THE MOLECULAR WEIGHTS OF TWO REDUCED AND CARBOXYMETHYLATED KERATINS BY DISK GEL ELECTROPHORESIS AND A COMPARISON OF TWO METHODS OF ANALYSING THE RESULTS

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[*Manuscript received February* 11, 1970]

Summary

Two methods of treating the results of disk electrophoresis experiments with proteins on gels of different porosities so as to estimate molecular weights are discussed. It is concluded that the method based on the slopes of the logarithm of relative mobility versus gel concentration plots (Hedrick and Smith 1968) is equivalent to that of Parish and Marchalonis (1970) but is simpler.

The conclusion of Hedrick and Smith that size isomeric proteins give rise to logarithm of relative mobility versus gel concentration plots which intersect at a common point in the vicinity of zero gel concentration is shown to be not generally true.

The molecular weights of the reduced and *S*-carboxymethylated low-sulphur wool protein, component 7, and seven components of *S*-carboxymethylated feather keratin from duck rachis have been estimated from their relative electrophoretic mobilities in acrylamide gels of different porosities containing 8M urea. This technique gave a molecular weight of 51,400 for component 7, in good agreement with high-speed sedimentation equilibrium experiments which gave a value of 51,300. The molecular weights of six of the feather keratin bands were in the range $10,500 \pm 1,500$ and these species are therefore apparently charge isomers of the unit whose molecular weight by sedimentation equilibrium is 11,000. The other feather keratin band which is only present in small amount had a molecular weight of 37,500 and is assumed to be an aggregate of the smaller unit.

I. INTRODUCTION

Component 7 is one of the two major constituents of the low-sulphur group of proteins which can be extracted from wool after reduction and carboxymethylation of its disulphide bonds. The other is component 8. Together these proteins, each of which runs as a single band on starch gel electrophoresis, make up 70–90% of the reduced and carboxymethylated group of low-sulphur wool proteins called SCMKA (Thompson and O'Donnell 1964, 1965). Both components 7 and 8 are known to be chemically heterogeneous (Frater 1968; O'Donnell 1969) but extensive sedimentation equilibrium studies of the molecular weight of component 8 both in the presence and absence of urea have indicated that about 95% of the protein in the samples studied had a molecular weight of 45,000 (Jeffrey 1968, 1969). The best estimate of the molecular weight of the remaining 5% or so of protein in the mixture was $14,000 \pm 2,000$ and it seems quite likely that this was high-sulphur or high-glycine protein not removed in the preparation of component 8. It is thought that component 8 probably consists of a family of related proteins differing slightly in amino acid composition but of uniform molecular weight.

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Considerable difficulty was experienced in interpreting the results of sedimentation equilibrium experiments with component 8 and in reconciling them with molecular weights from calibrated Sephadex columns. This was because component 8 is a mixture and it was thought that disk electrophoresis on polyacrylamide gels might be useful in studies of component 7 and other keratins, both in its own right and in conjunction with sedimentation equilibrium experiments. The present work is an investigation of disk gel electrophoresis as a method of measuring the molecular weights of the reduced and carboxymethylated keratins component 7 and feather keratin in 8M urea solution. In two recent papers (Hedrick and Smith 1968; Parish and Marchalonis 1970) on the measurement of the molecular weights of proteins, essentially the same technique of disk electrophoresis on polyacrylamide gels is used but there are some differences in the way the results are treated and these are discussed in Section II. The present work, for reasons given later, uses the method employed by Hedrick and Smith (1968).

