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Capillary sodium dodecyl sulfate gel electrophoresis of proteins II. On the Ferguson method in polyethylene oxide gels

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Abstract

Capillary sodium dodecyl sulfate (SDS) gel electrophoresis is demonstrated to be a powerful new analytical method for the separation of protein molecules based on their molecular mass. Standard curves of logarithm molecular mass *versus* reciprocal relative migration time give the estimated molecular mass of sample proteins within the acceptable 10% error for most proteins. Larger errors are possible, however, when special groups such as carbohydrates (glycoproteins) or lipids (lipoproteins) are present due to a different ratio of binding SDS molecules. An automated Ferguson method is shown that corrects for this non-ideal behavior in capillary SDS–polyethylene oxide gel electrophoresis.

1. Introduction

Capillary gel electrophoresis (CGE) containing sodium dodecyl sulfate (SDS) as an ionic detergent is a newly established separation tool for the size separation and purity assessment of protein molecules using cross-linked [1] and linear polyacrylamide [2–6], dextran [4] and polyethylene oxide [7,8] gels. Classical SDS gel electrophoresis of proteins [9–11] is based on the phenomenon that most proteins bind SDS in a constant mass ratio of 1:1.4 [12,13], thus different size protein–SDS complexes having similar mass-to-charge ratios can be separated by means of a sieving matrix via electrophoresis. However, some types of proteins having specific side groups, such as carbohydrates (glycoproteins), lipids (lipoproteins) or other prostetic groups may bind SDS differently [14]. The irregular binding of SDS causes a different charge-to-mass ratio for these molecules resulting in inaccurate estimates in the apparent molecular mass [13,14]. The so called Ferguson method [15] can be used to help correct for this non-ideal behavior. Ferguson plots are constructed by plotting the logarithm of reciprocal migration time of the individual proteins as a function of different gel concentrations. Linear regression provides slopes which are the negative of the retardation coefficients (K_R). In this way a more universal calibration curve can be drawn by simply plotting the logarithms of molecular masses as a function of the retardation coefficients. In fact, K_R is proportional to the effective molecular surface area (or to the radius of a spherical molecule with the same surface area) and not directly upon on molecular mass [14]. Chrambach [13] showed that a linear relationship is obtained when $K_R^{1/2}$ is plotted against the molecular radius for spherical molecules in slab polyacrylamide

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gel electrophoresis (PAGE). In capillary polyacrylamide gel electrophoresis, Werner *et al.* [16] attained a similar logarithm molecular mass *vs.* $K_R^{1/2}$ relationship.

Before the advent of SDS CGE the Ferguson method was extremely time consuming and labor intensive, due to the requirement of making different gel concentrations in slab format and the evaluation of the separated bands by regular staining/destaining procedures [7]. Here we describe a rapid automated Ferguson analysis method for protein molecular mass determination using a computer-controlled separation system.

Our data clearly show that the use of the automated Ferguson method in conjunction with capillary polyethylene oxide gel electrophoresis gives a more precise molecular mass estimate for those proteins with different SDS-binding levels.

2. Materials and methods

2.1. Apparatus

In the capillary electrophoresis 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 mass determination option of the System Gold software package (Beckman).

2.2. Procedures

In all the capillary electrophoresis experiments the eCAP SDS 14-200 (Beckman) capillary electrophoresis size separation kit for SDS proteins was used. The 27 cm long (20 cm to the detector) and 0.1 mm I.D. coated eCAP SDS 14-200 fused-silica capillary column (Beckman) was washed with 1 *M* HCl after each run. The appropriately pretreated samples were injected by pressure (typically: 30–60 s, 0.5 p.s.i.; 1 p.s.i. = 6894.76 Pa) into the replaceable polyethylene oxide gel-filled capillary column.

To perform the Ferguson experiments the test mix and the sample proteins were run on a 20 cm effective length eCAP SDS 14-200 capillary column using various concentrations of the eCAP SDS 14-200 gel buffer at a constant field strength of 300 V/cm. The gel buffer is used in concentration received as well as diluted to 75, 67 and 50% with a dilution buffer [17].

2.3. Chemicals

The SDS protein molecular mass test mixture (M_r 14 200–205 000) and all the other proteins were purchased from Sigma (St. Louis, MO, USA). Before injection, the samples were diluted to 0.2–2 mg/ml with the eCAP SDS 14-200 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 & Schuell, Keene, NH, USA) and carefully vacuum degassed at 100 mbar.

3. Results and discussion

In SDS CGE for protein molecular mass determination, the sieving matrix used was a low-viscosity gel formulation of polyethylene oxide which is not bound to the inside surface of a coated capillary. This permits replacement of the gel buffer system in the coated capillary column by means of the pressure rinse operation mode of the electrophoresis 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 non-specific adsorption of protein on the inner surface of the capillary [18].

