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MODEL CALCULATIONS OF CASEIN MICELLE SIZE DISTRIBUTIONS

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A previously proposed model for the formation and structure of casein micelles from subunits of variable composition is used to calculate theoretical micelle size distributions. Using the fractional content of κ-casein as the only variable but with a value near that observed in a sample of milk serum, the model successfully reproduces experimentally determined distributions. Predicted size distributions are quite sensitive to the value of the variable and shift toward smaller average size as the assumed fractional content of κ-casein gets larger. Also, there is a discontinuity in the distributions which predicts that there will be essentially no micelles with radii smaller than 25–30 nm. These predictions are all in accord with experimental observations. The good agreement between theory and experiment supports the micelle structure suggested by the model.

1. Introduction

The latest of many attempts to determine the size distribution of bovine milk casein micelles have been reported by Schmidt et al. [1], who used electron microscopy and light-scattering, and by Dewan et al. [2], who used the inelastic light-scattering techniques outlined earlier by Lin et al. [3]. Dewan et al. [2] also summarized and compared the results obtained previously by a number of workers. The observed differences were attributed mainly to technique although it was recognized that pooled milk from the same herd, collected on different days, could exhibit a different distribution even when using the same technique each time [3]. Thus, results obtained in different laboratories at different times may be subject to natural variation. The mechanism of micelle formation, using three distinctly different monomers and leading to a variation in casein micelle size distribution in an individual cow on different days, has been the subject of considerable study and speculation.

Experimentally observed casein micelle size distributions can be reproduced mathematically by a number of equations having no relationship to a mechanistic model for micelle formation but simply containing enough empirically determined parameters. Interestingly, the best fit of the data appears to have been obtained by Lin et al. [3] who used equations derived under the following assumptions. First, monomers combine to form relatively small polymers in a statistically determined range of sizes. These small polymers then aggregate to form large polymers with a characteristic size distribution. The concept of micelles being formed from submicellar particles of variable size has recently been proposed by Creamer and Berry [4] as a possible mechanism for casein micelle formation but no means of predicting the proper parameters in the equations has yet been devised. However, the fact that such equations can reproduce experimental data lends support to the idea that casein micelles are composed of aggregates of smaller polymers or submicelles.

There are a number of properties of casein micelle distributions that must be reproduced by a micelle model. Waugh and Talbot [5] have shown that there is an apparent discontinuity in the size distribution with no micelles being present with a radius of less than 25–30 nm. This is considerably larger than the postulated size of most submicelles. Ordinarily a smooth increase from submicellar size would be expected. It has also been shown that micelles reconstituted from α_s- and κ-caseins are an equilibrium system with a size distribution dependent upon the ratio of

κ-casein to α_s-caseins [5]. A higher percentage of κ-casein shifts the distribution toward a smaller average size. This also appears to be true of natural micelles [6] and they also apparently form an equilibrium system [1]. Consequently, some dependency of size distribution upon the casein constituency must be a part of the model.

Very few models of casein micelle structure can be used to predict a size distribution. In this paper I will examine the model proposed by Slattery and Evard [7] and show how it can be used to predict not only a micelle size distribution but also the other properties discussed above.

2. The model

2.1. Submicellar composition

2.1.1. Total

Slattery and Evard [7] suggested that a spherical submicelle can contain all three of the major caseins oriented radially from the center. For the calculations of this paper, there will be no differentiation between α_{s1}-casein and β-casein since each can provide interactive surface for submicellar aggregation. These interactions may be through the formation of calcium or calcium apatite bridges, through hydrophobic interactions or perhaps both. The α_{s1}- and β-casein monomers are probably of slightly different dimensions and could change the number of monomers possible in a submicelle if they were present in variable quantities [8]. It will be assumed, however, that the relative amounts of each remain constant throughout. It will also be assumed that every submicelle contains thirty monomers of one sort or another, and therefore vary not in size but in composition. If we let p equal the fraction of the casein present as monomers through which submicelles can interact and aggregate, then the probability, P_k, that a particular submicelle has k κ-casein monomers and $(30-k)$ α_{s1}- and/or β-casein monomers is given by

$$P_k = \frac{30!}{k!(30-k)!}(p)^k(1-p)^{30-k} \qquad (1)$$

The value of P_k should be directly proportional to the number (and concentration) of submicelles having k out of the thirty positions filled with κ-casein. Submicellar composition thus follows a binomial distribution of concentrations which is dependent upon the relative amount of κ-casein in the system, a quantity which can be measured.

