge to
mass ratios are nearly constant for different proteins. As shown in Fig. 3 a a single sharp peak here the number of theoretical plates was 155 000 plates/m which indicates no difference in mobility among the SDS-protein complexes meanwhile the SDS-complexes migrated very fast indicating that they were highly negatively charged. Both characteristics of SDS-protein complexes favored FESI-based preconcentration which not only eliminated the analyte discrimination effect of conventional FESI but also allowed high sensitivity enhancement. The SDS-protein complexes were hydrodynamically injected into the capillary and analyzed as shown in Fig. 3 b. The calculated area ratios of different proteins were 1 : 0.8 : 1.5 : 1.0 : 0.8 : 0.9 : 0.9 : 0.1. Compared with the calculated area ratios of different proteins in Fig. 3 d 1 : 0.8 : 1.3 : 0.9 : 0.9 : 1 : 0.9 : 0.1 and Fig. 3 e 1 : 1.0 : 1.0 : 1.4 : 1.3 : 1.6 : 1.5 : 0.1 the calculated areas of the proteins in Fig. 3 b were close to each other. This further indicated that the analyte discrimination effect of electro-injection was reduced to a great extent for SDS-protein complexes. In addition Fig. 3 d illustrates that FESI without a water plug was a simple and effective way for preconcentration which only needed to dissolve the sample in a dilute sample matrix while the sensitivity was improved more than 100 fold without degradation of the resolution.

2.4 Effect of water plug in FESI
As shown in Fig. 4 a and b by further diluting the original sample solution to 200 fold prolonged electro-injection time did not lead to significant improvement in sensitivity enhancement. When a water plug containing 10% dextran was pressure-introduced into the inlet end of the capillary the peak heights obviously increased with slight degradation of the resolution Fig. 4 c. It elucidated that the introduction of a low-conductivity solution containing polymer at head column made FESI much more effective for sample pre-concentration.

![Image](Fig. 3 Electropherograms of SDS-protein complexes inj a CZE mode and b d preconcentration of FESI without water plug in CSE mode.)

Sample a c the original denatured protein solution d 100-fold diluted denatured sample. Injection a 0.002 MPa 0.3 psi b 0.014 MPa 2 psi c d 10 kV 4 s d 6 kV 8 s. BGE a 100 mmol/L Tris-HCl buffer at pH 8.3 b d 100 mmol/L Tris-HCl buffer containing 0.1% SDS and 10% dextran at pH 8.3. Other conditions were the same as in Fig. 1.

![Image](Fig. 4 Effect of water plug in FESI Sample a 200-fold diluted with water. Injection a b 6 kV 8 s b 6 kV 24 s c a water plug containing 10% w/v dextran injected under 0.034 MPa 5 psi for 10 s before sample injection 6 kV for 24 s. Other conditions were the same as in Fig. 1.)

2.5 Effect of tr-ITP based on FESI with a water plug
In order to further compress the sample zone we introduced tr-ITP after FESI with a water plug containing polymer which was called modified EKS. The sample buffer and operational electrolytes used here in EKS are described in Table 1.
Table 1 Sample buffer and operational electrolytes used in EKS

<table>
<thead>
<tr>
<th>Sample buffer</th>
<th>Leading electrolyte and BGE</th>
<th>Terminating electrolyte</th>
<th>Water plug</th>
</tr>
</thead>
<tbody>
<tr>
<td>60 mmol/L Tris-HCl</td>
<td>100 mmol/L Tris-HCl</td>
<td>25 mmol/L Tris</td>
<td>10% w/v dextran</td>
</tr>
<tr>
<td>1% w/v w/v SDS</td>
<td>10% w/v dextran</td>
<td>192 mmol/L glycine</td>
<td></td>
</tr>
<tr>
<td>15 mmol/L DTT pH 6.8</td>
<td>0.1% w/v w/v SDS pH 8.3</td>
<td>0.1% w/v w/v SDS pH 8.3</td>
<td></td>
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</table>

The similar electrolyte components of concentrated gel in SDS-PAGE were transferred to the capillary tr-ITP as leading and terminating electrolytes e.g. Tris-HCl and Tris-glycine respectively. Compared with FESI with or without a water plug containing 10% dextran the peak heights in the modified EKS were the highest for 500-fold diluted sample as shown in Fig. 5. It was demonstrated that tr-ITP made a positive effect for pre-concentration and EKS with a water plug was the most effective method. Moreover the peak width decreased a little in EKS which suggested that the combination of tr-ITP can further compress the sample zone.

2.6 Preconcentration of SDS-protein complexes by EKS with a water plug

Fig. 6 represents the preconcentration of SDS-protein complexes for 200- 500- and 1000-fold diluted samples by EKS with a water plug containing polymer. The original sample solution was separated using conventional hydrodynamic injection. This property overcame the analyte discrimination effect of conventional electrokinetic injection. Moreover we transferred the principle and electrolyte components of concentrated gel for tr-ITP in SDS-PAGE into CSE and combined it with FESI which can be called modified EKS. Unlike traditional EKS a water plug was introduced before electrokinetic sample injection. It was a combination of 3 preconcentration methods including polymer sieving effect FESI and transient ITP.
sitivity enhancement of more than 1 000-fold was achieved.

References