"MOLECULAR-SIEVE" ELECTROPHORESIS IN CROSS-LINKED POLYACRYLAMIDE GELS

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(Received September 14th, 1962)

INTRODUCTION

Electrophoresis of proteins in starch gels according to the method developed by SMITHIES[1] shows in general a much higher resolution than electrophoresis in media like agar gel and paper. Probably, this is in part due to the fact that the dimensions of proteins are of the same order of magnitude as the pores in the starch gel. The proteins will therefore be more or less restricted in their migration through such a gel. The possibility of utilizing this molecular sieving action of the stabilizing medium was first pointed out by SYNGE AND TISELIUS[2], when separating uncharged molecules by forcing them through an agar gel with the aid of electroendosmosis.

As a complement to starch RAYMOND AND WEINTRAUB[3] and DAVIS AND ORNSTEIN[4] introduced a synthetic polymer, cross-linked polyacrylamide, as a stabilizing agent. Unaware of these papers and before they were published the author noticed the high resolving power of gels of polyacrylamide when he was searching for a chemically better defined gel than starch and one with a pore size that could be easily adapted to the sizes of the substances to be separated*.

It is now three years since the first papers[3,4] on electrophoresis in polyacrylamide gels appeared. It is therefore surprising that the potentialities of molecular sieving observed by the author, which the flexibility of the polyacrylamide gel offers, have not been more extensively pointed out and utilized. We, therefore, now wish to publish some earlier performed experiments which clearly indicate these potentialities. Besides showing that the migration velocities decrease with increasing concentration of the gel, these experiments indicate that the relative decrease in velocity is not the same for all proteins, but, as expected, is most pronounced for those of higher molecular weight. Similar experiments have recently been published by RAYMOND AND WANG[5], but as they used hemoglobins of the same molecular weight, no effect of the gel concentration was observed.

EXPERIMENTAL

Materials

R-Phycoerythrin and R-phycocyanin were extracted as described by KYLIN[6], only small modifications of the method being introduced[7]. The separation of phycoerythrin and phycocyanin was performed by electrophoresis in agarose suspensions[8,9].

* Dr. R. MOSBACH of this Institute proposed the use of polyacrylamide when consulted on gels suitable for "molecular-sieve" electrophoresis.

Human carboxyhemoglobin was prepared according to the method of ADAIR AND ADAIR[10].

Bovine serum albumin was an Armour product, containing both monomer and dimer molecules.

Preparation of the gel

Polymerization was performed in the buffer to be used for the electrophoresis, exactly as described in ref. 11. The quantities total concentration T and cross-linking concentration C, which are used in the following, are also defined in ref. 11.

Investigation of the relationship between gel concentration, migration velocity and molecular weight

The column tubes were ordinary glass tubes with an inner diameter of 0.6 cm and a length of 25 cm. They were closed with the aid of thin dialysis membranes moistened with buffer and fixed to the glass tube by means of rubber bands. The deaerated solution of acrylamide and N,N'-methylene-bisacrylamide containing the catalyst system was poured into the vertical column tube. Three ml of petroleum ether was layered above the monomer solution. After some minutes the polymerization was completed; the petroleum ether was then removed and replaced by buffer. The petroleum ether prevents air — which inhibits the polymerization—from entering the monomer solution. Furthermore, a sharp boundary is obtained between the gel and the buffer above, which is of importance in obtaining a narrow starting zone. The sample was applied by layering under buffer[11]. The lower end of the column tube was dipped into the anode vessel, and the upper end was connected to the cathode vessel by means of a piece of polyvinyl chloride tubing filled with buffer. To avoid deformation of the applied sample zone by heat convection, the current was kept at a comparatively low value (about 2 mA) during the migration of the sample into the gel. The current was then increased to about 5 mA.

Each sample applied contained two colored proteins, A and B. B consisted of R-phycoerythrin in all experiments. The distances these two proteins had migrated

TABLE I

THE INFLUENCE OF THE TOTAL CONCENTRATION T OF THE GEL UPON THE RELATIVE MIGRATION VELOCITIES OF PROTEINS OF DIFFERENT MOLECULAR WEIGHTS

The migration velocities are measured against R-phycoerythrin (mol. wt. 290,000).

