Aust. J. Biol. Sci., 1979, **32**, 423–35

Microfibrillar Proteins of Wool: Partial Specific Volumes and Molecular Weights in Denaturing Solvents

E. F. Woods

Division of Protein Chemistry, CSIRO, Parkville, Vic. 3052.

Abstract

The molecular weights of the reduced and *S*-carboxymethylated microfibrillar protein components of wool have been investigated by sedimentation equilibrium in 6 M guanidine hydrochloride and by sodium dodecyl sulfate–polyacrylamide gel electrophoresis. The microfibrillar proteins have molecular weights of 57 000 (component 5), 58 000 (component 7c); 50 000 (component 8c-1) with a range of values for the other component 8 polypeptide chains of 45 000–50 000. The proteins migrate anomalously in polyacrylamide gels in the presence of sodium dodecyl sulfate as seen from a comparison of the free mobilities and retardation coefficients of standard proteins with those of the wool proteins. More reliable molecular weights were obtained by plotting the retardation coefficients against molecular weights (Ferguson plot). The partial specific volumes of the microfibrillar proteins have been measured in dilute aqueous buffer solutions, 8 M urea and 6 M guanidine hydrochloride. The values are compared to those calculated from the amino acid compositions.

Introduction

The molecular weights of the microfibrillar proteins of wool keratin (low-sulfur fraction) have been the subject of numerous investigations and the measurements prior to 1971 have been reviewed by Jeffrey (1972). Since this time gel electrophoresis techniques have shown that the two major components of the low-sulfur fraction (7 and 8) give multiple bands. Component 7 has been resolved into three bands, 7a, 7b and 7c, and component 8 into four bands, 8a, 8b, 8c-1 and 8c-2 (Sparrow and Crewther 1972; Crewther *et al.* 1976). The other low-sulfur protein, component 5, has not been resolved further on gels.

The determination of the molecular weights of components 7 and 8 in the ultracentrifuge has been fraught with difficulty due to the presence of both low and high molecular weight contaminants (Jeffrey 1969, 1972). The meniscus depletion method of Yphantis (1964) is ideally suited for the determination of molecular weights by sedimentation equilibrium where only small amounts of material are required. In the case of non-ideal solvents such as 8 M urea or 6 M guanidine hydrochloride used for the study of subunits, complications arise and a protein solution may appear to be homogeneous and ideal, whereas it is actually heterogeneous and non-ideal. In these cases a weight-average molecular weight extrapolated to infinite dilution from conventional low-speed sedimentation equilibrium (Richards *et al.* 1968) may be more representative of the major component. This paper reports the results of both these kinds of measurements on components 5 and 7c which have not been characterized previously and component 8c-1. The apparent partial specific volumes in denaturing

solvents of these three polypeptide chains are also reported thus removing one uncertainty in the determination of molecular weights by the sedimentation equilibrium method.

Molecular weights were also determined by sodium dodecyl sulphate–polyacrylamide gel electrophoresis* (Shapiro *et al.* 1967; Weber and Osborn 1969). Some caution is necessary in the interpretation of the results in view of possible anomalous behaviour (Frank and Rodbard 1975; Rodbard 1976) and this is considered in relation to the results obtained with the low-sulfur wool proteins.

Materials and Methods

Materials

All reagents used were analytical grade. Urea solutions were de-ionized prior to addition of buffer salts by passage through a column of mixed-bed, ion-exchange resin. The guanidine hydrochloride was prepared from recrystallized guanidine carbonate (Nozaki 1972).

Protein Preparation

The proteins were prepared from Merino wool by the method of Crewther *et al.* (1976). A mixture of components 7b and 7c was used for partial specific volume and refractive index increment measurements; likewise the component 8 used was a mixture of 8b and 8c. For the molecular weight determination in the ultracentrifuge the purified components 7c and 8c-1 were used. They were given a further purification by passage through Sephacryl S200 and chromatography on DEAE-cellulose. This served to remove low molecular weight species and material originating from the Sephadex. The proteins were clarified by centrifugation at 80 000 *g* (30 000 r.p.m.) for 1 h in a model L ultracentrifuge before dialysis for measurements of density or sedimentation equilibrium.

