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Short Communication

Rapid capillary gel electrophoresis of proteins

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ABSTRACT

The rapid separation of sodium dodecyl sulphate–protein complexes according to their molecular masses (M_r) by capillary gel electrophoresis is described. Using commercial equipment, standard proteins with M_r in the range 29 000–97 400 were resolved to the baseline in less than 2 min by utilizing a separation distance of 7 cm. A linear relationship between migration time and log M_r was found and rapid determination of the molecular mass of light and heavy chains of human immunoglobulin G is reported. The results are compared with applications using longer separation distances, showing that rapid and efficient analysis and adequate resolution can be obtained by using short separation distances.

INTRODUCTION

Capillary gel electrophoresis of proteins has made tremendous advance in recent years. The first separations were performed in cross-linked polyacrylamide matrices which were covalently attached to the capillary inner wall [1]. Excellent resolution was achieved owing to the anticonvective effect of the gel and the method was applied to protein standards and to complex natural mixtures of proteins [2,3]. However, the production of these gel-filled capillaries turned out to be time consuming and the lifetime was limited to a few experiments owing to bubble formation and deterioration of the gel structure. While improvements in production and in increasing the lifetime of gel-filled columns [4–7] were made, the application of columns, filled with linear, non-cross-linked polyacrylamide, was examined. These columns were easier to produce and they had a longer lifetime. Successful approaches were made with both gels formed in the capillary [4,8] and gels that were introduced into the capillary after polymerization [9,10]. The advantage of introducing the gels after formation is that the capillary can easily be emptied and refilled, leading to flexible, easy-to-use systems. However, the UV absorption of polyacrylamide at 200 and 214 nm is significant, causing relatively high detection limits [10]. Thus, UV-transparent liquid polymers such as

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dextran, poly(ethylene glycol) (PEG) or poly(ethylene oxide) (PEO) were used for the separations of proteins based on molecular mass (M_r). Further, they have the advantage of low viscosity, which made introduction and removal of these gels easier to perform [9,11].

However, one of the main advantages of capillary electrophoresis, the speed of analysis, has not yet been fully employed in the capillary gel electrophoresis of proteins, although approaches have been made with oligonucleotides [12]. This paper describes high-speed separations of proteins according to their molecular mass.

EXPERIMENTAL

Chemicals

With two exceptions all reagents were bought from Sigma (St. Louis, MO, USA) and were of the following qualities.

Proteins. Carbonic anhydrase (M_r 29 000), ovalbumin (M_r 45 000), bovine serum albumin (M_r 69 000) and phosphorylase *b* (rabbit muscle; subunit M_r 97 400) were of electrophoresis reagent grade and human immunoglobulin G (IgG), purified, was of reagent grade.

Buffers. Tris(hydroxymethyl)aminomethane (Tris), crystalline 99%, and 2-(N-cyclohexylamino)ethanesulphonic acid (CHES) were of analytical-reagent grade, 2-mercaptoethanol, 98%, of electrophoresis reagent grade and sodium dodecyl sulphate (SDS), crystalline (Serva, Heidelberg, Germany) of research grade.

Coatings. γ-(Glycidoxypropyl)trimethoxysilane was of analytical-reagent grade and N,N,N,-N-tetraethylmethylethylendiamine (TEMED), 99%, acrylamide, 99%, and ammonium peroxodisulphate (APS) (Biometra, Göttingen, Germany) were of electrophoresis reagent grade.

Gel. Dextran (M_r 2 000 000) was of analytical-reagent grade.

Instrumentation

In all experiments a P/ACE 2000 system (Beckman, Palo Alto, CA, USA) was used with P/ACE system software controlled by an IBM PS/2 computer. Fused-silica capillaries were obtained from CS-Chromatographie Service (Langerwehe, Germany) with 27 cm total length and 50 or 100 μm I.D. The distance from the detector was either 7 or 20 cm. The capillary was thermostated at 25°C and the samples were injected on the cathodic side by electromigration for 10 s at 10 kV. Detection was performed by measuring UV absorption at 200 nm.