2.1.2. In the micelle surface

The composition of the submicelles in the micelle surface must now be examined to determine what fraction of the surface of these submicelles would have to be non-interactive in order to prevent further growth of the micelle. The situation is approximated by fig. 1 showing the surface submicelles of radius, r_s, as being close-packed with their centers at a distance of $(r_i - r_s)$ from the center of the micelle. This distance, rather than the micelle radius, r_i, will be used later to calculate the surface area to be covered by submicelles. The shaded circles represent submicelles which could be added during continued growth. The two-dimensional analysis given here will be assumed applicable in the three-dimensional situation.

The lines joining the centers of two surface submicelles and one of those added for growth form an equilateral triangle with sides equal to $2r_s$ and angles of 60°. To prevent growth, the surface of each submicelle would have to be noninteractive over an area

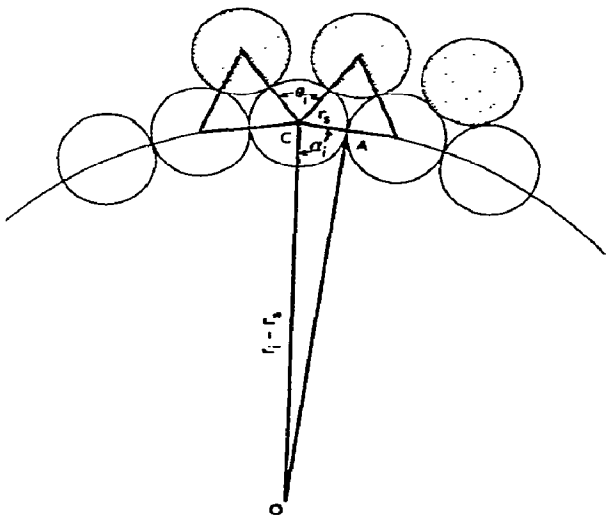

Fig. 1. Schematic of the casein micelle surface showing a layer of close-packed submicelles. The shaded circles represent submicelles which could be added as the micelle grows to a larger size.

equal to or larger than that subtended by a solid angle equal to θ_i. The maximum hydrophilic area, however, would be that subtended by a solid angle equal to $(120° + \theta_i)$. The value of θ_i depends upon r_i and is calculated by recognizing that

$$60° + 60° + \theta_i + 2\alpha_i = 360° , \quad (2)$$

so that

$$\theta_i = 240° - 2\alpha_i . \quad (3)$$

The angle α_i is determined by the right triangle AOC where AC equals r_s and CO equals $(r_i - r_s)$. In particular

$$\cos\alpha_i = r_s/(r_i - r_s) . \quad (4)$$

Therefore

$$\theta_i = 240° - 2 \arccos [r_s/(r_i - r_s)] . \quad (5)$$

Now, the fraction of the area of submicelle surface subtended by the solid angle equal to θ_i is given by

$$F_i(\min) = \tfrac{1}{2}(1 - \cos\tfrac{1}{2}\theta_i) . \quad (6)$$

This is the minimum fraction of the surface layer submicelles which must be κ-casein in order to prevent micelle growth. The maximum amount, $F_i(\max)$, can be calculated by replacing θ_i in eq. (6) with $(120° + \theta_i)$. If an amount of κ-casein greater than that were in a surface submicelle, it could not interact laterally to form a close-packed surface array. This value can be translated into the actual number of κ-casein monomers in a submicelle by recognizing that the values of k for these submicelles would be the values of the integers closest to $30F_i$.

