Biochem. J. (1972) **126**, 553–560
Printed in Great Britain

The Effect of Cross-Links on the Mobility of Proteins in Dodecyl Sulphate–Polyacrylamide Gels

By I. P. GRIFFITH
Commonwealth Serum Laboratories, Parkville, Vic. 3052, *Australia*

(*Received* 16 *August* 1971)

The effect of reduction of intramolecular disulphide bridges on the mobility of proteins in 5% (w/v) polyacrylamide gels in the presence of sodium dodecyl sulphate was investigated. A series of polypeptide polymers, containing up to 68 intramolecular disulphide bridges, was prepared by cross-linking proteins of known structure with glutaraldehyde. These model polypeptides were denatured with heat, sodium dodecyl sulphate and urea, and their mobilities in sodium dodecyl sulphate–polyacrylamide gels compared before and after reduction with dithiothreitol. The mobilities of polypeptides containing no cystine were unaffected by reduction. However, reduction generally decreased the mobilities of polypeptides containing cystine; the extent of this decrease depended on the number of cystine residues originally present in the polypeptide polymer, and on the protein from which the latter was derived. In contrast with their higher oligomers, the monomer of lysozyme and the dimer of ribonuclease increased in mobility after reduction. The reduced polypeptide oligomers formed by reaction with glutaraldehyde were generally found to migrate at a rate significantly faster than was expected from their calculated molecular weights. It was concluded that the use of unreduced proteins and protein aggregates for molecular-weight measurements by the sodium dodecyl sulphate–polyacrylamide-gel method may give erroneous estimates of the molecular weight of any protein being investigated.

Shapiro *et al.* (1967) reported that the rate of migration of a polypeptide in 5% (w/v) polyacrylamide gel in the presence of sodium dodecyl sulphate was proportional to its molecular weight. This finding has been confirmed for a large number of different proteins of well-characterized molecular weights (Shapiro & Maizel, 1969; Weber & Osborn, 1969; Dunker & Rueckert, 1969) and has been claimed to include covalently linked protein aggregates (Shapiro *et al.*, 1967; Dunker & Rueckert, 1969; Wolf *et al.*, 1970).

Pitt-Rivers & Impiombato (1968) reported that disulphide-stabilized proteins bound up to 50% more sodium dodecyl sulphate (by weight) after reduction. On the basis of this work and their investigation of the filtration of reduced and unreduced proteins in sodium dodecyl sulphate–Sepharose gels, Fish *et al.* (1970) suggested that unreduced proteins might migrate in an anomalous fashion during electrophoresis in sodium dodecyl sulphate–polyacrylamide gels. Dunker & Rueckert (1969) and Swank & Munkres (1971) reported that reduction had little effect on the mobility of certain proteins containing cystine, whereas Griffith (1970) found the opposite.

The present paper reports a more detailed investigation of the effect of cross-links on polypeptide mobility during sodium dodecyl sulphate–polyacrylamide-gel electrophoresis. As there are few well-characterized proteins with a high content of cystine, artificial oligomers of a number of proteins were prepared to serve as models. Compared with proteins containing no cross-links the behaviour of these model polypeptides suggests that the migration rate of proteins in sodium dodecyl sulphate–polyacrylamide gels may be significantly altered by the presence of several inter- and intra-chain cross-links.

Materials and Methods

Materials

Analytical-grade NaH_2PO_4,H_2O, $Na_2HPO_4,2H_2O$, EDTA, methanol and ammonium persulphate were obtained from British Drug Houses, Poole, Dorset, U.K.; analytical-grade acetic acid and NaN_3 from Ajax Chemicals (Vic.) Ltd., Melbourne, Vic., Australia; tris base from Sigma Chemical Co., St Louis, Mo., U.S.A.; acrylamide, *NN'*-methylenebisacrylamide, TEMED (*NNN'N'*-tetramethylethylenediamine) and β-mercaptoethanol from Eastman Organic Chemicals, Rochester, N.Y., U.S.A.; dithiothreitol and iodoacetamide from Calbiochem, Los Angeles, Calif., U.S.A.; sodium dodecyl sulphate (95%) from Matheson, Coleman and Bell, Norwood, Ohio, U.S.A.; urea from Mallinkrodt Chemical Works, New York, N.Y., U.S.A.; glutaraldehyde

(as a 25% soln.) from Koch-Light Laboratories Ltd., Colnbrook, Bucks., U.K.; Amido Black from Schmid & Co., Chroma-Gesellschaft, Stuttgart-Untertukheim, Germany; Procion Blue from Imperial Chemical Industries (Australia) Ltd., Melbourne, Vic., Australia; and Bromophenol Blue from G.T. Gurr Ltd., London S.W.6, U.K.

