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High-throughput protein analysis by multiplexed sodium dodecyl sulfate capillary gel electrophoresis with UV absorption detection

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Abstract

We have developed a novel, high-throughput approach for molecular mass determination of proteins from 14 200 to 116 000 based upon multiplexed, absorbance-based capillary electrophoresis. Via capillary multiplexing, 96 samples were analyzed simultaneously within 30 min. Detection with ultraviolet light obviates the need for protein staining or derivatization. The detection limit of the system was estimated at 5 μg/ml bovine serum albumin (BSA) when sampled from 12.5 mM Tris–HCl. The linear dynamic range was over two orders of magnitude from 5 μg/ml to 1000 μg/ml for BSA. Better than 5% sizing accuracy for protein molecular mass determination and excellent run-to-run and day-to-day reproducibility was obtainable with the described method.
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1. Introduction

Since its introduction 30 years ago [1], sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) in a slab gel format has been one of the most extensively used methods in protein chemistry for molecular weight-based separations of polypeptides and proteins. However, SDS-PAGE is time-consuming, labor-intensive, marginally quantitable and not readily amenable to automation. Hjerten [2] and Karger and coworkers [3,4] first introduced the use of capillary gel electrophoresis (CGE) for SDS-protein sizing (SDS-CGE). With the development of replaceable polymer sieving solutions [5–7], SDS-CGE provides a simple, fast and automated method for protein separation. Absorbance detection in the low UV range (200–220 nm) is often employed for detection because of the strong absorption of the peptide bonds and provides a simple means for protein quantification without the need for protein derivatization. For very low abundance protein detection, laser-induced fluorescence (LIF) is preferred but derivatization or labeling of the proteins is required [8,9]. However, the single capillary CGE format still lacks the throughput capacity of SDS-PAGE, which can analyze multiple samples (up to 26 samples) simultaneously.

Recently, SDS-CGE has also been performed using microchips [10], whereby ten samples can be analyzed in 30 min. However, the use of a single detection channel while reusing the sieving matrix to perform multiple sequential separations hinders separation consistency due to sample carryover and degradation of the sieving matrix. In addition, the sieving matrix and buffers require manual filling, which is of minimal operational benefit over slab gel analysis.

The recent development of multiplexed capillary zone electrophoresis with UV absorption detection [11–13] provides high throughput chemical analysis without the need for sample derivatization. This 96-capillary multiplexed CZE system has been applied towards the high throughput determination of drug compound acid dissociation constants (pK_a values) [14], octanol–water partition coefficients (log P values) [15], and for oligonucleotide analysis [16]. In addition, the use of eight-capillary array CGE to obtain a Ferguson plot for protein molecular mass determination was demonstrated [17]. The Ferguson method compensates for non-ideal conditions requiring multiple measurements under several different gel concentrations to estimate protein molecular mass. However, no high throughput CGE protein analysis and re-

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producibility results have been reported. As with multiplexed CE systems developed for DNA sequencing, the advancement of protein sizing from single column systems to highly multiplexed CE systems is an obvious goal. Conditions optimized for single capillary systems or small array systems may not be suitable for large array configurations. The scale-up to a 96-capillary array to, along with the associated automation, is highly challenging.

In this work, a 96-capillary multiplexed, absorbance-based capillary electrophoresis system for high throughput protein molecular mass determination and quantitative analysis is described. Up to 96 protein samples can be analyzed simultaneously within 30 min. A critical evaluation of this method is presented. The sizing accuracy and repeatability of this multiplexed system is comparable with conventional SDS-PAGE while providing a significant increase in throughput and automation. These results suggest that high speed, high throughput and automated molecular mass sizing determination of proteins can be achieved via multiplexed CGE with robust and reproducible long-term performance.

