

The Effects of Sucrose and Lactose on the Sizes of Casein Micelles Reconstituted from Bovine Caseins¹

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ABSTRACT

The mass distributions of reconstituted bovine casein micelles formed, in various media, with four preparations of whole casein were determined by sedimentation field flow fractionation. Two of the casein preparations were made from the milk of a single Jersey cow, the other two from the milks of Holstein cows, one being from a single cow, and the other from a herd. All media contained CaCl_2 , KCl, and piperazine- $\text{N,N}'$ -bis(2-ethanesulfonic acid) (PIPES) buffer, pH 6.75. Most of the experiments were conducted in a medium containing either no sugar, .3 *M* sucrose, or .3 *M* lactose. Micelle formation was initiated by adding an equal volume of buffered CaCl_2 to a solution of bovine sodium caseinate in a medium whose composition was identical except that it was free of Ca^{+2} . After 24 h at room temperature, the distribution of apparent micellar protein mass (M_{Prot} , grams of protein per mole micelles) was determined by sedimentation field flow fractionation. The values of apparent micellar protein mass at the maximum of the micelle peak were in the range 10^9 to 10^{10} for the Jersey casein preparations and 10^{10} and 10^{11} for the Holstein casein preparations. Lactose (.3 *M*) reduced the apparent micellar protein mass at the peak maximum by 45 to 90%, depending on the preparation. Sucrose reduced the apparent micellar protein mass at the peak maximum for one of the Jersey

preparations but increased it somewhat for the Holstein casein from the herd. The results suggest that lactose may affect the micelle size in milk and in products containing casein isolates of milk. We know of no other evidence suggestive of such an effect of lactose. In view of the known positive correlation between increased micelle size and decreased heat stability, the effects of sucrose and lactose on micelle size should be considered when making milk products such as concentrated sterilized milks and sweetened condensed milk. (Key words: micelles, casein, lactose, field flow fractionation)

Abbreviation key: FFF = field flow fractionation, H = preparations made from the milk of Holstein(s), J = preparations made from the milk of a single Jersey cow, M_{eff} = effective mass, M_p = particle mass, $M_{\text{Prot}}^{\text{Max}}$ = apparent micellar protein mass at the peak maximum, M_{Prot} = apparent micellar protein mass, SdFFF = sedimentation field flow fractionation.

INTRODUCTION

The size distributions of synthetic micelles formed from the proteins of various bovine nonfat dry milks (made from skim milk after subjecting it to different processing temperatures) were obtained in this laboratory by sedimentation field flow fractionation (SdFFF) (21). (When referring to a result obtained by SdFFF, the term "size" will sometimes be used to mean micellar protein mass.) The average size of the micelles observed in that work was more than an order of magnitude larger than the sizes of native casein micelles reported in the literature (7, 12, 13, 14, 18, 25). The large sizes could, of course, be attributed to the treatments used in preparing the nonfat dry

