# MOLECULAR WEIGHT ESTIMATIONS OF PROTEINS BY ELECTROPHORESIS IN POLYACRYLAMIDE GELS OF GRADED POROSITY

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## 1. Introduction

Polyacrylamide gel electrophoresis has been extensively used as a technique for the separation and characterization of proteins. The separation achieved is dependent on both the size and charge of the protein. Many workers have attempted to eliminate the charge effect [1–7] and thereby obtain a direct estimate of the size of the protein molecules. One method uses a buffer containing an anionic detergent [1, 3, 7] which is bound to the proteins so that they acquire similar charges and separation is primarily dependent on size. With this method there may however be variations in the binding of detergent to various proteins and values may be erroneous in certain cases.

The present paper describes a method for molecular weight estimations of proteins and protein subunits based on "Gradipore electrophoresis", electrophoresis in polyacrylamide gels of graded porosity [8, 9].

## 2. Materials and methods

The various plasma proteins used in this study were obtained from our ordinary fractionation procedure at AB Kabi. All other chemicals used were the purest commercially available, usually reagent grade.

The electrophoresis apparatus, "Gradipore electrophoresis", Universal Scientific Ltd., London, England, was obtained from AB Lambda, Stockholm, Sweden. In this apparatus electrophoresis is performed in a polyacrylamide gel sandwiched between vertical glass plates inserted between the cathodic chamber above and the anodic chamber below and having a continuous concentration gradient from 4–26%. The plates are commercially available from Universal Scientific Ltd., London, England.

The principle of the separation is that the proteins migrate through progressively smaller pores, the sizes of which are regulated by the gel concentration and finally tend to stop and concentrate where the pore size is too small to allow further migration. Separation is thus finally related to size although in the earlier stages of the run the separation is based on both charge and size.

The buffer was 0.09 M Tris, 0.003 M EDTA, 0.08 M borate, pH 8.3. Some runs were performed in 8 M urea or detergent solutions dissolved in the buffer. About 10 $\mu$l of protein solution was applied to the gel (usually 5 mg/ml, but higher concentrations when the samples contained many components.) Twelve samples could be run simultaneously on one plate. The temperature during the run was 6°. After the run the gel was stained with 0.5% amido black in methanol:$H_2O$:acetic acid, 5:5:1, for 5 hr and destained electrophoretically in 7% acetic acid for 1.5 hr at 6–12 V. The distances migrated were determined by direct measurements on the gel plates or on the photographs.

## 3. Results

Fig. 1 demonstrates the resolution obtained by "Gradipore electrophoresis". The samples run are: I. human serum, II. haptoglobin, III. haptoglobin + hemoglobin and IV. serum albumin.

In human serum it is possible to detect up to 30 components. The haptoglobin sample (II) contains several types of haptoglobin molecules which can be

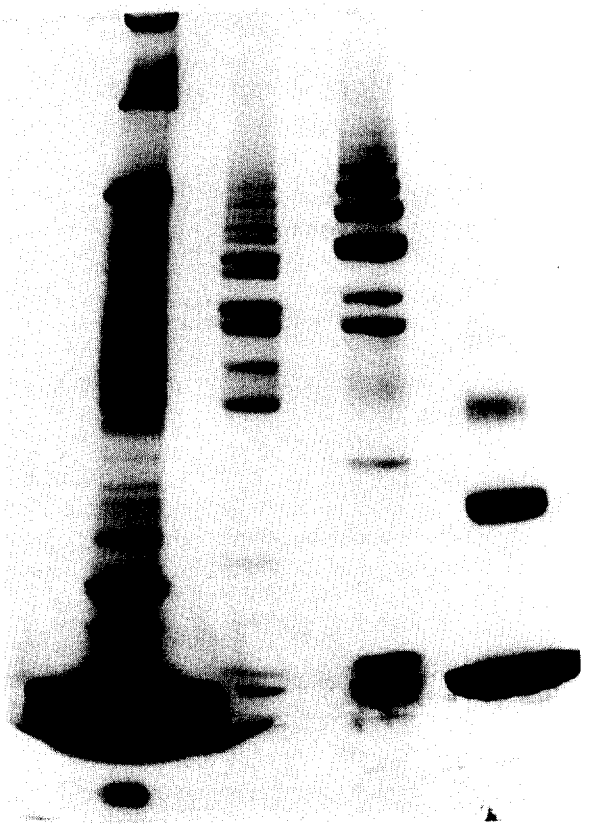

Fig. 1. "Gradipore electrophoresis" runs of some protein samples. From left to right: I. Human serum, II. Haptoglobin, III. Haptoglobin + hemoglobin, IV. Serum albumin.

classified by this method. When hemoglobin is added to a haptoglobin sample the complexes migrate shorter distances indicating an increase in molecular weights (fig. 1, 3). In the serum albumin sample (IV) dimeric, trimeric and tetrameric forms are readily detectable.