Buffer	*Protein*	*Molecular weight*	*Total conc. of the gel*	*Relative migration velocity* (v_A/v_B)
0.05 *M* sodium acetate buffer, pH 5.4	R-Phycocyanin	270,000	2.5	0.48
			6.0	0.50
			10	0.48
0.02 *M* sodium phosphate buffer, pH 7.3	R-Phycocyanin	135,000	2.5	0.53
			6.0	0.67
			10.0	1.1
Sodium veronal buffer, pH 8.6, $\Gamma/2 = 0.05$	Human carboxy-hemoglobin	68,000	2.5	0.48
			6.0	0.77
			10.0	1.4

were measured at different times and the ratio between them calculated. This ratio represents the migration velocity of the protein A, relative to that of R-phycoerythrin (v_A/v_B). These measurements were carried out at three different total concentrations, T, of the gel. The cross-linking concentration was the same in all experiments and equal to 5 %. The proteins used, their molecular weights, the buffer and the total concentration of the gel are listed in Table I, in which the calculated relative migration velocities are also given. In Fig. 1 these velocities are plotted against the total concentration T of the gel. No correction was made for electroendosmosis.

It should be pointed out that during electrophoresis in the 10 % gels, phycoerythrin was split into two zones. Judging from the color intensity, the concentration of the fastest moving component was 10–15 % of that of the slower component. The migration velocities given in Table I are related to the latter component. Examination of the phycoerythrin solution in the ultracentrifuge revealed the presence of two components, the smallest of them being present in a concentration approximately equal to that of the fastest electrophoresis component. The heterogeneity of R-phycoerythrin has earlier been observed during chromatography on calcium phosphate columns[12,7].

Separation of monomer and dimer of bovine serum albumin

The column was made of plexiglass and was of a type very similar to that used by PORATH, LINDNER AND JERSTEDT[9,13] and VARGAS, TAYLOR AND RANDLE[14] for migration-elution electrophoresis. The electrophoresis tube had an inner diameter of 1 cm and the water-cooled part a length of 30 cm. A plexiglass tube with an inner diameter of 1 mm was inserted into the electrophoresis column, 1.6 cm from the lower end of the column and glued into place. To this tube a piece of polyethylene tubing was attached, the outlet of which was placed above a fraction collector. The lower end of the electrophoresis tube was supplied with a disc of vyon*. The column was packed with Pevikon[15], a copolymer of vinyl chloride and vinyl acetate, to a height of 2 cm. This packing was performed in buffer to which some sucrose had been added in order to increase the density of the buffer. The electrophoresis tube was then closed with a dialysis membrane fastened by means of rubber bands. The solution of acrylamide and N,N'-methylene-bisacrylamide together with the catalyst system was carefully layered above the Pevikon column. Application of the petroleum ether** and layering of the sample was performed as in the preceding experiment. Owing to the higher density of the buffer in the Pevikon column, due to the presence of sucrose, a well-defined boundary was formed between this column and the gel; furthermore, the risk that polymerization would take place inside the Pevikon column was eliminated. The polyacrylamide gel had the composition $T = 6$ %, $C = 5$ %; the polymerization was performed in sodium borate buffer, pH 8.9, $I/2 = 0.03$. The length of the gel column was 22 cm. About 0.25 ml of a 2 % protein solution, containing monomers and dimers of bovine serum albumin, was layered under the buffer. The dialysis membrane was removed before the voltage was applied. After the sample had entered the gel, the current was increased to 22 mA (from 8 mA). The elution rate was adjusted to 1 ml/h.

* A porous plastic material[9], available from Porous Plastics Limited, Dagenham Dock, Essex, England.

** In many cases the petroleum ether can with advantage be replaced by deacerated distilled water[16].

After about 50 hours the experiment was stopped. The protein contents of the collected 0.5 ml fractions were determined by absorption measurements in a 1-cm cell. Owing to the elution procedure, the protein concentrations in the collected fractions are very low, and we chose, therefore, to carry out these measurements at 230 mμ, where the absorption coefficient for proteins is much greater than at 280 mμ. The electropherogram is given in Fig. 2. The ultraviolet-absorbing fractions in front of the albumin peaks are due to impurities and non-polymerized material etc. Analysis by ultracentrifugation showed that peak I contained the monomers and peak II the dimers.