CASEIN MICELLE SIZE

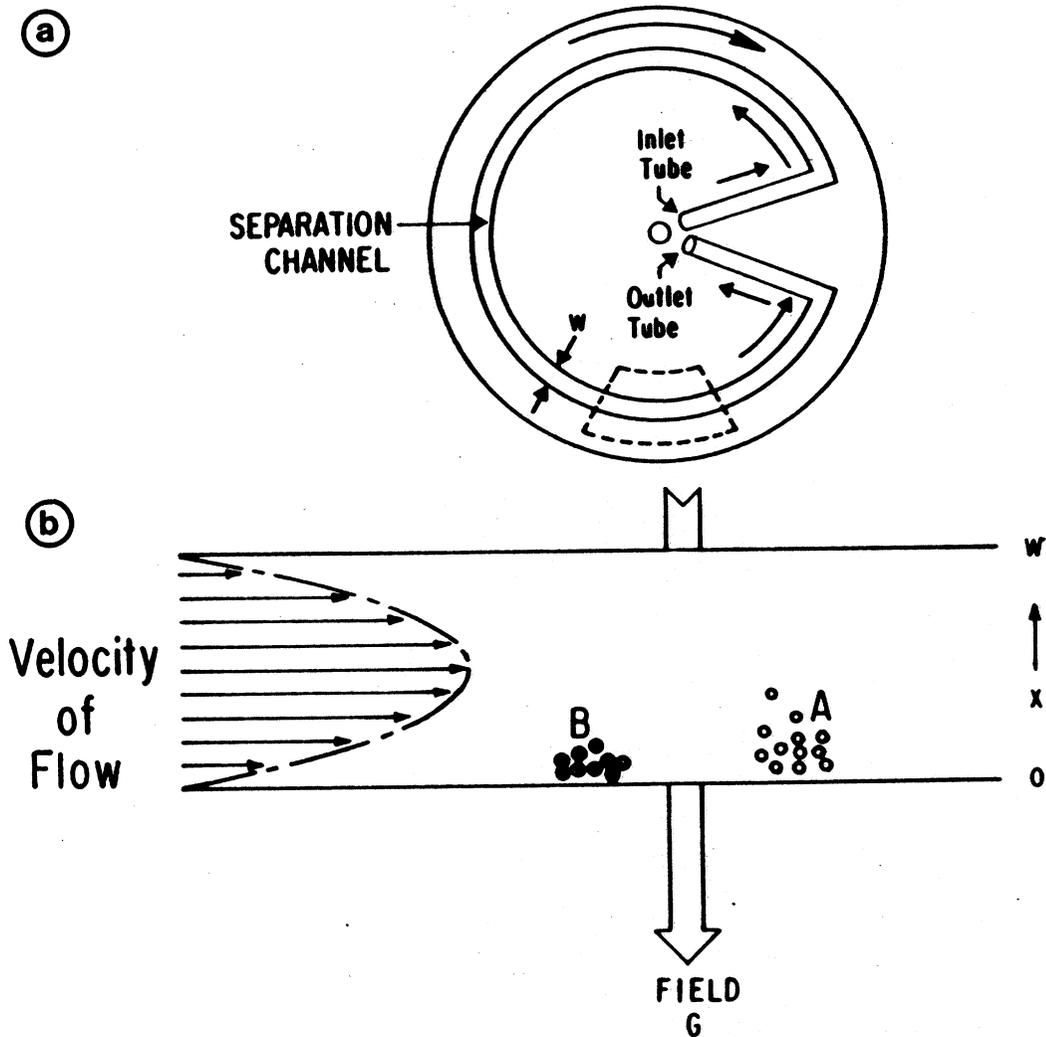


Figure 1. a. Cross-sectional view of the rotor of a sedimentation field flow fractionator. The "width" (or thickness) w is shown on the diagram. b. Enlarged view of a section of the separation channel showing the direction of the field, the flow profile, and the separation of two species of particles, A and B, the mass of B being greater than that of A.

milk powders as well as to the conditions of micelle reconstitution as opposed to *in vivo* micelle formation. However, because our reconstituted micelles formed and were examined in a medium containing sucrose, it appeared prudent to find out first whether replacement of the lactose of milk by sucrose would be expected to increase micelle size. To this end we have obtained, by SdFFF, the size distributions of micelles formed at room tem-

perature from isolated whole caseins in the presence of either lactose or sucrose as well as in the absence of sugar. The results of these studies are reported here.

Because SdFFF is a relatively new separation procedure, it will be described briefly. A more detailed description including references has been provided elsewhere (19). Separation takes place in a ribbon-like cavity or channel in the rotor of a centrifuge through which

carrier (solvent) is flowing at a uniform velocity in a direction anti-parallel to the direction of rotation (Figure 1a). In a commonly used design, the channel is located about 16 cm radially from the axis of rotation; it is about .25-mm wide ("width" being the dimension in the radial direction), ca. 2-cm broad (parallel to the axis of rotation), and ca. 95-cm long (circumferentially) from inlet to outlet. The inlet and outlet are connected to tubes located on the axis of the rotor, which, in turn, are connected through seals to stationary inlet and outlet tubes. The sample and carrier enter the rotor from the rear (behind the plane of the cross-section of Figure 1) and exit from the front (flowing towards the reader).

The sample is introduced into the channel as a band or zone. With the carrier flow turned off, the sample is subjected to the desired centrifugal field (acceleration) until sedimentation equilibrium is achieved. Unlike classical sedimentation equilibrium, this step, termed "relaxation", takes little time, because the channel is so narrow. Carrier flow is then started (this defines time zero), the field being held constant at its initial value. Particles (e.g., casein molecules and micelles) elute (Figure 1b) in the order of their effective masses (M_{eff}) where M_{eff} is the particle mass (in vacuo) corrected for the buoyancy effect of the solvent. This separation results from the different equilibrium distributions of particles of different M_{eff} across the width of the channel combined with the variation of carrier flow velocity with distance (x) from the channel wall towards which particles are driven. For particles of moderate and large effective mass,

$$M_{\text{eff}} = (6R_0T/wG_0t_0)t_c \quad [1]$$

where R_0 is the gas constant (erg/Kelvin mol); T , the absolute temperature (Kelvin); w , the channel width (cm); G_0 , the centrifugal field (cm/s^2); t_0 , the elution (retention) time for particles for which $M_{\text{eff}} = 0$; and t_c the elution (retention) time for particles of effective mass M_{eff} . The units of M_{eff} are given in grams per mole (daltons per particle). For low retention, Equation [1] must be replaced by the following pair of equations, cf., Equations [3] and [4] of reference (19):

$$\lambda[\coth(1/2\lambda) - 2\lambda] = t_0/6t_c. \quad [1a]$$

$$M_{\text{eff}} = R_0T/\lambda wG_0. \quad [1b]$$

When particles of widely different M_{eff} are to be separated, a field G_0 sufficient to retain the particles of moderate M_{eff} would result in excessively long retention times for the particles of large M_{eff} , with corresponding low, broad elution peaks. This problem can be circumvented by allowing the field to decay after particles of small M_{eff} have eluted. For a field allowed to decay exponentially,

$$M_{\text{eff}} = (6R_0T/wG_0t_0)\{t_c + \tau_g[\exp((t_c - t_c)/\tau_g) - 1]\}. \quad [2]$$

Here t_c is the time (min) during which the field was held at its initial value G_0 and τ_g is the field decay constant (min); other symbols have the same meaning as for Equation [1]. The field is controlled by a microprocessor, which permits selection of the relaxation time and the parameters G_0 , t_c , and τ_g .

