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HIGH-PERFORMANCE SODIUM DODECYL SULFATE POLYACRYLAMIDE GEL CAPILLARY ELECTROPHORESIS OF PEPTIDES AND PROTEINS

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SUMMARY

High-performance capillary sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) has been developed for the separation and molecular weight (MW) determination of peptides and proteins. In this work, acrylamide was polymerized in fused-silica capillaries of 75 μm I.D. and 10 or 20 cm length. On-line UV detection was employed by placing the capillary in the optical path of the detector. Rapid separations of myoglobin fragments and of a mixture of standard proteins were demonstrated with efficiencies of the order of 40 000 plates for a column, 20 cm in length. Linear plots of log MW *vs.* mobility for the SDS protein complexes were observed. Variation in the percentage monomer composition (% T) of the polyacrylamide yielded the expected common intercept of log mobility *vs.* % T, confirming the size separation mechanism in the gel capillaries. The separation of the A and B chains of insulin with a 10-cm column in less than 10 min was also demonstrated.

INTRODUCTION

Electrophoresis is an efficient separation method that is widely used in biochemical research. One of its main analytical applications is the determination of the molecular weight (MW) of polypeptides and proteins by means of sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)[1]. In this procedure, the protein mixture is denatured, and disulfide bonds are cleaved by heat in the presence of excess SDS and a reducing agent (*e.g.* β-mercaptoethanol). Under these conditions, most polypeptides bind SDS in a constant weight ratio to yield molecules of constant charge density and thus similar free electrophoretic mobility. In addition, the shapes of the SDS–polypeptide complexes are similar. Separation is then based on size or MW differences via sieving through the polyacrylamide gel matrix. This procedure is a standard approach to MW determination in slab gel operation.

In the past several years, high performance capillary electrophoresis has become a very effective new technique for separations at the trace level. Previously, electrophoresis has been conducted with tube diameters of roughly 6 mm I.D.[2], and subsequently 1–3 mm I.D.[3]. Currently, capillary tubing of less than 400 μm I.D. and often below 100 μm I.D. is employed. The use of capillaries in isotachophoresis[4],

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electroosmosis[5–8], free zone electrophoresis[9], and isoelectric focusing[10] has been demonstrated. Relative to columns of wider diameter, capillary columns permit higher potential fields to be employed to yield high-speed and high-performance separations. A major factor controlling performance is the ability of the system to dissipate the heat generated by the high potentials and currents. Capillary columns produce less current than wider tubes for a given mobile phase and potential drop (and thus less heat). In addition, temperature control is more efficient with narrow tubes. In this regard, fused-silica capillaries are of particular interest, since the walls of the capillaries can be made very thin; *e.g.* with 75 μm I.D., a wall thickness of 30 μm is possible. Such thin walls permit efficient heat removal from the capillary.

The goal of this paper is to combine SDS-PAGE with high performance capillary electrophoresis in which a polyacrylamide gel is contained within the fused-silica tubing. The rapid separation of SDS–protein complexes at the nanogram level is demonstrated, and the validation of the method for measurement of MW is shown. The method does not require staining which can at times be problematical, especially with respect to loss of protein bands from the slab gel matrix[11]. Earlier, a capillary electropherogram of the separation of membrane proteins, solubilized with SDS, has been shown[12].

EXPERIMENTAL

Apparatus

SDS-PAGE of peptides and proteins was performed in 75-μm I.D. fused-silica capillary tubing (Scientific Glass Engineering, Rigwood, Australia) with migration distances of 10 or 20 cm, depending on the experiment. A high-voltage d.c. power supply Model LG-30R-5 (Glassman, Whitehouse Station, NJ, U.S.A.) was used to produce a potential along the capillary. On-line UV detection was employed (Soma S-3702 IR&D, Kingston, MA, U.S.A.), with the detector modifications described elsewhere[7]. The capillary was thermostated at 25°C, using a liquid cooling system (Lexacal, Model EX-100D) bath with a Model FTC-350A cooler (Neslab Instruments, Portsmouth, NH, U.S.A.). A Nelson Analytical Model 762 SB A/D interface (Cupertino, CA, U.S.A.), attached to an IBM PC/XT computer, was used to record the electropherograms and to process the data.