The proteins used were: ovalbumin, ribonuclease and lysozyme (Nutritional Biochemicals Corp., Cleveland, Ohio, U.S.A.); pepsin and soya-bean trypsin inhibitor (Worthington Biochemical Corp., Freehold, N.J., U.S.A.); A and B variants of β-lactoglobulin (Koch-Light Laboratories, Colnbrook, Bucks., U.K.); glucagon (Lilly and Co., Indianapolis, Ind., U.S.A.); *Bacillus subtilis* α-amylase, type IIA (Sigma); bovine serum albumin and human γ-globulin (Commonwealth Serum Laboratories, Melbourne, Vic., Australia). Human $\alpha_2\beta_2$ haemoglobin was prepared from erythrocytes; the cells were washed three times with buffered saline (0.15M-NaCl in 0.01M-sodium phosphate buffer, pH7.2) and lysed with 10vol. of water; the haemoglobin was clarified by centrifugation for 4h at 82000*g* in a Spinco 40 rotor and then freeze-dried.

Preparation of samples for electrophoresis

Bovine serum albumin, ovalbumin, α-amylase, β-lactoglobulin, haemoglobin, lysozyme and ribonuclease (15mg of each) were dissolved in 1ml portions of sodium phosphate buffer (0.1M, pH7.0) in screw-cap vials. After addition of 2μl of the 25% (w/v) glutaraldehyde to each, the samples were agitated gently at room temperature (21°C) for 2h. Half of each sample was transferred to a fresh vial containing 0.05ml of 1M-iodoacetamide to block thiol groups exposed by denaturation; 0.05ml of 0.5M-dithiothreitol was then added to the remaining portion of each sample. To all samples 0.25ml of 2M-tris base, 0.25ml of 10% (w/v) sodium dodecyl sulphate and 0.05ml of 5% (w/v) EDTA (disodium salt) were added. The vials were capped and placed in cold water, which was brought to the boil. After treatment at 100°C for 2min, the vials were cooled and 1.0g of solid urea was added to each. All samples were incubated at 35°C for 3h. To the samples reduced with dithiothreitol, excess of iodoacetamide was then added (0.1ml of a 1M soln. in 1M-$NaHCO_3$). All samples were dialysed for 8h against 500vol. of water (one change), and then for 16h against 0.01M-sodium phosphate buffer, pH7.2, containing 0.1% sodium dodecyl sulphate, 6% (w/v) sucrose and 0.01% NaN_3 to prevent bacterial growth.

Polyacrylamide gels

Polyacrylamide gels were prepared as described by Summers *et al.* (1965); 10–12cm gels were cast in 14cm glass tubes (internal diam. 6mm) mounted vertically on Parafilm. Gel and electrophoresis buffers were 0.1M-sodium phosphate, pH7.2, containing 0.1% sodium dodecyl sulphate and 0.01% NaN_3. The gels were 5% (w/v) with respect to acrylamide, 0.13% with respect to *NN'*-methylenebisacrylamide, and were polymerized with 0.1% of ammonium persulphate and 0.05% of TEMED. Gels were overlayered with water. After polymerization, this water was replaced with electrophoresis buffer, and the gels were stored for 1–5 days at room temperature before use.

Electrophoresis

Protein samples were mixed with a few μl of a soln. of glucagon [5mg in 1ml of 1% (w/v) sodium dodecyl sulphate] which was used as an internal reference marker, and a trace of Bromophenol Blue which was used as a running guide. Between 5 and 35μl of each sample containing 15–150μg of protein was layered under the buffer on to the surface of the gel. Electrophoresis was carried out at constant current for various times (usually 9mA per tube for 4h).

Staining

After electrophoresis, gels were stained by agitation for 1h in acetic acid–methanol–water (1:9:10, by vol.) containing 1% (w/v) Procion Blue and 2% (w/v) Amido Black. Gels were destained by agitation in acetic acid–methanol–water, (1:9:10, by vol.) overnight, followed by 7% (v/v) acetic acid for 24h. Gels were photographed through a Wratten 23A filter.