Determination of Protein Concentrations

The protein concentrations of dilute aqueous buffer solutions were determined from the dry weights (110–115°C *in vacuo* for 48 h) of the solution and its diffusate after dialysis equilibrium with the usual corrections for buoyancy and for the volume of salt excluded by the protein in solution. Protein concentrations in denaturing solvents were determined spectrophotometrically with a Beckman DB spectrophotometer at 277 nm. The extinction coefficients were determined by gravimetric dilution of protein solutions of known concentration with guanidine hydrochloride or urea solutions of appropriate concentrations so that the final diluted solution was 6 M guanidine hydrochloride or 8 M urea.

Apparent Partial Specific Volumes

These were determined from the densities of dialysed solutions and their diffusates. The densities were measured with a precision density meter DMA-02 (Anton Paar, Graz) and the temperature was maintained at $20 \pm 0{\cdot}02$°C with a circulating water bath. The instrument was calibrated with sucrose solutions and was checked periodically during the course of the measurements. The measurement procedure was similar to that described by Lee and Timasheff (1974*a*). Protein concentrations were in the range 4–20 mg/ml. No concentration dependence of the calculated specific volumes was observed. For the denaturing solvents each protein concentration represented a separate dialysis experiment.

The apparent partial specific volume, ϕ_2', was calculated from the equation

$$\phi_2' = 1/\rho_0\{1-(\rho-\rho_0)/c\},$$

where c is the protein concentration in g/ml and ρ and ρ_0 are densities of solution and diffusate respectively in g/ml. The quantity ϕ_2' represents the apparent partial specific volume at fixed chemical potential of the added diffusible components, i.e. buffer salts, guanidine hydrochloride or urea. For

* Abbreviation: SDS gel electrophoresis.

multicomponent systems the insertion of ϕ_2' into the equilibrium molecuiar weight equations gives the molecular weight of the anhydrous protein component (Casassa and Eisenberg 1964). This component will include any buffer salts bound to the protein since the concentrations all refer to the dry weight of the protein component after dialysis.

Refractive Index Increment Measurements

Increments in refractive index (ml/g) of the low-sulfur wool proteins in aqueous buffer and denaturing solvents were measured at 20°C on the dialysed solutions with a Brice–Phoenix differential refractometer and these values are given in the following tabulation:

Solvent	Component 5	Component 7[A]	Component 8[A]
0·05 M sodium phosphate, pH 7·0	0·184	0·183	0·183
0·01 M sodium tetraborate, pH 9·2	0·186	0·186	0·186
8 M urea–0·01 M sodium tetraborate, pH 9·2	0·144	0·137	0·145
8 M urea–0·05 M sodium phosphate, pH 7·0	0·152	0·146	0·153
6 M guanidine hydrochloride	0·133	0·129	0·138

[A] Component 7 was a mixture of component 7b and 7c and component 8 a mixture of 8b, 8c-1 and 8c-2.

Sedimentation Equilibrium

Experiments were carried out at 20°C with a Beckman model E ultracentrifuge equipped with Rayleigh interference optics. Molecular weights were measured by the conventional low-speed equilibrium method (Richards *et al.* 1968) and the meniscus depletion method (Yphantis 1964). A six-channel centrepiece was employed for the conventional equilibrium runs with column heights of 1·6 mm; for the meniscus depletion experiments regular double-sector cells were used with column heights of 3 mm. The fluorocarbon, FC 43 (Beckman) was used as a base fluid in all cases.

For the conventional equilibrium experiments the apparent weight-average molecular weight over the whole cell was calculated from the equation:

$$M^{\text{app}}_{\text{w, cell}} = \frac{2(c_b - c_m)RT}{c_0(r_b^2 - r_m^2)} \times \frac{RT}{(1-\phi_2'\rho)\omega^2},$$

and the apparent point-average molecular weights from

$$M^{\text{app}}_{\text{w,r}} = \left[\frac{\text{d}(\ln c)}{\text{d}(\frac{1}{2}r^2)}\right]_r \times \frac{RT}{(1-\phi_2'\rho)\omega^2},$$

where c_b, c_m are the concentrations in interference fringe numbers at the base and meniscus respectively, c_0 is the initial concentration and r_b, r_m and r distances from the centre of rotation, ω the centrifuge speed in radians/sec, R the gas constant and T the temperature.