Fig. 1 shows the separation of the standard protein test mixture of α-lactalbumin, carbonic anhydrase, ovalbumin, bovine serum albumin, phosphorylase *b*, β-galactosidase and myosin as well as the tracking dye of orange-G by SDS CGE. The standard curve for molecular mass estimation was constructed by plotting the logarithms of the molecular masses as a function of the electrophoretic mobility (inset in Fig. 1). While these standards show a linear relationship, there are some proteins that behave differently and do not fall on the standard regression line. This unusual behavior can be attributed to special groups on the polypeptide chain such as carbohydrates side chains (glycosylations) or lipids (lipoproteins). These groups do not bind SDS in the usual manner resulting in a change in the charge-to-mass ratio of the SDS–protein complex. This in turn causes an increase or decrease in the migration time in CGE and therefore higher or lower estimated apparent molecular mass. In these instances the well established classical Ferguson method can be used to obtain data with higher precision. Fig. 2 shows separations of amylase and IgG light and heavy chains by SDS CGE. Simply using the calibration curve shown in Fig. 1 gives estimated molecular masses of the peaks of interest that are off by 21% for amylase and 87 and 25% for the light and heavy chains, respectively of IgG (Table 1). Therefore the Ferguson method should be used to achieve a more precise estimate of the molecular masses of these types of molecules.

In practice, the electrophoretic mobilities of standard proteins and the proteins of interest are be determined from a series of separations using several concentrations of sieving media. In our

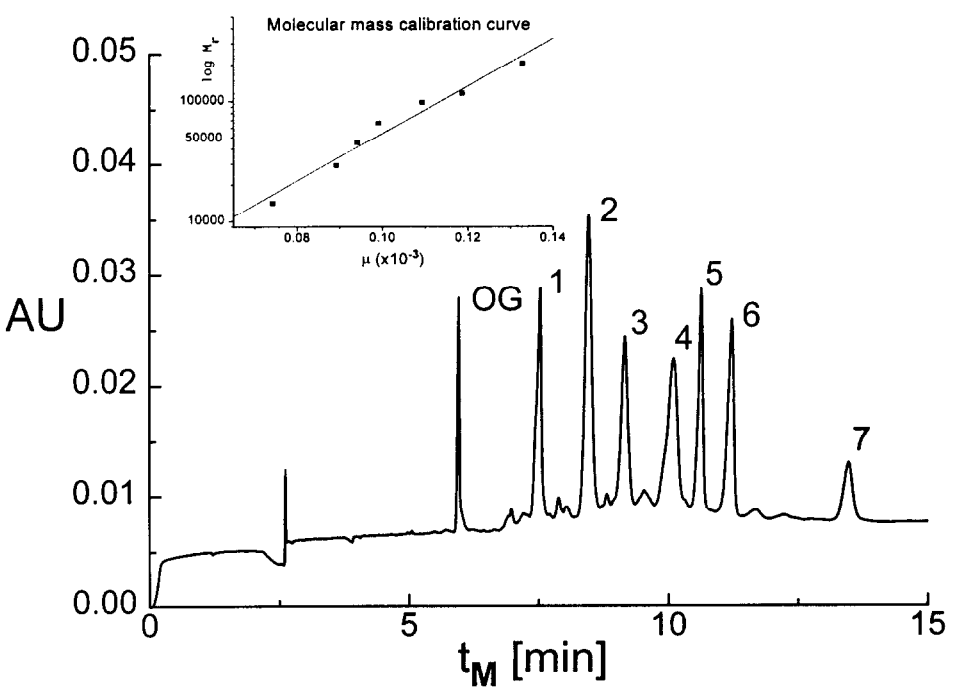

Fig. 1. SDS CGE pattern of the test mixture containing seven proteins on the eCAP SDS 14-200 gel. Peaks: 1 = α-lactalbumin (M_r 14 200); 2 = carbonic anhydrase (M_r 29 000); 3 = ovalbumin (M_r 45 000); 4 = bovine serum albumin (M_r 66 000); 5 = phosphorylase *b* (M_r 97 400); 6 = β-galactosidase (M_r 116 000); 7 = myosin (M_r 205 000). A tracking dye orange-G (OG) was added to the sample in the concentration of 0.005%. Conditions: injected amount: 0.1 μg protein; detection 214 nm; run temperature, 20°C; field strength, 300 V/cm; current, 25–30 μA. Inset: Calibration curve of mobility *vs.* logarithm molecular mass for protein molecular mass determination. t_M = Migration time.