Measurement of relative mobilities

The distance from the discontinuity at the top of the gel (visible as a refractile band, or as a change in degree of background stain) to the centre of each protein band was measured. The values were converted into relative mobilities by dividing each by the distance migrated by the glucagon marker.

Results

To compare small differences in mobility of proteins in sodium dodecyl sulphate–polyacrylamide gels, it was first necessary to ensure that the mobilities of the proteins relative to a given standard were constant. Bromophenol Blue has been used as a reference marker (Weber & Osborn, 1969) but is unsatisfactory as it is lost from the gel during the staining–destaining procedure. Glucagon was chosen as an internal standard, since its migration relative to Bromophenol Blue was found to be constant. Nine proteins were chosen to construct a standard

Table 1. *Proteins studied by sodium dodecyl sulphate–polyacrylamide-gel electrophoresis*

Published values for the cystine and cysteine content and molecular weights for various polypeptides are listed below. Where two references are cited, the first refers to cysteine content, and the second to molecular weight.

Protein	Cysteine	Half-cystine	Mol.wt. of polypeptide chain	Mol.wt. of iodoacetamide derivative*	Mol.wt. of reduced protein iodoacetamide derivative†	Reference
Bovine serum albumin	1‡	34	68530§	68590	70530	Cecil (1963); Castellino & Barker (1968)
γ-Globulin, H chain	11‖		51600		52230	Edelman *et al.* (1969); Rutishauser *et al.* (1969)
α-Amylase	0	0	48200	48200	48200	Connellan (1968); Kakiuchi *et al.* (1965)
Ovalbumin	5	2	43830§	44120	44220	Neuberger & Marshall (1966); Castellino & Barker (1968)
Pepsin	0	6	35500	35500	35840	Cecil (1963); Dunker & Rueckert (1969)
γ-Globulin, L chain	5‖		23500		23790	Edelman *et al.* (1969); Rutishauser *et al.* (1969)
Trypsin inhibitor	0	4	21500	21500	21730	Wu & Scheraga (1962)
β-Lactoglobulin	1	4	18400	18460	18680	McKenzie (1967); Weber & Osborn (1969)
Haemoglobin	1.5¶	0	15500¶	15585¶	15585¶	Cecil (1963); Weber & Osborn (1969)
Lysozyme	0	8	14310	14310	14760	Canfield & Liu (1965); Canfield (1963)
Ribonuclease	0	8	13700	13700	14160	Smyth *et al.* (1963); Weber & Osborn (1969)

* Protein denatured in the presence of iodoacetamide.
† Protein denatured in the presence of dithiothreitol, and later treated with iodoacetamide.
‡ After reduction of undenatured protein to mercaptalbumin (Cecil, 1963).
§ The mean of the values for the native proteins determined by Castellino & Barker (1968).
‖ After reduction of denatured protein.
¶ The mean value for a mixture of equal amounts of α and β chains.

curve. Fig. 1 shows the log of the molecular weight of each protein given in Table 1 plotted against its mobility measured relative to that of glucagon. The value for ribonuclease was not used for the standard curve, as this protein is reported (Shapiro *et al.*, 1967; Dunker & Rueckert, 1969) to behave anomalously in sodium dodecyl sulphate–polyacrylamide gels. The mobilities measured relative to glucagon were highly reproducible between experiments, provided that measurements were taken from the boundary of the gel proper and the visible discontinuity present at the top of the gel. This discontinuity, which varies from gel to gel, is probably the greatest source of error, especially for proteins of low mobility (Maizel, 1969). Fig. 1 also shows that deviations from the expected curve were reproducible, (e.g. compare γ-globulin L chain and soya-bean trypsin inhibitor).