Very low rotor velocities can give rise to mixing problems. To avoid these, we have made a practice of terminating the decay when the rotor velocity ω' reaches a preselected value, e.g., 60 rpm, and holding ω' constant at that value until elution is complete. For components eluting after field decay has been terminated, Mozersky showed

$$M_{\text{eff}} = \left(\frac{6R_0T}{wG_0t_0}\right) \left\{ t_c + \tau_g \left[\exp\left(\frac{t_{\text{Hold}} - t_c}{\tau_g}\right) - 1 \right] + \left(\frac{\omega'_0}{\omega_{\text{Hold}}}\right)^2 (t_c - t_{\text{Hold}}) \right\}. \quad [3]$$

Here ω'_0 is the initial rotor velocity; ω_{Hold} is the rotor velocity and t_{Hold} the time when field decay is terminated. Like Equations [1] and [2], Equation [3] is based on Equations [3] and [10] of reference (30). The three terms inside the braces correspond, respectively, to the initial constant field (G_0) phase of duration t_c , the decay phase of duration $t_{\text{Hold}} - t_c$, and the terminal ("hold") constant field phase of dura-

tion $t_e - t_{\text{Hold}}$ (at elution time t_e). The first and last terms are strictly analogous, because the field during the hold phase is $G_0 (\omega'_{\text{Hold}}/\omega_0)^2$. The second term inside the braces in Equation [3] is analogous to that in Equation [2], the duration of the decay phase being $t_{\text{Hold}} - t_c$ in the former and $t_e - t_c$ in the latter (at elution time t_e).

Once M_{eff} has been determined (by one of the equations presented), the particle mass (M_p) can be calculated:

$$M_p = \frac{M_{\text{eff}}}{\Delta\rho/\rho_p} = \frac{M_{\text{eff}}}{1 - \rho v_p} \quad [4]$$

ρ_p is the density (grams per cubic centimeter) of the particle; ρ , the density of the solution; $\Delta\rho = \rho_p - \rho$; and $v_p = 1/\rho_p$ is the partial specific volume of the particle.

If the particle of mass M_p contains solvent (carrier) in addition to the material of primary interest, in our case protein, ρ_p (and v_p) may be unknown, so that M_p cannot be calculated. However, the effective masses of the protein and the carrier-containing particle are identical; and the apparent mass of the protein (19) can be calculated. Assuming that the protein does not interact preferentially with any component of the solvent, the mass of protein in the particle (M_{prot}) is given by

$$M_{\text{prot}} = \frac{M_{\text{eff}}}{\Delta\rho/\rho_{\text{Prot}}} = \frac{M_{\text{eff}}}{1 - \rho v} \quad [5]$$

Here ρ_{Prot} is the density (grams per cubic centimeter) of the solvent-free protein; $\Delta\rho = \rho_{\text{Prot}} - \rho$; and $v = 1/\rho_{\text{Prot}}$ is the partial specific volume of the (solvent-free) protein, which is known. If the protein interacts preferentially with one or more solvent components, e.g., water or sugar, the partial specific volume v in Equation [5] should be replaced by ϕ'_2 , the partial specific volume at constant chemical potential (17). If $v(v_2)$, the partial specific volume at constant molality, is used instead of ϕ'_2 , one obtains an apparent value of M_{Prot} instead of the true value (17).

Sedimentation field flow fractionation is useful for particles in the mass range 10^6 to 10^{12} g/mol. The general features of a field

flow fractionation (FFF) system are similar to those of a chromatographic system, with the chromatographic column replaced by the FFF channel. An SdFFF run requires about an hour, during which time the effluent can be collected fractionally to provide samples of relatively homogenous mass. For particles smaller than 500 nm, particle sizes determined by FFF agree reasonably well with those determined by analytical ultracentrifugation (16), and it is recognized by practitioners of SdFFF that reproducibility is very good. These features of the technique suggested its use for the work reported here.

MATERIALS AND METHODS

Reagents and Solutions

The following reagents were used: 1) sucrose, reagent grade (J. T. Baker Chemical Co., Phillipsburg, NJ); 2) α -lactose monohydrate (Sigma Chemical Co., St. Louis, MO) the content of the β isomer being ca. 2%; and 3) piperazine- N,N' -bis (2-ethanesulfonic acid) (PIPES) and its disodium salt (Sigma Chemical Co.). Other reagents were reagent grade. The compositions of the solutions used are given in Table 1.

Sample Preparations

The method by which casein is prepared can influence its structure (10), and residual fat can influence the size distribution of casein micelles (14). Because the different preparations of whole bovine sodium caseinate exhibited significant differences in behavior, some details of the methods of preparation will be supplied. Other details are as given by Kumosinski et al. (15).

Two preparations (J1 and J2) were made from the fresh milk of a single Jersey cow as follows. The milk was skimmed at 37°C with a De Laval cream separator (Alfa-Laval Separation, Inc., Warminster, PA). The caseins were precipitated from the skim milk at room temperature by addition of HCl to pH 4.6, and the precipitate was washed at the same pH.

Preparation J1 was made from one portion of this precipitate and preparation J2 from another portion. The first portion was suspended in H_2O and redissolved by adding

TABLE 1. Composition of solutions.

Function	Solution Designation	Concentration (mM ¹)		
		CaCl ₂	Sucrose	Lactose
Casein	S1	0	0	0
	S2	0	300	0
Solvents	S3	0	0	100
	S4	0	0	300
	S5	0	150	0
Micelle	I1	40	0	0
	I2	40	300	0
Initiators	I3	40	0	100
	I4	40	0	300
	I5	40	150	0
FFF	C1	20	0	0
	C2	20	300	0
Carriers	C3	20	0	100
	C4	20	0	300
	C5	20	150	0

¹All solutions contained 80 mM KCl and 25 mM piperazine-N,N'-bis(2-ethanesulfonic acid) (PIPES) buffer and had a (final) pH of 6.75 (24°C). FFF = Field flow fractionation.