2.2. Micelle composition

2.2.1. Volume per submicelle

According to the proposed model [7], the micelle is composed of a mostly close-packed array of spherical submicelles. To determine the number of submicelles required to make a micelle of a particular size, the average volume occupied by a submicelle must be calculated. This can be done by assuming that the close-packed spheres form a face-centered cubic structure which contains four spheres per unit cell. In fig. 2, consider one face of the unit cell, designated ABCD. Looking at the right triangle DEF, it is apparent that DE = $2r_s$ and DF = EF = DC/2. Therefore,

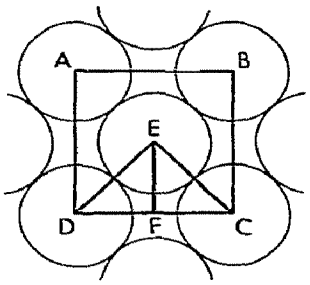

Fig. 2. Close-packed spheres in a face-centered cubic array. The square ABCD represents one face of the unit cell.

$$(\tfrac{1}{2}\,DC)^2 + (\tfrac{1}{2}\,DC)^2 = (2r_s)^2 , \quad (7)$$

or

$$DC = 2\sqrt{2}r_s , \quad (8)$$

which is the length of one edge of the cubic unit cell. The volume of the unit cell containing four submicelles is the cube of this which gives the volume per submicelle as $4\sqrt{2}r_s^3$. This can be divided into the volume of the micelle to determine the number of submicelles needed in its construction.

2.2.2. Surface area per submicelle

In order to be stable, a micelle must contain enough κ-casein in the surface to prevent the inter-submicellar interactions which result in further aggregation. Consequently, as a group, the surface submicelles are more restricted in their composition than are those in the micelle interior. The number of such submicelles

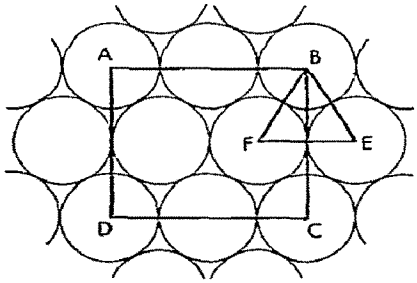

Fig. 3. A possible arrangement of close-packed spheres in a surface. Four spheres are included in the area enclosed by ABCD.

per micelle can be calculated if the average surface area occupied per submicelle is known. Consider a close-packed array as shown in fig. 3. Upon inspection, it is apparent that the area enclosed by ABCD contains four submicelles and that the length of side AB equals $4r_s$. However, the side BC is shorter and is equal to twice the height of the equilateral triangle BEF, or $2\sqrt{3}r_s$. Therefore,

$$(AB)(BC) = 8\sqrt{3}r_s^2 , \tag{9}$$

and the area per submicelle is thus one-fourth of this or $2\sqrt{3}r_s^2$.

2.2.3. Sequential construction

For a particular micelle with radius r_i, there are x_i interior submicelles for which $0 \leqslant k \leqslant 29$. This upper limit is purely arbitrary and simply states the belief that any submicelle which contains at least one α_{s1}- or β-casein monomer can interact with another submicelle and thus participate in micelle growth. Even if the upper limit were decreased to as low as 24 or 25, so that as much as one-fifth or one-sixth of the submicelle would need to be interactive in order for it to be in the interior, the calculated results would not be changed significantly. These interior submicelles must be followed by the addition of y_i surface submicelles for which $30F_i(\min) \leqslant k \leqslant 30F_i(\max)$. The probability of producing a certain size of micelle, then, is the probability that in $(x_i + y_i)$ trials or submicelle aggregations, the first x_i will be with submicelles having the larger range of k and the next y_i will be with those having the more restricted range. Remembering that eq. (1) is normalized so that the sum of the P_k values is equal to unity, the probability of getting the proper submicelle at a particular trial is equal to the sum of the P_k values over the selected interval. Consequently, the probability, P_i, of forming a micelle of radius r_i is [9]

$$P_i = \left(\sum_{k=0}^{29} P_k\right)^{x_i} \left(\sum_{k=30F_i(\min)}^{30F_i(\max)} P_k\right)^{y_i} , \tag{10}$$

where

$$y_i = \frac{4\pi(r_i - r_s)^2}{2\sqrt{3}r_s^2} = \frac{2\pi}{\sqrt{3}} \frac{(r_i - r_s)^2}{r_s^2} , \tag{11}$$

and

$$x_i = \frac{\frac{4}{3}\pi r_i^3}{4\sqrt{2}r_s^3} - y_i = \frac{\pi}{3\sqrt{2}} \frac{r_i^3}{r_s^3} - \frac{2\pi}{\sqrt{3}} \frac{(r_i - r_s)^2}{r_s^2} . \tag{12}$$

