

The Reliability of Molecular Weight Determinations by Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis*

(Received for publication, April 1, 1969)

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SUMMARY

Forty proteins with polypeptide chains of well characterized molecular weights have been studied by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate following the procedure of Shapiro, Viñuela, and Maizel (*Biochem. Biophys. Res. Commun.*, 28, 815 (1967)). When the electrophoretic mobilities were plotted against the logarithm of the known polypeptide chain molecular weights, a smooth curve was obtained. The results show that the method can be used with great confidence to determine the molecular weights of polypeptide chains for a wide variety of proteins.

Determination of the molecular weights of polypeptide chains in oligomeric proteins is an important problem. The most frequently used physicochemical method is equilibrium centrifugation in guanidine-HCl solution. For many purposes, a procedure which is experimentally less demanding but still yields reliable molecular weights would be of great value.

Shapiro, Viñuela, and Maizel (1) reported that the separation of proteins by polyacrylamide electrophoresis in the presence of the anionic detergent sodium dodecyl sulfate is dependent on the molecular weights of their polypeptide chains. Their results for 11 proteins were impressive, and in spite of some scatter, they showed the probable usefulness of this procedure.

We first made use of SDS¹ gel electrophoresis during studies on the structure of aspartate transcarbamylase of *Escherichia coli*. The molecular weights we obtained for aspartate transcarbamylase were in excellent agreement with chemical and X-ray data (2, 3), and we wondered how widely applicable and how accurate this method might be. To try to answer these questions we selected some 40 proteins with well characterized polypeptide molecular weights. When the electrophoretic mobilities were plotted against the logarithm of the known polypeptide chain molecular weights, a smooth curve was obtained. The results show that SDS gel electrophoresis can be used with

great confidence for a wide variety of proteins. It appears that by this technique polypeptide molecular weights may be determined with an accuracy of at least $\pm 10\%$.

MATERIALS AND METHODS

Analytical grade $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ and $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ were obtained from Mallinckrodt Chemical Works, New York. Glacial acetic acid and methanol were reagent grade chemicals. Acrylamide (for electrophoresis), methylenebisacrylamide, *N,N,N',N'*-tetramethylethylenediamine and β -mercaptoethanol were obtained from Eastman. Ammonium persulfate, analytical grade, was a Fisher product. Bromphenol blue and Coomassie brilliant blue (R-250) were obtained from Mann. Sodium dodecyl sulfate (95%), obtained from Matheson Coleman and Bell, was recrystallized from ethanol. Dialysis tubing 8/32 and 23/32, obtained from Union Carbide, was cleaned by standard procedures. Distilled water was used in all experiments.

The proteins used were obtained from Boehringer Mannheim: L-amino acid oxidase (*Crotalus terrificus*), D-amino acid oxidase (pig kidney), fumarase (pig heart), and glutamate dehydrogenase (bovine liver); from Calbiochem: cytochrome *c* (horse heart), enolase (rabbit muscle), glyceraldehyde phosphate dehydrogenase (rabbit muscle), β -lactoglobulin, and pyruvate kinase (rabbit muscle); from Mann: myoglobin (horse heart), lysozyme (egg white), pepsin (hog stomach), ribonuclease (bovine pancreas), serum albumin (bovine), and subtilisin (*Bacillus subtilis*); from Sigma: α -chymotrypsin (bovine pancreas), α -chymotrypsinogen A (bovine pancreas), hemoglobin (bovine), lactate dehydrogenase (bovine heart), liver alcohol dehydrogenase (horse liver), and yeast alcohol dehydrogenase; from Worthington: aldolase (rabbit muscle), carboxypeptidase A (bovine pancreas), ovalbumin (egg white), papain (papaya latex), and phosphorylase *a* (rabbit muscle).

Carbonic anhydrase B (human blood), creatine kinase (chicken muscle), β -galactosidase (*E. coli*), γ -globulin (rabbit), leucine amino peptidase (bovine eye balls), paramyosin, tropomyosin, and myosin (rabbit muscle) were gifts from Drs. Edsall, Quiocho, Weil, Pappenheimer, Carpenter, Riddiford, and Lowey. The bacteriophage coat proteins (R17 and Q β) and aspartate transcarbamylase (*E. coli*) were prepared as reported (4, 2).

Performic acid oxidation of papain, subtilisin, and ribonuclease was performed by the method of Hirs (5). Carboxymethylation of lysozyme, ribonuclease, γ -globulin, aspartate transcarbamylase

* This work was supported by Grant GM 16 132-01 from the National Institutes of Health.