Procedures

Columns. The capillaries were coated as described by Hjerten [13] using γ-(glycidoxypropyl)trimethoxysilane and 4% linear polyacrylamide. Prior to this coating procedure a detection window 2 mm long was made by removing the outer polyimide coating at a distance of 7 cm from one end of the capillary.

Buffer. Tris (0.1 *M*) containing 0.1% SDS was titrated to pH 8.6 with 0.1 *M* CHES which also contained 0.1% SDS. All buffer solutions were filtered through a 0.45-μm filter (Sartorius, Göttingen, Germany).

Gel. A 1-g amount of dextran was mixed with 10 ml of the buffer solution described above and stirred for about 10 min. The clear liquid polymer solution was degassed prior to use. The gel was then filled in one of the buffer vials and introduced into the capillary by pressure.

Samples. The proteins were dissolved in 0.05 *M* Tris–CHES buffer (pH 8.6) containing 1% SDS and 5% 2-mercaptoethanol and then heated for 15 min at 95°C.

RESULTS AND DISCUSSION

Fig. 1 shows the separation of the SDS–protein complexes of carbonic anhydrase (M_r 29 000), ovalbumin (M_r 45 000), bovine serum albumin (M_r 69 000) and phosphorylase *b* (M_r 97 400) in the 20-cm long part of the capillary. Baseline resolution of the four standard proteins was achieved and the migration time obviously depends on the molecular mass. For phosphorylase *b* two peaks can clearly be distinguished, indicating a degradation product of the protein with $M_r = 87\,000$. Using a field of 370 V/cm in the capillary of 100 μm I.D. the analysis was completed within 12 min.

In order to reduce the migration time of the

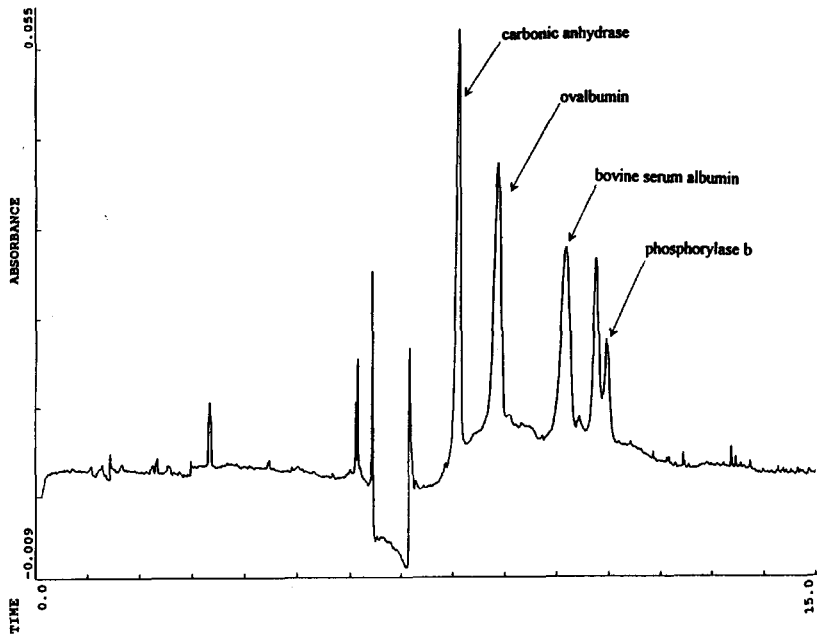

Fig. 1. Electropherogram of the SDS complexes of four standard proteins using the 20-cm long part of a 27 cm × 100 μm I.D. capillary. The proteins are separated according to their molecular masses. Other conditions: buffer, 0.1 *M* Tris–CHES containing 0.1% SDS and 10% (w/v) dextran (pH 8.6); applied field, 370 V/cm; UV detection at 200 nm, 0.025 a.u.f.s.; injection, electromigration, 10 s, 10 kV. Time in min.