The number of such micelles produced should be proportional to the value of P_i.

2.3. Weight fractions

A comparison between calculated and measured size distributions can be made if the values of P_i can be converted to weight fractions. If micelles of different size are assumed to have essentially equal densities, the weight fraction, $W(r_i)$, of a particular size is given by

$$W(r_i) = \frac{r_i^3 P_i}{\Sigma_{\text{all } i}\, r_i^3 P_i} . \tag{13}$$

And, as indicated by Dewan et al. [2], a cumulative weight distribution, $W(r_j)$, can be obtained, using eq. (14), for all micelles up to and including those with radius r_j.

$$W(r_j) = \sum_{i=1}^{j} W(r_i) . \tag{14}$$

From these a determination can be made as to the ability of the model to predict experimentally measured size distributions.

3. Results and discussion

3.1. The value of p

There is a variation in relative casein composition, not only in the milk from different cows but also in milks from the same cow obtained at different times [10]. A normal average is considered to be about 15% κ-casein ($p = 0.15$). Fig. 4 shows how P_k changes as k changes for various values of p. From this, it can be seen that the submicelle with the maximum concentration when $p = 0.15$ has four κ-casein monomers and 26 α_{s1}- and/or β-casein monomers. These are the most likely to aggregate to form the micelle interior. However, as those submicelles aggregate which are poor in κ-casein but have the highest concentrations, the overall composition of the remaining submicelles changes

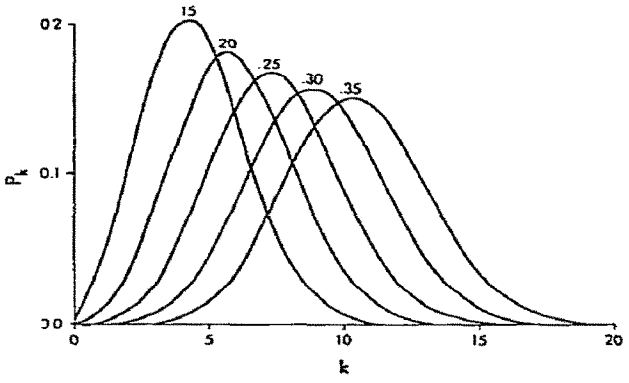

Fig. 4. Plots of P_k versus k for various fractional amounts of κ-casein in the system. Each curve represents a normalized compositional concentration distribution for the submicelles.

toward a larger fraction of κ-casein. Assuming that there is a dynamic equilibrium with reasonably rapid exchange between surface submicelles and those in the supernatant, all of the submicelles exposed to the solution would attain a distribution in P_k corresponding to a higher value for p. An examination of eq. (10) along with fig. 4 indicates that the sum of the P_k values for the x_i interior submicelles is always essentially equal to unity since P_k is appreciable only for $k < 20$. The P_k values used in the summation for the y_i surface submicelles, however, must correspond with the fraction of κ-casein in the milk serum. This value is given by Rose [11] as 0.29 for the milk from a single cow at a particular milking. Consequently, for the purposes of calculation, p is an adjustable parameter which should have a value near the experimental value of 0.29.