II. Materials and Methods

(*a*) *Gels*

The stock solutions from which the gels were prepared were of the same composition as those of Ornstein and Davis (1962) and using the nomenclature of those authors were denoted solutions A, B, C, D, and E. They were prepared from A.R. grade chemicals and glass-distilled water, filtered through 1·2 μm millipore filters, and kept in the dark at 20°C. The acrylamide (B.D.H.) was recrystallized from chloroform by the method of Loenig (1967) before use. The gels were prepared to have a final urea concentration of 8M and the running pH was about 9. Electrophoresis was carried out on running gels with concentrations of acrylamide monomer ranging from 6 to 15 g of acrylamide per 100 g of solution. These gels were all prepared by diluting the same stock solution (solution C) so that the ratio of the cross-linking agent *N,N'*-methylenebisacrylamide to acrylamide remained the same in all the gels. Hedrick and Smith (1968) found that changes in this ratio changed the sieving characteristics of the gel. The running gels were 4·5 cm long and the spacer gels (2·5% acrylamide) and the sample gels (3% acrylamide) 0·5 cm long. Electrophoresis was continued at a constant current of 5mA per tube in tubes of 6 mm internal diameter until the tracking dye bromothymol blue was about 1 cm from the end of the running gel (3 hr 15 min). At the conclusion of a run the gels were removed from the tubes and the position of the tracking dye marked by inserting a piece of 35-gauge copper magnet wire through the gel in line with the front of the dye band.

(*b*) *Proteins*

Bovine plasma albumin (Cohn fraction V, Commonwealth Serum Laboratories, Australia), ovalbumin (five times crystallized, lot 5, Pentex) and bovine β-lactoglobulin (lot 4814, Pentex) were reduced and carboxymethylated as described previously (Jeffrey 1968) and stored in the cold in 8M urea. *S*-Carboxymethylated (SCM) feather keratin was prepared from duck feather rachis by the method of Harrap and Woods (1964*a*). SCM rabbit tropomyosin was supplied by Mr. E. F. Woods (Woods 1967) and had been purified by passages through DEAE-cellulose and Sephadex G-200 in 8M urea. The component 7 was prepared by Mr. L. M. Dowling by fractional precipitation from an extract of low-sulphur wool proteins from Merino wool. It had been further purified by passages through DEAE-cellulose and Sephadex G-200 in 8M urea and the sample used in this study was from a single tube from the Sephadex experiment. The starch gel pattern showed a broad intense main band with a trace of slower moving material behind it. The feather keratin tropomyosin and component 7 were all stored in the cold in buffers containing 8M urea and before being used for disk electrophoresis were dialysed against 8M urea so that the buffer ions were removed. Unless otherwise stated the protein samples used for electrophoresis contained 20 μg of protein in 0·1 ml of 8M urea solution.

(c) *Ultracentrifuge Experiments*

The sedimentation equilibrium experiments with component 7 were of the high-speed, meniscus-depletion type (Yphantis 1964) and were carried out at 52,600 r.p.m., 25°C, 3 mm. solution columns in a Spinco model E ultracentrifuge. The technique used both for the experiments and measuring the Rayleigh interference fringe patterns was exactly as described previously (Jeffrey 1968). Component 7 was dissolved in a buffer solution of pH 7·00 consisting of 0·01M Tris, 0·001M EDTA, 0·2M sodium chloride, and 8M urea and its final concentration was 0·02 g/100 ml.

(d) *Gel Electrophoresis*

After electrophoresis the position of the tracking dye was marked as described above and the gels were stained overnight in 0·02% amido black in 7·5% acetic acid or 0·25% coomassie brilliant blue after overnight precipitation with 5% trichloroacetic acid in 7·5% acetic acid. Amido black was used for all the proteins except feather keratin which did not stain under these conditions. Coomassie brilliant blue was used for feather keratin and also for component 7. Component 7 stains well with amido black but high-sulphur wool proteins do not and coomassie blue was used to check for their presence. The gels were destained by washing from 1 to 2 days with several changes of 10% acetic acid and were stored in this solvent in stoppered Pyrex tubes. The distance moved by the protein and dye bands from the junction of the spacer gel and running gel was measured with a Nikon microcomparator fitted with a projection screen. The gel in a Pyrex tube was held in a Perspex rack which was constructed to fit into the stage of the microcomparator and allow light to be transmitted through the gel. Measurements were made to the leading edges of the bands and were reproducible to better than 0·05 mm. The migration of a band was expressed as a relative mobility R_m defined as the ratio of distance moved by protein band to the distance moved by dye band, i.e.

$$R_m = d_{\text{protein}}/d_{\text{dye}}. \tag{1}$$

Relative mobilities were measured for all of the proteins at gel concentrations of 6, 8, 10, 12, and 14% and experiments were usually done in duplicate. It was found that when the logarithm of the relative mobility was plotted against the gel concentration a linear relationship was obtained for all of the proteins investigated over the gel concentration range mentioned above.* This is in agreement with the results of Hedrick and Smith (1968) and Parish and Marchalonis (1970). The slopes of these plots were determined by fitting the points with linear regressions by the method of least squares.