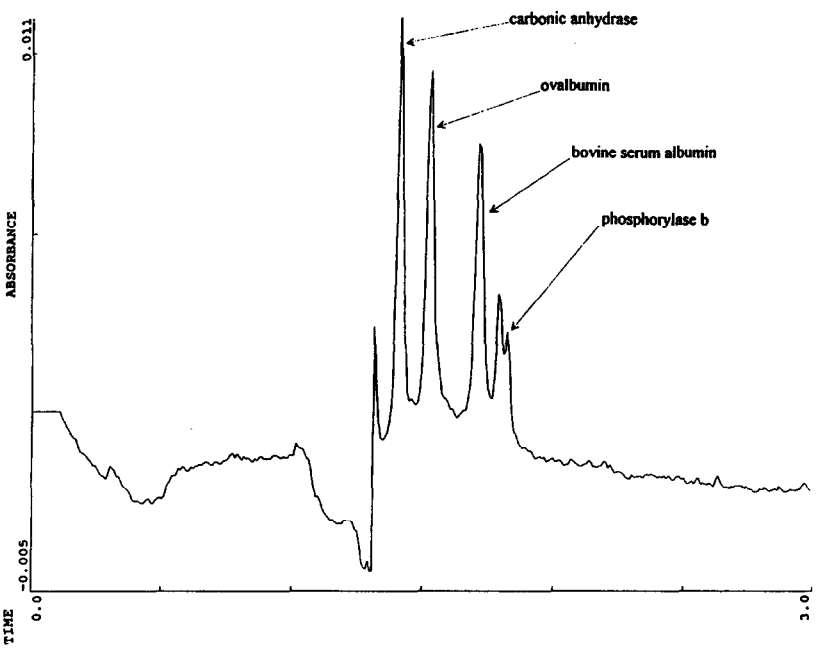

Fig. 2. Rapid separation of SDS complexes of four standard proteins according to their molecular masses using the 7-cm long part of a 27 cm × 50 μm I.D. capillary. Other conditions as in Fig. 1 except applied field, 740 V/cm. Time in min.

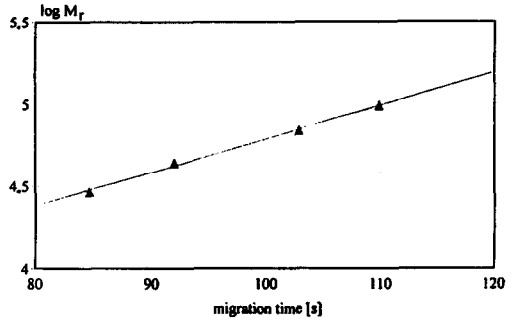

Fig. 3. Linear dependence of the migration time of the SDS–protein complexes on log M_r with $r = 0.998$. Data from Fig. 2.

proteins, higher field strengths of 740 V/cm were applied to the capillary. Baseline separation was achieved within 6 min, but the resolution was 7% lower than that obtained with the lower field. For a further decrease in migration time, the short, 7-cm long end of the capillary was used for the separation. Further, a field of 740 V/cm was applied to the column, which of course led to enhanced generation of Joule heat in the capillaries. For better heat dissipation capillaries of 50 μm I.D. were used, which have the additional advantage that the electrical resistance increases with decreasing inner diameter and thus less heat is generated in the electrophoresis process. However, the decrease in optical path length leads to poorer detection limits.

In Fig. 2 the rapid separation of the four standard SDS–protein complexes is demonstrated. All four proteins are resolved to the baseline in less than 2 min, although the resolution decreases under these conditions to 72% of that achieved with a 20-cm separation distance and 370 V/cm field strength. This decrease in resolution might be a drawback when dealing with very complex mixtures, which might contain several hundred proteins. In such cases longer separation distances will certainly be advantageous. However, the impurity in the phosphorylase *b* can still be distinguished from the protein, which means that proteins with differences of 11% in molecular mass can be resolved. Moreover, the correlation between migration time and

