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The Molecular Weight in 8 M Urea of a Low-Sulfur Protein from Wool*

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ABSTRACT: The low-sulfur protein fraction from wool consists of two major and several minor components as revealed by starch gel electrophoresis. The present paper is a study of the molecular weight of one of the major components, component 8, in 8 M urea solutions by the technique of sedimentation equilibrium. Two samples of component 8 have been examined, one prepared by acetone precipitation and the other prepared by the same method but fractionated further by gel filtration on Sephadex G-200. Conventional low-speed sedimentation equilibrium and high-speed Yphantis meniscus depletion methods have been used. It is concluded that component 8 exists as units of mol wt 46,000 in 8 M urea solutions and that further dissociation or aggregation at high pH (pH 11) or salt concentration (ionic strength 1.0) does not occur. The sample fractionated on Sephadex is essentially homogeneous with respect to molecular weight while the unfractionated sample appears to contain about 10% or less of lower molecular weight material.

The molecular weight of the low-sulfur fraction from reduced and alkylated wool keratin, SCMKA,[1] has been measured by several techniques and under a variety of conditions (Harrap, 1955, 1957; O'Donnell and Woods, 1956; DeDeurwaerder and Harrap, 1965). However SCMKA has been shown to consist of a number of components of which the two major ones, components 7 and 8 (Thompson and O'Donnell, 1964, 1965), have recently been isolated in sufficient quantity to permit studies to be made of their individual properties. This paper, which reports measurements of the molecular weight of component 8 by the technique of sedimentation equilibrium in solutions containing 8 M urea, is part of an investigation with the aim of clarifying the results obtained with SCMKA and ultimately the role of the low-sulfur proteins in the wool fiber.

Two samples of component 8 have been examined, one prepared by acetone precipitation, denoted A, the other by acetone precipitation followed by fractionation on Sephadex G-200, denoted S. The sedimentation equilibrium experiments were performed at two different speeds. The lower speed experiments (LS) produce the type of concentration distribution normally encountered where molecular weights can be evaluated across the entire solution column; the higher speed experiments (MD) produce a concentration distribution in which the concentration of solute in the region near the solution meniscus is essentially zero and allow the application of the theory developed by Yphantis (1964). The meniscus depletion method has higher sensitivity for the detection of low molecular weight material which may be present in small amounts and the combination of experiments at two speeds facilitates the detection of heterogeneity. Some experiments with mixtures of reduced and alkylated ovalbumin and β-lactoglobulin were carried out to test the sensitivity of the meniscus depletion method to small proportions of material of lower molecular weight than the major species in solutions containing 8 M urea.

Materials

Buffers. Analytical grade reagents and glass-distilled water were used in the preparation of all buffer solutions. Solutions containing 8 M urea were freshly prepared and filtered before use. The composition of the buffer solutions of 0.01 ionic strength are: pH 7.00, 0.0016 M NaH_2PO_4–0.0028 M Na_2HPO_4; pH 9.20, 0.01 M sodium borate; and pH 11.00, 0.005 M NaCl–0.005 M NaOH–0.01 M glycine. The pH values of the buffer solutions were adjusted to the values given above where necessary by the addition of acid or alkali. Where experiments were done at ionic strengths other than 0.01 the necessary adjustment was made by the addition of NaCl.

Ovalbumin and β-Lactoglobulin. The ovalbumin (Pentex lot 5) and β-lactoglobulin (Pentex lot 4814) were reduced and alkylated in 10 M urea–0.01 M sodium borate at pH 10 with mercaptoethanol followed by iodoacetic acid. When alkylation was complete excess iodoacetic acid was removed by the addition of mercaptoethanol. The protein solutions were then dialyzed against glass-distilled water to remove excess reagents and finally against 8 M urea–0.01 M sodium borate (pH 9.2) and stored in the cold. These reduced and alkylated proteins gave single peaks on Sephadex G-200 in the presence of 8 M urea and the elution volumes corresponded to molecular weights of 45,000 for SCM-oval-

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[1] Abbreviation used that is not listed in *Biochemistry 5*, 1445 (1966), is: SCMKA, *S*-carboxymethylkerateine A. LS denotes low-speed sedimentation equilibrium experiments and MD denotes meniscus depletion (high-speed) experiments.