III. Treatment of Results

Hedrick and Smith (1968) studied a number of native proteins with molecular weights ranging from 45,000 to 500,000 at a pH about one unit below that used in this work and found that a linear relationship was obtained when the slopes of the log R_m versus gel concentration graphs were plotted against molecular weight. This plot was used as a calibration line for determining the molecular weight of an unknown protein from its rates of migration on acrylamide gels of different porosities. Parish and Marchalonis (1970) obtained their calibration graph by a different method. They used the linear relationship between log R_m and gel concentration to obtain a "frictional ratio" for each protein for increments of gel concentration ranging from 1 to 8% and found that when these frictional ratios were plotted against molecular weight a series of curves was obtained. The form of each curve depended on the increment in gel concentration for which the frictional ratio was evaluated and a straight line was obtained for a gel increment of 4%. This line was used as the calibration graph. Parish and Marchalonis (1970) used a series of reduced and

* In general, logarithms to base "e" are used for theoretical discussions, but for convenience logarithms to base "10" are used for plotting experimental results.

carboxymethylated proteins with molecular weights ranging from 18,000 to 138,000 in concentrated urea solutions at acid pH as their calibrating proteins. It is interesting to see how the curves which Parish and Marchalonis (1970) obtained when the frictional ratio was plotted against molecular weight arise and how this method of treating the log R_m plots is related to that used by Hedrick and Smith (1968).

Since the logarithm of the relative mobility is found to be linearly related to the gel concentration over the range of gel concentrations and molecular weights which have been investigated, we can write

$$R_m = a\mathrm{e}^{kg},$$

where g is the gel concentration in g/100 g and a and k are constants for a particular protein. From equation (1),

$$d_{\text{protein}}/d_{\text{dye}} = a\mathrm{e}^{kg}. \tag{2}$$

The distance moved through a medium by a charged particle of electrophoretic mobility m in time t under a potential gradient of V volts/cm is given by

$$d = mtV.$$

Also,

$$m = QX/f,$$

where Q is the net charge of the particle, X is a factor by which the net charge is modified depending on the ionic environment, and f is the frictional resistance of the particle and is dependent on its size and shape (Ornstein and Davis 1962). Thus

$$d = QXtV/f. \tag{3}$$

Equation (3) applies to both the protein and the dye and if the migration is occurring in a gel of concentration g_1, equation (2) becomes

$$(Q_pX_pf_d/Q_dX_df_p)_1 = a\mathrm{e}^{kg_1}, \tag{4a}$$

where the subscripts p and d refer to protein and dye respectively. Similarly for a gel of concentration g_2

$$(Q_pX_pf_d/Q_dX_df_p)_2 = a\mathrm{e}^{kg_2}. \tag{4b}$$

The ionic environment is the same in the two gels and the same protein and dye are used so

$$(X_pQ_p)_1 = (X_pQ_p)_2,$$

$$(X_dQ_d)_1 = (X_dQ_d)_2,$$

and

$$(f_d/f_p)_1(f_p/f_d)_2 = a\mathrm{e}^{kg_1}/a\mathrm{e}^{kg_2} = \mathrm{e}^{k\Delta g}. \tag{5}$$

If we assume that the frictional resistance experienced by the tracking dye is the same in the two gels,

$$(f_p)_2/(f_p)_1 = \mathrm{e}^{k\Delta g}. \tag{6}$$

In general the frictional resistance experienced by the tracking dye will be different in gels of different concentration but this only means that what is defined as the frictional ratio contains the dye frictional ratio as well as that of the protein. Parish and Marchalonis (1970) defined the frictional ratio as $f_2/f_1 = m_1/m_2$ and as we have shown above, this is equal to $\mathrm{e}^{k\Delta g}$. Thus when these workers plotted frictional

ratio for various increments of gel concentration versus molecular weight they were plotting $e^{k\Delta g}$ versus molecular weight for various values of Δg and k. The constant k is characteristic of each protein and is not explicitly evaluated when frictional ratios are used as above. Equation (2) was based on the empirical fact that the logarithm of the relative mobility of a protein is linear with gel concentration or $\ln R_m = \ln a + kg$. The slope of the plot of $\ln R_m$ versus gel concentration is k and Hedrick and Smith (1968) found that when the values of k for a series of proteins of known molecular weight were plotted against the molecular weight, a linear relationship was obtained. The same result with a different set of proteins under different conditions is found in the present work (see Section IV) and it is apparent from equation (6) that the value of k for a given protein can be evaluated from its frictional