2. Experimental

Protein standards ranging in molecular masses from 14 200 to 116 000 and all other chemicals were obtained from Sigma (St. Louis, MO, USA). A seven-protein standard mixture was prepared comprised of: α-lactalbumin (14 200), trypsin inhibitor (21 500), carbonic anhydrase (31 000), ovalbumin (45 000), bovine serum albumin (BSA, 66 200), phosphorylase b (97 000), and β-galactosidase (116 000). Protein samples were dissolved in a buffer composed of 12.5 mM Tris–HCl, 0.5% SDS, and 5 mM dithiothreitol (DTT) and placed in a 96-well microtiter plate. The final concentration of each protein was ∼100 μg/ml. Protein samples were denatured first by heating the titer plate at 95 °C for 20 min before sample injection. The sieving matrix used for SDS capillary gel electrophoresis consisted of 5% Dextran (M_r= 2 000 000) in 0.1% SDS and 50 mM Tris–borate buffer with a proprietary positively charged polymer for dynamic coating to reduce electroosmotic flow. To reduce the possibility of generating excessive Joule heating from the total current of the closely-spaced capillary array, the buffers were made at a lower ionic strength than that typically used for single column systems.

A cePRO 9600 96-capillary electrophoresis instrument utilizing UV absorbance detection at 214 nm (CombiSep, Ames, IA, USA) was used for all protein separations. The system was fitted with 75 μm i.d. × 150 μm o.d. uncoated silica capillaries (Polymicro Technologies, Phoenix, AZ, USA) with 28 cm effective and 54 cm total lengths. The protein samples were electrokinetically injected into the capillary array simultaneously for 50 s at 4.5 kV. Separations were performed at −12 kV. ProSize data analysis software (CombiSep) was utilized for automated sizing and concentration analysis.

The capillary array was initially conditioned with a 0.1 M NaOH solution for 10 min followed by 5 min flushes with 0.1 M HCl and deionized water. The sieving matrix was then flushed through the capillary array for 15 min before initial sample injection. Between separations, fresh sieving matrix was flushed through the capillary array for 5 min. No other capillary treatment was required. Up to ten consecutive separations could be performed before re-conditioning of the capillary array was necessary.

3. Results and discussion

Fig. 1 shows a typical output of the 96-capillary SDS-CGE separation of a seven protein standard mixture displayed as a gel-like image. Bands at M_r 14 200 (α-lactalbumin) and 116 000 (β-galactosidase) were used as internal standards for automated sizing to normalize variations in migration times between individual capillaries of the array. The 116 000 protein peak was detected in less than 30 min with good resolution and signal-to-noise ratio. The migration time variations were reduced from over 7% to ∼1% across the capillary array following normalization. We note that if a different range of molecular mass proteins were analyzed, one could use other low and high molecular mass proteins as internal markers to normalize the data.

Fig. 1 demonstrates for the first time that a significant increase in sample throughput for SDS-protein separations can be obtained by using a 96-capillary array. Importantly, the same capillary array has been continuously used for several months without displaying any significant degradation of separation efficiency. In addition, we have been able to perform ten consecutive SDS-protein separations (over 900 protein samples) in less than 7 h with over a 98% success rate in terms of obtaining similar resolution and sensitivity for all capillaries in each separation. These results indicate that the system and separation protocol is extremely robust for long-term operation.

One capillary in the array was chosen to establish the reference molecular mass calibration curve by plotting the logarithmic molecular mass of the standard proteins against their migration times. As shown in Fig. 2, an excellent linear correlation was obtained over the M_r 14 200 to 116 000 sizing range ($R^2 = 0.995$). In addition, the average theoretical plate number for these seven proteins is estimated to be 33 000, which is slightly better than those reported using LIF detection [8,9].

To evaluate the sizing accuracy and reproducibility of the multiplexed CE technique for protein sizing, the migration times for each sample peak from all the capillaries were utilized to predict the corresponding molecular mass from the calibration curve of Fig. 2. These results are summarized in Table 1. The estimated molecular mass of the five proteins determined by multiplexed CE were comparable to published single column CE values [18] and data obtained previously with SDS-PAGE [19]. The deviation in predicted molecular