Materials

Peptides and proteins were purchased from Sigma (St. Louis, MO, U.S.A.). The other reagents were of ultrapure grade and obtained from Schwartz/Mann Biotech (Cleveland, OH, U.S.A.). All solutions were filtered through a Nylon-66 filter unit of 0.2 μm pore size. The water was deionized and triply distillled. The samples were kept frozen at −20°C or were freshly used.

Procedure

SDS–peptide and protein samples were prepared in a conventional manner[1]. Acrylamide polymerization was accomplished within the capillary, using the appropriate amount of monomer and bis-acrylamide cross-linking agent, with ammonium persulfate and N,N,N′,N′-tetramethylethylenediamine (TEMED) as the free-radical initiator and catalyst, respectively. The polymer was covalently attached to the walls

of the fused silica capillary using a bifunctional reagent[13]. After polymerization, both ends of the capillary tube were dipped into separate 5-ml reservoirs, filled with the desired buffer. The reservoir at the injection end contained a platinum electrode, which was attached to the negative side of the power supply. The reservoir at the detector end contained a second platinum electrode, attached to the positive side. Samples were electrophoretically introduced into the gel capillary by inserting the tubing into the sample reservoir and applying a constant current for a fixed period of time, *e.g.* 10 s at 6 μA. After the current was switched off, the gel capillary was replaced into the buffer reservoir for electrophoretic separation.

RESULTS AND DISCUSSION

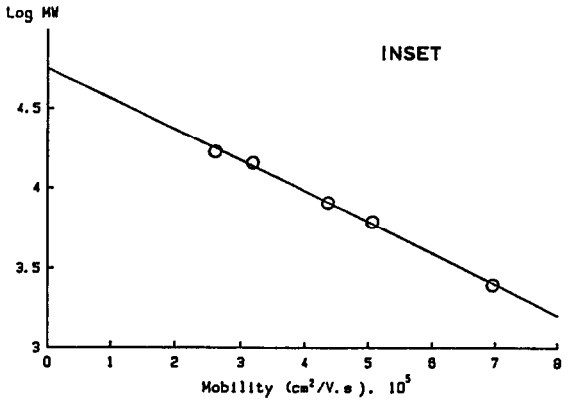
Fig. 1 shows the separation of fragments of myoglobin from MW 2500 to 17000. SDS-PAGE gave a linear relationship between the logarithm of MW and mobility (see inset of Fig. 1). The separation was achieved on a 20 cm fused-silica capillary (75 μm diameter), with a potential field of 400 V/cm, corresponding to a current of 36 μA. The buffer contained 8 *M* urea and the polymer characteristics[3] were T = 12.5%, C = 3.3%. Urea was selected, since it is well known that SDS-PAGE of proteins and peptides with relatively low MW can be improved by addition of this denaturant to the buffer[14]. As can be observed, relatively sharp peaks are obtained for each component; for example, peak 1 in Fig. 1 corresponds to approximately 40000 theoretical plates. With such sharp peaks in narrow capillaries, detection limits in the low nanogram range are readily obtainable by this procedure.

We next turned to the separation of larger MW proteins. Fig. 2 shows the SDS-PAGE of a mixture ranging from α-lactalbumin (MW 14200) to pepsin (MW 34700). The gel characteristics were T = 10% and C = 3.3%. Relatively sharp peaks were again obtained in less than 1 h. Separation times can be lowered by increasing the electric potential, provided the heat generated by the increased current can be dissipated. Another approach is to use shorter columns (see later).

In order to explore more completely the high performance capillary SDS-PAGE method of MW determination and to examine further the influence of gel porosity on separation, we next explored gels with a smaller proportion of monomer. Fig. 3A shows the separation of proteins by SDS-PAGE with T = 7.5%, C = 3.3%. Fig. 3B shows the same separation with T = 5%, C = 3.3%. As expected, under identical field strength, the proteins were eluted faster as the pore size increased, *i.e.* lower percentage T[1]. Interestingly, the peak widths also increased as the percentage T decreased just as in slab gels[15].