NaOH to pH 7.0. The solution was centrifuged at 10,000 × *g* (at *r*_{max}, the maximal radial distance of centrifuged solution from the axis of rotation) for 30 min (4°C), and the upper fat-containing layer was discarded. The caseins were reprecipitated and redissolved as before; the resulting solution was adjusted to pH 7.2 and centrifuged at 25,000 rpm (4°C) for 1 h in a Beckman SW 28 rotor (Beckman Instruments, Palo Alto, CA) (112,000 × *g* at *r*_{max}). After skimming again, the solution was filtered through several layers of fine cheesecloth and lyophilized. The lyophilized product is sodium caseinate preparation J1.

To make preparation J2, the second portion of the precipitate was treated in the same way as the first except that the last centrifugation was at 10,000 × *g* instead of 112,000 × *g*. The caseinate solution was then skimmed twice more, once after centrifuging for 30 min at 12,000 × *g* (5°C) and again after centrifuging for 60 min at 14,500 × *g* (5°C); the pellets were discarded. The intermediate layer, i.e., the liquid between the pellet and the upper fat-containing "layer", was lyophilized; the resulting product is preparation J2.

Caseinate preparations (H1 and H2) were made from Holstein milk; H1 was from the milk of a single cow, H2 from herd milk. Preparation H1 was made in the same way as J1 except as follows: phenylmethylsulfonyl

fluoride was added to the fresh milk. The milk was held for 1 d at 5°C before processing was begun. The initial skimming was done by centrifuging at 4000 × *g* at 5°C. After the initial skimming, the milk (1 L) was dialyzed against 18 L of water containing 10 mM EDTA for 24 h at 5°C and skimmed again at 10,000 × *g* for 10 min. The milk was then dialyzed against 18 L of water. The twice-precipitated and redissolved caseins were dialyzed overnight against water at 5°C; after adjusting to pH 7.2, the solution was skimmed at 75,000 × *g* and 5°C. Ethanol was added to a concentration of ca. 5% (vol/vol) before lyophilization.

Preparation H2 was made in the same way as H1, except phenylmethylsulfonyl fluoride (PMSF) was not used, the milk was processed fresh and was not dialyzed before skimming off the cream, and ethanol was not used.

Micelle reconstitution was carried out as follows. Whole bovine caseinate was dissolved in one of the five casein solvents (Table 1) to give a concentration of 13.65 g of caseinate/L of solution; the casein solvent was selected for its sugar content. To a 3-ml aliquot of this solution in a round-bottom culture tube having an internal diameter of 14 mm were added 3 ml of the micelle initiator (Table 1), while stirring vigorously at room temperature (24 ± 1°C) with a magnetic stirrer. The micelle initiator contained the same kind and concentration

of sugar as the selected casein solvent (as indicated by the same numerical designation), and, in addition, 40 mM CaCl₂. The resulting suspension had the same composition as the carrier (Table 1) later used for SdFFF. As an example, to study the size distribution of micelles in a medium containing 100 mM lactose, whole caseinate was dissolved in solution S3, micelle formation was initiated by addition of solution I3, and medium C3 was used as the carrier for FFF. The suspension was incubated at room temperature for 24 h prior to FFF.

Sedimentation Field Flow Fractionation

Sedimentation FFF was carried out at room temperature as previously described (19). The accumulation (outer) wall of the channel was lined with Kapton Polyimide Temp-R-Tape K-250 (CHR Industries, Hew Haven, CT) to minimize protein adsorption. A Rheodyne 7120 injection valve (Rheodyne, Inc., Cotati, CA) was inserted into the carrier line just upstream from the point of entry into the fractionator. Carrier solution was pumped by a Waters model 6000A HPLC pump (Waters Division, Millipore Corp., Milford, MA) at a rate of 1.00 ml/min. The sample was injected into the flowing stream of carrier solution after accelerating the rotor to its initial rotational velocity ω_0 (rpm). The load volume was 1 ml, the injection volume 327 μ l, and the run-in time 60 s. The rotor velocity was held constant during injection, run-in, relaxation, and the first phase of elution.

After injection and run-in, carrier flow was stopped for a relaxation period (t_{rx}) of 10 min. Resumption of flow defines the beginning ($t_e = 0$) of the elution program. This consisted of three phases: 1) an initial constant field (G_0) provided by the rotor velocity of ω_0 for t_c min, 2) exponential decay of the field (G) with a time constant τ_g , and 3) a constant terminal field (G_{Hold}) beginning at time t_{Hold} when the rotor reached a preselected speed ω_{Hold} . All of the runs except the one on preparation H1 were made with the following program parameters: $G_0 = 3.95 \times 10^5$ cm s⁻², provided by $\omega_0 = 1500$ rpm; $\tau_c = 10.0$ min; $\tau_g = 5.0$ min; and $\omega_{Hold} = 60$ rpm, which provides $G_{Hold} = 6.32$

$\times 10^2$ cm s⁻², beginning at $t_{Hold} = 42.19$ min. For preparation H1, $G_0 = 5.37 \times 10^3$ cm s⁻², provided by $\omega_0 = 175$ rpm; $t_{Hold} = 20.70$ min. All other parameters were as described.

Detection and Recording

The detector was a Waters model 450 variable wavelength absorbance detector set at a wavelength (λ) of 280 nm and range .04 AUFS (sensitivity of .25 V absorbance⁻¹ cm). The detector output was amplified 10-fold. The amplifier output (0 to 5 V) was connected to an autoranging scale expander (6), which separated the amplified signal into its integral portion (0, 1, 2, 3, or 4 V) and the decimal remainder (0 to .999 V). The latter was passed through a stepwise-variable power supply, which was used to offset negative signals. The output of the power supply was input to a Hewlett-Packard model 680 strip-chart recorder (Hewlett-Packard, Avondale, PA) set on the 1-V range, with a chart speed of 1/8 inch/min. Areas of the strip-chart record where the detector signal had exceeded 1 V were corrected by adding the integer outputs of the scale expander to the recorded signal. The retention time (t_e) scale of raw fractograms was converted into a log₁₀ M_{Prot} scale in accordance with Equations [1a], [1b], [2], [3], and [5] of the present paper.