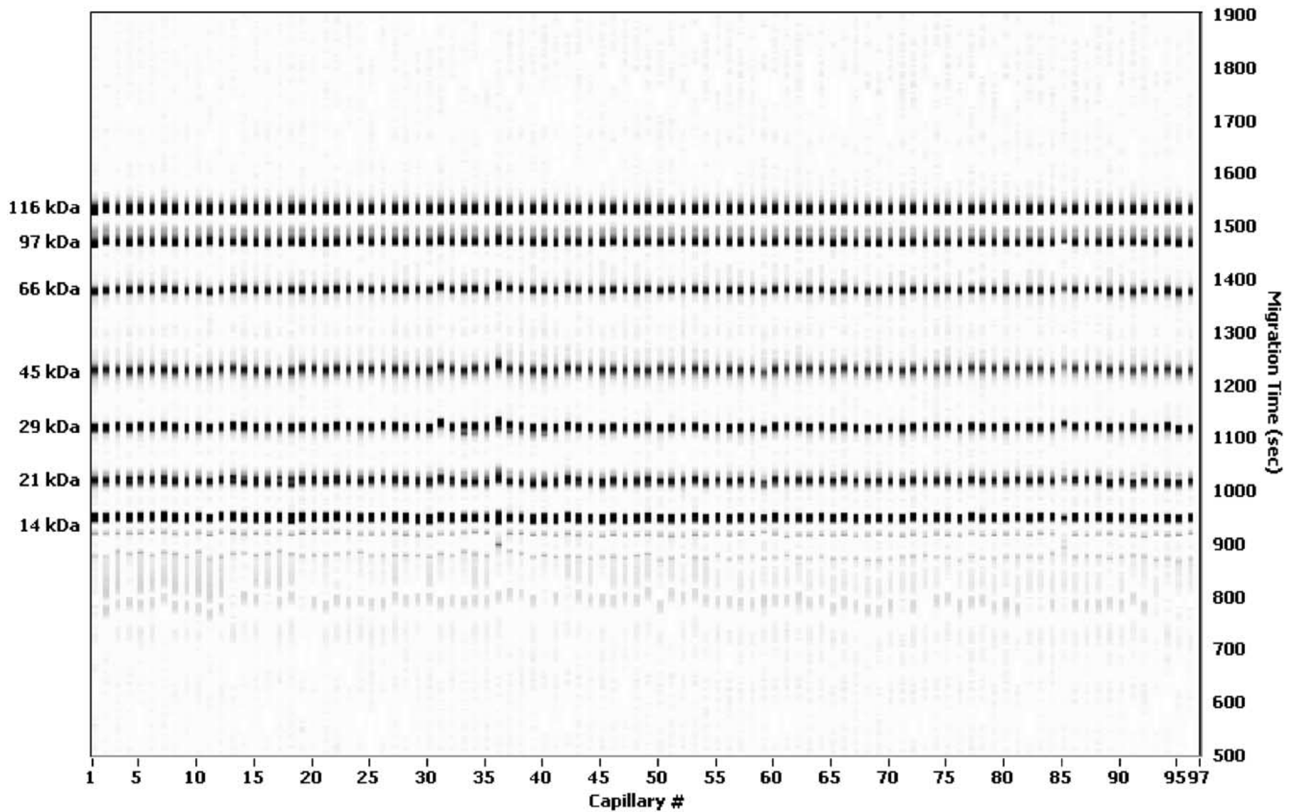

Fig. 1. SDS-protein separation of a seven protein standard mixture using a 96-capillary array. A gel-like image of the CE data is displayed after normalization with α-lactalbumin (peak 1, M_r 14 200) and β-galactosidase (peak 7, M_r 116 000).

mass for the proteins tested here is less than 5%. The R.S.D. values for the sizing of proteins by multiplexed CE are 2% or less and are comparable to or slightly better than SDS-PAGE. The day-to-day reproducibility for molecular mass determination was also evaluated. The molecular mass determination had less than 4% error for day-to-day operations. However, in order to accurately determine the molecular mass of glycoproteins with the multiplexed CGE system, the Ferguson method, as described in ref. [17], or deglycosylation is still required.