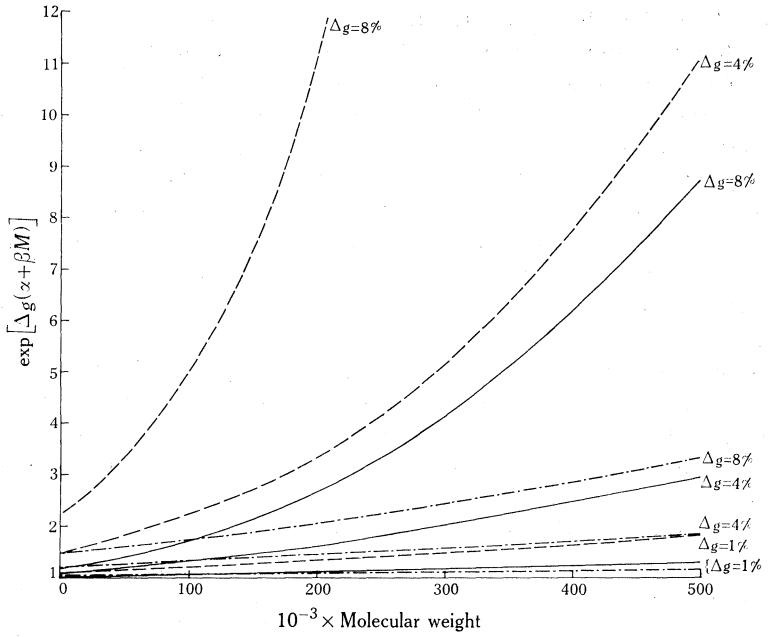

Fig. 1.—Plot of $\exp[\Delta g(\alpha+\beta M)]$ versus M. Dashed lines ($\alpha = 0{\cdot}1$, $\beta = 0{\cdot}001$) correspond approximately to the results of Parish and Marchalonis (1970). Solid lines ($\alpha = 0{\cdot}02$, $\beta = 0{\cdot}0005$) correspond approximately to the present work. Dashed and dotted lines ($\alpha = 0{\cdot}05$, $\beta = 0{\cdot}0002$) correspond approximately to the results of Hedrick and Smith (1968).

ratio if Δg is known. When this was done from the results of Parish and Marchalonis (1970) it was found that k was also linear with molecular weight for their calibrating proteins and conditions. Thus (again empirically) we can write

$$k = \alpha+\beta M, \tag{7}$$

where M is the molecular weight and α and β are constants whose values depend on the group of proteins used for the calibration and the experimental conditions. Plots of frictional ratio versus molecular weight can therefore be regarded as plots of $\exp[\Delta g(\alpha+\beta M)]$ versus M. The form taken by such plots for different values of Δg, α, and β is shown in Figure 1 where the values of the parameters have been chosen