† Recipient of a Postdoctoral Fellowship from the Damon Runyon Fund for Cancer Research.

¹ The abbreviation used is SDS, sodium dodecyl sulfate.

ylase, tropomyosin, and paramyosin was performed using iodoacetamide in sodium dodecyl sulfate or 8 M guanidine-HCl (6).

The following procedure is based on the original descriptions by Shapiro *et al.* (1), by Ornstein (7), and Davis (8).

Preparation of Protein Solutions

The proteins were incubated at 37° for 2 hours in 0.01 M sodium phosphate buffer, pH 7.0, 1% in SDS, and 1% in β -mercaptoethanol. Tropomyosin, paramyosin, and myosin were dissolved in this buffer in the presence of 8 M urea. The protein concentration was normally between 0.2 and 0.6 mg per ml. After incubation the protein solutions were dialyzed for several hours at room temperature against 500 ml of 0.01 M sodium phosphate buffer, pH 7.0, containing 0.1% SDS and 0.1% β -mercaptoethanol. In most cases the dialysis step may be omitted and the protein dissolved directly in dialysis buffer.

Preparation of Gels

Gel buffer contained 7.8 g $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$, 38.6 g of $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$, 2 g of SDS per liter. For the 10% acrylamide solution, 22.2 g of acrylamide and 0.6 g of methylenebisacrylamide were dissolved in water to give 100 ml of solution. Insoluble material was removed by filtration through Whatman No. 1 filter paper. The solution was kept at 4° in a dark bottle. Gels with increased and decreased cross-linker contained twice and half the concentration of cross-linker, respectively.

The glass gel tubes were 10 cm long with an inner diameter of 6 mm. Before use they were soaked in cleaning solution, rinsed, and oven-dried. For a typical run of 12 gels, 15 ml of gel buffer were deaerated and mixed with 13.5 ml of acrylamide solution. After further deaeration, 1.5 ml of freshly made ammonium persulfate solution (15 mg per ml) and 0.045 ml of N,N,N',N' -tetramethylethylenediamine were added. After mixing, each tube was filled with 2 ml of the solution. Before the gel hardened a few drops of water were layered on top of the gel solution. After 10 to 20 min an interface could be seen indicating that the gel had solidified. Gels with normal amount of cross-linker remain clear, those with doubled cross-linker turn opaque. Just before use the water layer was sucked off, and the tubes were placed in the electrophoresis apparatus.

Preparation of Samples

For each gel, 3 μl of tracking dye (0.05% Bromphenol blue in water), 1 drop of glycerol, 5 μl of mercaptoethanol, and 50 μl of dialysis buffer was mixed in a small test tube. Then 10 to 50 μl of the protein solution were added. After mixing, the solutions were applied on the gels. Gel buffer, diluted 1:1 with water, was carefully layered on top of each sample to fill the tubes. The two compartments of the electrophoresis apparatus were filled with gel buffer, diluted 1:1 with water. Electrophoresis was performed at a constant current of 8 ma per gel with the positive electrode in the lower chamber. Under these conditions the marker dye moved three-quarters through the gel in approximately 4 hours. The time taken to run the gel may be decreased by decreasing the molarity of the gel buffer.²

After electrophoresis, the gels were removed from the tubes by squirting water from a syringe between gel and glass wall and by using a pipette bulb to exert pressure. The length of the gel and the distance moved by the dye were measured.

² A. Burgess, personal communication.

Staining and Destaining—The gels were placed in small tubes filled with staining solution prepared by dissolving 1.25 g of Coomassie brilliant blue in a mixture of 454 ml of 50% methanol and 46 ml of glacial acetic acid, and removing insoluble material by filtration through Whatman No. 1 filter paper. Staining was at room temperature. The time varied from 2 to 10 hours. The gels were removed from the staining solution, rinsed with distilled water, and placed in destaining solution (75 ml of acetic acid, 50 ml of methanol, and 875 ml of water) for a minimum of 30 min. The gels were then further destained electrophoretically for 2 hours in a gel electrophoresis apparatus using destaining solution. The length of the gels after destaining and the positions of the blue protein zones were recorded. The gels were stored in 7.5% acetic acid solution.

The gels swell some 5% in the acidic solution used for staining and destaining. Gels with lower amount of cross-linker show more swelling. Therefore the calculation of the mobility has to include the length of the gel before and after staining as well as the mobility of the protein and of the marker dye. Assuming even swelling of the gels, the mobility was calculated as

$$\text{Mobility} = \frac{\text{distance of protein migration}}{\text{length after destaining}} \times \frac{\text{length before staining}}{\text{distance of dye migration}}$$

The mobilities were plotted against the known molecular weights expressed on a semi-logarithmic scale.

