Journal of Chromatography, 632 (1993) 171-175
Elsevier Science Publishers B.V., Amsterdam

CHROMSYMP. 2589

Capillary sodium dodecyl sulfate gel electrophoresis of proteins

András Guttman, Judith A. Nolan and Nelson Cooke

Beckman Instruments, Inc., Fullerton, CA 92634 (USA)

ABSTRACT

In recent years, there has been considerable activity in the separation and characterization of protein molecules by sodium dodecylsulfate (SDS) gel electrophoresis with particular interest in using this technique to separate on the basis of size and to estimate molecular mass. In this paper we report a new improved and automated electrophoretic method in the form of high-performance capillary SDS gel electrophoresis. Rapid separations of protein molecules in the molecular mass range of 20 000–200 000 daltons were demonstrated with excellent linearity and intra- and inter-day reproducibility of the migration time. Monomer–dimer forms of the recombinant human ciliary neurotrophic factor were examined by the use of this method under reducing and non-reducing conditions.

INTRODUCTION

Electrophoresis in polyacrylamide or other gels containing ionic detergents, such as sodium dodecyl sulfate (SDS), has proven to be a powerful tool for the size separation of protein molecules, the estimation of their molecular mass and assessment of their purity [1]. In the presence of reducing agents such as mercaptoethanol, the detergent (SDS) dissociates proteins into their constituent subunits and binds to the polypeptide chains, so that similar charge-to-mass ratios of the proteins are obtained [2]. In gel electrophoresis, the polymer network structure creates a sieving effect so that separation can be performed based on the size of the molecules [3]. An approximately linear relationship is obtained if the logarithm of the molecular mass of standard polypeptide chains are plotted against their electrophoretic mobilities [4]. The method is reliable and reproducible in the molecular mass range of 20 000–200 000 daltons generally within 10% of those obtained by other techniques [2]. Prior to the application of the sample to the gel, the proteins are denatured by heat treatment in the presence of a thiol-reducing agent such as β-mercaptoethanol or dithiothreitol. Rod or slab gels of a variety of cross-linked polyacrylamides were conventionally used as sieving matrices to separate these polypeptide chains according to their molecular masses [5].

In the past several years capillary gel electrophoresis has been successfully utilized for the size separation and purity assessment of synthetic oligonucleotide probes and primers [6] and polymerase chain reaction (PCR) products [7]. Early attempts to apply capillary gel electrophoresis for protein separation by SDS polyacrylamide gel electrophoresis (PAGE) have involved highly concentrated cross-linked polyacrylamide as sieving medium [8]. Later, others employed lower-concentration cross-linked polyacrylamide [9] and linear polyacrylamide in the capillary as sieving material [10]. Recently, Karger's group [11] introduced non-polyacrylamide-type, low-viscosity branched polymers such as dextrans with excellent UV transparency at 214 nm to obtain size separation with enhanced detectability.

In this paper we report the use of a non-polyacrylamide-based, hydrophilic linear polymer

Correspondence to: Dr. A. Guttman, Beckman Instruments, Inc., Fullerton, CA 92634, USA.

0021-9673/93/$06.00

network for capillary SDS gel electrophoresis of proteins. This polymer network is advantageous in that capillary gels are easily prepared and are easily replaceable if necessary. Examples are shown of the usefulness of this system in the demonstration of dimer formation of the recombinant human ciliary neurotrophic factor.

EXPERIMENTAL

Apparatus

In all these studies, the P/ACE System 2100 capillary electrophoresis apparatus (Beckman Instruments, Fullerton, CA, USA) was used in reversed polarity mode (cathode on the injection side). The separations were monitored on-column at 214 nm. The temperature of the gel-filled capillary columns was controlled at 20°C by the liquid cooling system of the P/ACE instrument. The electropherograms were acquired and stored on an Everex 386/33 computer. Molecular masses of the protein samples were estimated by using the Molecular Weight Determination Option of the System Gold software package (Beckman Instruments).

Procedures

In all the capillary electrophoresis experiments the eCAP SDS-200 (Beckman Instruments) capillary electrophoresis size separation kit for SDS proteins was used. In this kit the sieving matrix is a low-viscosity gel formulation which is not bonded to the capillary wall. This permits replacement of the gel-buffer system in the coated capillary column by means of the pressure rinse operation mode of the P/ACE apparatus (i.e., replaceable gel). It is important to note, that a coated capillary column should be used in these experiments to eliminate the electroosmotic flow and minimize adsorption of proteins on the inner surface of the column. The 47 cm long (40 cm to the detector) and 0.1. mm I.D. coated eCAP SDS-200 fused-silica capillary column (Beckman Instruments) was washed with 1 *M* HCI after each run. In some specific applications a longer or a shorter column may be required to extend a useful range and/or to shorten the analysis time. The samples were injected by pressure (typically: 30–60 s, 0.5 p.s.i.) into the replaceable gel-filled capillary column.