bumin and 18,000 for SCM-β-lactoglobulin. Mixtures of the two proteins in the ratios 10% SCM-β-lactoglobulin–90% SCM-ovalbumin and 20% SCM-β-lactoglobulin–80% SCM-ovalbumin in 8 M urea were prepared by adjusting the concentrations of the individual protein solutions to the required values by dilution with 8 M urea–0.01 M sodium borate solution of pH 9.20 and then mixing suitable volumes of the solutions using optical density at 278 mμ as a measure of concentration. The mixture was then dialyzed against 500 × its volume of buffer for 24 hr on a rocking dialyzer at room temperature with one change of buffer.

Component 8. The sample denoted A was prepared from merino top (merino wool 148) by the acetone precipitation method of Dowling and Crewther (L. M. Dowling and W. G. Crewther, unpublished data) and was stored in the cold in a solution of 8 M urea–0.01 M sodium borate at pH 9.2. The sample denoted S was prepared from a single merino fleece (merino wool 157) by the technique referred to above but was subsequently fractionated in pH 10.1 buffer containing 8 M urea on Sephadex G-200. A single peak with a small trailing edge was obtained. The major portion of the peak was taken, concentrated, and freed of dissolved Sephadex by binding to DEAE-cellulose at pH 7.4 followed by elution with 0.15 M KCl solution. The sample was stored in the cold in a solution of 8 M urea–0.15 M KCl–Tris buffer at pH 7.4. Changing the composition of the solution in which the protein was dissolved was accomplished by dialysis over 48 hr on a rocking dialyzer against 1000 × the volume of the new buffer solution with two changes of buffer.

Dialysis tubing was Visking $^{18}/_{32}$ which had been boiled in 1% sodium bicarbonate and then in distilled water.

Methods

Technique of Sedimentation Equilibrium Experiments. For the low-speed experiments protein solutions of concentration about 1% were dialyzed against the appropriate buffer and were transferred directly from the dialysis sac to one sector of the cell of a Brice-Phoenix differential refractometer by means of a syringe. The other sector was filled with diffusate. Direct transfer from the dialysis sac is necessary with concentrated urea solutions to prevent loss of dialysis equilibrium between solution and solvent by evaporation. Blank measurements were made with distilled water and the net refractive index increment converted to a concentration in Rayleigh interference fringes in the 12-mm ultracentrifuge cell. Dilutions of the protein solution to concentrations of approximately 0.3, 0.2, and 0.12% (10, 7, and 4 Rayleigh interference fringes) were accurately made with diffusate by means of pipets graduated in 0.01 ml. The concentrations were chosen to give overlap between concentration distributions under the experimental conditions used. The solutions of accurately known initial concentration were transferred immediately to three channels of a six-channel ultracentrifuge cell. The other three channels contained diffusate. The volumes used were solution, 0.10 ml; solvent, 0.11 ml; and FC 43 (washed with urea), 0.01 ml. Such volumes gave column heights of just under 3 mm.

For the meniscus depletion experiments the dialyzed protein solution of concentration about 0.15% was transferred directly to one sector of a double-sector Kel F-coated cell. The other sector contained diffusate. The volumes of solution, solvent, and FC 43 were the same as those used in the low-speed experiments.

At the conclusion of the meniscus depletion experiments blanks were carried out following exactly the procedure suggested by Yphantis (1964). The low-speed experiments were done at 16,200 rpm, the meniscus depletion experiments at 39,460 rpm, in a Spinco Model E analytical ultracentrifuge, and the actual speed was taken from odometer readings over the last few hours of the experiment. The temperature was controlled by means of the RTIC unit and the refrigerator. Photographs were taken on Kodak IIG spectroscopic plates after 24 hr for the LS experiments and after 48 hr for the MD experiments using the Rayleigh interference optical system of the ultracentrifuge with a symmetrically positioned upper limiting aperture. The patterns were measured on a Nikon two-dimensional microcomparator at 1-fringe or 0.5-fringe increments for the LS experiments and at 0.1-mm increments (on the photographic plate) for the MD experiments. For the latter a blank photograph taken with distilled water in both sectors of the cell was measured at the same radial positions. The attainment of sedimentation equilibrium was checked by comparison of measurements of the fringe positions near the bottom of the column at 20 and 24 hr for the LS experiments and 44 and 48 hr in the MD experiments. The measurements agreed within experimental error indicating that equilibrium had been reached in each case at the shorter time. In all of the MD experiments a region of horizontal fringes was present at the meniscus end of the cell, indicating that within the limits of detection all protein had been removed from that region. Solvent densities were measured with calibrated 50-ml pycnometers at the temperature appropriate to the sedimentation equilibrium experiment.