Time Required for Molecular Weight Determination—It is possible to obtain the molecular weight of a particular protein within a day: preparation of gels and samples, 2 hours; electrophoresis, 3 to 4 hours; removing the gels, $\frac{1}{2}$ hour; staining, 2 hours; destaining, 2 to 3 hours.

Amount of Protein

Usually 0.01 mg of protein was applied per gel. The amount could be lowered if the gel was stained for a longer period. If 0.1 mg was applied although the trailing edge of the band was diffuse, the leading edge was still very sharp and molecular weights could still be found very accurately.

Elution of Proteins from Gels

On each of two gels, 0.1 mg or more was run. One gel was stored wrapped in Saran Wrap at 4°. The other gel was stained and destained to localize the protein band. The corresponding volume of the first gel was cut into smaller pieces. The gel material was suspended in a small amount of 0.1% SDS solution and kept for several hours at 37°. The solution was withdrawn and a second elution was performed. The combined eluents were lyophilized in a conical centrifuge tube. Distilled water was added to obtain a 1% SDS solution (usually about 50 to 100 μl). Then nine parts of ice-cold acetone were added for one part of solution. The protein precipitated and could be centrifuged off, whereas the detergent stayed in solution. The precipitate could be transferred to a hydrolysis tube by dissolving it in 5% piperidine solution or in 12 N HCl.

RESULTS

Two procedures were used to examine the relationship between electrophoretic mobilities and molecular weights of various proteins. Either several SDS-denatured proteins were mixed

and run together on one gel or the proteins were run in a parallel manner on different gels. Identical results were obtained by both methods.

Fig. 1, *A*, *B*, and *C*, shows examples of the separation of several different polypeptide chains on a single gel. Remarkably good separation is obtained for the polypeptide chains of catalase, fumarase, aldolase, glyceraldehyde phosphate dehydrogenase, carbonic anhydrase, and myoglobin (Fig. 1*A*). The identity of each band was established by running each protein on a different gel. In each case a single protein band with an electrophoretic mobility corresponding to the one assigned to it in the mixture was observed. Fig. 1*B* shows a similar mixture which was run on a different occasion, but without myoglobin. In Fig. 1*C* the same proteins were run as in Fig. 1*A*, but substituting liver alcohol dehydrogenase for aldolase. Again, separation of all six polypeptide chains was observed. The control gel for liver alcohol dehydrogenase indicated only one band with a mobility corresponding to that assigned to it in the mixture. The molecular weights for the different polypeptide chains are taken from Table I, in which the proteins we studied by SDS gel electrophoresis are listed. The pictures illustrate the dependence of the mobility on the logarithm of the molecular weight of the polypeptide chains (1). The separation in the molecular weight range of 30,000 to 60,000 is excellent. It can be improved further either by increasing the length of the gel or by decreasing the amount of protein applied.

As an example of the method in which each protein is run on a different gel, Fig. 2 illustrates the determination of the molecular