The value of c_0 required for the calculation of the apparent weight-average molecular weight over the whole cell was determined from the refractive index difference between the solution and its diffusate. As with the partial specific volume measurements special care was required in transfering solutions of guanidine hydrochloride and urea to the refractometer cell in order to maintain dialysis equilibrium. In the evaluation of the equilibrium patterns the fringes were labelled by the procedure based on conservation of mass in the cell employing the computer program of Teller (1965). A polynomial was then fitted to the ln c versus r^2 data for the calculation of apparent point-average molecular weights. A quadratic equation adequately represented the data in most cases but occasionally a cubic equation gave a marginally better fit. The computer program of Roark and Yphantis (1969) was used to calculate the number-, weight- and z-average molecular weights for points throughout the cell in the meniscus depletion experiments.

SDS Gel Electrophoresis

Electrophoresis at pH 7 in 0·1% (w/v) SDS–0·05 M phosphate buffer was carried out by the technique of Weber and Osborne (1969). The samples were incubated for 2 min in the presence of 1% (w/v) SDS and 1%(w/v) 2-mercaptoethanol at 95°C before loading on to the gels. Bromphenol blue was used as a tracking dye and the position of the dye band marked with a wire after electrophoresis. Gels were stained with Coomassie Brilliant Blue G-250 (I.C.I.) and destained with 5% (w/v) methanol–7·5% (w/v) acetic acid. Gels were scanned with a Shimadzu dualwavelength TLC scanner at 590 nm. Relative mobilities, R_F, were calculated in the usual way. The following proteins were used as molecular weight standards: α-tropomyosin, 32800; yeast alcohol dehydrogenase, 36700; fructose bisphosphate aldolase, 39000; ovalbumin, 44100; fumarate hydratase, 48500; bovine liver glutamate dehydrogenase, 56100; catalase, 57500; bovine serum albumin, 66200. With the exception of fumarate hydratase the molecular weights are from published amino acid sequences. The retardation coefficient, K_R, was estimated from measurements at several gel concentrations from the Ferguson (1964) equation

$$\log R_F = -K_R T + \log Y_0,$$

where T is the total percentage (w/v) acrylamide concentration and Y_0 is the relative mobility at zero gel concentration, i.e. the free electrophoretic mobility. Gel concentrations from 5 to 10% at intervals of 0·5% were investigated but only those in the range 6–9% were used for calculations since for some proteins non-linearity in the log R_F versus T plot was observed outside this range. Each R_F value was the mean of at least two runs with duplicate gel tubes for each protein in each run. The molecular weights, M, of the wool proteins were estimated from the plot of K_R versus M for the calibrating proteins.

Table 1. Apparent specific volumes, ϕ_2', of low-sulfur wool proteins in aqueous buffer and in denaturing solvents

Numbers in brackets are the number of measurements, i.e. individual dialysis equilibrium experiments involving both a density and concentration measurement

Solvent	Specific volumes (ml/g)			
	SCMKA	Component 5	Component 7[A]	Component 8[A]
0·05 M sodium phosphate, pH 7·0	0·707 (2)	0·718 (3)	0·718 (1)	0·714 (4)
0·01 M sodium tetraborate, pH 9·2		0·712 (2)	0·707 (2)	0·700 (3)
8 M urea–0·05 M sodium phosphate, pH 7·0	0·705 (4)	0·709 (4)	0·716 (14)	0·708 (3)
8 M urea–0·01 M sodium tetraborate, pH 9·2		0·711 (2)	0·713 (5)	0·705 (5)
6 M guanidine hydrochloride		0·706 (7)	0·710 (14)	0·698 (8)

[A] Component 7 was a mixture of 7b and 7c and compound 8 a mixture of components 8b, 8c-1 and 8c-2.

Results and Discussion

Apparent Partial Specific Volume Measurements

The apparent partial specific volumes of the whole low-sulfur complex (*S*-carboxymethylkerateine A, SCMKA) and components 5, 7 and 8 are given in Table 1. The standard deviation of measurements in phosphate and borate buffers was 0·002 ml/g. The main contribution to the standard deviation arises from errors in the measurement of protein concentration since replicate density measurements on the same solutions indicated a standard deviation of 0·0003 ml/g arising from the measurement of density.

An error of 1% in the determination of the protein concentration by dry weight leads to an error of 0·003 ml/g in the value of the specific volume. The standard deviation of measurements in urea and guanidine hydrochloride was 0·005 ml/g. The greater error in denaturing solvents is due to the difficulty of maintaining equilibrium in the handling and transfer of concentrated solutions of urea and guanidine hydrochloride Also the concentration determination involves an extinction coefficient based on a dry weight determination plus another absorbance measurement on the solution under investigation.