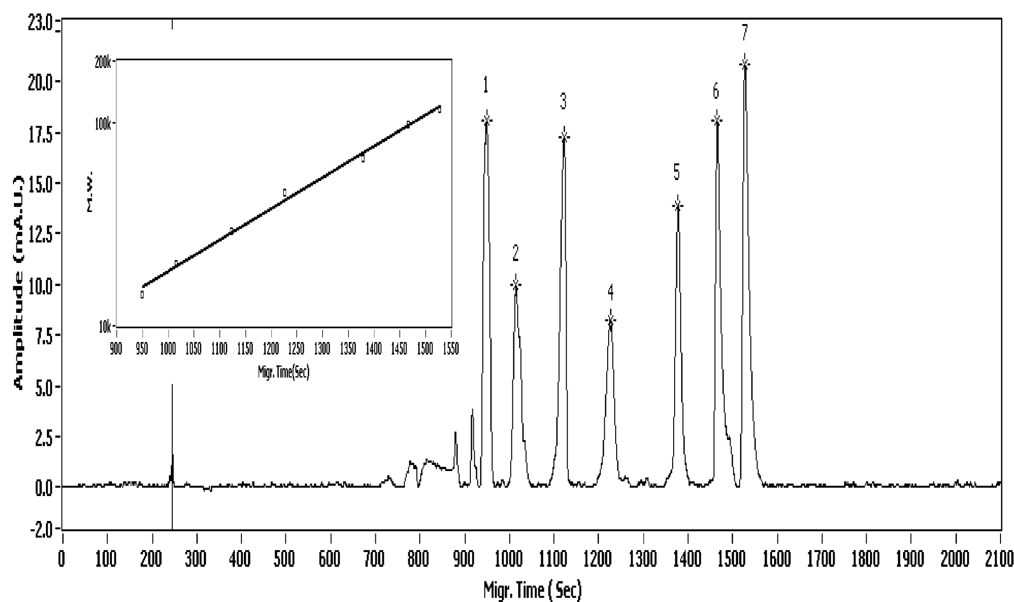

Fig. 2. Electropherogram and calibration curve generated from a mixture of seven standard proteins covering a molecular mass range from 14 200 to 116 000. Protein peaks: (1) α-lactalbumin; (2) trypsin inhibitor; (3) carbonic anhydrase; (4) ovalbumin; (5) BSA; (6) phosphorylase b; (7) β-galactosidase.

Table 1
Comparison of protein sizing accuracy and reproducibility between SDS-PAGE and the 96-capillary multiplexed CE system

Protein	Literature [18] (M_r)	SDS-PAGE [19]			Multiplexed CE–UV		
		M_r	S.D.	R.S.D. (%)	M_r	S.D.[a]	R.S.D. (%)[a]
Trypsin inhibitor	20 100	19 100	0.6	3.4	20 200	0.4	1.8
Carbonic anhydrase	29 000	27 200	0.6	2.1	29 500	0.6	2.0
Ovalbumin	45 000	46 100	1.1	2.3	45 100	0.9	1.9
Serum albumin	66 000	N/A[b]			67 900	1.4	2.0
Phosphorylase b	97 000	N/A[b]			96 400	1.4	1.5

[a] The values given are the average of 60 separations for each protein.
[b] Ref. [19] did not include these two proteins for analysis.

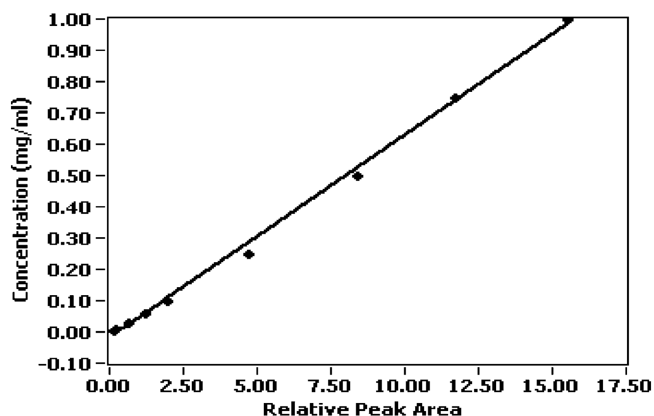

Fig. 3. Calibration curve for BSA demonstrating the detection sensitivity and linear dynamic range of the multiplexed CE–UV method. The normalized peak area was obtained by dividing the peak area of BSA by the peak area of β-galactosidase internal standard.