Having established that the technique was reproducible, the effect of intact disulphide bonds on the mobility of a number of proteins and their glutaraldehyde-linked aggregates was investigated. Reduced and unreduced samples of glutaraldehyde-treated proteins were analysed by electrophoresis in parallel as described in the Materials and Methods section. The molecular weights of the polypeptide oligomers were calculated from the values given in Table 1 together with the amount of iodoacetamide and glutaraldehyde known to have reacted with each. The log of the molecular weight of each polypeptide was plotted against its mobility relative to that of glucagon, the mean value obtained from four to six gels being used for each point. Fig. 2 shows the plots for β-lactoglobulin and its oligomers. It is apparent that the molecules in which intramolecular disulphide bridges have been cleaved migrate more slowly than their unreduced counterparts. The differences were significant at the 5% level, as were all other comparisons in Figs. 2–5 unless otherwise stated. The mobilities for ovalbumin oligomers, both reduced and unreduced after reaction with glutaraldehyde, are also shown in Fig. 2. The mobilities of both monomer and dimer of denatured ovalbumin that had not been treated with either glutaraldehyde or a reducing agent are also shown in Fig. 2; the mobility of this monomer is significantly different from both that of the glutaraldehyde-treated but unreduced ovalbumin, and that of the reduced ovalbumin used

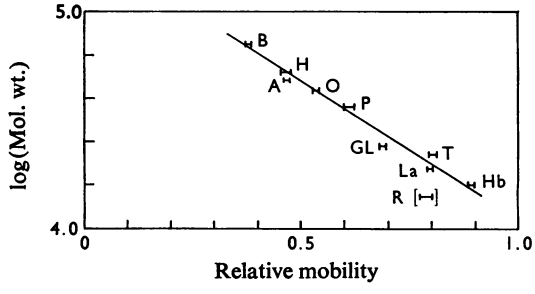

Fig. 1. *Plot of log(molecular weight) against mobility relative to glucagon for various proteins denatured with reduction*

Proteins were analysed by electrophoresis in the presence of sodium dodecyl sulphate in 5% polyacrylamide gels as described in the Materials and Methods section. Migration (towards the anode) was from left to right. Each point represents the mean of a number of measurements (given below in parentheses for each protein) and the 99% confidence limits are indicated. The line of best fit for the mean values (excluding ribonuclease, R) was determined by the method of least squares by using an IBM 7044 computer. B = bovine serum albumin (34), H = γ-globulin heavy chain (10), A = α-amylase (13), O = ovalbumin (11), P = pepsin (13), GL = γ-globulin light chain (10), T = trypsin inhibitor (11), La = β-lactoglobulin (13), Hb = haemoglobin (11), R = ribonuclease (10).

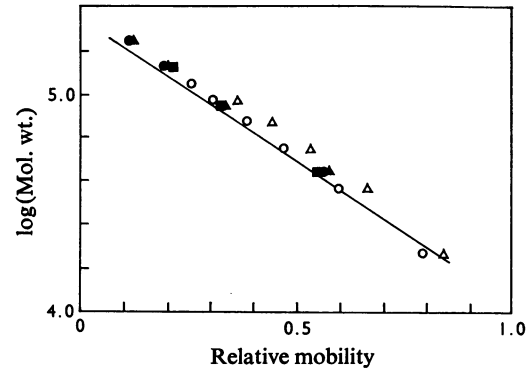

Fig. 2. *Plots of log(molecular weight) of β-lactoglobulin and ovalbumin and their oligomers against mobility relative to glucagon*

■, Proteins denatured without reduction or reaction with glutaraldehyde; ○, ●, proteins treated with glutaraldehyde before denaturation with reduction; △, ▲, proteins treated with glutaraldehyde before denaturation without reduction; ○, △, β-lactoglobulin; ■, ●, ▲, ovalbumin. The continuous line (——) represents the extrapolated standard curve plotted in Fig. 1. Details of reaction of proteins with glutaraldehyde and their subsequent denaturation and analysis by sodium dodecyl sulphate–polyacrylamide-gel electrophoresis are described in the Materials and Methods section. Migration (towards the anode) was from left to right.

for the standard curve. In contrast neither the mobilities of the various dimers nor those of the various trimers of ovalbumin differed significantly.

Fig. 3 shows the plots for haemoglobin and its oligomers. With this protein, no difference was seen in the migration rate of reduced and unreduced glutaraldehyde-reacted molecules. However, both the monomer and oligomers of glutaraldehyde-treated bovine serum albumin showed a marked decrease in mobility on reduction. Denatured monomer and dimer of bovine serum albumin, not treated with either glutaraldehyde or dithiothreitol, migrated less rapidly than their unreduced glutaraldehyde-treated equivalents.

