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SHORT COMMUNICATION

MEASUREMENT OF PROTEIN MOBILITY IN SODIUM DODECYL SULPHATE POLYACRYLAMIDE GEL ELECTROPHORESIS

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Introduction

Sodium dodecyl sulphate - polyacrylamide gel electrophoresis (SDS-PAGE), with the Laemmli discontinuous buffer system[1] is widely used for molecular weight (m.wt) estimation and separation of proteins. The determination of m.wts. and identification of specific proteins often relies on accurate measurement of the mobilities of proteins. When the mobilities of proteins in cell and tissue lysates are being determined, it is not possible to ensure that all the proteins are present in approximately the same concentration that permits optimal resolution within the loading capacity of the gel. In seeking to identify minor components in a mixture, the more prevalent proteins may be loaded in amounts greater by one or even two orders of magnitude. Consequently, the question arises as to whether the leading edge, middle or trailing edge of a broad protein band detected by protein staining gives a more accurate estimation of its real mobility.

Materials and Methods

High m.wt marker proteins (myosin, β-galactosidase, phosphorylase b, bovine serum albumin, ovalbumin and carbonic anhydrase), low m.wt marker (bovine serum albumin, ovalbumin, glyceraldehyde 3-phosphate dehydrogenase, carbonic anhydrase, trypsinogen, trypsin inhibitor and lactalbumin) and human immunoglobulin (IgG) were obtained from Sigma, St. Louis, Mo, USA. The marker proteins were dissolved in reducing Laemmli buffer[1] at a concentration of 0.5 mg ml^{-1}. IgG was dissolved in the buffer at 4 mg ml^{-1}. Serial dilutions of the proteins in Laemmli buffer were made and equal aliquots carefully loaded into wells of the same capacity in a 4% Laemmli stacking gel and resolved by SDS-PAGE using a 10% separating gel. Polyacrylamide gel slabs were prepared as described by Laemmli.[1] Gels were prepared from a stock solution containing 30% by weight of acrylamide and 0.8% by weight of N,N^1 - bis-methylene acrylamide. The separating gel contained 0.375M Tris-HCl (pH 8.8) and 0.1% SDS. The gels were polymerized using 0.025% by volume of tetraethyl ethylenediamine (TEMED) and ammonium persulphate. The electrode buffer contained 0.025M Tris-HCl (pH 6.8), 0.192M glycine and 0.1% SDS. The sample

buffer contained 0.0625M Tris-HCl (pH 6.8), 2% SDS, 10% glycerol, 5% of 2-mercaptoethanol and 0.001% bromophenol blue as a dye. Stacking was performed at 30V and separation at 120V in a Hoeffer slab gel electrophoresis apparatus. The gels were stained in 0.5% Coomassie blue, destained in 10% acetic acid with 45% methanol, dried and photographed.

Results

Figure 1 shows the resluts of a typical experiment with mixtures of marker proteins. Significant overloading and hence band distortion becomes evident with approximately 1.25 μg of protein in each band under the conditions used for electrophoresis. Increasing the amount of loaded proteins form 600 ng to 10 μg (high m.wt. mixture) and 150 ng to 20 μg (low m.wt. mixture) leads to increasing mobility of the leading edge. The mobility of the trailing edge of the protein bands on the other hand remains relatively constant in comparison. Similar observations were made with the heavy chain of IgG (Figure 2). The m.wts. estimated for the IgG heavy chain using mobilities of the leading and trailing edges of the band in the lane containing 50 μg IgG, and sufficiently small quantities of m.wt standards to avoid overloading, were 44 and 55 kDa respectively. The use of the middle of the heavy chain band gave a value of 48.5 kDa for the m.wt. A commonly accepted value for the m.wt. of the human IgG heavy chain, based on the electrophoresis of small quantities of heavy chains when overloading effects are absent, is 55kDa.

A general upward skewing of bands towards the sides of the gel, due to differences in current flowing between the middle of the gel and the sides, is sometimes seen in electrophoresis eg. Lanes 1,2 and 3 in Figure 1. However, such effects are not relevant to the main observation reported here since the migration of all bands, including the dye front, are similarly affected at the sides of the gel.

Discussion

When protein mobilities are measured after PAGE authors do not clearly state which part of a broad protein band is being used.[1] This is because it is an accepted practice to measure the centre of the band for calculating m.wts. The results presented here show that the trailing edge of stained bands of nine different proteins viz. myosin, β-galactosidase, phosphorylase b, bovine serum albumin, glyceraldehyde 3-phosphate dehydrogenase, ovalbumin, carbonic anhydrase, trypsinogen and heavy chains of IgG are best for estimating mobilities and hence m.wts. Significant errors, as illustrated in the case of the IgG heavy chain, can be introduced by using either the middle or the leading edge of overloaded proteins bands for this purpose. The reasons for the observed phenomenon may be complex and are probably related to overloading the sieving capacity of the gel. The interpretation of the mobilities of similarly overloaded protein from autoradiography and enzymatically stained Western blots will depend on the degree of band broadening caused by the radiation and diffusion of coloured