Table 2. Comparison of calculated[A] and measured values of ϕ_2' (ml/g) in sodium phosphate buffer and in guanidine hydrochloride

Calculations of ϕ_2' were made by the procedure of Lee and Timasheff (1974*b*)

Protein	0·05 M sodium phosphate, pH 7·0			6 M guanidine hydrochloride		
	ϕ_2' (meas.)	$\bar{v}_2$[B]	$\bar{v}_2$[C]	ϕ_2' (meas.)	ϕ_2' (calc.)[B]	ϕ_2' (calc)[C]
SCMKA	0·707	0·718	0·706			
Component 5	0·718	0·722	0·711	0·706	0·715	0·703
Component 7[D]	0·718	0·721	0·710	0·710	0·713	0·702
Component 8c[D]	0·714	0·719	0·709	0·698	0·716	0·705

[A] Since the specific volumes of the amino acids are given for 25°C the calculated values of $\bar{v}_2$ were corrected to 20°C utilizing the mean temperature dependence of the specific volumes for five proteins given by Bull and Breese (1973).

[B] Calculations using values of Cohn and Edsall (1943) for the specific volumes of amino acid residues.

[C] Calculation using modified Cohn and Edsall's method (Zamyatnin 1972).

[D] The calculated values are for components 7c and 8c-1. Calculated values for the subcomponents of 7 and 8 differ by approximately 0·001 ml/g.

Table 2 gives partial specific volumes, $\bar{v}_2$, calculated from the amino acid compositions (Sparrow *et al.* 1979). Two values for $\bar{v}_2$ are given, one based on the specific volumes of amino acid residues given by Cohn and Edsall (1943) and the other based on the revised values of Zamyatnin (1972). The values of the apparent specific volumes in guanidine hydrochloride were calculated by means of the procedure of Lee and Timasheff (1974b). Their equations (8), (9) and (9a) were used to calculate the number of water and guanidine hydrochloride molecules bound to the proteins. The value of ϕ_2' was then derived from their equation (6) utilizing the calculated values of $\bar{v}_2$. The experimental values for ϕ_2' (Table 2) are between the two sets of calculated values with the exception of component 8 in guanidine hydrochloride. Most published calculations of the partial specific volumes of proteins (e.g. Lee and Timasheff 1974*b*) utilize the values for the apparent specific volumes of the amino acids given by Cohn and Edsall (1943) whereas the values of Zamyatnin (1972) are rarely employed. The difference in calculated molecular weights arising from the two calculated partial specific volumes is about 4% in dilute aqueous buffers and 7% in guanidine hydrochloride.

The only reported measurements for the apparent partial specific volumes for low-sulfur wool proteins are those of DeDeurwaerder and Harrap (1964) on SCMKA. These authors obtained values of 0·715 ml/g in 0·05 M aqueous phosphate at pH 6·9 and 0·728 ml/g in 60% (w/v) formamide–water. Jeffrey (1968*a*, 1968*b*) used these values for his measurements on component 8 in dilute aqueous buffers and denaturing solvents respectively but the present results show that the value in formamide–water

does not apply to 8 M urea or 6 M guanidine hydrochloride. The residual error in the molecular weights in denaturing solvents arising from partial specific volume measurements is estimated to be ± 1500 for molecular weights in the range 50 000–60 000. This is derived from the calculated standard error of ϕ_2' (95% confidence limits).