3.2. The value of r_s

The size of the submicelles is difficult to determine accurately. Hydrodynamic measurements, under conditions which lead to maximum submicellar size, indicate that r_s is about 10 nm [7, 8]. This value is supported by at least one study with the electron microscope [12] which places r_s between 7.5 nm and 10 nm. Gel filtration suggests either a 5 nm radius [13, 14] or a wide range of sizes [4] depending upon the method of pre-treatment of the micelles. Most electron microscopy, beginning with the work of Shimmin and Hill [15, 16], also places r_s at about 5 nm. However, it should be noted that in these studies, conditions were such as to cause casein dissociation and/or dehydration. A lower apparent value for r_s might therefore be expected. For the calculations of this paper, a value of 8.7 nm was used for r_s. This value is most consistent with the assumed number of monomers per submicelle and with the micelle surface area per κ-casein monomer, calculated by Waugh and Talbot [5]. It represents the distance of closest approach for the submicelles which could either be larger and have some interpenetration of monomers, as suggested by Waugh et al. [8], or smaller, as suggested by some of the methods mentioned above, and be connected by salt bridges across the open space between.

3.3. Calculated distributions

Fig. 5 contains the experimental points of Dewan et al. [2] and Lin et al. [3], showing how $W(r_j)$ varies with r_j for different pooled milk samples. The solid lines show the calculated distributions at the indicated values for p. It can be seen that two of the milk samples with coincident distributions are represented quite well by the distribution calculated at $p = 0.33$. Two other coincident samples are well represented by the distribution calculated at $p = 0.32$. The separate pooled milk sample is not as well represented by a theoretical distribution but could probably be fit, to within experimental error, by a curve calculated with p between 0.32 and 0.31. It should be noted that the model predicts a shift in the micelle size distribution toward smaller size as the fraction of κ-casein present increases, as represented by an increased value for p. The data indicate, in fact, that a fairly small change in the relative amount of κ-casein in the system would cause a considerable change in the micelle size distribution. This would be reflected in only a small variation in overall composition between different milk samples. It is particularly encouraging to see such a close correspondence between the values of p which allow the model to reproduce experimental results and the value predicted by the measurements of Rose [11]. Another point worth noting is that in the size distribution calculations, based on the model of Slattery and Evard [7], there emerges a large difference in $W(r_j)$ between micelles with a radius of 20 nm and those with a radius of 30 nm. This difference is of the order

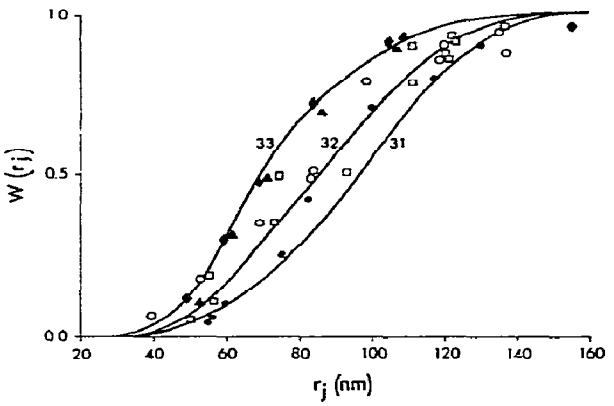

Fig. 5. A comparison of calculated and experimental cumulative micelle size distributions. The solid points are experimental distributions of three different pooled milk samples as determined by Lin et al. [3]. Two are essentially coincident while the third is different. The open points are experimental distributions of two different pooled milk samples as determined by Dewan et al. [2]. These two are essentially coincident. The solid curves represent distributions calculated by the methods of this paper using the values indicated for the fractional κ-casein content of the final system.

of 10^6 to 10^9. This means that while the model predicts some micelles of 30 nm radius and larger to be present, there are essentially none of 20 nm radius. As mentioned in the introduction, this result is consistent with experimental observation [5].

4. Conclusions

Most models for casein micelle structure do not lend themselves to the calculation of a size distribution. One which does [17] has been examined [18] and found inadequate in predicting experimental distributions. The results of this paper, however, show that the model proposed by Slattery and Evard [7] will reproduce a number of experimental micelle size distributions, supposedly containing different relative amounts of κ-casein. It would be interesting to determine various micelle size distributions and the corresponding κ-casein content of the milk serum to see just how close a correlation there may be. While not in any way proving the accuracy of their model, the results reported here give support to the concept of casein micelle formation and structure as outlined by Slattery and Evard [7].

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