Comparison of Fig. 1 with Fig. 4 shows that the mobility of the α-amylase monomer was little affected by reaction with glutaraldehyde. Fig. 4 shows that the mobilities of both the monomer and the dimer of α-amylase were unaffected by reduction. By contrast, the reduced oligomers of lysozyme were less mobile than their unreduced counterparts, a difference which increased with increasing molecular weight and cystine content. Surprisingly, the unreduced monomer of lysozyme migrated less rapidly than the reduced monomer. Similarly, Fig. 5 shows that the unreduced dimer of ribonuclease migrated less rapidly than the reduced dimer, but that the reduced and unreduced monomers of ribonuclease had mobilities that were not significantly different. The higher oligomers of ribonuclease, as with lysozyme, migrated more slowly after reduction, and showed a divergence in mobility between the unreduced and reduced equivalents which increased with molecular weight and cystine content.

The results show that generally, the reduced glutaraldehyde-treated polypeptides migrated more rapidly than one might have expected from the standard curve. It is also evident that reduction had no effect on the mobility of polypeptides derived from haemoglobin and α-amylase, which contain no cystine. Polypeptides containing few cystine residues showed either little change in mobility on reduction, as was found with ovalbumin and its oligomers, or a marked change in mobility, as was found with β-lactoglobulin and its oligomers. The other polypeptides derived from bovine serum albumin, lysozyme and ribonuclease, which contain many cystine bridges, generally showed marked decreases in mobility on reduction. A plot of mobility

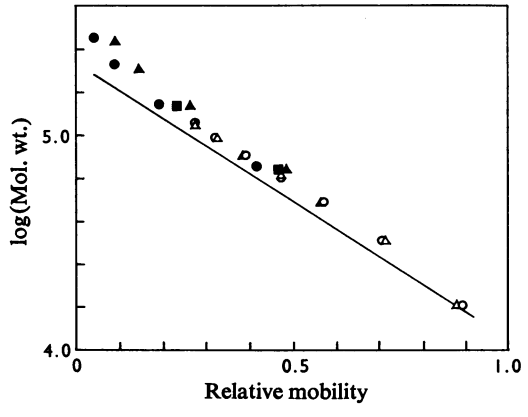

Fig. 3. *Plots of log(molecular weight) of haemoglobin and bovine serum albumin and their oligomers against mobility relative to glucagon*

Proteins were denatured without reduction or reaction with glutaraldehyde (■), or were treated with glutaraldehyde before denaturation with reduction (○, ●) or without reduction (△, ▲). ○, △, Haemoglobin; ■, ●, ▲, bovine serum albumin. The continuous line (——) represents the extrapolated standard curve plotted in Fig. 1. Details of reaction of proteins with glutaraldehyde and their subsequent denaturation and analysis by sodium dodecyl sulphate–polyacrylamide-gel electrophoresis are described in the Materials and Methods section. Migration (towards the anode) was from left to right.

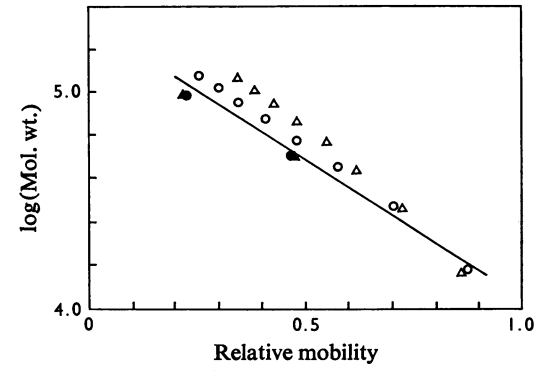

Fig. 4. *Plots of log(molecular weight) of lysozyme and α-amylase and their oligomers against mobility relative to glucagon*

Proteins were treated with glutaraldehyde before denaturation with reduction (○, ●) or without reduction (△, ▲). ○, △, Lysozyme; ●, ▲, α-amylase. The continuous line (——) represents the extrapolated standard curve plotted in Fig. 1. Details of reaction of proteins with glutaraldehyde and their subsequent denaturation and analysis by sodium dodecyl sulphate–polyacrylamide-gel electrophoresis are described in the Materials and Methods section. Migration (towards the anode) was from left to right.