To evaluate the detection sensitivity and linear dynamic range of the multiplexed CE system, a set of BSA samples with concentrations from 5 μg/ml to 1000 μg/ml were prepared and analyzed. The peak area of BSA was divided by the peak area of a β-galactosidase internal standard (116 000) in order to obtain a normalized peak area for calibration. The use of normalized peak areas compensated for any variations during electrokinetic sample injection and for any spatial variations in detector response across the imaged capillary array. Fig. 3 shows the calibration curve obtained for BSA. From the figure, the detection limit was estimated to be less than 5 μg/ml (76 nM) BSA when prepared in a 12.5 mM Tris–HCl buffer solution. Importantly, the detection limit was affected by the sample matrix in a similar fashion to other SDS-CGE based approaches. Specifically, high salt contents in the sample matrix significantly reduced the sensitivity due to a decrease in sample stacking and the preferential electrokinetic injection of salts. For example, the sensitivity was reduced to 50 μg/ml when using a 25 mM NaCl concentration in the sample matrix. Therefore, a desalting procedure may be required prior to sample injection to maximize detection sensitivity. The linear dynamic range was more than two orders of magnitude from 5 μg/ml to 1000 μg/ml of protein ($R^2 = 0.998$) as shown in Fig. 3. Taken together, these results demonstrate that multiplexed SDS-CGE can provide good detection sensitivity and quantification for protein analysis while achieving high sample throughput.

In conclusion, high throughput molecular mass sizing analysis of proteins can be performed using 96-capillary multiplexed CE with UV detection. Protein sizing accuracy, sensitivity and repeatability are comparable to conventional SDS-PAGE, while providing higher throughput and automated operation. The detection limit was approximately 5 μg/ml (76 nM) for BSA when prepared in a 12.5 mM Tris–HCl sample solution. The linear dynamic range extended over two orders of magnitude, from 5 μg/ml to 1000 μg/ml for BSA. The day-to-day reproducibility of protein sizing indicates that multiplexed CE is highly suitable for routine assays of protein samples.

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References

[1] A. Shapiro, E. Vinuela, J. Maizel, J. Biochem. Biophys. Res. Commun. 28 (1967) 815.
[2] S. Hjerten, J. Chromatogr. 270 (1983) 1.
[3] A.S. Cohen, A. Paulus, B.L. Karger, Chromatographia 24 (1987) 15.
[4] A.S. Cohen, B.L. Karger, J. Chromatogr. 397 (1987) 409.
[5] K. Benedek, A. Guttman, J. Chromatogr. A 680 (1994) 375.
[6] W. Werner, D.M. Demorest, J. Stevens, J.E. Wiktorowicz, Anal. Biochem. 212 (1993) 253.
[7] K. Benedek, S. Thiede, J. Chromatogr. A 676 (1994) 209.
[8] S. Hu, J. Jiang, L.M. Cook, D.P. Richards, L. Horlick, B. Wong, N.J. Dovichi, Electrophoresis 23 (2002) 3136.
[9] S. Hu, Z. Zhang, L.M. Cook, E.J. Carpenter, N.J. Dovichi, J. Chromatogr. A 894 (2000) 291.
[10] L. Bousse, S. Mouradian, A. Minalla, H. Yee, K. Williams, R. Dubrow, Anal. Chem. 73 (2001) 1207.
[11] X. Gong, E.S. Yeung, Anal. Chem. 71 (1999) 4989.
[12] Y. Zhang, X. Gong, H. Zhang, R. Larock, E.S. Yeung, J. Comb. Chem. 2 (2000) 450.
[13] P. Britz-McKibbin, T. Nishioka, S. Terabe, Anal. Sci. 19 (2003) 99.
[14] Application Note APP-111200, CombiSep, Ames, IA, 2002.
[15] K. Wong, J. Kenseth, R. Strasburg, J. Pharm. Sci. 93 (2004) 916.
[16] Application Note APP-207500, CombiSep, Ames, IA, 2002.
[17] Y. He, E.S. Yeung, J. Proteome Res. 1 (2002) 273.
[18] A. Guttman, Electrophoresis 17 (1996) 1333.
[19] M. Kuschel, Publication 5988-0975EN, Agilent Technologies, 2000.