The value used for the partial specific volume of component 8 was 0.728 ml/g. This was measured in 60% formamide–water (DeDeurwaerder and Harrap, 1964) and is thought to be a good approximation to the value in 8 M urea inasmuch as the helix content of component 8 in 8 M urea is zero and is very close to zero in 60% formamide–water (B. S. Harrap, private communication). It does not take into account any selective binding of urea. The partial specific volume of SCM-ovalbumin in 8 M urea is 0.738 ml/g (Charlwood, 1957). The number of groups on reduced and alkylated ovalbumin is 7 (Warner, 1954), and it is possible to calculate roughly the decrease in the partial specific volume caused by the electrostriction associated with the ionization of these groups, assuming a decrease with volume of 18 ml/mole per charge pair (Charlwood, 1957), of 0.003 ml/g. Thus the value of $\bar{v}$ for SCM-ovalbumin was taken as 0.735 ml/g. For β-lactoglobulin the value of $\bar{v}$ in aqueous solution is 0.751 ml/g (Pedersen, 1936). Christensen (1952) found a volume constriction of 612 ml/mole (molecular weight taken as 42,000) in 6 M urea at

30°, which is equivalent to 0.015 ml/g. The number of SCM groups per molecule of reduced and alkylated β-lactoglobulin (mol wt 18,000) is 5 (Piez *et al.*, 1961), leading to a constriction on the same basis as above of 0.005 ml/g. The value of $\bar{v}$ for SCM-β-lactoglobulin in 8 M urea was taken as 0.751 − 0.015 − 0.005 = 0.731 ml/g.

Calculation of Molecular Weight. Apparent weight-average molecular weights were evaluated from eq 1 or 2, where R is the gas constant, T the temperature, $\bar{v}$ the

$$\bar{M}_{\mathrm{w\,app}(r)} = \frac{2RT}{(1-\bar{v}\rho)\omega^2}\,\frac{\mathrm{d}\ln c}{\mathrm{d}(r^2)_r} \tag{1}$$

$$\bar{M}_{\mathrm{w\,app}\frac{(c_\mathrm{b}+c_\mathrm{a})}{2}} = \frac{c_\mathrm{b}-c_\mathrm{a}}{c_0(r_\mathrm{b}^2-r_\mathrm{a}^2)}\,\frac{2RT}{(1-\bar{v}\rho)\omega^2} \tag{2}$$

partial specific volume of the solute, ρ the density of the solvent, ω the angular velocity, c_0 the initial solute concentration, and c_a and c_b the concentrations at the meniscus and bottom r_a and r_b, respectively. The protein concentration was always left in terms of Rayleigh interference fringes, j, in the 12-mm cell for the purpose of calculating molecular weights. In the LS experiments the apparent weight-average molecular weight was evaluated as a function of j for each initial concentration used by drawing tangents at a number of points to a large-scale plot of ln j *vs.* r^2. The best smooth curve through the plotted points was drawn using a French curve and the tangents were carefully drawn using a transparent ruler, at values of ln j where the curve intersected a graticule line. The precision of this procedure as determined by repeating the whole process on the same set of points was about ±1% in terms of molecular weight. Molecular weights were also evaluated from eq 2 for these experiments. The value of the molecular weight at zero concentration was obtained from the intercept of a linear least-squares regression to the $1/\bar{M}_{\mathrm{w\,app}(r)}$ *vs.* j data.

