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Short Communication

Capillary zone electrophoresis with a linear, non-cross-linked polyacrylamide gel: separation of proteins according to molecular mass

ALEXANDRA WIDHALM and CHRISTINE SCHWER
Institute for Analytical Chemistry, University of Vienna, Währingerstrasse 38, A-1090 Vienna (Austria)
DIETER BLAAS
Institute for Biochemistry, Faculty of Medicine, University of Vienna, Währingerstrasse 17, A-1090 Vienna (Austria)
and
ERNST KENNDLER*
Institute for Analytical Chemistry, University of Vienna, Währingerstrasse 38, A-1090 Vienna (Austria)
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ABSTRACT

The separation of proteins according to their molecular masses using capillary zone electrophoresis was demonstrated in a buffer containing a linear, non-cross-linked polyacrylamide gel as a sieving medium. The four test proteins, covering a molecular mass range of between 17 800 and 77 000, were applied as sodium dodecyl sulphate (SDS) complexes. The separation was carried out at pH 5.5 (phosphate buffer, 0.5% SDS, 10% liquid, linear polyacrylamide gel). The retention times of the proteins correlated with the logarithm of their molecular masses.

INTRODUCTION

The use of polyacrylamide gels in capillary zone electrophoresis (CZE) was first described by Hjertén [1]. Karger and co-workers [2,3] introduced this method mainly for the separation of oligonucleotides. Only a few results have been reported for the separation of proteins [2,4], and in all these instances an immobilized, cross-linked gel was used.

The use of linear, non-cross-linked polyacrylamide gels in CZE has been reported for the separation of polynucleotides [5]. These analytes (and also the subunits of serum albumin) have also been separated using other linear polymers such as methylcellulose or polyethylene glycol as additives to the buffer solutions [6]. In contrast, for

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the separation of proteins, liquid, linear polyacrylamide has been reported so far only for classical zone electrophoresis, which was carried out with agar-agar [7,8], cellulose acetate [9], or glass beads as stabilizers [10].

In contrast to the cross-linked gel, linear polyacrylamide offers a number of advantages. These include the following: the preparation of the capillary system, containing the linear gel, is very simple, which is not the case for the cross-linked gel; the capillary can easily be emptied and refilled; the gel–buffer system can be replaced after each run, which leads to a great flexibility with respect to the application of different gels or buffers; the column is insensitive to sample components contaminating the separation system; and all types of capillaries from commercial equipment can be used.

In this paper, the applicability of linear, non-cross-linked polyacrylamide gels for the separation of proteins according to their molecular mass is demonstrated.

EXPERIMENTAL

Chemicals

The chemicals and reagents used were of the following qualities: sodium hydroxide and phosphoric acid, analytical-reagent grade (E. Merck, Darmstadt, Germany); sodium dodecyl sulphate (SDS), *N,N,N',N'*-tetramethylethylenediamine (TEMED) and ammonium persulphate, analytical-reagent grade (Serva, Heidelberg, Germany); acrylamide, research grade (doubly crystallyzed, Serva); egg albumin, research grade (five times recrystallyzed, Serva); myoglobin and conalbumin, research grade (lyophilyzed, Serva); and bovine serum albumin, analytical-reagent grade (Serva).

The water used was doubly distilled from a quartz apparatus before use.

Apparatus

The measurements were carried out with an instrument (P/ACE System 2000, Beckman, Palo Alto, CA, USA) equipped with a fused-silica capillary (100 μm I.D.; total length 57 cm; length to the detector 50 cm). The capillary was thermostated at 25°C.

A constant voltage of 20 kV, resulting in a current of about 120–160 μA for the SDS buffers, was applied. Injection into the capillary was carried out hydrodynamically for 4 s.

UV absorption was measured at 254 nm.

Procedures

Gel preparation [7]. For the preparation of 25 ml of a linear, non-cross-linked liquid polymer acrylamide gel of medium chain length, 1.88 g of acrylamide were dissolved in 15 ml of water; 19 mg of TEMED were added and the solution was degassed with a water-jet pump for 10 min. Polymerization was initiated with 23 mg of ammonium persulphate (dissolved in 10 ml of water). No cross-linker was applied. The resulting polyacrylamide gel solution was degassed for 15–20 min using a water-jet pump.

Buffer solution. The buffer was prepared by adjusting a solution of phosphoric acid (0.05 mol/l) to the appropriate pH with sodium hydroxide solution.

SDS buffer. To the phosphate buffer (0.05 mol/l), solid SDS was added to give a final concentration of 0.5% (w/w).

Running buffer. A 2-g mass of gel (prepared as just described) was mixed with the SDS buffer and diluted to 20 ml, giving a running buffer solution with 10% (w/v) gel. This buffer solution was used to fill the capillary and the electrolyte vessels.

Protein samples for SDS electrophoresis. Masses of 1–1.5 mg of the proteins were dissolved in 1 ml of SDS buffer and heated to about 100°C for 5 min in a water-bath.