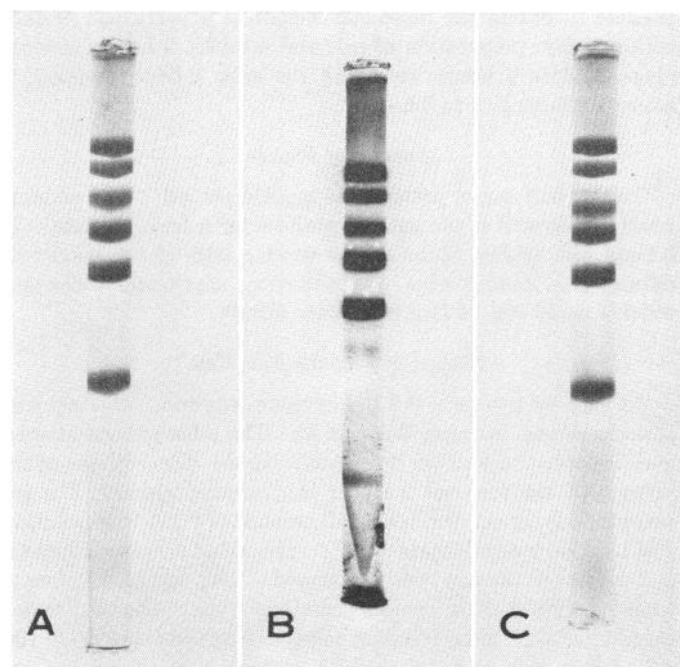


FIG. 1. Separation of the polypeptide chains of different proteins in gels with the normal amount of cross-linker. The proteins are listed from top to bottom, and the molecular weights given for the different polypeptide chains are taken from Table I. *A*, catalase (60,000), fumarase (49,000), aldolase (40,000), glyceraldehyde phosphate dehydrogenase (36,000), carbonic anhydrase (29,000), and myoglobin (17,200); *B*, same as *A*, but omitting the myoglobin. This gel was run on a different occasion; *C*, catalase, fumarase, liver alcohol dehydrogenase (41,000), glyceraldehyde phosphate dehydrogenase, carbonic anhydrase, and myoglobin.

TABLE I

Proteins studied by SDS-electrophoresis

The table lists molecular weights of the polypeptide chains taken from the literature. Proteins which under native conditions exist as oligomers are indicated by an asterisk.

Protein	Mol wt of polypeptide chain	Reference
Myosin*	220,000	9, 10, 11
β -Galactosidase*	130,000	12
Paramyosin	100,000	13
Phosphorylase a*	94,000	12, 14
Serum albumin	68,000	15
L-Amino acid oxidase	63,000	16
Catalase*	60,000	17, 18
Pyruvate kinase*	57,000	19
Glutamate dehydrogenase*	53,000	12, 20, 21
Leucine amino peptidase	53,000	^a
γ -Globulin, H chain*	50,000	22
Fumarase*	49,000	23
Ovalbumin	43,000	24
Alcohol dehydrogenase (liver)*	41,000	24, 25 ^b
Enolase*	41,000	24, 26
Aldolase*	40,000	24, 27, 28
Creatine kinase*	40,000	29
D-Amino acid oxidase*	37,000	30
Alcohol dehydrogenase (yeast)*	37,000	31 ^c
Glyceraldehyde phosphate dehydrogenase*	36,000	32, 33
Tropomyosin*	36,000	13, 34
Lactate dehydrogenase*	36,000	24
Pepsin	35,000	35
Aspartate transcarbamylase, C chain*	34,000	2, 3
Carboxypeptidase A	34,600	36
Carbonic anhydrase	29,000	37
Subtilisin ^d	27,600	38
γ -Globulin, L chain*	23,500	22
Chymotrypsinogen	25,700	^e
Trypsin	23,300	^e
Papain (carboxymethyl)	23,000	^{e, f}
β -Lactoglobulin*	18,400	15
Myoglobin	17,200	^e
Aspartate transcarbamylase, R chain*	17,000	2, 3
Hemoglobin*	15,500	^e
Q β coat protein	15,000	40 ^g
Lysozyme	14,300	^e
R17 coat protein	13,750	^e
Ribonuclease	13,700	^e
Cytochrome c	11,700	^e
Chymotrypsin*, 2 chains	11,000 and 13,000	^e

^a K. Weber, unpublished results.

^b JORNVAL, H. AND HARRIS, J. I., *Abstracts of the Federation of European Biochemical Sciences, Praha, 1968*, abstract 759.

^c BUTLER, P. J. G. AND HARRIS, J. I., *Abstracts of the Federation of European Biochemical Sciences, Praha, 1968*, abstract 741.

^d After performic acid oxidation.

^e Calculated from the amino acid sequences given in Dayhoff and Eck (39).

^f Corrected according to the X-ray structure (J. Drenth, personal communication).

^g W. Konigsberg, personal communication.