to cover the range of k found experimentally (Hedrick and Smith 1968; Parish and Marchalonis 1970; this work). It is apparent from Figure 1 that all of the curves are exponential in form and do not show the tendency to become concave to the abscissa for low values of Δg which Parish and Marchalonis (1970) found for values of Δg less than 4%. This behaviour may have arisen from experimental errors in evaluating frictional ratios at low values of Δg. The curves with $\alpha = 0{\cdot}1$ and $\beta = 0{\cdot}001$ should be closely similar to the frictional ratio curves of Parish and Marchalonis (1970) for which I obtain values of about $0{\cdot}09$ for α and $0{\cdot}001$ for β from their diagram. Figure 1 shows that the curve with these parameters is not linear when $\Delta g = 4\%$ if an extended range of molecular weights is considered although it is approximately linear as Parish and Marchalonis (1970) found if only the range of molecular weights below 140,000 is considered. Figure 1 also emphasizes the need, when using the technique of acrylamide gel electrophoresis for measuring molecular weights, of studying calibrating proteins of the same characteristics and under the same conditions (i.e. the same α and β) as the proteins of unknown molecular weight. Since one cannot know this with certainty the possibility of getting incorrect molecular weights by this method must be considered and such molecular weights should be checked by an independent method. Finally it seems that the method of plotting frictional ratios versus molecular weights to get a calibration graph is an unnecessary complication because as shown above such plots arise because of the linear dependence of k on molecular weight. It is therefore simpler to use the plot of k versus molecular weight as the calibration graph as Hedrick and Smith (1968) did and as done in the present work.

Hedrick and Smith (1968) concluded from a study of the ln R_m versus gel concentration graphs for a number of proteins that such graphs would be: (1) a family of parallel lines for proteins which were charge isomers; (2) a family of non-parallel lines intersecting at gel concentrations other than zero for proteins which differ in size and charge; and (3) a family of non-parallel lines intersecting at or near zero gel concentration for proteins which were size isomers.

Point (1) follows from equation (7), i.e. proteins with the same molecular weight have the same values of k. Point (2) is apparent by combining equations (2) and (3):

$$[(QXtV/f)_{\text{protein}}]/[d_{\text{dye}}] = ae^{kg}. \tag{8}$$

This equation is a statement of the fact that proteins of different charge (Q) and different size (f) will give rise to plots of ln R_m [the left-hand side of equation (8)] versus gel concentration (g) which have different intercepts (ln a) and different slopes (k). From equation (8), at $g = 0$,

$$[(QXtV/f)_{\text{protein}}]/[d_{\text{dye}}] = a.$$

Note that $g = 0$ corresponds to the gel concentration where the protein has the same mobility as in free solvent and this is not necessarily equal to a weight/weight gel concentration of 0%. For two proteins of different size and charge

$$[Q_1X_1tV/f_1]/[d_{\text{dye}}] = a_1,$$

and

$$[Q_2X_2tV/f_2]/[d_{\text{dye}}] = a_2.$$

If the effective charges on the two proteins are the same but their sizes are different [point (3) above], $Q_1X_1 = Q_2X_2$ but $f_1 \neq f_2$ so $a_1 \neq a_2$. Therefore, in general, proteins which are size isomers will not give rise to a family of lines intersecting at a common point when ln R_m is plotted against g. It is possible that with a group of proteins which are polymers of the same unit, the size and charge factors might vary in such a way that QX/f remains constant or at least constant within experimental error, over a limited degree of polymerization and then the ln R_m versus g lines would intersect at a common point. This apparently occurs with plasma albumin monomers, dimers, and trimers as Hedrick and Smith (1968) found a common intersection of the three lines for these polymers at a gel concentration near 2%. These authors claimed that the ferritin monomer, dimer, and trimer plots also intersect at a common point near 1% gel concentration; however, from their Figure 4 there appears to be a difference of about 1% in gel concentration between the points where the monomer and dimer and trimer lines intersect.

It is concluded from the above discusssion that when log R_m is plotted versus g, a group of proteins which differ in charge but are of the same size will give rise to a family of parallel lines, but proteins which differ in size or in size and charge will in general give rise to a family of lines which do not intersect at a common point. This is another way of saying that the intercept of log R_m versus g plots depends on both size and charge whereas the slope is independent of charge.

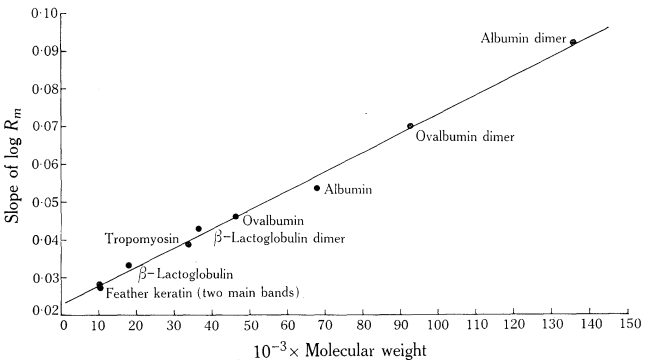

Fig. 2.—Calibration graph of the slope of the log R_m versus gel concentration graphs against molecular weight. The proteins were in the reduced and alkylated form in 8M urea and the slopes were obtained from least squares linear regressions. The line is the least squares regression line through the points.

