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Original article

Simulating the self-association of caseins: towards a model for the casein micelle

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Abstract – A Monte Carlo computer simulation is used to simulate the self-association of model casein-like block copolymers. One-component studies of α_{s1} and β-casein show a strong tendency to form large micelles, with some evidence of flower-like and worm-like micelles in α_{s1}-casein systems. α_{s2}-Casein forms small micelles in a one-component system, and has difficulty forming mixed micelles in binary and three-component mixtures. This is attributed to its tetra-block structure (alternating hydrophobic and hydrophilic blocks), where one of the hydrophobic blocks is attached at both ends to a hydrophilic block, which reduces its ability to self-associate with other hydrophobic blocks. The order of increasing micelle size depends on whether hydrophobic association is allowed between blocks on the same chain or not. When allowed, the chains tend to fold up on themselves which makes micellization more difficult. The order of increasing micelle size is then $\alpha_{s2} < \alpha_{s1} < \beta$. If intra-chain hydrophobic association is not allowed (increased rigidity of the blocks) the order of increasing micelle size changes to $\alpha_{s2} < \beta < \alpha_{s1}$. In the latter case two hydrophobic blocks at the ends of the α_{s1}-chain are able to associate with more than one hydrophobic micellar core, and thus act as a bridge between two micelles. In binary systems only α_{s1} + β-casein mixtures show a strong preference to form micelles where the hydrophobic blocks of the two chains are mixed relatively evenly in the hydrophobic core, with an approximate 1:1 ratio of the two chains. When α_{s2}-casein is present in the binary simulations it dominates the micellization behavior by limiting the growth of micelles. This is most likely due to the second hydrophobic block of α_{s2}-chains having a low propensity to associate with other hydrophobic blocks, and thus having a low ability to propagate micelle growth. A similar situation is seen in three component mixtures of $\alpha_{s1} + \alpha_{s2} + \beta$ at ratios of 1:1:1 and 4:1:4.

casein / association / computer simulation / micelle

摘要 – 模拟酪蛋白的自聚作用：酪蛋白胶束形成模型。采用蒙特卡洛计算机模拟方法建立了酪蛋白样嵌段共聚物自聚作用的模型。单一化合物 α_{s1}和 β-酪蛋白表现出非常强的形成大胶束的趋势, 在 α_{s1}-酪蛋白体系中能够形成明显的花状和蠕虫状的胶束。在单一化合物体系中, α_{s2}-酪蛋白形成的胶束小, 而且难于形成二元和三元的混合物; 原因是其具有四嵌段结构 (相互疏水和亲水的嵌段), 其中一个疏水嵌段被附着在亲水嵌段的两端, 因而降低了与其他疏水嵌段的自聚作用。酪蛋白胶束尺寸增加的顺序取决于相同或不同链长的嵌段是否能够发生疏水聚合作用。如果能够发生聚合作用, 链自身折叠起来则很难发生胶束化作用, 因而胶束尺寸增加的顺序是 $\alpha_{s2}<\alpha_{s1}<\beta$。如果不发生链内的疏水聚合作用 (增加了嵌段的刚性) , 胶束尺寸增加的顺序则是 $\alpha_{s2}<\beta<\alpha_{s1}$。在后者的情况下, 在 α_{s1}-链端点的两个疏水嵌段可以结合成为多个疏水胶束的核心, 因而 α_{s1}-酪蛋白在两个胶束之间的起着桥梁作用。对于只有 α_{s1}+β-酪蛋白的二元混合体系中表现出了非常强的优先形成胶束的能力, 这个胶束是由两个链的疏水嵌段均匀地混合形成的疏水中心,

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两个链的比例基本上是1:1。当 α_{s2}-酪蛋白出现在二元混合物模型中时, α_{s2}-酪蛋白决定着胶束作用的行为并限制着胶束的生长。原因是 α_{s2}-酪蛋白链上的次级疏水嵌段与其他疏水嵌段结合的能力较低, 因而胶束增长的能力也较低。在 $\alpha_{s1}+\alpha_{s2}+\beta$混合物的比例在1:1:1 和 4:1:4 的三元混合体系中也得到了类似的结果。