In the MD experiments the apparent weight-average molecular weight as a function of j was obtained by evaluating

$$\frac{\mathrm{d}\ln j}{\mathrm{d}(r^2)_{r_0}} = \frac{0.1}{2r_0\Delta r}(2\ln j_{r_2} + \ln j_{r_1} - \ln j_{r_{-1}} - 2\ln j_{r_{-2}}) \tag{3}$$

where $\ln j_{r_2} \ldots \ln j_{r_{-2}}$ are five data points spaced at equal increments, Δr. The value of $\mathrm{d}\ln j/\mathrm{d}(r)^2$ at r_0, the central point, is equal to the slope of a least-squares straight line through the five points (Yphantis, 1964). No fringe displacements of less than 100 μ were used and the value of the molecular weight at zero concentration was obtained from a straight line through the $1/\bar{M}_{\mathrm{w\,app}(r)}$ *vs.* j data.

In a homogeneous solution of a nonelectrolyte where the solution is incompressible and the dependence of activity coefficient on pressure is negligible it may be shown (Fujita, 1962) that

$$\frac{1}{M_{\mathrm{app}\frac{(c_\mathrm{b}+c_\mathrm{a})}{2}}} = \frac{1}{M_1} + (B_1 + \bar{v}_1/M_1)\left(\frac{c_\mathrm{b}+c_\mathrm{a}}{2}\right) + \ldots \tag{4}$$

where M_1 is the true molecular weight of the solute species, $\bar{v}_1$ is its partial specific volume, and $B_1 + \bar{v}_1/M_1$ is the second virial coefficient. It is also possible to show that

$$\frac{1}{M_{\mathrm{app}(r)}} = \frac{1}{M_1} + (B + \bar{v}_1/M_1)c_{1,r} + \ldots \tag{5}$$

Thus in such a system the reciprocal of the molecular weight evaluated from eq 1, when plotted against the concentration at which it was evaluated, will lie on a straight line of intercept $1/M_1$ if concentration terms of higher order than one can be neglected. Similarly the reciprocal of the molecular weight evaluated from eq 2 when plotted against $(c_\mathrm{b} + c_\mathrm{a})/2$ will yield $1/M_1$ at $C = 0$. In a heterogeneous nonideal system in general if a plot of the reciprocal of $\bar{M}_{\mathrm{w\,app}(r)}$ from eq 1 is made against c_r the value of the intercept at infinite dilution will be the reciprocal of the molecular weight of that species (if any) which is present virtually alone at low concentrations. Such a condition may be realized in a paucidisperse system when the meniscus depletion technique is used.

Yphantis (1964) has shown that, for a disperse ideal system if the meniscus concentration can be neglected with respect to that at some point r' further toward the base of the cell and if the molecular weight of the smallest species is large enough that the number of molecules per unit volume at the meniscus is also negligible, a number-average quantity (eq 6) can be evaluated from points

$$\sigma_{n(r)} \cong c(r)\bigg/\int_{r=a}^{r} c(r)\mathrm{d}(r^2/2)\ (r > r') \tag{6}$$

centrifugal to r'. The number-average molecular weight is given by

$$M_{n(r)} = \sigma_{n(r)}\frac{RT}{(1-\bar{v}\rho)\omega^2} \tag{7}$$

It is easy to show that if eq 6 and 7 are applied in a monodisperse nonideal system to which the conditions given above apply, one obtains

$$1/'M'_{n\,\mathrm{app}(r)} = 1/M_1 + {}^1\!/_2(B_1 + \bar{v}_1/M_1)c_{1,r} + \ldots \tag{8}$$

where the prime marks signify that a number-average quantity has been evaluated although the system is monodisperse. One could equally write $1/'M'_{\mathrm{w\,app}(r)}$ in