RESULTS AND DISCUSSION

The size distributions of casein micelles formed at room temperature from whole bovine (Jersey) caseinate preparation J2 in the presence of lactose (carrier solution C4 in Table 1), in the presence of sucrose (carrier C2), and in the absence of disaccharide (carrier C1) are shown in Figure 2. The curves are the raw fractograms as obtained with the strip-chart recorder. The abscissa is therefore linear in time, as indicated by the 20-min time interval at the top of the figure. The fractograms were lined up in the horizontal direction after the time scale was converted into a log₁₀ M_{Prot} scale (see Materials and Methods, Detection and Recording). Log M_{Prot} is chosen because most of the micelles elute during the decay phase, when M_{eff} is an exponential function of elution (retention) time t_e (Equation [2]); during this period, the exponential term

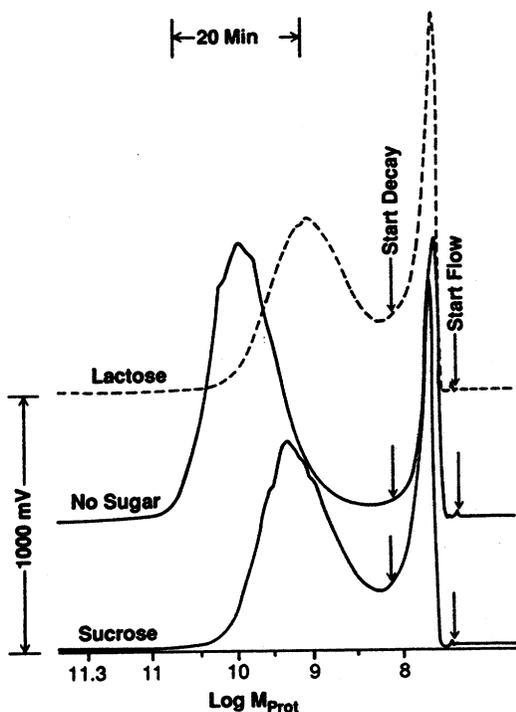


Figure 2. Sedimentation field flow fractograms of micelles formed from caseinate preparation from Jersey cow (J2) in the absence of sugar and in the presence of 300 mM lactose or sucrose, showing the decrease in apparent micellar protein mass (M_{Prot}) associated with the presence of the disaccharides. The time scales (abscissae) of the original raw fractograms have been replaced by $\log_{10} M_{Prot}$. The micelle peak is the second, broad peak in each fractogram.

predominates, and $\log M_{Prot}$ is nearly linear with elution time. Replacement of the time scale of the raw fractogram by a scale linear in $\log M_{Prot}$ would have little effect on the appearance of the fractogram. The micelle peak is the second peak in each of the fractograms and is broad; the first (void-volume) peak, which is much narrower, consists of nonmicellar casein. The micellar peak has a maximum in the absence of sugar at an apparent M_{Prot} of 9.3×10^9 (grams of protein per mole micelles) (M_{Prot}^{Max}); in the presence of 300 mM sucrose $M_{Prot}^{Max} = 2.0 \times 10^9$, and in the presence of 300 mM lactose $M_{Prot}^{Max} = 1.1 \times 10^9$. Micellar peak heights are also reduced somewhat in the presence of the disaccharides, and the concentra-

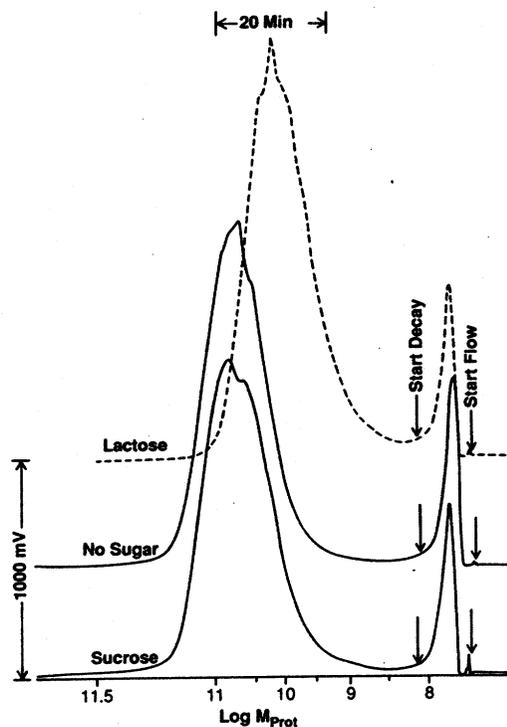


Figure 3. Sedimentation field flow fractograms of micelles formed from caseinate preparation from Holstein cows (H2) in the absence of sugar and in the presence of 300 mM lactose or sucrose, showing the divergent effects of the two disaccharides with this preparation. M_{Prot} = Apparent micellar protein mass.

tion of particles with M_{Prot} in the neighborhood of 2×10^8 is increased (Figure 2). The M_{Prot} distribution (not shown) for 150 mM sucrose (carrier C5 in Table 1) is intermediate between those for 0 and 300 mM sucrose, and that for 100 mM lactose (carrier C3) is intermediate between those for 0 and 300 mM lactose.

