MOLECULAR WEIGHT ESTIMATION OF POLYPEPTIDE CHAINS BY ELECTROPHORESIS IN SDS-POLYACRYLAMIDE GELS[1]

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Introduction.

Electrophoresis in polyacrylamide gels in the presence of the anionic detergent sodium dodecyl sulfate (SDS) has proven to be a useful tool for the separation and identification of polypeptide chains (Maizel, 1966; Shapiro et al., 1966; Viñuela et al., 1967). It is the purpose of this communication to indicate that the same technique may be used for the rapid and simple estimation of the molecular weights of proteins and their subunits.

Materials and Methods.

Ribonuclease A, lysozyme, carboxypeptidase A, and pepsin were obtained from the Worthington Biochemical Corporation. Ovalbumin (3x crystallized) was a gift from Dr. R. Warner; bovine hemoglobin was purchased from Pentex and trypsin from Calbiochem. Thyroglobulin type II was obtained from Sigma and bovine serum albumin from Armour. Half molecules of gamma globulin (H and L dimer) were produced by the MPC-11 mouse plasma cell tumor (Scharff et al., 1967).

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Fifty microgram samples of each protein were denatured and reduced at pH 7.1 in 1% SDS and 1% 2-mercaptoethanol (2-Me) at 37° for 3 hours. The samples were then dialyzed for 16 hours against 0.01M phosphate pH 7.1 with 0.1% SDS and 0.1% 2-Me. Electrophoresis was performed at 8 volts/cm. for two hours in 125 m.m. 5% polyacrylamide gels containing 0.1M phosphate and 0.1% SDS (Maizel, 1966). The electrophoresis buffer was 0.1M phosphate pH 7.1 with 0.1% SDS.

Two samples of DEAE-purified rabbit gamma globulin (Fahey, 1959) were included in this study. One was treated as described above to yield heavy (H) and light (L) chains. The second was not reduced but was incubated with 1% SDS. Iodoacetamide (0.05M) was added to block sulfhydryls exposed as a result of disruption of secondary and tertiary structure by the SDS. This sample was dialyzed against 0.01M phosphate with 0.1% SDS but without 2-Me.

The gels were fixed in 20% sulfosalicylic acid for 16 hours, stained with 0.25% Coomasie Blue for 5 hours, and destained with 7% acetic acid. The relative migration of each band was then correlated with the known molecular weights[4] of the polypeptide chains for each protein. The migration of bromphenol blue was used as the reference point within each gel. The data were then tabulated, using the migration of the alpha and beta chains of hemoglobin as a standard of relative migration.

In preliminary studies performed on some of these proteins, we found no differences in electrophoretic migration in 0.1%, 0.5%, and 1.0% SDS, suggesting that under these conditions of incubation and dialysis, 0.1% SDS is sufficient.

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[4]The molecular weights used were obtained from The Proteins, ed. H.Neurath (New York and London: Academic Press, 1963); references listed in the Worthington Biochemical Corp. catalog; Edelhoch, H., and De Crombrugghe, B., J. Biol. Chem., 241, 4357 (1966); and Fleischman, J. B., Ann. Rev. Biochem., 35, 835 (1966).

Results.

A typical experiment performed under the conditions described is shown in Fig. 1.

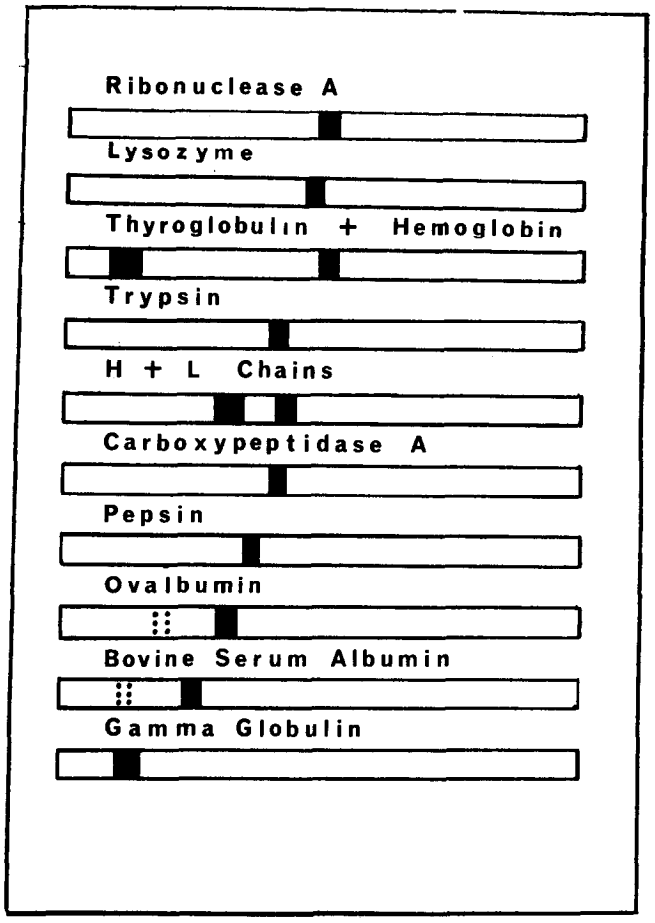

Figure 1 - Stained 5% polyacrylamide disc gels prepared and electrophoresed as described in the text to show the relative migrations of the polypeptide chains of a number of proteins. The anode is on the right. The bands of bromphenol blue are not shown in this illustration.