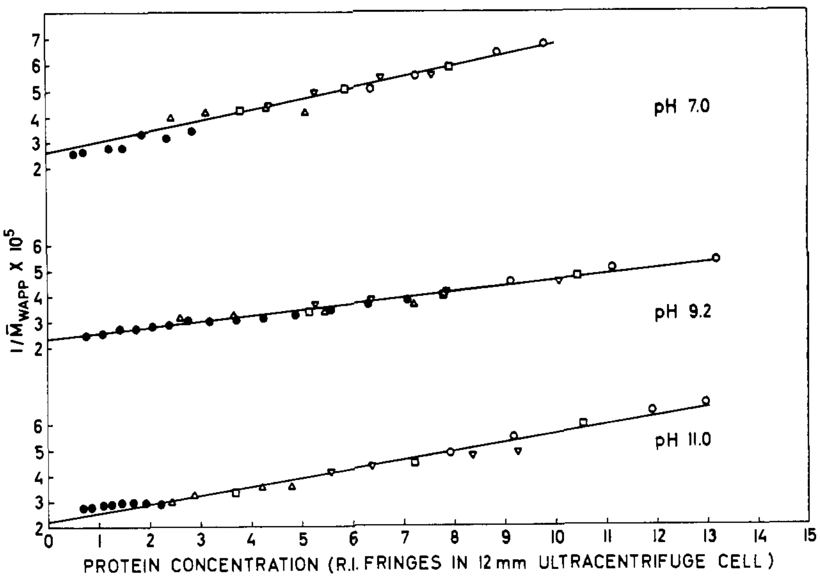

FIGURE 1: Sedimentation equilibrium experiments with component 8 at ionic strength 0.01, 25°, and three pH values in 8 M urea. (●) MD experiments, (△, ▽, and ⊙) LS experiments of different initial concentrations, and (□) $1/M_{w\text{app}(c_b + c_a)/2}$. The solid line is a linear least-squares regression through the LS data.

eq 5. Combining eq 5 and 8 gives

$$\frac{'M'_{n\text{app}(r)}\,'M'_{w\text{app}(r)}}{2'M'_{w\text{app}(r)} - 'M'_{n\text{app}(r)}} = M_1$$

Thus if one evaluates molecular weights from equations 1 and 7 in a meniscus depletion experiment, a plot of the left-hand side of eq 9 *vs.* concentration will yield a horizontal straight line of value M_1 if the system is monodisperse but nonideal providing only one virial coefficient is required.

Results

Plots of $1/M_{w\text{app}}$ *vs.* concentration for sample A at ionic strength 0.01 and three different pH values are shown in Figure 1 and at pH 11.0 and 2 temperatures in Figure 2. The values of the molecular weight at infinite dilution under these conditions as well as that at ionic strength 1.0 and pH 7.0 are summarized in Table I.

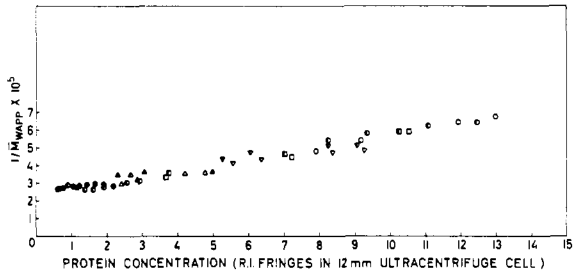

FIGURE 2: Sedimentation equilibrium experiments with component 8 at pH 11.0, ionic strength 0.01 and two temperatures in 8 M urea. (●) MD experiments at 25°, (△, ▽, and ○) LS experiments of different initial concentrations at 25°, and (□) $1/M_{w\text{app}(c_b + c_a)/2}$ at 25°. Half-filled symbols have the same significance but refer to experiments at 10°

The agreement between the molecular weights obtained from the LS and MD experiments and between the weight- and number-average molecular weights, under a given set of experimental conditions, indicates that sample A of component 8 is homogeneous. Figures 1 and 2 show that the solutions are thermodynamically nonideal, but this is to be expected in solutions of 8 M urea. Plots of $\bar{M}_{n\text{app}}\bar{M}_{w\text{app}}/(2M_{w\text{app}} - M_{n\text{app}})$ (eq 9) *vs.* protein concentration (Figure 3) are sufficiently horizontal over the range investigated to support the conclusion of apparent homogeneity. A comparison of the mean values of $\bar{M}_w$(LS) and $\bar{M}_w$(MD) for different experimental conditions shows that all fall within the range 40,000 ± 3000, a reasonable experimental error for experiments of this kind. There seems to be no dissociation to units of molecular weight lower than 40,000. An increase of pH from 7 to 11, which might be expected to promote dissociation if electrostatic effects were important does not do so. Similarly an increase in the ionic strength by a factor of 100 at pH 7 where the electrostatic charge will have its lowest value under the conditions investigated, does not cause aggregation. The agreement between the molecular weights under different conditions of charge is also evidence that an ionic strength of 0.01 is sufficient to suppress any charge effects which would affect the measured molecular weight. Finally lowering the temperature by 15° which would lead to increased dissociation if hydrophobic bonding were important apparently has no effect on the molecular weight at pH 11, the pH most likely to favor dissociation (see also Figure 2). No correction was applied for the possible effect of temperature on the partial specific volume as the variation of $\bar{v}$ with temperature is likely to be of the order of 5×10^{-4} ml/g per °C (Reithel and Sakura, 1963) and this would lead to about a 5% correction to the molecular weights. Such a correction is not significant in relation to the scatter of the measured molecular weights at the two temperatures (Figure 2).