The size distributions of casein micelles formed from bovine (Holstein) caseinate preparation H2 made from herd milk are shown in Figure 3. The fractograms for preparations J2 (Figure 2) and H2 (Figure 3) are very different. With both preparations, lactose gave rise to a substantial reduction in the apparent M_{Prot} of micelles formed in its presence. In the case of H2, however, the M_{Prot} values of the micelles are an order of magnitude larger than for J2, and sucrose had little effect on the M_{Prot} distribution. With H2, there was also a substantial

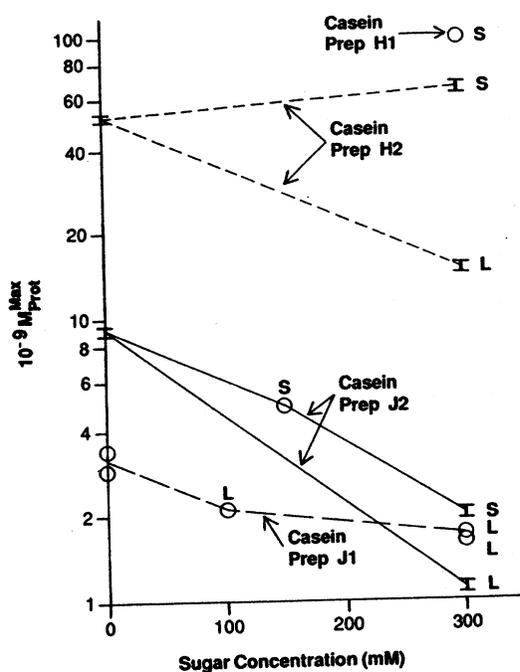


Figure 4. The apparent micellar protein mass at the peak maximum (M_{Prot}^{Max}) as a function of the concentration of disaccharide for the four caseinate preparations (Prep) used. Preparations J2 (from Jersey cow) and H2 (from Holsteins) were run in triplicate; a vertical bar indicates the standard deviation for a set of three runs. The limited amount of J1 available precluded replication in triplicates for this preparation, and there was only enough of preparation H1 for a single run. A circle indicates the results of a single run. S = Sucrose; L = lactose.

increase in peak height in the presence of lactose and little or no change in the concentration of particles in the neighborhood of $M_{Prot} = 2 \times 10^8$. Further differences between the fractograms of Figures 2 and 3 will be discussed.

The M_{Prot}^{Max} values for all experiments are plotted as a function of disaccharide concentration in Figure 4. The effect of lactose on Jersey caseinate preparation J2 was greater than its effect on J1. This is largely a reflection of the fact that J1 forms smaller micelles, in the absence of disaccharide, than does J2. The effect of sucrose on Jersey preparation J2 contrasts strikingly with its effect on Holstein caseinate preparation H2. The apparent M_{Prot}^{Max} values, in the presence of sucrose, for H1 and

H2, which were prepared from individual and herd Holstein milks, respectively, are similar, especially considering that the M_{Prot}^{Max} values were obtained with different elution programs (Materials and Methods). Weight average values [reference (27), chapter 3] of the M_{Prot} distributions for the micelle peaks showed the same trends as the M_{Prot}^{Max} values.

Perhaps the most striking result apparent from Figure 4 is that, both in the presence and absence of disaccharides, the M_{Prot}^{Max} values for the H preparations are about an order of magnitude larger than for the J preparations. Several factors could contribute to the observed relative differences in apparent micelle size; these include residual fat, method of preparation, and relative distributions of the individual caseins. The total lipid contents of the J2 and H2 preparations were .7 and .8%, respectively. These values, although substantially lower than those for laboratory caseins not subjected to ultracentrifugation [1.5%, reference (4)], are not significantly different from each other. Graham et al. (10) have shown that the conformation of casein can be influenced by the method of preparation, but H1 and H2, which are most divergent in preparation methods, are not different in physical properties. The most likely explanation could be differences in relative content of the various caseins. Casein distributions were estimated by the HPLC method of Parris et al. (22). The H1 and H2 samples were not significantly different from each other, averaging 34, 10, 38, and 18%, respectively, for the α_{s1} -, α_{s2} -, β -, and κ -caseins. In contrast, the J samples, prepared from the same cow, were significantly lower in relative β -casein content (25 vs. 38%) and somewhat higher in κ -casein content (20 vs. 18%). In micelles reformed from individual casein components, such ratios (24) would favor smaller micelles for the J samples, as observed.

The mechanisms responsible for the effects of sucrose and lactose observed here (Figure 4) are not known. Both sugars would be expected to favor association of molecularly dispersed caseins and, presumably, the formation of large micelles over small ones (1, 17). The result obtained with preparation H2 in the presence of sucrose is consistent with this ex-

pectation. The reduction in the concentration of free Ca^{+2} resulting from binding by lactose, if large enough, would explain both the decrease in micelle size and the decrease in light scattering observed in our work (20). Significant binding of Ca^{+2} by lactose is well established (8, 11) and may explain the enhancement by lactose of intestinal absorption of Ca^{+2} (5). However, assuming an association constant for the Ca^{+2} lactose complex of .20 (26), .3 M lactose would bind only 1.1 mM of the 20 mM Ca^{+2} present. This is far too small an effect to explain our observations with this sugar.