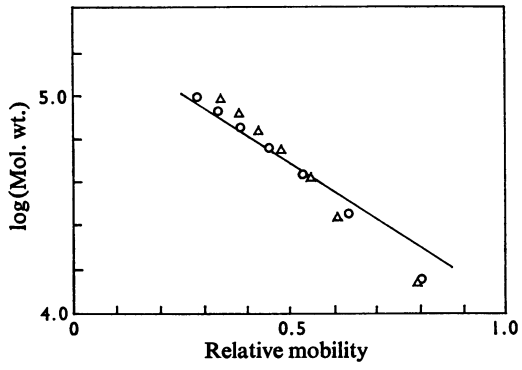

Fig. 5. *Plots of log(molecular weight) of ribonuclease and its oligomers against mobility relative to glucagon*

Ribonuclease was treated with glutaraldehyde before denaturation with reduction (○) or without reduction (△). The continuous line (——) represents the extrapolated standard curve plotted in Fig. 1. Details of reaction of ribonuclease with glutaraldehyde and its subsequent denaturation and analysis by sodium dodecyl sulphate–polyacrylamide-gel electrophoresis are described in the Materials and Methods section. Migration (towards the anode) was from left to right.

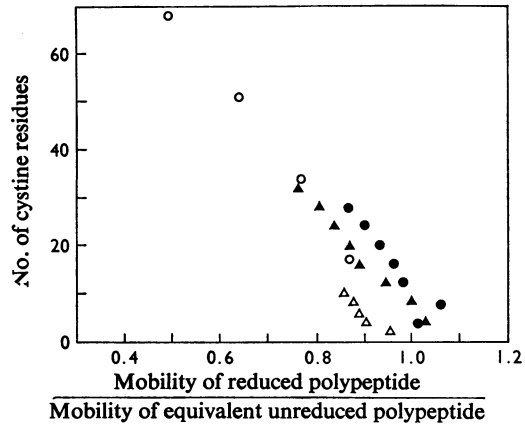

Fig. 6. *Plot of cystine content of protein oligomers versus the ratios of the relative mobilities of the reduced to the corresponding unreduced protein oligomer*

○, Bovine serum albumin; ●, ribonuclease; △, β-lactoglobulin; ▲, lysozyme.

of reduced relative to unreduced polypeptide versus cystine content showed that a relationship existed between change in mobility and cystine content (Fig. 6). Although the plots obtained for the four proteins differed, the slopes of the curves for the higher-molecular-weight oligomers were almost parallel. Fig. 6 also shows that for a given protein there was an almost linear relationship between the cystine content of its oligomers and their decrease in mobility on reduction.

Discussion

Most reduced and fully unfolded proteins migrate during sodium dodecyl sulphate–polyacrylamide-gel electrophoresis at a rate proportional to molecular weight (Shapiro *et al.*, 1967; Shapiro & Maizel, 1969; Weber & Osborn, 1969) and in a reproducible manner (Fig. 1 of the present paper). Reynolds & Tanford (1970*a*) showed that when the sodium dodecyl sulphate–monomer concentration is above a certain value, reduced and fully unfolded proteins bind about 1.4g of sodium dodecyl sulphate/g of protein irrespective of the total sodium dodecyl sulphate concentration or ionic strength of the medium. They also suggested (Reynolds & Tanford, 1970*b*) that in excess of sodium dodecyl sulphate the protein is present as a rod-like particle the length of which varies directly with the molecular weight of the protein, although Fish *et al.* (1970) showed that this applies only to proteins lacking cystine. However, the molecular size and shape of a protein are not the only factors affecting mobility during electrophoresis in the presence of dodecyl sulphate anions, since the latter impart a negative charge to proteins (Shapiro & Maizel, 1969). Pitt-Rivers & Impiombato (1968) found that proteins containing intramolecular disulphide links bound much less sodium dodecyl sulphate when these bonds were left intact. Thus, compared with its fully reduced equivalent, a protein with intact disulphides should have a lower negative charge and be less mobile during electrophoresis. Conversely, the unreduced proteins having a smaller Stokes radius (presumably a result of less bound sodium dodecyl sulphate as well as the presence of cross-links) should offer less frictional resistance and be more mobile during electrophoresis through the small pores of polyacrylamide gels. No difference in mobility between reduced and unreduced proteins would be expected where these opposing tendencies exactly balanced each other.