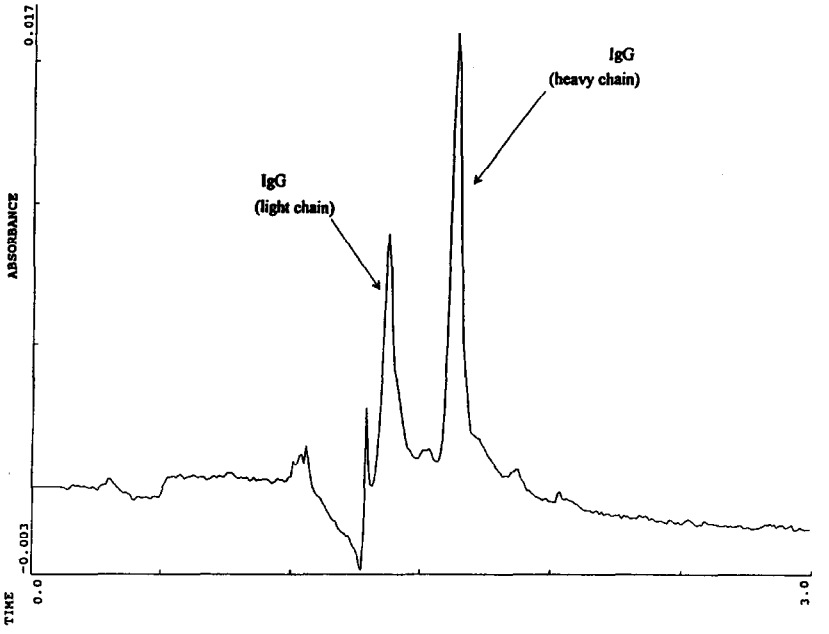

Fig. 4. Rapid separation of the SDS complexes of light and heavy chains of human IgG in the short, 7-cm end of the 27 cm × 50 μm I.D. capillary. Other conditions as in Fig. 2. Time in min.

log M_r is excellent ($r = 0.998$), as can be seen from Fig. 3. Hence this method can be used for high-speed molecular mass determinations of proteins.

In Fig. 4, the analysis of a commercial human IgG using rapid capillary gel electrophoresis is shown. Owing to the use of the reducing agent, the immunoglobulin is fractionated into its light and heavy chains, which are clearly separated according to their molecular masses. From the linear calibration graph of migration time *versus* log M_r, molecular masses of 25 600 for the light chain and 55 100 for the heavy chain of IgG were calculated. As a control experiment, the same determination was carried out, using the long end of the capillary. The differences in the molecular masses were less than 1%. In repetitive analysis the migration times proved to be highly reproducible, as can be seen in Table I. For twenty consecutive runs the SDS complexes of the standard proteins and the IgG were analysed by capillary gel electrophoresis. Between the runs the column was emptied and refilled with the dextran gel. The reproducibility of the migration times was excellent, with a relative standard deviation of 0.77%. Hence this method can be routinely used for rapid determinations of molecular masses of proteins.

CONCLUSIONS

Rapid separations of proteins according to their molecular mass can be performed by using a short separation distance of 7 cm and high electric field strengths. Baseline resolution of protein standards can be achieved in less than 2 min with a relative standard deviation of 0.77% in repetitive analyses. The capillary gel system

TABLE I

MIGRATION TIMES OF THE SDS COMPLEXES OF FOUR STANDARD PROTEINS MEASURED IN ALTERNATING ORDER WITH HUMAN IgG FOR 20 SUBSEQUENT RUNS

Run no.	Migration time (s)					
	Carbonic anhydrase	Ovalbumin	Bovine serum albumin	Phosphorylase *b*	IgG (light chain)	IgG (heavy chain)
1	85.7	94.5	104.8	111.4		
2					84.2	99.6
3	86.5	94.3	105.2	111.8		
4					83.9	100.9
5	85.3	92.3	103.4	109.7		
6					83.0	99.4
7	85.1	92.9	104.4	110.6		
8					84.6	99.7
9	86.5	93.6	104.4	111.4		
10					83.8	100.1
11	86.1	92.3	103.3	109.5		
12					82.8	98.6
13	85.7	92.9	103.4	109.5		
14					83.2	98.6
15	86.4	92.9	104.0	110.8		
16					84.0	98.6
17	85.7	92.9	104.0	110.7		
18					85.7	100.1
19	85.7	92.0	102.9	109.5		
20					83.8	98.6
R.S.D. (%)	0.60	0.85	0.67	0.76	0.95	0.77

using the UV-transparent dextran gel as a matrix is easy to handle and offers the possibility of high-speed molecular mass determinations.

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