IV. Results

Figure 2 shows the linear relationship obtained when the slope of log R_m versus gel concentration was plotted against the known molecular weights of a number of reduced and alkylated proteins with molecular weights ranging from 10,000 to 138,000. This graph was used to determine the molecular weights reported below.

SCM feather keratin from fowl rachis has a molecular weight of 11,000 by sedimentation equilibrium after a small amount of aggregate has been removed by preliminary centrifugation (Harrap and Woods 1964*b*) and 10,400 by osmotic pressure (Jeffrey 1969). It is known to be electrophoretically heterogeneous by

moving boundary electrophoresis (Harrap and Woods 1964*b*). In the present work 200 μg of reduced and carboxymethylated feather keratin which had been given a preliminary centrifugation was loaded on the gels and on some gels 10 bands could be counted after staining with coomassie brilliant blue. One was always predominant (Fig. 3) and this was used for the calibration and assigned a molecular weight of 10,500. Figure 2 shows this assignment to be correct as the slopes of the log R_m plot of the band referred to lies on the same line as the rest of the calibrating proteins. Six other bands could be detected and measured at enough gel concentrations to allow meaningful plots of log R_m versus gel concentration to be made and the resulting lines are shown in Figure 4(*a*). Six of the lines in this figure are very nearly parallel and their regression slopes give molecular weights ranging from 9,000 to 12,000, that is 10,500$\pm$1,500. The six components giving rise to these lines therefore are

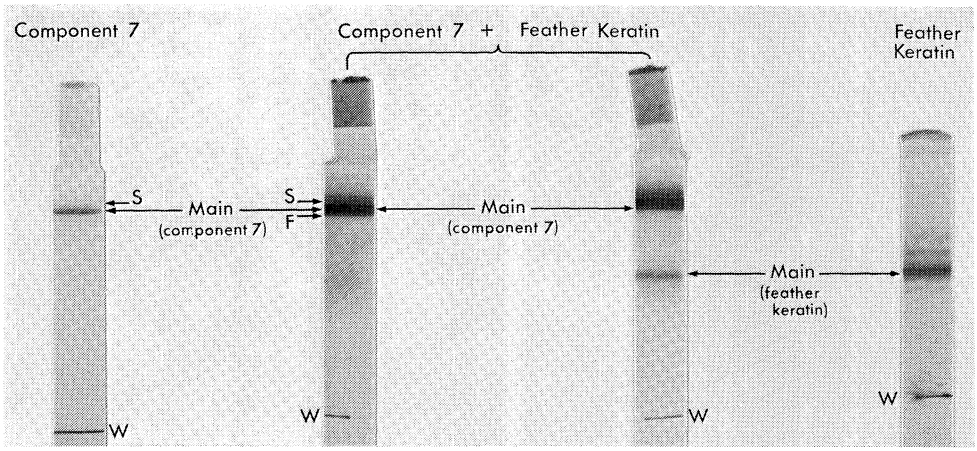

Fig. 3.—Disk gel electrophoresis of component 7 and feather keratin, singly and in mixture, in 12% acrylamide gels containing 8M urea. The two left-hand gels were stained with amido black and the two right hand gels with coomassie brilliant blue. The mixture loaded on the two central gels contained 60 μg of component 7 and 100 μg of feather keratin while the amounts of component 7 and feather keratin loaded in the individual experiments were 20 μg and 200 μg respectively. The position of the tracking dye (bromothymol blue) is shown by the position of the copper magnet wire (W) inserted immediately after electrophoresis was completed. Slow (S) and fast (F) bands of component 7 are also indicated.

charge isomers of the feather keratin molecule of molecular weight 11,000. The slope of the other line gives a molecular weight of 37,500 and seems to represent a trimer or tetramer of the basic unit, i.e. a trace of the aggregate referred to above which was not removed by the preliminary centrifugation. These results are therefore in agreement with those of Harrap and Woods (1964*b*) in that reduced and carboxymethylated feather rachis keratin is essentially homogeneous with respect to molecular weight except for the presence of a small proportion of aggregate which they estimated to have a molecular weight of 40,000.