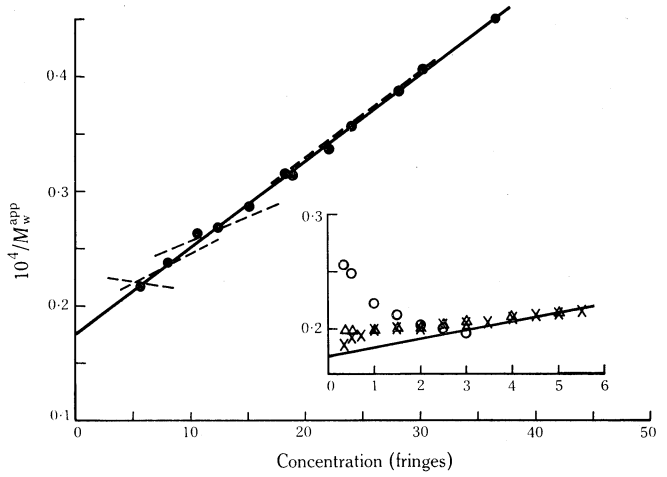


Fig. 1. Sedimentation equilibrium of component 5 in 6 M guanidine hydrochloride. ● Reciprocal of apparent weight-average molecular weight over the whole cell as a function of concentration, $(c_m+c_b)/2$, for low-speed equilibrium runs (16 000 r.p.m.). The concentration is in Rayleigh interference fringes in a 12-mm ultracentrifuge cell. From the refractive index increment measurements (tabulation, p. 425) it can be calculated that a solution of 10 mg/ml in guanidine hydrochloride is equivalent to 29 interference fringes. In Figs 1–3 the broken lines show trends of the reciprocal of point-average molecular weight with concentration for individual cell channels for various cell loading concentrations. *Inset*: Reciprocal of apparent point-average molecular weight for meniscus depletion equilibrium at c_0 values and speeds, respectively, of 1·2 mg/ml and 40 000 r.p.m. (○), 1·2 mg/ml and 30 000 r.p.m. (△), and 0·3 mg/ml and 30 000 r.p.m. (×). The full line represents low-speed experiments.

Sedimentation Equilibrium Measurements

The results of low-speed equilibrium experiments in 6 M guanidine hydrochloride are shown in Figs 1–3 for components 5, 7c and 8c-1, as plots of the reciprocal of apparent weight-average molecular weights over the whole cell versus concentration. Molecular weights were determined by linear extrapolation since statistical analysis showed that a higher degree polynomial did not give any significant improvement in fit to the data. All three components show a similar second virial coefficient of $1{\cdot}1 \times 10^{-3}$ cc mole g^{-2} which is in the range found for other proteins in 6 M guanidine hydrochloride (Lapanje and Tanford 1967). The broken lines in each figure represent the reciprocals of the apparent point-average molecular weights as a function of concentration down the solution column for individual c_0 values. For a homogeneous protein these plots should coincide with the values for the apparent weight-average molecular weights over the whole cell. For each component the point-average molecular weights deviate from the line corresponding to the whole cell values and at the lowest initial concentration the non-ideality is outweighed by the heterogeneity. This heterogeneity is also evident in meniscus depletion experiments (shown in the insets to the figures) where the non-ideality is less than expected from the reciprocal of the weight-average molecular weight over the whole cell versus concentration plots.

Moreover, the molecular weights are dependent on speed and initial cell loading concentration which again is evidence of heterogeneity.

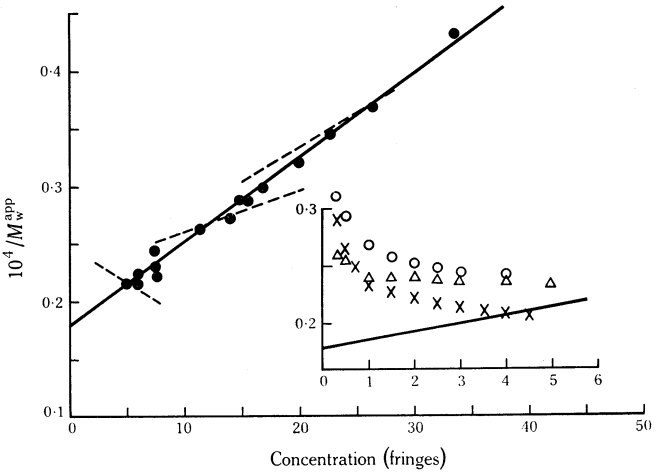


Fig. 2. Sedimentation equilibrium of component 7c in 6 M guanidine hydrochloride. ● Reciprocal of apparent weight-average molecular wiight over the whole cell for low-speed equilibrium (12 000 and 16 000 r.p.m.). *Inset*: Reciprocal of apparent point-average molecular weight for meniscus depletion equilibrium at c_0 values and speeds of 1·4 mg/ml and 40 000 r.p.m. (○), 1·4 mg/ml and 32 000 r.p.m. (△), and 0·3 mg/ml and 32 000 r.p.m. (×).