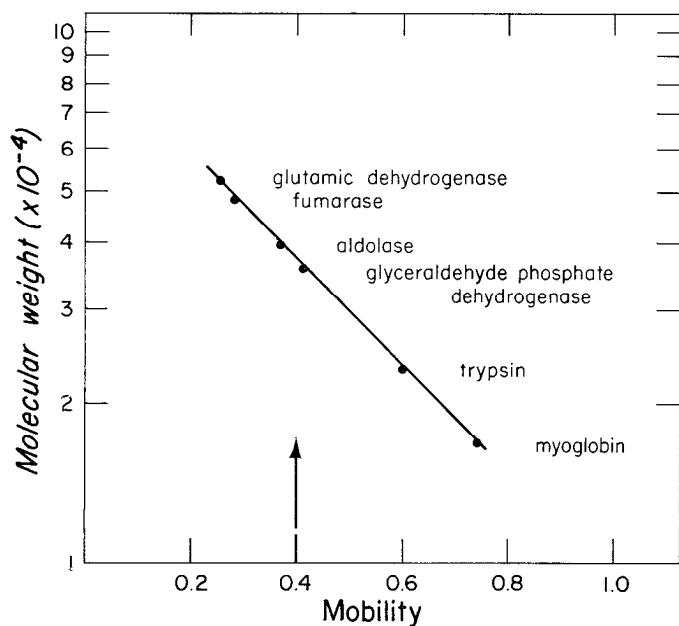


FIG. 2. Determination of the molecular weight of the polypeptide chain of D-amino acid oxidase from a set of 12 individual standard gels. The six marker proteins used were glutamic acid dehydrogenase, fumarase, aldolase, glyceraldehyde phosphate dehydrogenase, trypsin, and myoglobin. All proteins were run on duplicate gels except glutamic dehydrogenase and myoglobin. The arrow indicates the mobility of D-amino acid oxidase from two different gels (0.398; 0.402). The molecular weights of the marker proteins are taken from Table I. The extrapolated value for D-amino acid oxidase is 37,000.

weight of D-amino acid oxidase. Again the electrophoretic mobilities for marker polypeptide chains are plotted against the log of their molecular weights. From the electrophoretic mobility of D-amino acid oxidase, a molecular weight of 37,000 is found.

The reproducibility of the system can be illustrated by the following experiment. Aspartate transcarbamylase from *E. coli* was run in a parallel manner on 12 individual gels. Two protein bands were obtained which correspond to the two different polypeptide chains of this enzyme (2). The electrophoretic mobilities were 0.44 to 0.46 for the C Band and 0.74 to 0.76 for the R Band. The average mobilities calculated from these 12 gels were $0.445 \pm 3\%$ and $0.745 \pm 3\%$, respectively. From these results the reproducibility appears better than 5%. However, the absolute values of the mobility of the same polypeptide chain run on separate occasions sometimes shows a deviation of 5 to 10%. This slight deviation seems to occur with new batches of polyacrylamide. However, in all cases, the plot of the mobilities for a given set of standards gives the same value for the molecular weight of a particular protein.

The identity of a particular protein band has been repeatedly established by the elution procedure given in the previous section, followed by amino acid analysis. Greater than 75% recovery may be obtained by this method. The method allows easy separation and recovery of small quantities of proteins that are sufficiently different in molecular weight.

The procedure may be adapted to study different molecular weight ranges. This can be seen in Fig. 3. Six different polypeptide chains were run using 10% acrylamide solution, but changing the amount of methylenebisacrylamide. A drastic change in the mobilities occurs. For example, the mobility of

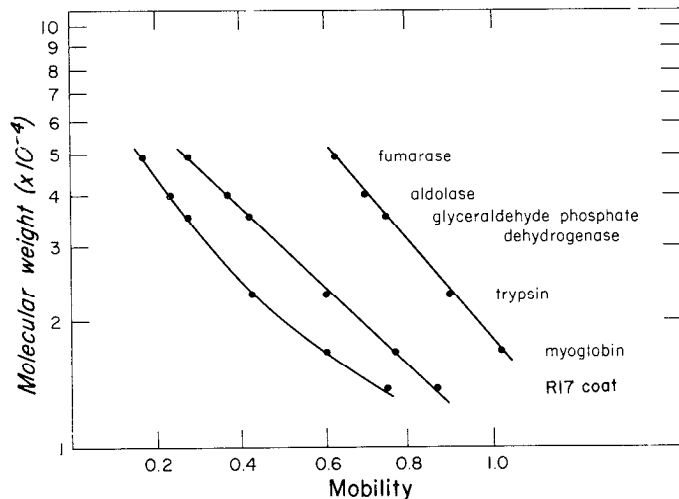


FIG. 3. Electrophoretic mobility as a function of the amount of cross-linker. The mobility is shown for the polypeptide chains of fumarase, aldolase, glyceraldehyde phosphate dehydrogenase, trypsin, myoglobin, and R17 coat protein using the normal amount of cross-linker (middle curve), 0.5 (right curve), and 2 times the amount (left curve).

fumarase with a molecular weight of 50,000 changes from 0.28 to 0.63 if the amount of cross-linker is reduced from the normal amount to half of this value. This suggests that gels with half the normal amounts of cross-linker would be useful to study proteins over a wide range of molecular weights (see below).