The gel electrophoresis experiments with component 7 all showed the presence of an intense main band and sometimes one faint band could be discerned just in front of the main band and one faint band just behind it. These are referred to as

the main band, the fast band, and the slow band (Fig. 3). Figure 4(*b*) shows the log R_m versus gel concentration plots for the main band and the slow band of component 7. The molecular weights which the calibration plot gave for these two bands were 52,500 and 50,200 respectively so they are probably charge isomers of the same-sized unit. The fast band could not be measured at sufficient gel concentrations to derive a molecular weight in these experiments. However, in experiments with mixtures of feather keratin and component 7 where the amount of component 7 loaded was greater than that used above, measurements could be made and gave a molecular weight for the fast band of 43,000. The fast band is probably a trace of component 8 which does run ahead of component 7 on starch gels and has been shown to have a molecular weight of 45,000 in 8M urea solutions (Jeffrey 1968). Two meniscus-depletion, sedimentation-equilibrium experiments were done on the

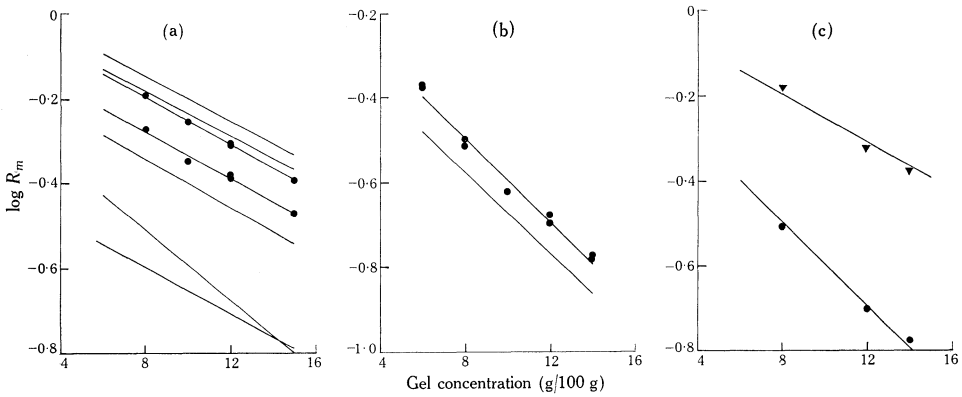

Fig. 4.—(*a*) Logarithm of R_m versus gel concentration for the seven bands measured for feather keratin from duck rachis in 8M urea. The individual points are shown only for the two most prominent bands. The lines are the least squares regression lines and the slopes of the six nearly parallel ones give molecular weights (from Fig. 2) ranging from 9,000 to 12,000, while that of the remaining line corresponds to a molecular weight of 37,500. (*b*) Logarithm of R_m versus gel concentration for the main band and the slow band of component 7 in 8M urea. The individual points are shown only for the main band. Both lines are least squares regression lines and their slopes give molecular weights of 52,500 (main band) and 50,200 (slow band). (*c*) Logarithm of R_m versus gel concentration for the main bands of component 7 (●) and feather keratin (▼) in 8M urea, measured in a mixture of the two, are shown as points. The least squares regression lines obtained for the main bands of the two proteins measured separately are shown as solid lines.

sample of component 7 used for the disk gel electrophoresis and point average weight and number average molecular weights were evaluated as described previously (Jeffrey 1968). Reciprocal plots of weight average and number average molecular weight versus concentration were extrapolated to infinite dilution and gave values of $M_w = 50{,}000$ and 52,600 and $M_n = 50{,}000$ and 50,000 for the two experiments. These runs were at high speed and the agreement between the weight and number average molecular weights together with the fact that no dependence of molecular weight on concentration was detectable is good evidence for the absence of species of lower molecular weight. The absence of any increase in apparent molecular weight near the bottom of the solution columns indicated the absence of any aggregated

material. The concentration ranges covered in the experiments were too small for any thermodynamic non-ideality to be detectable. The molecular weight of component 7 in 8M urea is thus between 50,000 and 52,600 by sedimentation equilibrium and between 50,200 and 52,500 by disk gel electrophoresis. The extremely good agreement between these values indicates that disk gel electrophoresis in 8M urea should be a useful and valid technique for measuring the molecular weights of reduced and alkylated wool proteins.