The retention followed the expected trends with increasing porosity. Fig. 4 shows calibration plots for the four proteins on the three gel columns. In all cases, good linear behavior is observed. It is to be noted that the plots are roughly parallel to one another, as would be expected on the basis of size separation[2].

Further validation of the size separation mechanism is provided by Fig. 5, which shows Ferguson plots[1] of the log mobility of an individual SDS protein *versus* the percent monomer composition, T. As expected, extrapolation of the plots for the four proteins to 0% T shows an approximately constant log mobility. Since each intercept represents the mobility of that SDS-protein complex in free solution, *i.e.* without any gel present, the intercepts of the four proteins must be MW independent,


Log MW
INSET
4.5
4
3.5
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2
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4
5
6
7
8
Mobility (cm²/V.s). 10⁵

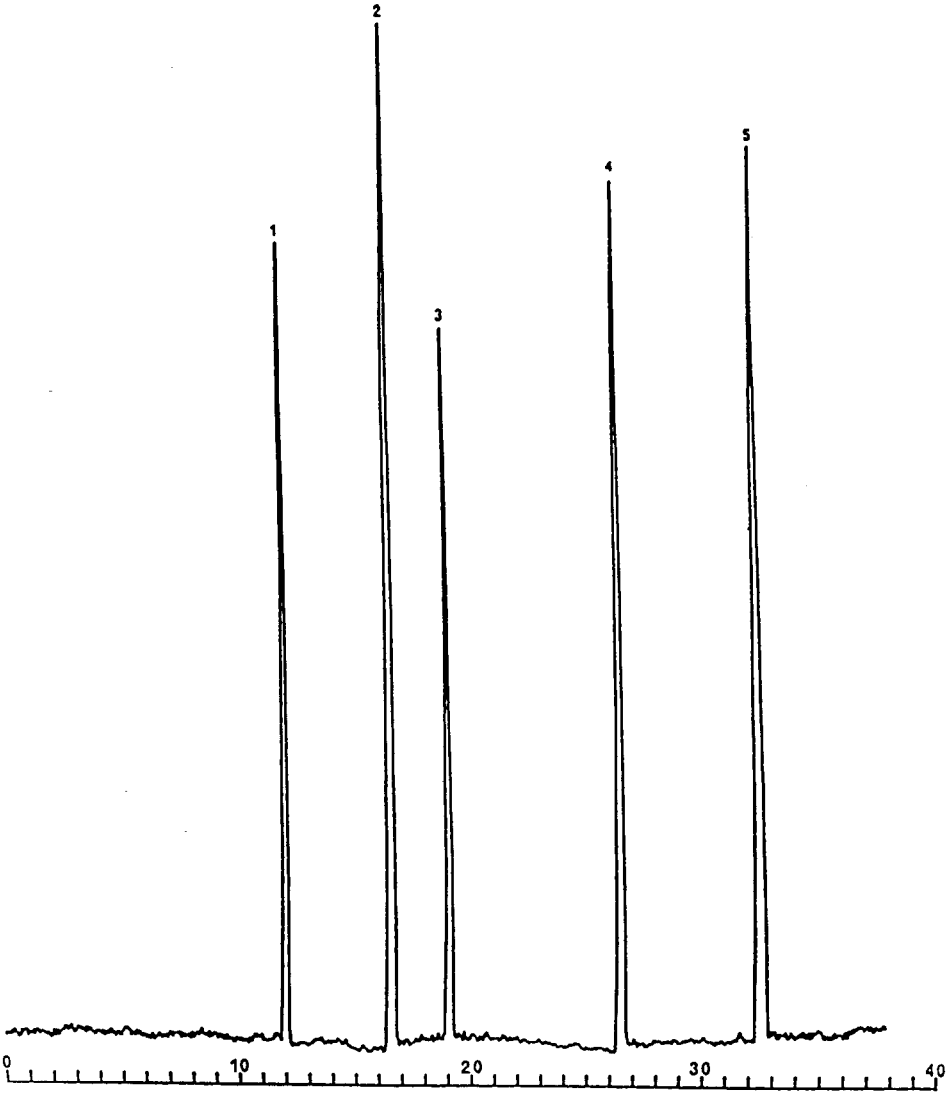
1
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Fig. 1.