DISCUSSION

Curve I in Fig. 1 shows that at pH 5.4, where R-phycoerythrin and R-phycocyanin have about the same molecular weight (290,000 and 270,000 respectively), changes in gel concentration, *i.e.* the pore size of the gel, have no observable influence upon the

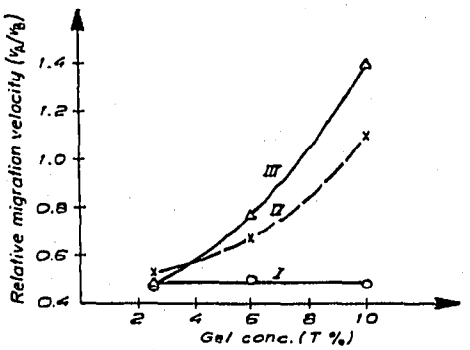

Fig. 1. The influence of the total concentration T of the gel upon the relative migration velocities of proteins of different molecular size. Curve I represents whole molecules of R-phycocyanin (mol. wt. 270,000), curve II dissociated molecules of R-phycocyanin (mol. wt. 135,000), and curve III human carboxyhemoglobin (mol. wt. 68,000). The migration velocities are measured against R-phycoerythrin (mol. wt. 290,000).

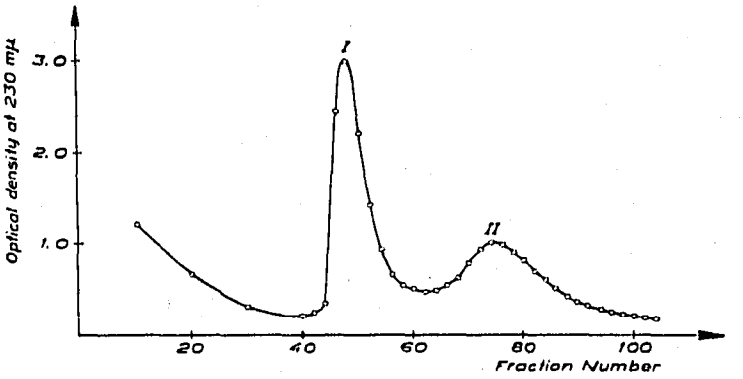

Fig. 2. Separation of monomers (I) and dimers(II) of bovine serum albumin on a gel of cross-linked polyacrylamide. The zones were continuously eluted during the electrophoresis.

relative migration velocities of these proteins. However, at pH 7.4, R-phycocyanin is dissociated into half molecules and the slope of curve II (Fig. 1) indicates that these have a lower value for the "friction coefficient" than the undissociated R-phycoerythrin molecules and even migrate faster than the latter at high gel concentrations ($v_A/v_B > 1$). For carboxyhemoglobin, which has a lower molecular weight (68,000) than dissociated R-phycocyanin, this effect is still more pronounced as curve III is steeper than curve II.

From the above one can expect a high resolution if the starting material is first purified by electrophoresis in a bed completely devoid of "molecular sieving" properties, such as cellulose powder or agarose suspensions. The fractions containing the material to be isolated, are then rerun on a polyacrylamide gel of suitable composition;

if these fractions consist of substances of different molecular size, they can in many cases be resolved. With "molecular-sieve" electrophoresis performed in this way, one can thus achieve separations similar to those obtainable with "molecular-sieve" chromatography[11]. The experiment corresponding to Fig. 2 may serve as an example of this.

Too high a gel concentration should be avoided, since the migration velocities of all proteins decrease as the concentration of the gel is increased.

ACKNOWLEDGEMENTS

The author wishes to thank Mr. S. JERSTEDT for performing part of the experimental work.

This work has been aided by grants to the Institute of Biochemistry, Uppsala, from the U.S. Department of Army through its European Office under contract number DA-91-591-EUC-1462, and from the National Science Foundation, U.S.A. (G-18702).

SUMMARY

The migration velocity of a protein in cross-linked polyacrylamide gels is dependent not only on the charge which the protein carries, but also to a large extent on the molecular size of the protein. The latter factor is so pronounced that reversal of the migration velocities of two proteins of different molecular weights can often be accomplished by mere changes in the gel concentration, *i.e.* the pore size. If this "molecular-sieve" electrophoresis is combined with electrophoresis in a medium without molecular sieving properties a high resolution can be expected. "Molecular-sieve" electrophoresis, which can be used both for analytical and small scale preparative purposes, can in some cases give separations similar to those obtained in "molecular-sieve" chromatography.

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