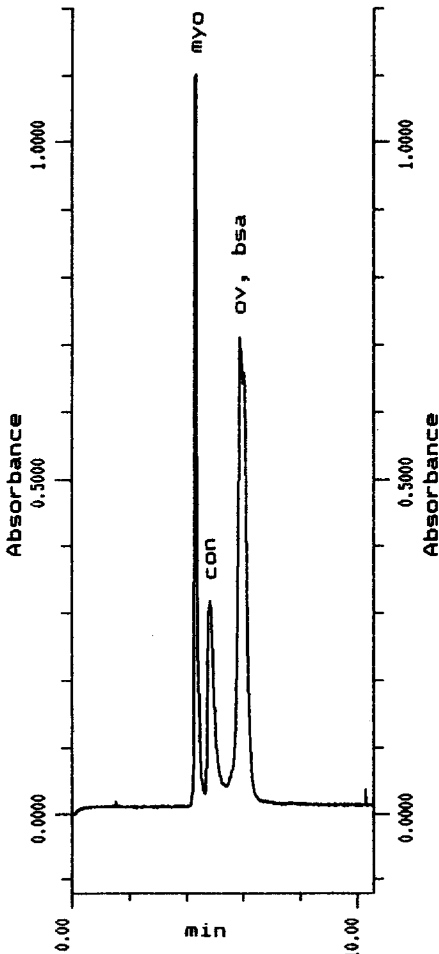

Fig. 1. Electropherogram of untreated proteins by free CZE (without polyacrylamide gel). The electropherogram was obtained at a pH of 7 (phosphate buffer, 0.05 mol/l). The proteins were moving towards the cathode. myo = Myoglobin, ov = ovalbumin, bsa = bovine serum albumin, con = conalbumin. The horizontal axis gives the migration time in minutes; UV absorbance at 254 nm.

TABLE I

REFERENCE PROTEINS USED FOR CZE

Protein	Molecular mass
Myoglobin	17 800
Ovalbumin	45 000
Bovine serum albumin	62 000
Conalbumin	77 000

RESULTS AND DISCUSSION

The zone electropherogram of a mixture of myoglobin, ovalbumin, bovine serum albumin and conalbumin, obtained at pH 7 without SDS and polyacrylamide gel is shown in Fig. 1. The molecular masses of the reference proteins are given in Table I. Owing to the electro-osmotic flow of the system, the proteins move towards the cathode. Under these conditions, the migration is a function of the effective mobilities of the proteins, but is not correlated with their molecular masses. It can be seen that myoglobin exhibits the lowest mobility (it has an isoelectric point, p*I*, of 6.8), having about the same velocity as a neutral compound (not shown in the electropherogram), followed by conalbumin. Ovalbumin and bovine serum albumin (with a p*I* of 4.8 and 5.0, respectively), migrating as anions with the highest mobility, are not separated under the given conditions.

After conversion of the proteins into their (anionic) SDS complexes, the elution order of myoglobin and the other proteins is reversed, as shown in Fig. 2. This electropherogram was obtained with a gel-free buffer, containing 0.5% SDS. It can be seen that the binding of SDS to the protein minimizes the difference in the mobilities, at least for ovalbumin, conalbumin and bovine serum albumin, which are eluted in a single, non-resolved peak. In contrast to Fig. 1, myoglobin is eluted (as a result of electroosmosis) with the largest migration time, because it has the highest mobility. However, as can be seen from Figs. 1 and 2, in both cases the migration sequence is not related to the molecular masses of the different proteins.

When liquid polymer acrylamide was added to the running buffer, the electropherogram shown in Fig. 3 was obtained. A reduction of the pH to 5.5 was necessary, because in the presence of the polyacrylamide gel the electroosmotic flow nearly opposes the electrophoretic velocity of the proteins, resulting in extremely long and irreproducible retention times. At pH 5.5 the electroosmotic flow is reduced so far that the anionic protein complexes can be detected within a reasonable time at the side of the anode. The migration sequence of the SDS–protein complexes is independent of the pH in this range.

Fig. 3 shows that all four proteins are well separated and that a correlation between the molecular mass and the migration time can be seen. The SDS complex of the smallest protein, myoglobin, has the shortest retention time, followed by ovalbumin, bovine serum albumin and conalbumin.