The results of our measurements on close to 40 different proteins are shown in Figs. 4 and 5 where the mobilities are plotted against the log of the molecular weight taken from Table I. Each protein has been run on at least four different occasions. Fig. 4 shows results for 37 different polypeptide chains in the molecular weight range from 10,000 to 70,000. In no case has a serious deviation from the molecular weights given in Table I been shown. The maximum deviation from the predicted values in this range is less than 10% and for most proteins the agreement is better. This accuracy is expected because, as shown in Fig. 1, polypeptide chains differing by only 10% in weight can be separated on the same gel.

Fig. 5 shows the results for 10 different polypeptide chains in the higher molecular weight range from 50,000 to 200,000. Gels with half the normal amount of cross-linker were used in these experiments. In spite of the hyperbolic curve obtained (see also Shapiro *et al.* (1) and Fig. 3), the mobilities follow the known molecular weights. Although fewer standards are available for this range, an accuracy of $\pm 10\%$ still seems possible.

Our results show that meaningful molecular weights can be obtained by SDS gel electrophoresis for the three muscle proteins tropomyosin, paramyosin, and myosin. We were interested in these proteins because they are known to have a high helical content. Tropomyosin yields a molecular weight of 36,000 in good agreement with the values given in the literature (13, 34). Using very low protein concentrations we found that the protein actually did not give a single band, but a doublet that was hardly separated. At the present it is unclear whether this is an artifact with the preparation we have available or whether the polypeptide chains of tropomyosin are not identical. Paramyosin yielded the expected polypeptide chain of molecular weight 100,000 (13). For myosin we obtained a polypeptide chain

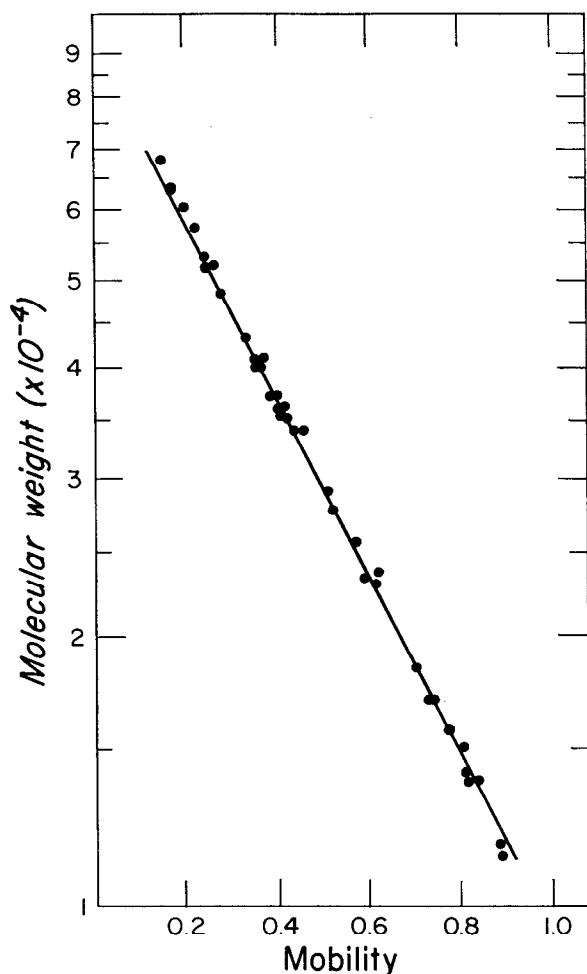


FIG. 4. Comparison of the molecular weights of 37 different polypeptide chains in the molecular weight range from 11,000 to 70,000 with their electrophoretic mobilities on gels with the normal amount of cross-linker. The references to the molecular weights are given in Table I.

with a molecular weight close to 200,000, in agreement with the reported values for this protein (9, 10). However, we also detected three minor components of lower molecular weights (16,000 to 23,000). At this time, we don't know if these components are impurities in the preparation we had available or if they are inherent components of myosin. The latter assumption is supported by recent results obtained in different laboratories.³

In a few cases we studied the proteins after performic acid oxidation or carboxymethylation. The electrophoretic mobilities of papain and ribonuclease after performic acid oxidation were identical with the ones obtained before. No differences were seen after carboxymethylation for lysozyme, aspartate transcarbamylase, tropomyosin, and paramyosin. This indicates that the β -mercaptoethanol prevents the formation of interchain cystine bonds. This assumption is supported by the fact that we did not find dimers of polypeptide chains. The possibility that some rare protein will not be denatured by the SDS treatment in the presence of β -mercaptoethanol suggests that a control should be performed using an alkylated or oxidized derivative.