In order to see whether the migration of component 7 on acrylamide gels was affected by the presence of another protein, a mixture of component 7 and feather keratin was loaded on gels of different concentrations and the relative mobilities of the bands measured. The loading was 60 μg of component 7 and 100 μg of feather keratin and one set of gels was stained with amido black and one with coomassie blue. Since feather keratin did not stain with amido black this procedure allowed the bands to be identified (Fig. 3). The logarithms of the relative mobilities of the main bands of feather keratin and component 7 as measured in the mixture are compared with those measured alone in Figure 4(*c*) where it is evident that the migration of neither protein has been affected by the presence of the other.

V. Discussion

The ways in which the results of disk gel electrophoresis experiments on gels of different porosities are treated to obtain the molecular weights of charged proteins are discussed in Section III. It is emphasized that as with the use of gel filtration on Sephadex the method relies on the unknown proteins having the same conformation in solution as the calibrating proteins. Acrylamide gel electrophoresis has two advantages over Sephadex gel filtration in the estimation of the molecular weights of proteins or peptides: the small quantity of material required (micrograms instead of milligrams) and the potential extra resolving power offered by an electric field acting on a mixture of charged particles. It takes about the same time to measure a molecular weight by the two methods. Molecular weights obtained by either should always be checked by some other method which is more soundly based theoretically such as sedimentation equilibrium.

The experimental error of the method was assessed from the error in the slopes of the log R_m versus gel concentration plots for the six bands of feather keratin which apparently represent charge isomers of a unit of the same molecular weight and which should therefore all have the same slope. The maximum error in the molecular weights was 3000 and the root mean square error $\pm$900. The same errors were obtained when the method of Parish and Marchalonis (1970) was used. The absolute error in the slopes of the log R_m plots should remain the same over a range of molecular weight providing the gel concentrations are chosen to give R_m values lying between about 0·1 and 0·8. In the experiments reported here this covered the molecular weight range from 10,000 to 140,000. The root mean square error in a molecular weight of 50,000 would therefore be expected to be $\pm$1000 or $\pm$2% and this is in good agreement with the idea that the main band and the slow band of component 7 both have the same molecular weight, namely 51,400$\pm$1000. The molecular weight of component 7 has not been measured before but a value of 51,000 is consistent with the observation that component 7 runs slightly ahead of component

8 (molecular weight 45,000) on Sephadex G-200 (Thompson and O'Donnell 1965). The errors discussed above, however, refer to the precision of the experimental technique and do not take into account possible inaccuracies in the molecular weights due to the failure of a given protein to behave in the same way as the calibrating proteins.

Some solutions of component 8 have been found to contain small amounts (5% or less) of material of molecular weight about 14,000 even after repeated fractionation and it is suspected that these are high-sulphur or high-glycine proteins (Jeffrey 1969). Neither the sedimentation equilibrium nor the gel electrophoresis experiments showed any sign of low molecular weight material in the present sample of component 7 although sedimentation equilibrium on other samples prepared in the same way have done. Probably the low molecular weight material which is present in some preparations of component 7 is the same as that found in component 8 solutions. In either case its presence is unpredictable and the possibility of it being fragments split off the major proteins somehow in the course of their preparation has not been ruled out.

The extraction of unique solutions from the results of sedimentation equilibrium experiments on mixtures of proteins is extremely troublesome and sometimes impossible. The gel electrophoresis technique discussed above should be very helpful in conjunction with such experiments by showing how many components one is dealing with and providing estimates of their molecular weights which can be used as starting points in the analysis of the sedimentation equilibrium data.

VI. Acknowledgments

I thank Mr. E. F. Woods and Mr. L. M. Dowling for gifts of proteins and Dr. C. R. Parish for a prepublication copy of his paper. I also thank Dr. R. D. B. Fraser for helpful advice.

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