The broad peaks in Fig. 3 result partially from inhomogeneities in the proteins, especially the albumins, a fact which is well known from SDS-polyacrylamide gel

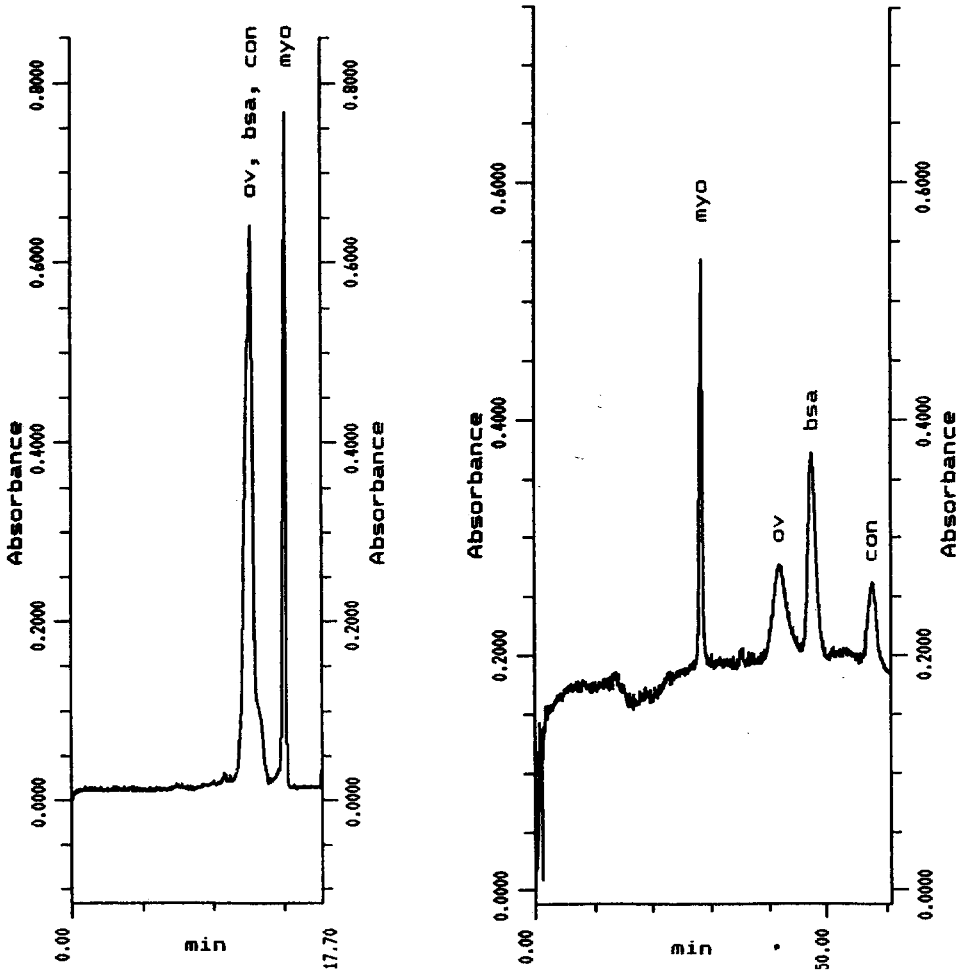

Fig. 2. Electropherogram of the protein–SDS complexes by free zone electrophoresis (without polyacrylamide gel). Buffer: phosphate, pH 7, 0.05 mol/l; 0.5% SDS. Proteins recorded at the side of the cathode. Symbols as in Fig. 1.

Fig. 3. Electropherogram of protein–SDS complexes obtained in a capillary filled with linear polyacrylamide. The capillary was filled with liquid, non-cross-linked polyacrylamide gel (10%) in a pH 5.5 running buffer (phosphate, 0.05 mol/l; 0.5% SDS). The proteins are separated according to their molecular mass. Symbols as in Fig. 1.

electrophoresis in slab gels. Furthermore, an excessive Joule's heat is produced by the high electric current, owing to the relatively high concentration of the SDS buffer. These effects explain to some extent the poor efficiency of the separation.

The relationship between the retention time and the molecular mass of the proteins is shown in Fig. 4. The shape of this curve indicates that the polyacrylamide acts as a sieving medium for the protein complexes, leading to a migration order

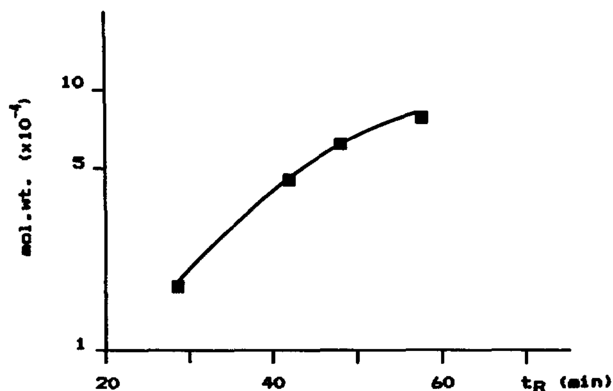

Fig. 4. Semi-logarithmic plot of the dependence of the molecular mass (mol/wt.) of the reference proteins on the retention times (t_R) in CZE with linear polyacrylamide. Data from Fig. 3.

according to their size. This is similar to the results obtained with the cross-linked gels commonly used in SDS-polyacrylamide gel electrophoresis.

These promising preliminary results on the use of linear, non-cross-linked polyacrylamide gels for the separation of proteins according to their molecular mass need further optimization. Studies in which parameters such as the pH of the buffer electrolyte and the percentage and chain length of the polyacrylamide gel are varied are being carried out.

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