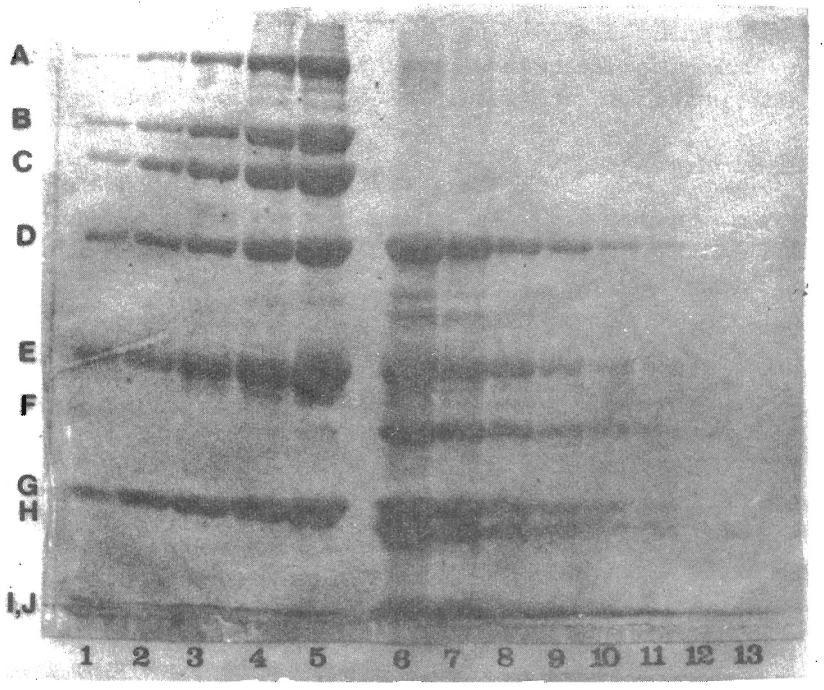

Figure 1: **Variation in mobility of A-Myosin, B-β galactosidase, C-phosphorylase b, D-serum albumin, E-ovalbumin, F-glyceraldehyde 3-phosphate dehydrogenase, G-carbonic anhydrase, H-trypsinogen, I-trypsin inhibitor and J-lactalbumin with protein concentration. Lanes 1-5 were loaded with 600 ng, 1.25 μg, 2.5 μg, 5 μg, and 10 μg respectively of each of the high m.wt. marker proteins while lanes 6-13 were loaded with 20 μg, 10 μg, 5 μg, 2.5 μg, 1.25 μg, 600 ng, 300 ng and 150 ng respectively of each of the low m.wt. marker proteins. The marker dye comigrated with lactalbumin in the gel.**

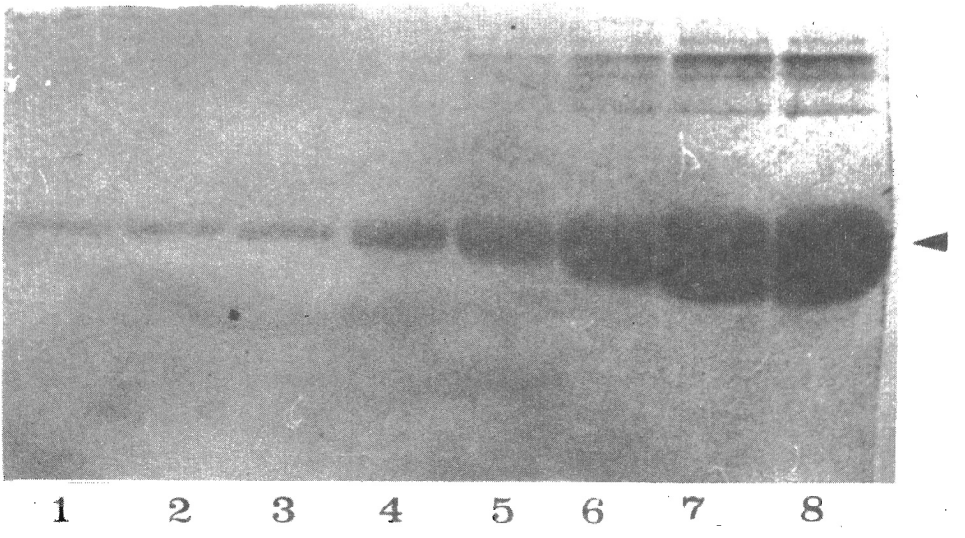

Figure 2: **Variation in the mobility of the heavy chain of human IgG with protein concentration. Lanes 1-8 were loaded with 375 ng, 750ng. 1.5 μg, 3.1 μg, 6.25 μg, 12.5 μg, 25 μg, and 50 μg of IgG respectively. The migration of the IgG heavy chain is indicated by an arrow.**

products of enzymatic reactions. However in both these procedures neither the middle or the leading edge of the detected bands will yield the best estimate of mobility where proteins are overloaded.

Acknowledgements

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Reference

1. Laemmli U.K. (1970). Cleavage of structural proteins during the assembly of the head of the bacteriophage T4. *Nature* 227:680-685.