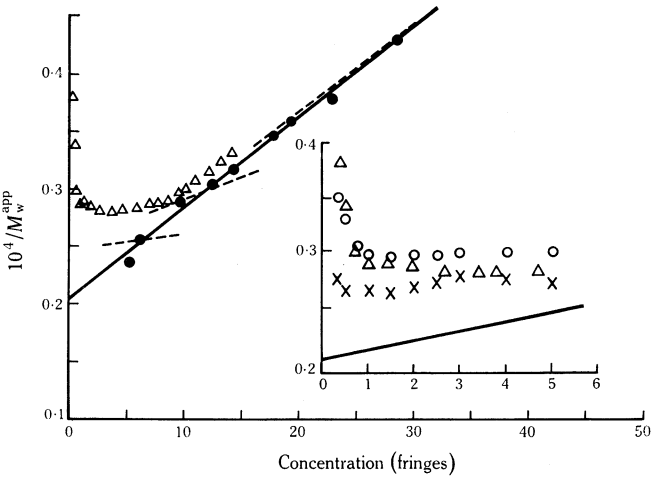


Fig. 3. Sedimentation equilibrium of component 8c-1 in 6 M guanidine hydrochloride. ● Reciprocal of apparent weight-average molecular weight over the whole cell for low-speed equilibrium (16 000 r.p.m.). △ (main part of figure and also inset) Reciprocal of apparent point-average molecular weight for meniscus depletion equilibrium at a c_0 value of 1·3 mg/ml and speed of 32 000 r.p.m. *Inset*: Meniscus depletion equilibrium molecular weights for c_0 values and speeds of 1·3 mg/ml and 44 000 r.p.m. (○) and 0·3 mg/ml and 32 000 r.p.m. (×).

For component 5 the extrapolated molecular weight from the plot in Fig. 1 is 56 800. The meniscus depletion experiment gave minimum values of 52 000 (inset,

Fig. 1) decreasing to 38 000 at higher speeds and concentrations. SDS gel electrophoresis showed that the major component comprised more than 94% of the stained bands with a small percentage of species of both higher and lower molecular weight. This supports the assessment from sedimentation equilibrium but because of the small amounts of contaminants present the extrapolated value of 56 800 (Fig. 1) should be representative of the main component.

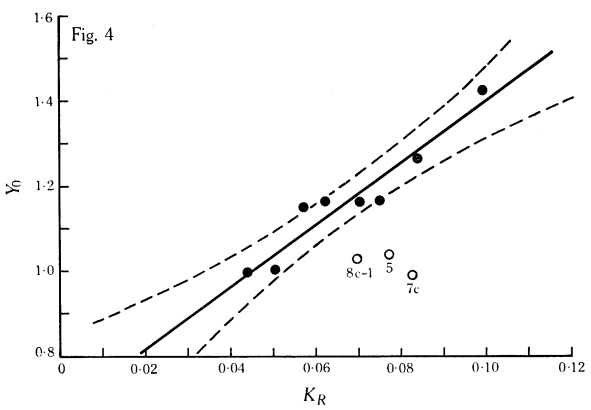
For component 7c there is a wider deviation between the low-speed sedimentation equilibrium and meniscus depletion experiments. The extrapolated value for weight-average molecular weight over the whole cell is 55 800 but meniscus depletion experiments indicate molecular weights as low as 27 000. SDS gel electrophoresis showed one major component but when very high loadings were applied to the gel there were two slower moving bands and at least six bands moving faster than the main component. The minor components present account for 10–15% of the total protein and since the predominant contribution is from lower molecular weight species the molecular weight value of 55 800 would be a lower limit for the main species.

For components 8c-1 the molecular weight from Fig. 3 is 48 500. Meniscus depletion experiments give values as low as 27 000. In one experiment (triangles in Fig. 3) where the fringes are well resolved the expected non-ideality was apparent towards the base of the solution column at high fringe displacements and heterogeneity at low fringe displacements. SDS gel electrophoresis showed one major component (mol. wt ~50 000) and three faster moving minor components of approximate molecular weights 27 000, 40 000 and 45 000; the gel scans showed these to be present to the extent of about 5% each. Thus a value of 50 000 for the molecular weight of component 8c-1 would be in accord with the sedimentation equilibrium results. Only a small amount of component 8a was available. From a meniscus depletion experiment in 8 M urea at 0·2 mg/ml and 32 000 r.p.m. a molecular weight of 45 300 at zero fringe displacement was found and the expected non-ideality was observed. At a higher cell loading of 1·5 mg/ml and higher speed (44 000 r.p.m.) there was a slight decrease to 39 000 but no evidence of any lower molecular weight species. SDS gel electrophoresis indicated about 5% of material of molecular weight lower than that of the main component.