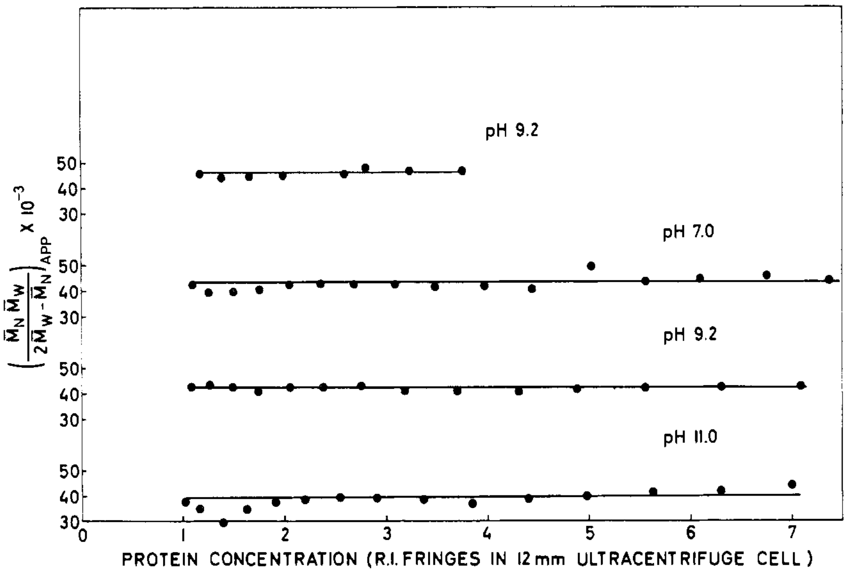

FIGURE 3: Plots of $(\bar{M}_n\bar{M}_w/2\bar{M}_w - \bar{M}_n)_{app}$ *vs.* protein concentration from MD experiments with samples A and S at ionic strength 0.01, 25° in 8 M urea. The upper plot is for samples S, the lower three for sample A. The ordinate is the true molecular weight (see eq 9).

TABLE I: Molecular Weight of Component 8 at Infinite Dilution in Solutions Containing 8 M Urea (Sample A).

pH	Ionic Strength	Temp (°C)	Method[a]	$\bar{M}_w$	$\frac{\bar{M}_w(\text{MD}) + \bar{M}_w(\text{LS})}{2}$	$\bar{M}_n$	$\left(\frac{\bar{M}_n\bar{M}_w}{2\bar{M}_w - \bar{M}_n}\right)_{app}$[b]
7.0	0.01	25	LS	38,300			
7.0	0.01	25	MD	41,500	39,900	40,500	43,000
7.0	1.00	25	LS	37,000			
7.0	1.00	25	MD	37,000	37,000		
9.2	0.01	25	LS	42,200			
9.2	0.01	25	MD	43,000	42,600	42,000	42,000
11.0	0.01	25	LS	45,500			
11.0	0.01	25	MD	38,300	41,900	38,000	39,000
11.0	0.01	10	LS	40,400			
11.0	0.01	10	MD	39,300	39,800		

[a] All of the solutions for the MD experiments were of initial concentration approximately 0.15%. [b] Obtained as the best horizontal line by eye through the points shown in Figure 4.

Table II shows the results of the experiments with sample S of component 8.