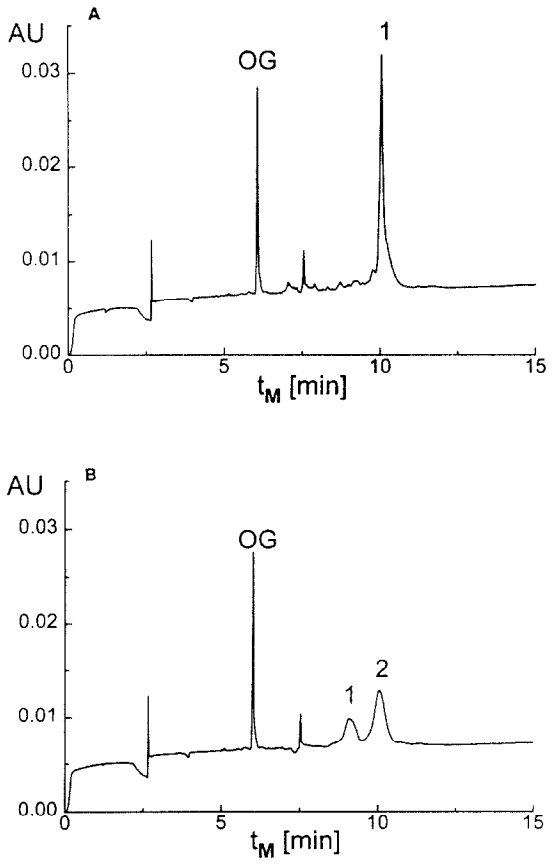

Fig. 2. SDS CGE of amylase (A) and the light and heavy chains of human IgG (B). Peaks: OG = orange-G tracking dye; (A) 1 = amylase; (B) 1 = IgG light chain; 2 = IgG heavy chain. Separation conditions as in Fig. 1.

Table 1
Comparison of estimated molecular mass data of amylase and light and heavy chain subunits of human IgG of Fig. 2, using regular standard curve method and the automated Ferguson method

Protein	Molecular mass		
	Lit. [19]	SDS 14-200	Ferguson
Amylase	56 500	68 200 (21%)	54 900 (3%)
IgG			
Light chain	23 000	4 280 (87%)	27 200 (17%)
Heavy chain	55 000	68 500 (25%)	49 300 (10%)

The values in parentheses after the SDS 14-200 and Ferguson methods show the difference between the measured and literature values.

case, CGE experiments were performed by the use of the original gel concentration in the eCAP SDS 14-200 kit and subsequent dilutions to 75, 67 and 50% of the original concentration. The logarithm of electrophoretic mobilities for each protein *versus* gel buffer concentration are then plotted to generate a series of curves forming the Ferguson plots [7,14]. The negative slopes associated with the proteins are converted to positive numbers which are equal to K_R (retardation coefficient) values for all proteins in the experiments. A plot of the logarithm of protein molecular mass *versus* $K_R^{1/2}$ can then be generated (Fig. 3) and used to determine the molecular masses of proteins, including those that do not bind with SDS in the regular 1 to 1.4 ratio. The exponent of K_R is 1/2 for spherical shaped molecules SDS-PAGE [13]. The same value was found for the polyethylene oxide-based eCAP SDS 14-200 gel buffer system using the suggested 300 V/cm field strength [17]. However, it is important to note that using extremely high field strengths, such as > 1000 V/cm, the exponent of K_R can be different than 1/2. This phenomenon is probably due to the use of different sieving material (linear polyethylene oxide *versus* cross-linked polyacrylamide) and the higher field strength accompanied with CGE. This hypothesis is supported by our earlier results showing that high field strengths employed in CGE might cause orientation effects such as stretching of the

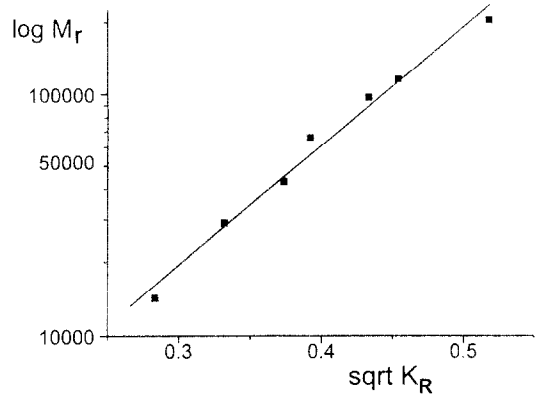

Fig. 3. Square root (sqrt) of retardation coefficient (K_R) *vs.* logarithm molecular mass plot of the standard proteins of Fig. 1.

random coil shaped SDS–protein complexes [20].

More detailed examination of these effects as well as other CGE parameters, *e.g.*, temperature, on the exponent of K_R is under further investigation.

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