The slab gel experiments were performed on SDS-PAGE (10% polyacrylamide) according to the procedure of Laemmli [12] applying 30 V/cm constant field on a Bio-Rad Mini-Protean II dual-slab cell system (Bio-Rad Labs, Richmond, CA, USA). The separated bands were visualized by Commassie Brilliant Blue R250 staining [13].

Chemicals

The high-molecular-mass SDS protein test mixture (29 000–205 000 daltons) and the lysozyme were purchased from Sigma (St. Louis, MO, USA). Before injection, the samples were diluted to 0.2-2 mg/ml with the eCAP SDS-200 sample buffer (final concentration: 60 m*M* Tris–HCl, 1% SDS, pH 6.6) and were boiled in a water bath for 5 min after adding 2.5% β-mercaptoethanol as reducing agent and 0.005% Orange G as internal standard. The samples were stored at -20°C or freshly used. All buffer and gel solutions were filtered through a 1.2 μm pore size filter (Schleicher and Schuell, Keene, NH, USA) and carefully vacuum-degassed.

RESULTS AND DISCUSSION

Fig. 1 shows the complete separation of a mixture of six proteins (size range 29 000–205 000 daltons) in only 22 min. The inset in Fig. 1 shows the polyacrylamide slab gel trace of the same protein test mixture. Detected amounts for the two systems were 120 ng total protein on the SDS-200 column and 10 μg total protein on the slab gel. The differences in relative migration times and relative migration distances for the two techniques are explained by the use of different sieving polymer matrices. A linear relationship between the logarithm of molecular mass and mobility were found in both instances. The relative standard deviation (R.S.D.) in capillary gel electrophoresis (CGE) gave high linearity for the range of 20 000-200 000 daltons ($R.S.D._{CGE}$ = 0.995%) *versus* the slab gel ($R.S.D._{SLAB}$ = 0.986%, dat not shown). The calibration curve can be used for molecular mass estimation of unknown proteins with 10% accuracy. As can be observed, well resolved peaks are obtained for each component; however, some peaks seem to be broader than others. It is postulated that this phenomenon is caused by small differences in the complexation of SDS with the different protein molecules [1–3]. Table I is a comparison of the individual steps and