Ovalbumin, ribonuclease and β-lactoglobulin bind over 50% more sodium dodecyl sulphate by weight after reduction (Pitt-Rivers & Impiombato, 1968) and might be expected to have greatly altered mobilities during electrophoresis. However, the ribonuclease monomer (Fig. 5) and glutaraldehyde-treated ovalbumin oligomers did not change in mobility on reduction (Fig. 2). This suggests that, in 5% polyacrylamide gels, the opposing factors altered by

reduction that control the mobility of these proteins in the presence of sodium dodecyl sulphate roughly balance each other. On the other hand the mobilities of β-lactoglobulin and its oligomers decreased after reduction (Fig. 2) as did those of bovine serum albumin and its oligomers (Fig. 3), and the higher oligomers of lysozyme (Fig. 4) and ribonuclease (Fig. 5). These findings suggest that the increase in frictional resistance accompanying unfolding of these proteins has a greater effect on their mobility than the increase in their negative charge through binding of more sodium dodecyl sulphate; presumably the opposite occurs with the lysozyme monomer and the ribonuclease dimer as their mobilities increase after reduction. It is clear (Fig. 6) that a given cystine content affects the mobilities of different proteins to varying degrees, presumably because the shape of the protein after total denaturation without reduction depends on the position (as well as the number) of disulphide cross-links.

The measured molecular weights of some protein aggregates used as markers in sodium dodecyl sulphate–polyacrylamide-gel electrophoresis appear to be close to their theoretical values (Shapiro *et al.*, 1967; Dunker & Rueckert, 1969; Wolf *et al.*, 1970). Although this agreement is good for fully reduced proteins (Wolf *et al.*, 1970) the results reported here show that this may not be so with unreduced proteins. That the reduced bovine serum albumin tetramer migrated at about half the rate of the unreduced tetramer (Fig. 6) is particularly significant in view of the use of unreduced oligomers of this protein as standards for molecular-weight determination (Dunker & Rueckert, 1969). Generally even the reduced oligomers of the various proteins tested here migrated marginally faster than expected from the standard curve, perhaps because each was a branched rather than a linear polypeptide. Alternatively, during the reaction between glutaraldehyde and the lysine residues of these proteins (Wang & Tu, 1969) it is possible that some intramolecular glutaraldehyde cross-links may have been formed. The presence of such cross-links would be expected to increase the mobility of the reduced glutaraldehyde-linked protein aggregates compared with the standard curve, since the presence of several intramolecular cystine cross-links generally resulted in greater protein mobility. The reduced glutaraldehyde-linked oligomers differed from their unreduced equivalents only in that their intramolecular cystine residues had been reduced and alkylated. Thus, whatever the tertiary structure arising from the glutaraldehyde treatment, the observed difference in mobilities of each corresponding pair of glutaraldehyde-linked oligomers must have been a consequence of their different tertiary structure owing to the presence or absence of intramolecular disulphide bridges.

The anomalous behaviour of ribonuclease has been observed by others (Shapiro *et al.*, 1967; Dunker & Rueckert, 1969) and may be a result of its small size, since low-molecular-weight proteins more closely resemble spheres than rods in the presence of sodium dodecyl sulphate (Fish *et al.*, 1970). Although this might account for the anomalous behaviour of the ribonuclease monomer (Figs. 1 and 5) it does not explain why the dimer also migrates more slowly than expected from the standard curve (Fig. 5). It is possible that the amino acid composition of ribonuclease is responsible for its anomalous behaviour. It is known that the chemical nature of a protein may significantly affect the amount of sodium dodecyl sulphate it binds (Pitt-Rivers & Impiombato, 1968) and its electrophoretic mobility in sodium dodecyl sulphate–polyacrylamide gels (Swank & Munkres, 1971; Tung & Knight, 1971). Although when reduced, ribonuclease binds as much sodium dodecyl sulphate as other reduced proteins (Pitt-Rivers & Impiombato, 1968), the sodium dodecyl sulphate may be less firmly associated, since it is bound to proteins by hydrophobic interaction (Reynolds & Tanford, 1970*a*) and ribonuclease is relatively deficient in the large hydrophobic side chains of leucine, isoleucine and tryptophan.