The interpretation of SdFFF data involves a problem that is not generally emphasized but that should at least be pointed out. The problem comes to SdFFF from sedimentation equilibrium, because SdFFF consists of the achievement of sedimentation equilibrium in a separation chamber followed by elution from the chamber. Equation [5] is based on the assumption (see text discussing Equation [5] in the Introduction) that the particle (micelle) to which the equation refers contains only protein and carrier (solvent), i.e., that the solvent components (water, sugar, salt, and buffer) contained in the particle are present therein in the same ratio as in the surrounding medium. Because the voluminosity of casein micelles is very high, and micelle structure is probably quite loose (9, 15, 23), the intramicellar solvent is probably very similar to the extramicellar solvent (carrier). However, if the protein interacts preferentially with one or more components of the solvent, and if (as we have done in our calculations) \bar{v} in Equation [5] is taken to be \bar{v}_2 , the partial specific volume at constant molality, the apparent M_{Prot} can be in error by as much as 20 to 35% (17). For β -lactoglobulin in .4 M lactose, where a large preferential hydration is observed (1), it can be shown that the error in calculated molecular weight [Equation [15] in reference (17)] is approximately -14%. However, the decreases of apparent M_{Prot} that we have observed with .3 M lactose and, for J2, with .3 M sucrose vary from 45 to almost 90%. Assuming that the preferential hydration of the caseins is not larger than that of β -lactoglobulin, these results appear to reflect primarily decreases in the true M_{Prot} values. This interpretation is supported by the data on the micelles of caseinate prep-

aration H2; $M_{\text{Prot}}^{\text{Max}}$ is slightly larger in the presence of sucrose than in the absence of sugar, yet M_{Prot} decreases substantially in the presence of lactose.

In addition to differences in the positions of the micelle peaks with respect to elution time and $\log_{10}M_{\text{Prot}}$, differences in shape between the micelle peaks of Figures 2 and 3 may also be noted. The micelle peaks of caseinate preparation H2 are obviously taller and relatively narrower than those of J1 and J2, whereas the nonmicellar peaks of H2 are smaller. The areas of the casein micelle peaks are plotted in Figure 5b, and those of the nonmicellar casein peaks in Figure 5a. The ratios A1:A2 of the peak areas (not shown) are two to four times as large for J1 and J2 as for H2. Thus, nonmicellar (including molecularly dispersed) casein constitutes a much greater proportion of the total protein for the Jersey caseinate preparations than for the Holstein preparation. The relative widths of the micelle peaks are plotted in Figure 6.

These observations cannot be attributed to differences in protein concentration. The weight concentration was identical in all experiments, and, from measurements made on dilute solutions (1 g/L) at 278 nm, the absorptivity of H2 is 5.3% lower than that of J2.

Because the detector used detects transmitted light, its output is influenced by scattering by casein micelles. Scattering has the advantage of increasing the sensitivity of micelle detection substantially. However, light scattering varies with particle diameter and, therefore, with the micellar protein mass, M_{Prot} . Comparisons of apparent protein concentrations in different regions of a fractogram must therefore be made with caution. However, comparison of the relative values under two sets of conditions for evaluating a change in particle size distribution is justifiable.

The average diameter of the bovine micelle is frequently taken to be 1400Å (2). A spherical particle of this diameter has a volume $v_p = 1.44 \times 10^{-15} \text{ cm}^3$. Assuming, very conservatively (15, 23), 3 g of intramicellar carrier/g of casein, a carrier density of 1.02 (as for our medium containing 100 mM lactose), and a partial specific volume for the protein of .736 cm^3/g , the total volume of the micelle $v_{\text{Micelle}} = 3.677 m_{\text{Prot}}$, where m_{Prot} is the mass of the micellar protein. Setting $v_{\text{Micelle}} = v_p$, we ob-

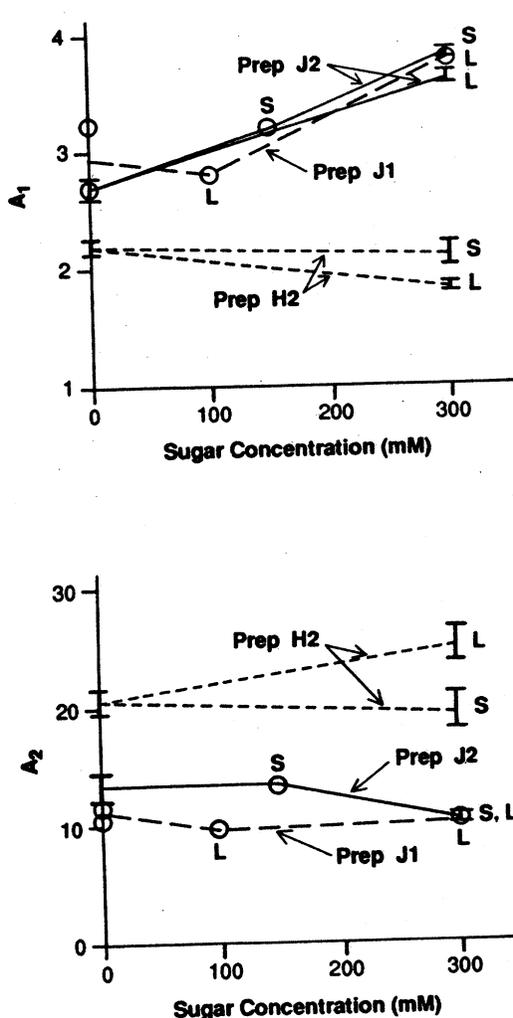


Figure 5. The areas (A1 and A2, respectively) of a) the nonmicellar casein (void-volume) peaks and b) the micelle peaks of sedimentation field flow fractograms as a function of disaccharide concentration. Area was calculated as peak height multiplied by the peak width at half-height. It is given in arbitrary units. To facilitate reading, the data for a given casein preparation at different sugar concentrations are connected by line segments. J = Casein preparations (Prep) from milk of a Jersey cow; H = casein preparations from milk of Holsteins; S = sucrose; L = lactose.