³ S. Lowey, personal communication.

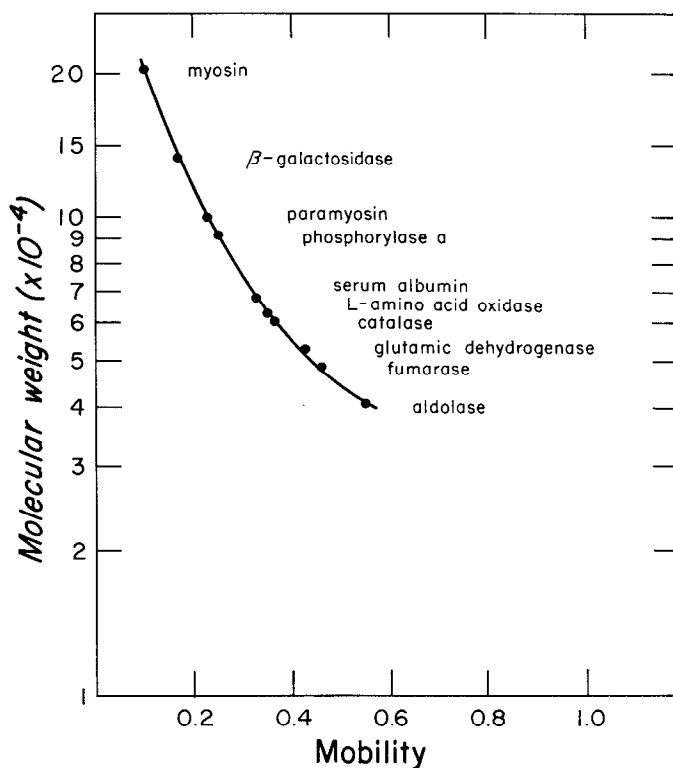


FIG. 5. Comparison of the molecular weights of different polypeptide chains (see Table I) in the molecular weight range from 40,000 to 200,000 with their electrophoretic mobilities on gels with half the amount of cross-linker.

DISCUSSION

Separation of native proteins on polyacrylamide gels was shown by Ornstein (7) and Davis (8) to be dependent not only on the charge but very strongly on the size of the molecules. The binding of dodecyl sulfate ions to proteins has been shown for several protein molecules (see Tanford (41) for a recent review), and was assumed to be the basis of the separation of the denatured proteins upon SDS electrophoresis on polyacrylamide (1). If so, one must assume that the individual charge pattern of each protein is totally changed by the binding of SDS anions, rendering all molecules negatively charged. At present it is difficult to see why proteins that differ widely in amino acid composition and isoelectric points should all follow the general pattern. It is possible that the sieving effect, which is an exponential function, overcomes the charge effect which may be of minor importance. Because of these theoretical difficulties we will discuss the results only from a practical point of view.

Two questions were raised during this study. How reproducible are the results obtained by SDS gel electrophoresis and how reliable are the molecular weights obtained? The results presented above quite clearly show that a high degree of reproducibility in the determination of the electrophoretic mobility can be obtained whether the proteins are run on different gels or on the same gel.

We have shown that close to 40 different proteins have electrophoretic mobilities which are independent of the isoelectric point and the amino acid composition and seem governed solely by the molecular weights of their polypeptide chains. Since the majority of the polypeptide chains used are either characterized by molecular weights known from amino acid sequence analysis,

or X-ray crystallography, or by very careful studies in guanidine-HCl with similar results obtained in different laboratories, we feel confident that this study is a very stringent test for the technique of Shapiro *et al.* (1). All the proteins studied yielded molecular weights within the experimental error of the known values. We have not studied proteins containing large amounts of carbohydrate or lipid material. It is possible that such proteins, because of their large nonprotein part, will behave differently on gel electrophoresis. It is, however, noteworthy that SDS gel electrophoresis yields not only excellent results for proteins, which are globular in the native state, but also for the highly helical, rod-shaped molecules, myosin, paramyosin, and tropomyosin. For all three proteins we obtained polypeptide chains with molecular weights in agreement with the values from the literature (see "Results").