Molecular Weights from SDS Gels

Molecular weights of the low-sulfur proteins determined from plots of log M versus R_F at single gel concentrations were higher than found in previous investigations by physicochemical techniques which indicated a value of 45 000 for component 8 and 50 000–55 000 for component 7c (Jeffrey 1972). There was also a trend towards lower molecular weights at high gel concentrations. For gel concentrations in the range 6–9% the mean values of the molecular weights were 63 000, 69 000 and 59 000 for components 5, 7c and 8c-1 respectively. To resolve this discrepancy molecular weights were estimated by determining K_R and Y_0 from experiments at different gel concentrations. If the standard proteins and unknowns bind the same amount of SDS and carry the same charge they should share a common intercept, Y_0, which is related to the net charge. This has been shown for some systems (Frank and Rodbard 1975; Steinert and Idler 1975) but in other cases (Banker and Cotman 1972) a positive correlation is found between Y_0 and K_R. Fig. 4 shows the relation between Y_0 and K_R obtained in the present investigation where it is seen that the free mobility is

related to molecular size as found by Banker and Cotman (1972). The points for components 5, 7c and 8c-1 deviate significantly from the linear relationship between Y_0 and K_R for the standard proteins. This suggests that the conditions necessary for the valid determination of molecular weights have not been met. These are that the SDS complexes of the standards and unknowns have the same charge density and free mobility and that the molecular conformation of the SDS complexes of the standards and unknowns are identical.



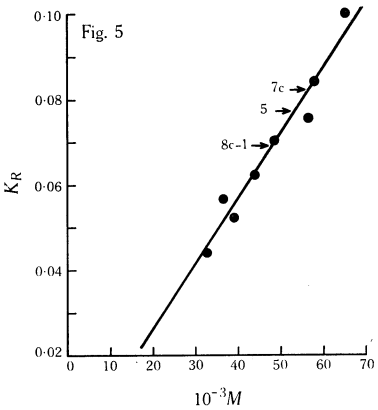


Fig. 4. Relation between free electrophoretic mobility (Y_0) and retardation coefficient (K_R) for standard protein (●) and components 5, 7c and 8c-1 (○). The dotted line gives the 95% confidence limits.

Fig. 5. Relation between retardation coefficient (K_R) and molecular weight (M) for standard proteins (●); K_R values for components 5, 7c and 8c-1 are given by horizontal arrows.

Many factors affect the order of migration of proteins in SDS gel electrophoresis and hence derived molecular weights. Factors shown to affect migration are: chemical modification—for example, esterification (McCumber and Clem 1976), maleylation (Banker and Cotman 1972), method of modification of sulfhydryl groups (Takagi *et al.* 1975); conditions of electrophoresis (Capaldi *et al.* 1977) and freeze-drying of proteins (Steinert and Idler 1975). More recently de Jong *et al.* (1978) have shown that a single amino acid substitution influenced the mobility of closely related proteins. The migration of the low-sulfur wool proteins has been shown to be influenced by the time, temperature and pH of storage (L. Dowling, unpublished observations in this laboratory). Thus numerous explanations may be postulated for the anomalous behaviour shown in Fig. 4.

The anomalous migration of the low-sulfur wool components suggests that more reliable molecular weights may be obtained from plots of K_R versus molecular weight, if one assumes that deviation from linearity in Fig. 4 is solely due to low Y_0 values and that K_R is appropriately related to molecular weight. The plot of K_R versus M for the standard proteins is linear (Fig. 5) from which the molecular weights of the unknowns are estimated to be 48 000, 53 000 and 57 000 for components 8c-1, 5 and 7c respectively (Table 3). The precision and accuracy of SDS gel electrophoresis has been analysed statistically by Rodbard (1976) and he concluded that the errors were far greater than most workers realize. The values of molecular weights of the wool

proteins determined from retardation coefficients agree with those from the ultracentrifuge within the precision and accuracy of the SDS gel electrophoresis method.

The molecular weights from SDS gel electrophoresis are summarized in Table 3 which includes some unpublished results obtained in gradient gels by Dr R. Blagrove in this laboratory. These values are lower than the values from single gel concentrations (see earlier) and closer to those found from the retardation coefficients.