tain $M_{Prot} = 3.9 \times 10^{-16}$ (grams of protein per micelle), which gives a molar micellar protein mass $M_{Prot} = 2.4 \times 10^8$ (grams of protein per mole micelles). This corresponds to the smallest particles in our micelle peaks and is smaller than the average micellar protein

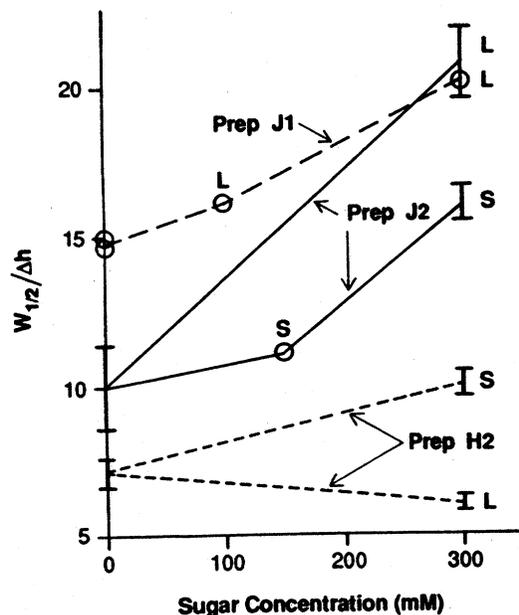


Figure 6. The ratio (in arbitrary units) of the peak width as half-height ($w_{1/2}$) to the peak height (Δh) for the micelle peaks of the sedimentation field flow fractionation fractograms as a function of disaccharide concentration. Note the relative sharpness of the micelle peaks of caseinate preparation H2. J = Casein preparations (Prep) from milk of a Jersey cow; H = casein preparations from milk of Holsteins; S = sucrose; L = lactose.

masses (molecular weights) reported by others (12, 18, 25). It must therefore be concluded that the average diameter of the bovine micelle is probably substantially larger than 1400Å.

Comparisons of the values of M_{Prot} obtained by different investigators (and frequently called "molecular weight") must be made cautiously, because, as stated by Dalglish (7), "... there are considerable differences between milks from different sources, from different animals, and even from the same animal at different stages of lactation." It is, however, of particular interest to compare the M_{Prot}^{Max} values that we obtained by SdFFF for synthetic micelles in media containing lactose (Table 2) with values for natural micelles obtained by others by sedimentation velocity measurements. Our values of 2.1×10^9 and 1.6×10^9 for caseinate preparation J1 are close to the value 1.9×10^9 obtained by Holt et al. (13) for one of two bovine skim milk samples and

TABLE 2. Dependence of micelle size (M_{Prot}^{Max})¹ on sugar content.

Casein preparation ²	Sugar		M_{Prot}^{Max}
	Type	Concentration (mM)	
J1	None	0	3.15×10^9
J1	Lactose	100	2.1×10^9
J1	Lactose	300	1.65×10^9
J2	None	0	9.3×10^9
J2	Sucrose	150	4.8×10^9
J2	Sucrose	300	2.0×10^9
J2	Lactose	300	1.1×10^9
H1	Sucrose	300	1.0×10^{11}
H2	None	0	5.2×10^{10}
H2	Sucrose	300	6.6×10^{10}
H2	Lactose	300	1.4×10^{10}

¹Apparent micellar protein mass at the peak maximum, grams of protein per mole of micelles.

²J = Preparation from Jersey cow; H = from Holsteins.

to the value 1.8×10^9 obtained by Morr et al. (18) for one of two casein micelle fractions isolated from native skim milk. [The other of Holt's samples gave a much lower value, 3.0×10^8 ; for the second micelle fraction of Morr et al. (18), the value was 2.3×10^8 .] Our caseinate preparation J2 had an apparent M_{Prot}^{Max} value in the presence of 300 mM lactose of 1.1×10^9 . For preparation H2 in the same medium, M_{Prot}^{Max} was 1.5×10^{10} . Many of the average M_{Prot} values in the literature lie in the range .5 to 2×10^9 (7, 12, 13, 14, 15, 18, 23, 24, 25).

Carroll et al. (2) and Thompson et al. (28, 29) have demonstrated that large micelle size is positively correlated with low solvation and low stability of milk products to heat and aging. This has been discussed recently by Farrell (9). In addition, as suggested by Holt (14), other environmental factors, such as pH, urea, and salts, can influence the size and thus the physical properties of native micelles. Our observations on the effects of sucrose and lactose on micelle size suggest that it might sometimes be possible to replace some of the sucrose in milk products, such as sweetened condensed milk, with additional lactose, thus altering functionality. Micellar protein mass can serve as a guiding parameter in studies designed to optimize composition. Sedimentation FFF provides a reasonably rapid and convenient means of ascertaining the distribution

of M_{Prot} under a variety of experimental conditions.

CONCLUSION

The M_{Prot} distributions of reconstituted micelles formed from whole bovine caseins in media of different sugar content have been determined by SdFFF. The relative amounts of micellar and nonmicellar casein have also been compared. The strongest determinant of micelle size (apparent M_{Prot}) was the casein preparation from which the micelles were reconstituted. Lactose (.3 M) reduced M_{Prot}^{Max} by 45 to 90%, depending on the casein preparation. Sucrose had a substantial but lesser effect on one preparation but very little effect on another. The relative amounts of micellar and nonmicellar (including molecularly dispersed) casein were also strongly preparation dependent, as was the sharpness of the micelle peaks. It is suggested that the difference observed may reflect primarily differences between Jersey and Holstein caseins, in particular the relative contents of β - and κ -caseins in the milks.

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