酪蛋白 / 聚合 / 计算机模拟 / 胶束

Résumé – Simulation de l'auto-association des caséines : vers un modèle de la micelle de caséines. Une simulation informatique Monte Carlo a été utilisée pour modéliser l'auto-association de copolymères modèles comme les caséines. Dans un système à un composant, la caséine α_{s1} et la caséine β montrent une tendance lourde à former de grosses micelles avec, pour la caséine α_{s1}, des micelles en forme de fleur ou de ver. La caséine α_{s2} forme des petites micelles dans un système à un composant, et a des difficultés à former des micelles mixtes dans des mélanges de 2 ou 3 composants, ce qui est attribué à sa structure tétra-bloc (alternant des blocs hydrophobes et des blocs hydrophiles), où un des blocs hydrophobes est attaché aux deux extrémités d'un bloc hydrophile, ce qui réduit son aptitude à s'auto-associer aves les autres blocs hydrophobes. L'ordre d'accroissement de la taille de la micelle dépend du fait que l'association hydrophobe est autorisée ou non entre blocs sur la même chaîne. Quand elle est autorisée, les chaînes tendent à se replier sur elles-mêmes, ce qui rend la micellisation plus difficile. L'ordre d'accroissement de la taille de la micelle est donc $\alpha_{s2} < \beta < \alpha_{s1}$. Dans le dernier cas, deux blocs hydrophobes aux extrémités de la chaîne α_{s1}sont capables de s'associer avec plus d'un noyau central hydrophobe de la micelle et agissent ainsi comme un pont entre deux micelles. Dans les systèmes binaires, seuls les mélanges caséine α_{s1}-caséine β montrent une préférence forte à former des micelles quand les blocs hydrophobes des deux chaînes sont mêlés relativement régulièrement dans la partie centrale, avec un rapport 1:1 des deux chaînes. Quand la caséine α_{s2} est présente dans les simulations binaires, elle a un rôle prédominant dans la micellisation en limitant la croissance des micelles. Ceci est probablement dû au second bloc hydrophobe des chaînes α_{s2} qui ont une faible propension à s'associer avec les autres blocs hydrophobes et ainsi une faible capacité à propager la croissance de la micelle. Une situation similaire est observée dans les mélanges à 3 caséines $\alpha_{s1} + \alpha_{s2} + \beta$ à des taux de 1:1:1 et de 4:1:4.

caséine / association / modélisation / micelle

1. INTRODUCTION

Computer simulation techniques have long been used to study the structure of colloidal and polymer systems [3]. Their use in food systems, however, is less common, although not unknown [5, 6]. This is due to the very complex and non-ideal nature of foods systems, which means that any model of the system must be highly simplified. Even with simple models much useful information can be gleaned from simulations. In this paper, we will report a simple model to simulate the self-association behaviour of bovine casein molecules which are treated as linear block copolymers. We will show that even when we ignore the complexities of the protein structure we can reproduce self-association behaviour that is qualitatively similar to that observed for casein molecules in solution.

The ability of the caseins to form self-association structures similar to surfactant micelles has been known for many years, and has been linked to the amphiphilic nature of the caseins. It has been noted that the primary sequence of the caseins shows a marked segregation of residues into regions rich in hydrophobic amino acids, and those rich in charged amino acids. Horne [13] has suggested that as a first approximation the caseins can be thought of as block copolymers, containing separate hydrophobic and hydrophilic blocks. In this scheme α_{s1}-casein is a triblock copolymer with hydrophobic blocks at the C- and N-terminal ends taking up one-third of the molecule, and a hydrophilic block between in the middle. α_{s2}-Casein becomes

a tetra-block copolymer with alternate hydrophobic and hydrophilic blocks starting from the N-terminal end, each occupying a quarter of the molecule. β-Casein and κ-casein are both di-block copolymers with β-casein having a hydrophilic block at the N-terminal end and a hydrophobic block at the C-terminal end, with the hydrophobic block taking up about two-thirds of the molecule. κ-Casein has a hydrophobic block at the N-terminal end, (a quarter of the molecule) with the remainder as a hydrophilic block.