From the experiments at 39,460 rpm, the speed at which the MD experiments with sample A were carried out, the molecular weight of sample S is 46,000. For an error of ±3000 in molecular weight, the results with samples A and S can just be made to agree; however the results for sample A seem to be consistently lower and consequently some experiments with mixtures of known proportions of SCM-β-lactoglobulin (mol wt 18,000) and SCM-ovalbumin (45,000) were done to assess the sensitivity of the MD technique to heterogeneity in 8 M urea solutions. SCM-ovalbumin was chosen as a protein having a molecular weight close to that of component 8 while the molecular weight of SCM-β-lactoglobulin is probably near to that of possible contaminants of component 8 preparations, as judged from the tail of the Sephadex G-200 elution profile of component 8. For the MD technique to be applicable the speed must be chosen to give $\sigma = \omega^2M(1 - \bar{v}\rho)/RT$ a value of about 5 (Yphantis, 1964). A speed of 39,460 rpm satisfies this requirement for a molecular weight of 45,000 and gives a value of σ of about 2 for a molecular weight of 18,000.

The results of sedimentation equilibrium experiments at 39,460 rpm with mixtures containing 10% and 20% of SCM-β-lactoglobulin are shown in Figure 4. Curves of $\bar{M}_w$ *vs.* concentration at 39,460 rpm calculated for ideal solutions initially having these proportions of

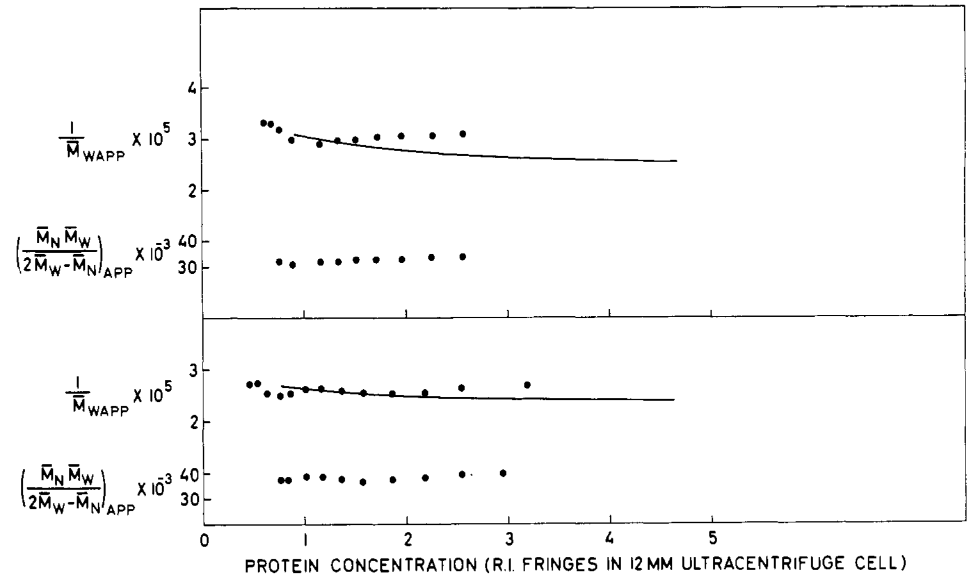

FIGURE 4: MD experiments with mixtures of SCM-β-lactoglobulin and SCM-ovalbumin at ionic strength 0.01, pH 9.2, 25°, 39,460 rpm in 8 M urea. The upper two graphs are for 20% SCM-β-lactoglobulin–80% SCM-ovalbumin; the lower two for 10% SCM-β-lactoblobulin–90% SCM-ovalbumin (w/v). The solid lines are the calculated distributions for ideal solutions of the above compositions at 39,460 rpm.

SCM-β-lactoglobulin are shown for comparison. Experimentally the molecular weights obtained by extrapolation of the low concentration regions of reciprocal plots to infinite dilution are for 10% SCM-β-lactoglobulin 37,000 and for 20% SCM-β-lactoglobulin 25,000. The only evidence of heterogeneity in the former case is the fact that ln j *vs.* r^2 is linear where one might expect it to be concave to the abscissa for such nonideal solutions, while with 20% SCM-β-lactoglobulin the ln j *vs.* r^2 plot consists of two distinct quite linear regions, the lower of which yields a molecular weight of 31,000, the upper one of 34,000. Thus on these criteria it would probably be concluded with unknown solutions that the one containing 10% of the smaller protein was of a homogeneous material with a molecular weight of about 37,000. With 20% however the heterogeneity would readily be detected. It is also apparent from Figure 4 that evaluation of $\bar{M}_{n\,\mathrm{app}}\bar{M}_{\mathrm{wapp}}/(2M_{\mathrm{wapp}} - M_{n\,\mathrm{app}})$ is not of much help in detecting heterogeneity as even the presence of as much as 20% of the lower molecular weight material does not produce any great deviation from a constant value.