With these results one is inclined to assume that the technique of Shapiro *et al.* (1) yields molecular weights with an accuracy of better than $\pm 10\%$ for polypeptide chains with molecular weights between 15,000 and 100,000. This range can be covered with numerous commercially available proteins as standards. The difficulty with higher molecular weights is probably only the fact that fewer markers are available for the range 90,000 to 200,000. Polypeptide chain markers commercially available for this range are phosphorylase with a molecular weight of 94,000 (12, 14) and thyroglobulin with a molecular weight of 160,000 (42). The preparation of β -galactosidase from *E. coli* can be easily accomplished (43) and yields a marker band with 135,000 molecular weight (12). Myosin and paramyosin can usually be obtained from the numerous laboratories working on these proteins and yield additional markers with molecular weights of 200,000 and 100,000. With enough markers an accuracy of about 10% in the determination of the molecular weight of an unknown protein falling in this molecular weight range may be possible.

Four examples will be used to illustrate these points further. Phosphorylase α gave a single polypeptide chain of molecular weight 94,000 without any indication of heterogeneity. This is in agreement with the results of Ullmann *et al.* (12), using the approach to equilibrium centrifugation in the presence of 6 M guanidine-HCl. Seery, Fischer, and Teller (14) found a similar value by equilibrium centrifugation in the same solvent only after accounting for some heterogeneity and preferential hydration. The latter, however, is an unlikely phenomenon in view of the results obtained by Kirby Hade and Tanford (44), Reithel and Sakura (45) and Ullmann *et al.* (12).

A further sample is liver alcohol dehydrogenase. The technique of Shapiro *et al.* (1) yields a value of 40,000 in agreement with results obtained by osmotic pressure measurements in guanidine-HCl (24), X-ray analysis (25), and preliminary sequence analysis⁴, but contrary to prior sedimentation analysis studies (46).

The separation of the polypeptide chains of fumarase, aldolase, and glyceraldehyde phosphate dehydrogenase on one gel is very striking. The molecular weights of these polypeptide chains are today well established. Aldolase was for some time assumed to have a molecular weight of 50,000. This value was mainly based on preferential hydration in guanidine-HCl (47). However by using glyceraldehyde phosphate dehydrogenase with a known amino acid sequence as one marker and fumarase, which

is well characterized, as the other, it can be concluded that the molecular weight of the aldolase polypeptide chain is 40,000. Meanwhile, this is the value accepted for careful measurements by ultracentrifugation (27) and osmotic pressure (24) in guanidine-HCl, as well as from chemical studies (28).

Conflicting values for the size of the polypeptide chain of D-amino acid oxidase are found in the literature and have been summarized recently by Henn and Ackers (30). They showed a value of 35,000 to 40,000 by gel filtration. The SDS electrophoresis yields 37,000. From all the evidence presented for the other proteins we think that the two values indicating 37,000 are much better than the one of 50,000 reported by Fonda and Anderson (48). Their value is based mainly on fingerprint analysis and a gel filtration study, which is less extensive than the one by Henn and Ackers (30).

Since the method appears to yield values in agreement with the best current estimates for all the proteins studied, it seems fair to compare SDS gel electrophoresis with other methods. The excellent resolving power of the gels over a wide range of molecular weights has been illustrated. In this respect the method is much superior to gel filtration patterns on Sephadex in a denaturing solvent like guanidine-HCl (49). The good resolution and the fact that an estimate of the molecular weight can be obtained within a day, together with the small amount of protein needed, makes the method strongly competitive with others commonly employed. The theoretically fully developed methods of osmotic pressure and sedimentation equilibrium in 6 M guanidine-HCl are still superior. However, osmotic pressure measurements suffer from the large amount of protein needed and the fact that extremely accurate protein determination is necessary. Both limitations do not apply for sedimentation equilibrium using interference optics. However this method is very demanding experimentally if good results are to be obtained. But even in the optimal case the calculation depends on the value chosen for the partial specific volume \bar{v} . An uncertainty of 0.02 in this value introduces a deviation of up to 10%, even if all the centrifuge measurements are performed well. Also, in some cases, in spite of the fact that there is good evidence for no major change in \bar{v} in guanidine-HCl (12, 44, 45), the assumption of preferential hydration is still quite often used (14, 47) to account for deviations.

It is by no means intended to assume that the accuracy of SDS gel electrophoresis will be comparable with the well developed physicochemical methods. Also, certain theoretical aspects of SDS gel electrophoresis are still not clearly understood. In spite of these limitations, however, the ease with which the method can be applied, together with the results presented above, encourage its wider use, especially in case of conflicting data obtained by other methods.

Acknowledgments—We thank Elizabeth Bloomquist for excellent and skillful assistance with many of these experiments. Discussions with Dr. R. Burgess, Dr. G. Guidotti, and Ann Burgess were most helpful.

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J. Biol. Chem. 1969, 244:4406-4412.

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