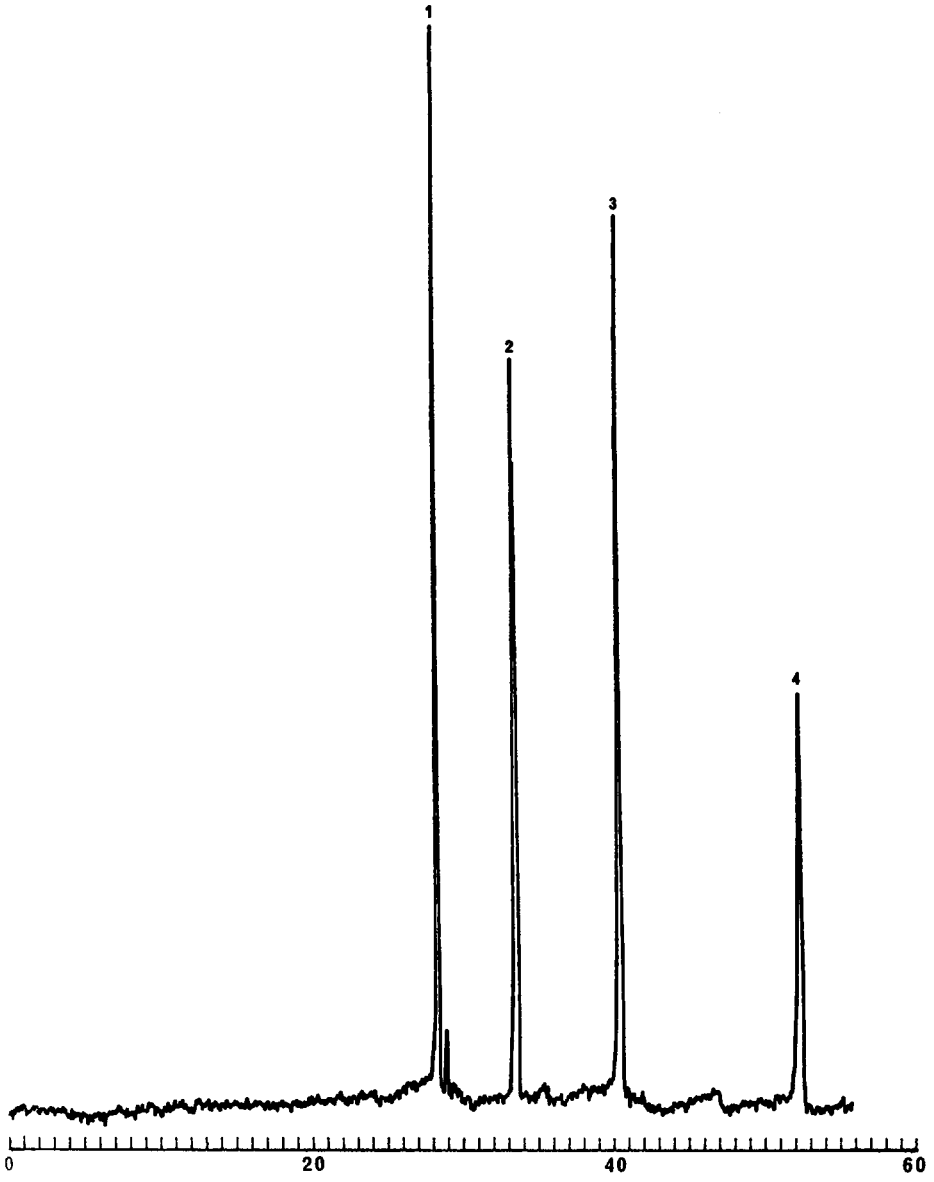

Fig. 2. High-performance capillary SDS-PAGE separation of proteins. Conditions: 400 V/cm, 24 μA, 27°C; T = 10%, C = 3.3%. Buffer, 90 m*M* Tris–NaH_2PO_4 (pH = 8.6), 8 *M* urea, 0.1% SDS. Samples: 1 = α-lactalbumin; 2 = β-lactoglobulin; 3 = trypsinogen; 4 = pepsin.