Both ovalbumin and bovine serum albumin revealed a second, slow moving component. Carboxymethylation of reduced samples resulted in disappearance of the slow component in each case, suggesting that aggregation into dimers by disulfide bonding during electrophoresis had occurred.

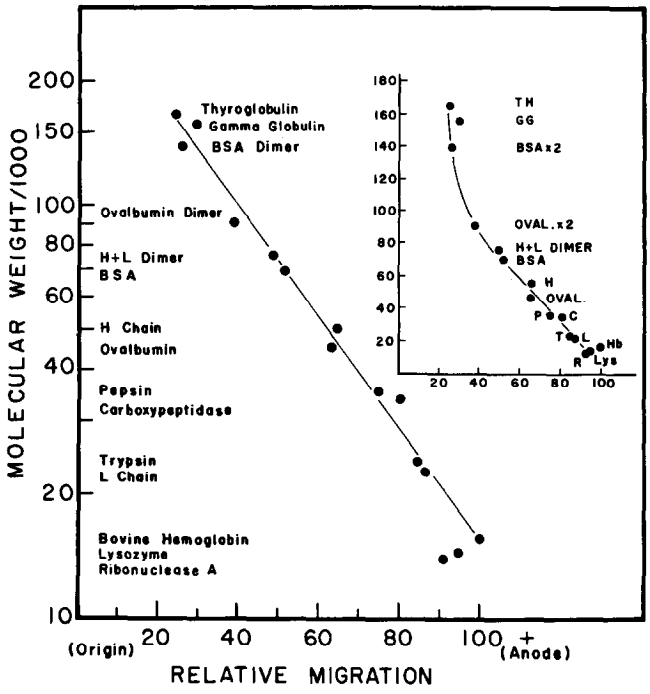

Figure 2 - Semi-log plot of molecular weight against distance of migration relative to the alpha and beta chains of hemoglobin. A line of best fit has been drawn through the data points. The insert represents a rectilinear plot of the same data.

A composite of three such experiments is shown in Fig. 2. If relative migration is plotted against the log of molecular weight (ordinate), a straight line can be fitted from molecular weights 15,500 to 165,000. The relationship between molecular weight and migration can thus be expressed as:

$$M.W. = k\ (10^{-bx})$$

where x is the distance of migration and b is the slope.

The rectilinearly plotted insert in Fig. 2 emphasizes the almost linear relationship between rates of migration and molecular weights in the range of 15,000 to 75-90,000 for these 5% gels. Over the entire range of molecular weights studied, however, the true function is hyperbolic.

A limited number of experiments indicated that other gel concentrations could be used in a similar way. A 5% gel was chosen for illustration because

many polypeptides of biological interest have molecular weights which fall within the almost linear 15,000 to 90,000 range.

The deviations of ribonuclease A and lysozyme from the straight line were repeatable, and, as yet, not completely explained.

Discussion.

As suggested by Smithies (1962) and Allison and Humphrey (1959), electrophoresis can be a useful tool for the approximation of molecular weight. Despite the choice of a group of proteins with a range of isoelectric points from 4 to 11 (Alberty, 1953), all points approximated the fitted curves, suggesting that SDS minimizes the native charge differences and that all proteins migrate as anions as the result of complex formation with SDS. The extensive disruption of hydrogen, hydrophobic, and disulfide linkages by SDS and 2-Me results in the quantitative solubilization of many relatively insoluble proteins. These factors and the ease of the polyacrylamide technique strongly recommend it as the electrophoretic method of choice.

It can be seen from Fig. 1, that the method is as applicable to a mixture of proteins (e.g. thyroglobulin and hemoglobin) as to a single polypeptide or protein. Thus, one can readily approximate the molecular weight of each component of a mixture of unknown polypeptides simply by running a suitable marker of known size in a second, parallel gel. In many cases, it may be necessary to carboxymethylate both the intrachain sulfhydryls exposed by SDS and the interchain disulfide bonds disrupted by 2-Me to prevent aggregation during electrophoresis.

Finally, the sensitivity, resolving power, and usefulness of this method can be increased by combined use of radioactive amino acids, double labeling, and a gel fractionator (Maizel, 1966) to detect and estimate the molecular weights of minute amounts of newly synthesized material, all within a single gel (Shapiro, in preparation).

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