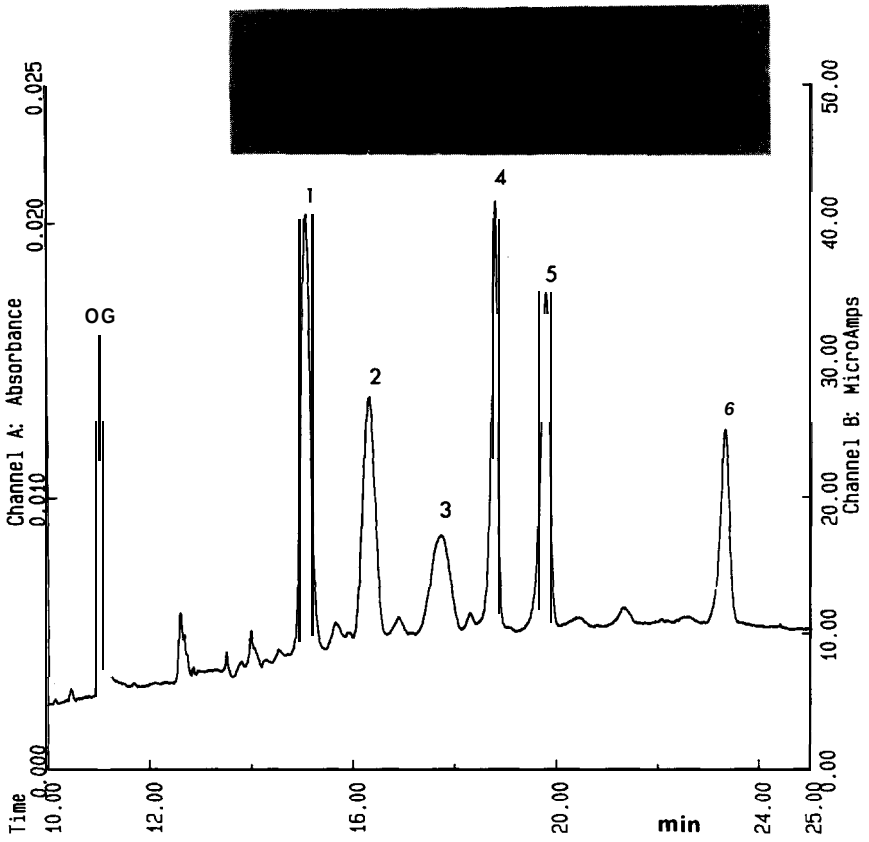

Fig. 1. Capillary SDS gel electrophoresis of six proteins on an eCAP SDS-200 capillary. Inset: SDS-PAGE pattern of the same sample mixture. Peaks: 1 = carbonic anhydrase (molecular mass, M_r 29 000); 2 = ovalbumin (M_r 45 000); 3 = bovine serum albumin (M_r 66 000); 4 = phosphorylase B (M_r 97 400); 5 = β-galactosidase (M_r 116 000); 6 = myosin (M_r 205 000). A tracking dye Orange G (OG) was added to the sample. Conditons: injected amount, 100 ng protein; detection, 214 nm; run temperature, 20°C; applied electric field, 300 V/cm; current, 25-30 μA.

TABLE I

COMPARISON OF eCAP SDS-200 AND SDS-PAGE ANALYSIS

Step	Time (min)	
	eCap SDS-200	SDS-PAGE
Gel preparation	6	80
Sample preparation	10	10
Gel run time	25	35
Staining	–	90
Destaining	–	720
Drying	–	720 (without dryer)
Quantitation	2	20 (densitometer)
Total	43	1675 (for sixteen lanes)

required time for both the traditional slab SDS-PAGE system and eCAP SDS-200 techniques. These data clearly show that even in an optimal case running sixteen different samples on the slab gel system, the automated capillary electrophoresis system is less time-consuming. It is important to note that capillary SDS gel electrophoresis is more easily quantitated (data is shown later) while in slab gel electrophoresis the frequently used visualization dye Coomassie Brilliant Blue R250 does not bind in a stoichiometric fashion for all proteins, making quantitative interpretation difficult [14]. It is important to note, that with the inclusion of an internal standard (Orange G) for each sample it is unnecessary to bracket samples with standard runs. The existing sieving phenomena in capillary SDS gel

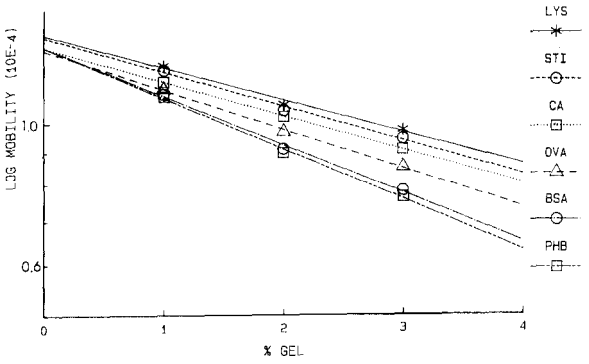

Fig. 2. Ferguson plot of logarithm mobility *versus* percent gel concentration for six proteins. LYS = lysozyme: STI = soybean trypsin inhibitor; CA = carbonic anhydrase: OVA = ovalbumin; BSA = bovine serum albumin; PHB = phosphorylase B.

electrophoresis was demonstrated by the Ferguson method [15]. As Fig. 2 shows, the plots of log mobility *versus* %(w/v) gel (Ferguson plots) were linear (R.S.D. = 0.995) with an intercept at 0% gel concentration which represents approximately the free solution mobility of the SDS-protein complex. Other variables such as polymer molecular mass, degree of polymer branching and column temperature will also affect the separation [16].

Using the pressure injection mode of this capillary electrophoresis system the injected amount is *ca.* 1 nl/s for this particular capillary and gel-buffer system based on the migration velocity of the internal standard using the low-pressure rinse (injection pressure) mode [17]. As an example, the amount for phosphorylase B (molecular mass 97 400 daltons) detected on column is 10^{-9} g (10^{-14} mol). The peak area was a linear function of the injected amount for lysozyme over a range of 10 μg/ml ($7 \cdot 10^{-7}$ *M*) to 1 mg/ml with R.S.D. = 0.999. Identical volumes of samples with different protein concentration were injected in this experiments. The capillary column was washed with 1 *M* HCl after each run to remove absorbed proteins. if any, from the capillary wall.

Initial characterization of the capillary SDS gel electrophoresis technique involved the determination of reproducibility for migration time. The R.S.D. of the uncorrected migration times for eighteen runs of the standard proteins range from 0.4 to 0.8%. These data represent a capillary and buffer that was cycled for 100 runs (intra- and inter-day variability) by altering nine replicate injections of the high-molecular-mass Sigma standard and nine replicate injections of untreated calf serum diluted