Fig. 1. High performance capillary SDS-PAGE separation of myoglobin and several of its fragments. Conditions: 400 V/cm, 34 μA, 25°C, migration distance 20 cm; fused-silica capillaries; 75 μm I.D., T = 12.5%, C = 3.3%. Buffer, 0.1 *M* Tris–H_3PO_4 (pH = 6.9), 0.1% SDS, 8 *M* urea. Samples: 1 = Fragment III, MW 2510; 2 = Fragment II, MW 6210; 3 = Fragment I, MW 8160; 4 = Fragment I & II, MW 14400; 5 = myoglobin, MW 17000. Inset: calibration plot of log MW *vs.* mobility for the above species.

if separation is solely based on size or MW. In addition, the slopes of the four lines in Fig. 5 were found to be directly proportional to MW, as would be expected for separation according to pure size[1]. Thus, in this MW range and with these polyacrylamide compositions in fused-silica capillaries, high-performance SDS-PAGE can be employed as a rapid method of MW determination.

As noted above, it is possible to speed up separation by decreasing the column length. Fig. 6 shows the separation of the A and B chains of insulin in a Tris–dihydrogenphosphate buffer containing 8 *M* urea. The electropherogram is again characterized by sharp peaks, and separation is completed in less than 10 min. It should be noted that while shortening the column from 20 to 10 cm, the field has been maintained constant at 400 V/cm.