The rate of migration of polypeptides during sodium dodecyl sulphate–polyacrylamide-gel electrophoresis is probably dependent on a complicated interplay of intrinsic charge, size and shape of polypeptides, and the variable binding of sodium dodecyl sulphate to them. It is clear from the evidence presented in this paper that the presence of several cross-links within a protein may substantially affect its mobility during sodium dodecyl sulphate–polyacrylamide-gel electrophoresis to an extent dependent on the protein and the number of links present. Thus, inaccurate molecular weights may be obtained by the sodium dodecyl sulphate–polyacrylamide-gel method where standards used are chemically linked protein aggregates or unreduced proteins such as bovine serum albumin and γ-globulin and their cystine-linked oligomers.

I thank Dr. D. C. Shaw for gifts of proteins and Dr. W. H. Sawyer for gifts of proteins and for helpful discussion. I am grateful to Mr. J. Ride for the computer analysis and to Mr. C. J. Durrant for outstanding technical assistance.

References

Canfield R. E. (1963) *J. Biol. Chem.* **238**, 2698
Canfield, R. E. & Liu, A. K. (1965) *J. Biol. Chem.* **240**, 1997
Castellino, F. J. & Barker, R. (1968) *Biochemistry* **7**, 2207
Cecil, R. (1963) in *The Proteins* (Neurath, H., ed.), 2nd. ed., vol. 1, p. 388, Academic Press, London
Connellan, J. M. (1968) Ph.D. Thesis, Australian National University, Canberra

Dunker, A. K. & Rueckert, R. R. (1969) *J. Biol. Chem.* **244**, 5074
Edelman, G. M., Cunningham, B. A., Gall, W. E., Gottlieb, P. D., Rutishauser, U. & Waxdal, M. J. (1969) *Proc. Nat. Acad. Sci. U.S.* **63**, 78
Fish, W. W., Reynolds, J. A. & Tanford, C. (1970) *J. Biol. Chem.* **245**, 5166
Griffith, I. P. (1970) Ph.D. Thesis, Australian National University, Canberra
Kakiuchi, K., Hamaguchi, K. & Isemura, T. (1965) *J. Biochem. (Tokyo)* **57**, 167
Maizel, J. V., Jr. (1969) in *Fundamental Techniques in Virology* (Habel, C. & Salzman, N. P., eds.), vol. 1, p. 334, Academic Press, New York
McKenzie, H. A. (1967) *Advan. Protein Chem.* **22**, 55
Neuberger, A. & Marshall, R. D. (1966) in *Glycoproteins: their Composition, Structure and Function* (Gottschalk, A., ed.), 1st. edn., p. 301, Elsevier, Amsterdam
Pitt-Rivers, R. & Impiombato, F. S. A. (1968) *Biochem. J.* **109**, 825
Reynolds, J. A. & Tanford, C. (1970*a*) *Proc. Nat. Acad. Sci. U.S.* **66**, 1002
Reynolds, J. A. & Tanford, C. (1970*b*) *J. Biol. Chem.* **245**, 5161
Rutishauser, U., Cunningham, B. A., Bennett, C., Konigsberg, W. H. & Edelman, G. M. (1969) *Proc. Nat. Acad. Sci. U.S.* **61**, 1414
Shapiro, A. L. & Maizel, J. V., Jr. (1969) *Anal. Biochem.* **29**, 505
Shapiro, A. L., Viñuela, E. & Maizel, J. V., Jr. (1967) *Biochem. Biophys. Res. Commun.* **28**, 815
Smyth, D. G., Stein, W. H. & Moore, S. (1963) *J. Biol. Chem.* **238**, 227
Summers, D. F., Maizel, J. V., Jr. & Darnell, J. E., Jr. (1965) *Proc. Nat. Acad. Sci. U.S.* **54**, 505
Swank, R. T. & Munkres, K. D. (1971) *Anal. Biochem.* **39**, 462
Tung, J.-S. & Knight, C. A. (1971) *Biochem. Biophys. Res. Commun.* **42**, 1117
Wang, J. H.-C. & Tu, J.-I. (1969) *Biochemistry* **8**, 4403
Weber, K. & Osborn, M. (1969) *J. Biol. Chem.* **244**, 4406
Wolf, B., Lausarot, P. M., Lesnaw, J. A. & Reichmann, M. E. (1970) *Biochim. Biophys. Acta* **200**, 180
Wu, Y. V. & Scheraga, H. A. (1962) *Biochemistry* **1**, 698