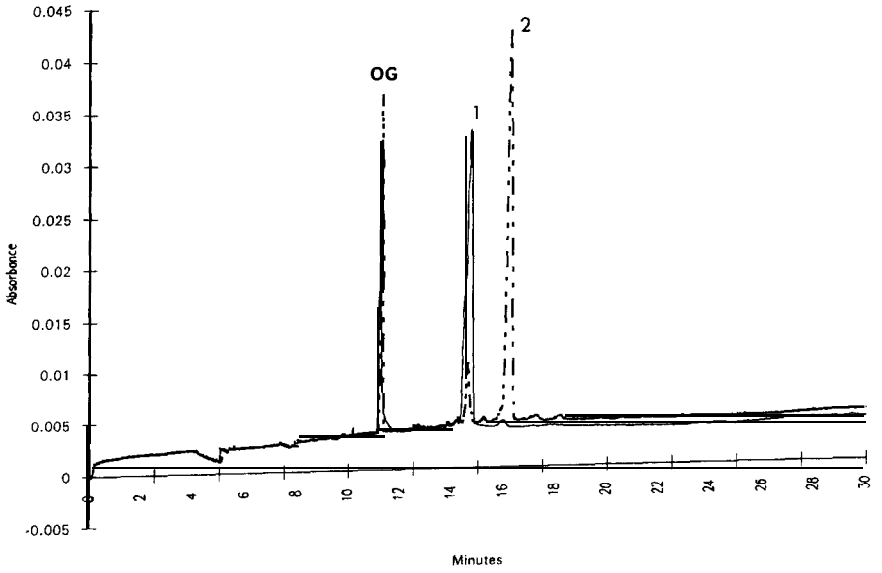

Fig. 3. Capillary SDS gel electrophoretic separation of the reduced (monomer, solid line) and unreduced (dimer, dotted line) proprietary protein, referred to as SFP. Conditons are the same as in Fig. 1.

1:50 with water. The good reproducibility of the results may be attributed to the direct visualization of the proteins during the separation and the ability to replace the polymer network with a rinsing step after each run. If a particulate sample was applied to the gel, or a sample containing a contaminant too large to be analyzed in the standard run time, the replacement of the low-viscosity gel alleviates any damage to the gel that would have occurred in a non-replaceable system.

As an example, the issue of disulfide bond formation for a proprietary protein, referred to as SFP, was addressed. The quantity of dimerization of the product was analyzed by preparing two samples, with and without disulfide reduction (Fig. 3). The larger peak (peak 2) in the run under non-reducing conditions (dotted line) is a dimer of a predicted molecular mass of 46 000 daltons. Upon addition of β-mercaptoethanol to the sample, the peak moved to the location of the monomer (molecular mass 23 000 daltons, peak 1, solid line). The two peaks were verified by molecular mass estimation. Predicted masses for the monomer and dimer were 21 700 and 5 1 000 daltons, respectively, in good agreement with the known values.

ACKNOWLEDGEMENT

The authors gratefully acknowledge Professor Barry L. Karger for his stimulating discussions.

REFERENCES

1 A. T. Andrews, *Electrophoresis,* Claredon Press, Oxford, 2nd ed., 1986.
2 K. Weber and M. Osborn, *J. Biol. Chem.*, 244 (1969) 4406.
3 B. D. Hames and D. Rickwood (Editors), Gel *Electrophoresis of Proteins*, IRL, Washington, DC, 1983.
4 J. A. Reynolds and C. Tanford, *Proc. Natl. Acad. Sci. USA, 66 (1970) 1002.*
5 A. Chrambach, *The Practice of Quantitative Gel Electrophoresis* VCH, Deerfield Beach, FL, 1985.
6 A. Guttman, A. S. Cohen, D. H. Heiger and B. L. Karger, *Anal. Chem.*, 62 (1990) 137.
7 H. E. Schwartz, K. J. Ulfelder, F. J. Sunzeri, F. J. Busch and R. G. Brownlee, *J. Chromatogr., 559 (1991) 267.*
8 A. S. Cohen and B. L. Karger, *J. Chromatogr., 397 (1987) 409.*
9 K. Tsuji, *J. Chromatogr., 550 (1991) 823.*
10 A. Widhalm, C. Schwer, D. Blass and E. Kenndler, *J. Chromatogr., 546 (1991) 446.*
11 B. L. Karger, presented at the *4th International Symposium on HPCE, Amsterdam, Feb. 9-13, 1992,* LW-I
12 U. K. Laemmli, *Nature, 227 (1970) 680.*
13 D. Freifelder, *Physical Biochemistry*, W.H. Freeman, New York, 2nd ed., 1982.
14 S. Fazekas de St. Groth, R. G. Webster and A. Datyner, *Biochim. Biophys. Acta, 71 (1963) 377.*
15 K. A. Ferguson, *Metab. Clin. Exp.*, 13 (1964) 985.
16 A. Guttman, J. Horvath and N. Cooke, presented at the *6th Symposium, Protein Society, San Diego, July 25-29, 1992, PT-78.*
17 J. Harbough, M. Collette and H. E. Schwartz, *Technical Bulletin*, TBIC-103, Beckman Instruments, Fullerton, CA, 1990.