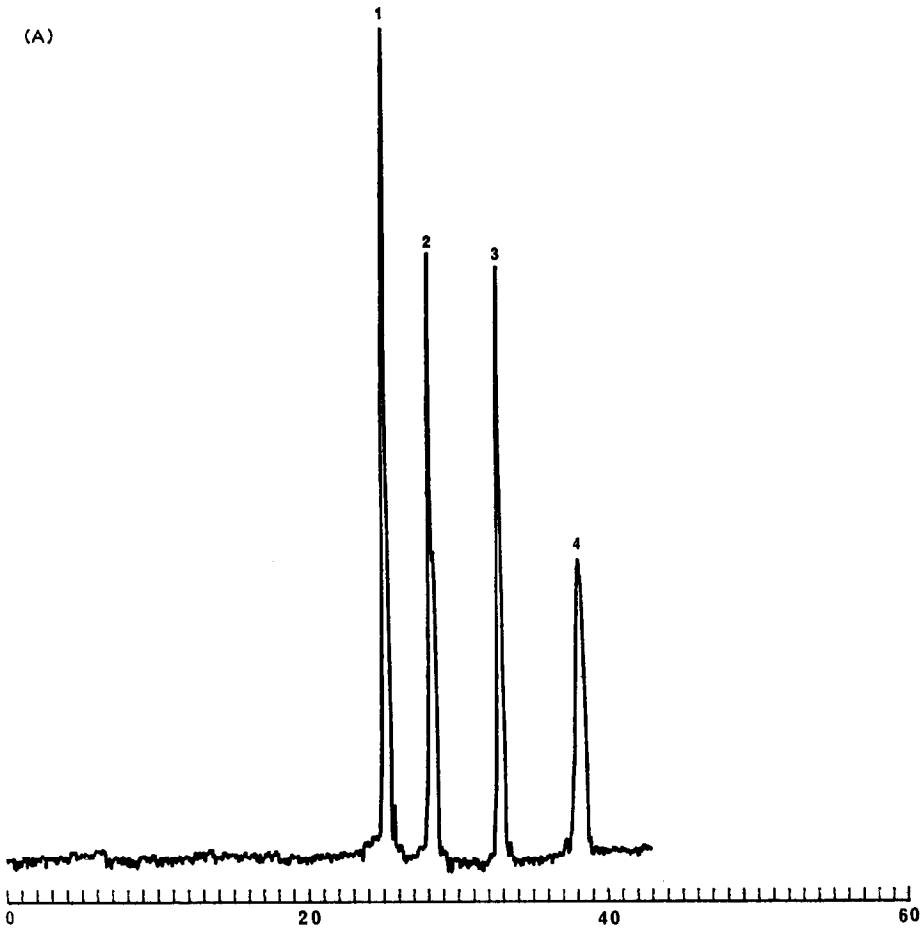

Fig. 3.

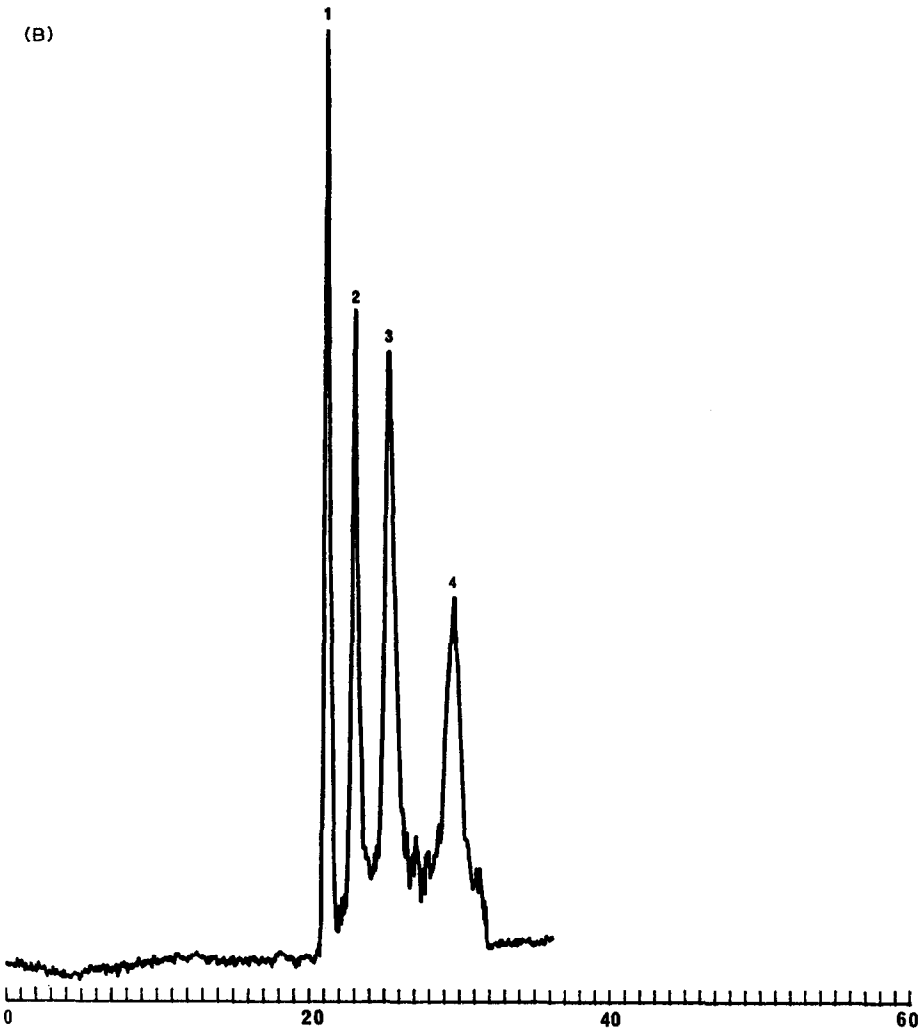

Fig. 3. High-performance capillary SDS-PAGE separation of proteins. Conditions: see Fig. 2, except (A) T = 7.5%, C = 3.3%; (B) T = 5%, C = 3.3%.