Table 3. Molecular weights of low-sulfur wool subunits

LS, conventional (low-speed) sedimentation equilibrium; MD, meniscus depletion; GuHCl, guanidine hydrochloride

Method of measurement	Mol. wt.	Reference
Component 5		
Sedimentation equilibrium (LS) in 6 M GuHCl	57 000	This study
SDS gel electrophoresis	53 000	This study
SDS gradient gel electrophoresis	59 000	Blagrove (unpubl. data)
Controlled pore glass chromatography in 6 M GuHCl	57 000	Blagrove and Frenkel (1977)
Component 7c		
Sedimentation equilibrium (LS and MD) in 8 M urea	60 600[B]	Jeffrey (unpubl. data)
Sedimentation equilibrium (LS) in 6 M GuHCl	56 000	This study
SDS gel electrophoresis	57 000	This study
SDS gradient gel electrophoresis	64 000[A]	Blagrove (unpubl. data)
Controlled pore glass chromatography in 6 M GuHCl	57 000	Blagrove and Frenkel (1977)
Component 8a		
Sedimentation equilibrium (MD) in 8 M urea	45 000	This study
SDS gradient gel electrophoresis	44 000[A]	Blagrove (unpubl. data)
Component 8b		
SDS gradient gel electrophoresis	47 000[A]	Blagrove (unpubl. data)
Component 8c-1		
Sedimentation equilibrium (LS) in 6 M GuHCl	48 000	This study
SDS gel electrophoresis	48 000	This study
SDS gradient gel electrophoresis	52 000[A]	Blagrove (unpubl. data)

[A] Run for 4 h at 50 mA in 0·05% (w/v) SDS, 0·025 M phosphate buffer pH 7, using Gradipore, variable cross-linkage gradient gels.

[B] Jeffrey's unpublished data have been recalculated with the values of ϕ_2' reported in Table 1.

Conclusion

The data for component 5 (Table 3) are probably the most reliable since this subunit can be purified most readily. The value of 57 000 from sedimentation equilibrium is supported by controlled pore glass chromatography (Blagrove and Frenkel 1977) and SDS gel electrophoresis results.

The assignment of a subunit size to components 7c and 8c-1 is more difficult because of persistence of species of lower molecular weight in samples which have had extensive fractionation. Many preparations of component 7 have been investigated in this laboratory and Table 3 includes some unpublished results of Dr P. D. Jeffrey on component 7c recalculated using the values of ϕ_2' in Table 1. The experiments

were carried out in 8 M urea by both the conventional sedimentation equilibrium and meniscus depletion methods. For one sample, a value of 60 600 was found over a wide range of speeds (8 000–44 000 r.p.m.). For other samples values varied from 50 000 to 56 000. In the present work a value of 55 800 was found from sedimentation equilibrium and this is considered to be low due to low molecular weight contaminants. Controlled pore glass chromatography and SDS gel electrophoresis indicate a value of 57 000. The SDS gels indicate a higher molecular weight than for component 5 and taking all the results together a value of 58 000 would be appropriate for this component.

A molecular weight of 50 000 for component 8c-1 is compatible with the sedimentation equilibrium and gel electrophoresis results. A value of about 45 000 is indicated for the molecular weight of 8a but from the present results it is not possible to say if there is any difference between 8b and 8c-1. SDS gradient gel electrophoresis (Table 3) indicates that 8b is intermediate in molecular weight between 8a and 8c-1. There must be some reservation about the values because of a lack of a theoretical model for the calculation of molecular weights from gradient gels (Rodbard 1976). Component 8c-2 was not resolved from 8b in the phosphate buffer systems used so no estimate of its molecular size could be obtained.

The range of molecular weights (45 000–60 000) found for the polypeptide chains of the wool microfibril is comparable to that found for the α-keratin from the filaments of bovine epidermis (Skerrow 1974; Lee and Baden 1976; Steinert and Idler 1975). The α-helical contents of components 5, 7 and 8 are 25%, 45% and 56% respectively (Crewther *et al.* 1968; Dowling unpublished data) and these are also similar to the values found for the three groups of polypeptide chains from bovine epidermal α-keratin (Steinert and Idler 1975).

Acknowledgments

The author is indebted to Dr L. Sparrow, Dr W. G. Crewther, Mr N. McKern and Mr L. Dowling for the protein samples; Dr P. D. Jeffrey, formerly of this laboratory, Dr R. J. Blagrove and Mr L. Dowling made available unpublished results; Miss H. E. Simpson carried out the gel electrophoresis experiments and assisted with the density measurements.

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Manuscript received 8 March 1979, accepted 15 May 1979