The relationship between the logarithm of the molecular weight and the distance migrated in 18 hr runs is shown in fig. 2 for a series of plasma proteins. Most of the proteins fall on the straight line with the exceptions of fibrinogen and $\alpha_2$-macroglobulin. Deviation of the former is attributed to its extended structure and larger hydrodynamic volume than would be expected for a globular protein with the corresponding molecular weight. Accordingly, the fibrinogen is found on the left side of the line indicating a much larger molecular

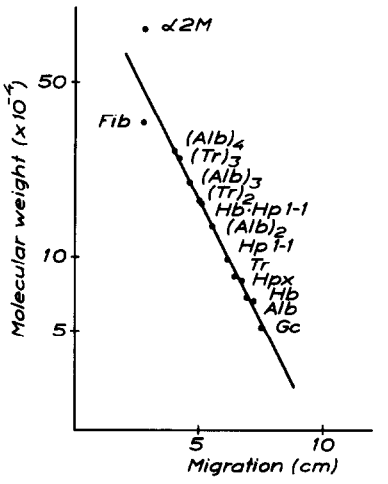


Fig. 2. Distances of migration for different human proteins as a function of the logarithm of their molecular weights. Alb = serum albumin monomer, $(Alb)_2$ = serum albumin dimer, $(Alb)_3$ = serum albumin trimer, $(Alb)_4$ = serum albumin tetramer, Tr = serum transferrin monomer, $(Tr)_2$ = serum transferrin dimer, $(Tr)_3$ = serum transferrin trimer, Gc = serum Gc-globulin, Hb = hemoglobin, Hpx = serum hemopexin, Hpl–1 = serum haptoglobin type 1–1, Fib = fibrinogen, $\alpha_{2M}$ = serum $\alpha_2$-macroglobulin.

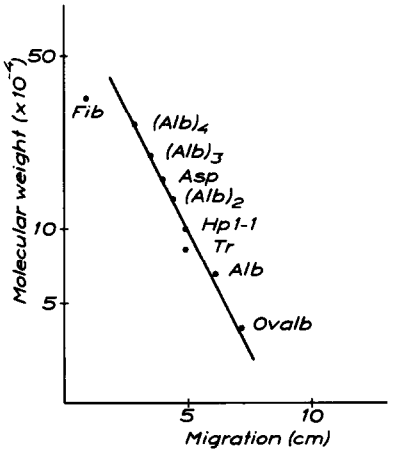


Fig. 3. Distances of migration in 8 M urea for different proteins as a function of the logarithm of their molecular weights. Ovalb = ovalbumin, Alb = human serum albumin monomer, $(Alb)_2$ = human serum albumin dimer, $(Alb)_3$ = human serum albumin trimer, $(Alb)_4$ = human serum albumin tetramer, Tr = human serum transferrin, Hpl–1 = human serum haptoglobin type 1–1, Asp = asparaginas, Fib = human fibrinogen.

weight than 340,000. The $\alpha_2$-macroglobulin deviates in the other direction, which suggests separation into subunits during the run.

Comparison of electrophoresis for 18, 41 and 64 hr showed that the proteins with lower molecular weight did migrate further during the prolonged runs, but for proteins with higher molecular weights there was very little increase. The linear relationship between the logarithm of the molecular weight and the distance migrated was maintained although the slope of the line was somewhat changed. Fibrinogen now appeared fairly close to the line suggesting that the elongated fibrinogen molecules are progressively forced further into the gel to a larger extent than are spherical proteins. This should yield a broader band than a spherical macromolecule, and in fact fibrinogen is found to have the greatest band width of the proteins studied, except IgG.

In 8 M urea solutions containing PCMB (fig. 3) a linear relationship is also obtained but fibrinogen now moves more slowly. This is difficult to explain but it is possible that aggregation of fibrinogen has taken place in urea. Molecular sizes are apparently larger in 8 M urea, which is to be expected and the bands are broader than those shown in fig. 1 which is probably a consequence of there being several different conformations with different molecular sizes in 8 M urea solutions.

Tanford et al. [10–12] has shown that proteins containing disulfide bonds do not behave as completely random coils in urea or guanidinium hydrochloride unless their disulfide bonds are broken. To study the influence of disulfide bonds, completely reduced and carboxymethylated proteins were run in 8 M urea solution. The results obtained were similar to those shown in fig. 3 except that a small increase in molecular volume could be detected, consistent with constraining effects of disulfide bonds on conformation in 8 M urea solution. However, the increased charge caused by carboxymethylation might also be responsible for the apparently increased molecular size by increasing electrostatic repulsion between the chain segments. Another difference was that fibrinogen and haptoglobin were dissociated into smaller pieces as a result of the splitting of disulfide bonds between various peptide chains. The molecular weight of the $\beta$-chain of haptoglobin was estimated to be 39,000.

## 4. Discussion

As pointed out recently [13] there is no "dead stop" in the migration of the proteins in "Gradipore electrophoresis", but nevertheless it is possible to perform molecular weight estimations by this method because migration after 18 hr is sufficiently slow to be of little significance for most globular proteins. With this method it is advisable to perform 18 hr and 40–60 hr runs in Tris-borate buffer. Proteins showing pronounced further migration with time are likely to have an elongated shape and the lower molecular weight value is probably more reliable. In this case an 18 hr run in 8 M urea is recommended for further assessment of the molecular weight. The procedure can also be used to study the subunit structure of proteins by performing electrophoresis in 8 M urea using reduced and unreduced proteins. Interactions between proteins can be studied as exemplified in this investigation by the formation of complexes between haptoglobin and hemoglobin.

A limitation with the present technique is the fact that the polyacrylamide plates available do not allow studies on proteins of molecular weights less than 50,000 when runs are performed in aqueous buffer. Proteins of molecular weights less than this migrate out of the gel. However, by using buffers containing 8 M urea it is possible to measure molecular weights down to 20,000.

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