In conclusion, this paper demonstrates that high-performance SDS-PAGE can be conducted in narrow, fused-silica capillaries for a rapid MW determination of proteins and polypeptides. The new method can be operated at trace levels to analyze nanogram amounts of material in nanoliter volumes of sample. It should be noted that further decreases in detection limits can be achieved by using other spectroscopic methods, such as laser induced fluorescence[16]. The high-performance capillary electrophoretic method is analogous to high-performance liquid chromatography and could, in principle, be automated. Moreover, since detection is on-line, there is no

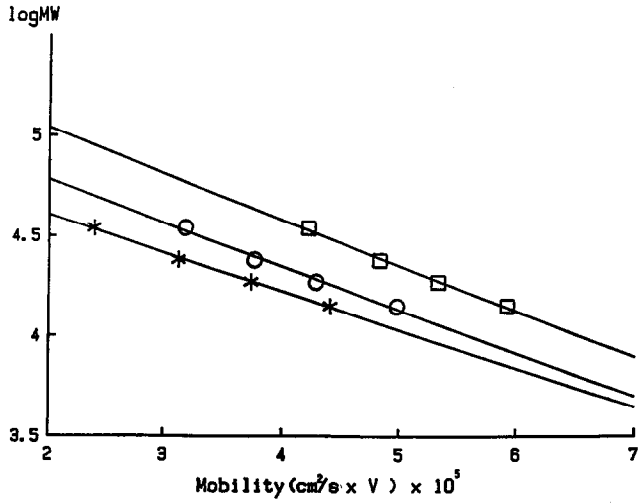

Fig. 4. Plot of log MW (proteins of Fig. 2) *vs.* mobility, as a function of polyacrylamide composition. (*) T = 10%, C = 3.3%; (○) T = 7.5%, C = 3.3%; (□) T = 5%, C = 3.3%.

need to spray or blot in order to determine solutes. Indeed, as demonstrated by others[17], quantitative analysis can be readily achieved with the capillary system. Work is continuing in this laboratory on the development of high performance capillary electrophoretic procedures.

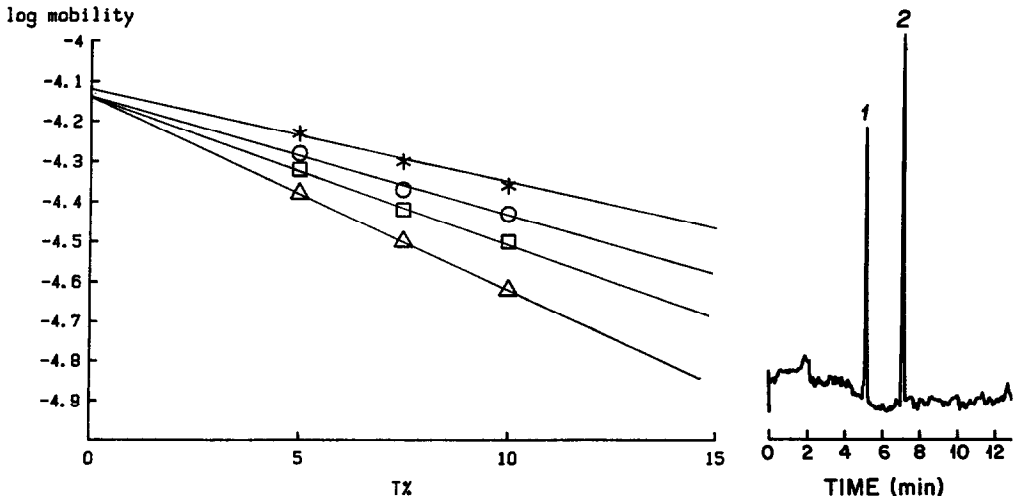

Fig. 5. Ferguson plot of log mobility *vs.* percent T for four proteins. (*) α-Lactalbumin; (○) β-lactoglobulin; (□) trypsinogen; (△) pepsin.

Fig. 6. High-performance capillary SDS-PAGE separation of the two chains of insulin. Conditions, see Fig. 2, except migration distance = 10 cm. 1 = A chain; 2 = B chain.

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