TABLE II: Molecular Weight of Component 8 at Infinite Dilution in Solutions Containing 8 M Urea. Meniscus Depletion Experiments with Sample S.[a]

Initial Protein Concn (%)	Speed (rpm)	$\bar{M}_w$	$\bar{M}_n$	$\left(\frac{\bar{M}_n\bar{M}_w}{2\bar{M}_n - \bar{M}_n}\right)_{\mathrm{app}}$
0.18	39,460	45,600		
0.09	39,460	46,000	45,500	46,000
0.15	52,640	42,500		

[a] The experiments were carried out at pH 9.2, 25°, and ionic strength 0.01 (sodium borate).

For ideal aqueous solutions Yphantis (1964) considers the sensitivity of detection of a contaminant of σ one-quarter that of the major species to be about 5% for an initial concentration of 0.1% and to be slightly less when the contaminating species is one-half the size of the major species. The experiments with SCM-β-lactoglobulin and SCM-ovalbumin were at an initial concentration of 0.15% and the sensitivity thus seems to be considerably lower in these nonideal solutions of 8 M urea. It can be seen from Figure 4 that there is good agreement between the experimental and calculated curves at low concentration, and the deviation at higher concentrations is due to the fact that the calculated curves are for ideal solutions.

The results obtained with sample A of component 8 indicate a molecular weight of 40,000 ±3000 in 8 M urea solutions. The molecular weight of sample S is 46,000 ±3000. The value for the latter sample which is more highly fractionated is more likely to be the true molecular weight of component 8, and the value of 40,000 can be satisfactorily explained as being due to the presence of about 10% of material of molecular weight about 20,000, or less than 10% of material of molecular weight lower than 20,000. Some indications of heterogeneity in the ln j *vs.* r^2 plots support this conclusion. One experiment was done on sample S at a speed of 52,640 rpm which corresponds to $\sigma = 5$ for a species of mol wt 20,000. The result of this experiment (Table II) indicates sample S may contain some low molecular weight material but from the speed necessary to detect its presence the quantity must be 5% or less.

A value of 46,000 for component 8 is in good agreement with that of 43,000 obtained in a limited study by Yphantis' midpoint method (1960) by Thompson and

O'Donnell (1965) and of 45,000 obtained by the same authors from gel filtration. The agreement between the sedimentation equilibrium and gel filtration molecular weights may be taken as evidence that the value used for the partial specific volume of component 8 in 8 M urea is substantially correct.

The experiments on sample S show that the species of mol wt 46,000 does not aggregate or dissociate further in 8 M urea solutions under the wide range of conditions investigated. It is unlikely that these conclusions would be affected by the presence of 10% or so of lower molecular weight contaminant thought to be present.

Studies on the mixed low-sulfur protein fraction from wool (SCMKA) have yielded a number of different values for the molecular weight of the minimum unit depending upon the technique and conditions used (Crewther *et al.*, 1965).

The lowest of these is of 9000–10,000 obtained by Harrup with the surface balance (Harrap, 1957). There is no evidence for dissociation into units as small as this from the present experiments, and it seems likely that they did not arise from component 8. Recently it was shown in a sedimentation equilibrium and osmotic pressure study of SCMKA in 50% formamide (DeDeurwarder and Harrap, 1965) that a reversible equilibrium exists between units of mol wt 23,000–25,000 and possibly dimers and trimers of this. No evidence for such an equilibrium in component 8 in 8 M urea solutions has been found (see, however, Jeffrey, 1968). This is in accord with the findings of Thompson and O'Donnell (1965) who were unable to dissociate component 8 into units of molecular weight less than 45,000 in 8 M urea, 14 M formamide, or 5 M guanidine hydrochloride. Further studies on component 8 in the absence of urea are reported in the following paper.

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