The self-association behaviour of the caseins is well documented, and shares some similarities with synthetic block copolymer systems which are also known to form self-association structures [1].

α_{s1}-Casein has been observed to form linear polymer-like chains where the hydrophobic regions of the individual chains join end to end [16, 18, 19, 23]. Horne [13] has also hypothesized that α_{s1}-casein might form a micelle where the two hydrophobic ends of the same molecule self-associate to form a petal-like loop, a number of which then join together to form a flower-like micelle. β-Casein has been observed to form ellipsoidal micelles [14]. κ-Casein has a micellization behaviour similar to β-casein [24, 25].

α_{s2}-Casein is the least studied of the caseins, and although it is known to form association structures in solution [20] the structure of these is not known. Horne [12] has speculated that α_{s2}-casein will form linear micelles similar to α_{s1}-casein.

Due to the simplicity of the model it can easily be extended to look at self-association of mixtures of model caseins, systems which have not been as widely studied experimentally. Eventually the model can be extended to investigate self-association of all four caseins into micelle-like structures. This paper is an extension of our earlier work on simulating micellization in one-component casein systems [5–7]. Although the model and methodology are still in the early stages of development, eventually we hope that this type of study will give us insight into the mechanisms of formation of the casein micelle.

2. MATERIALS AND METHODS

Casein chains were defined in a mesoscopic model as block copolymers comprised of 24 segments each. Each segment corresponds to approximately 7-8 amino acid residues, and thus the model represents a highly coarse-grained representation of the casein primary sequence. Two types of segment were defined, with these being equivalent to hydrophilic and hydrophobic amino acid residues. The two segment types were arranged in blocks. The length and number of these blocks was varied from casein to casein to reflect the known tendency for hydrophobic and charged residues to be grouped close together in these molecules. Table I gives details of the block structure used for the four different casein types.

Simulations were carried out on a 200×200 2-D 8-choice square lattice with periodic boundary conditions at all four sides. Each segment of a casein chain occupies one lattice site. For all simulations the number of chains was chosen to give an area fraction occupied by the chain segments of approximately 12%. A highly efficient partial reptation Monte Carlo algorithm was used to move the chain segments [4, 7, 10]. The implementation of this algorithm has been discussed in detail by Haire et al. [10], and is outlined below.

(i) Choose at random a vacant lattice site that is not occupied by a chain segment.
(ii) Choose at random one of the eight nearest neighbours of the vacant lattice site. The move is rejected if this lattice site is vacant (not occupied by a chain segment) otherwise a move is attempted.

Table I. Assignment of blocks in the mesoscopic model for caseins. The total number of polymer chain segments is 24.

	Number of chain segments per block			
Protein	1st hydrophobic	1st hydrophilic	2nd hydrophobic	2nd hydrophilic
α_{s1}-casein	8 (C-terminal)	8	8 (N-terminal)	
α_{s2}-casein	6 (N-terminal)	6	6	6 (C-terminal)
β-casein	6 (C-terminal)	18 (N-terminal)		
κ-casein	8 (N-terminal)	16 (C-terminal)		

(iii) To start the movement routine the positions of the vacant and occupied lattice sites are exchanged.
(iv) If this exchange leads to the chain being broken in two places the move is rejected.
(v) If the exchange can be achieved without breaking the chain the move is allowed and counts as a single Monte Carlo move.
(vi) If the exchange leads to a single break in the chain the move is allowed, and this constitutes the start of a sequence of multiple segment moves. The movement sequence is continued by exchanging the position of the vacant lattice site with subsequent segments along the chain until the chain is reconnected, or until the end of the chain is reached. This multiple segment movement sequence is counted as a single Monte Carlo movement.

Since chain movement involves exchange of segment positions with a vacancy, double occupancy of lattice sites is avoided. One of the big advantages of this method is that it can be used for systems at high area/volume fractions with high efficiency. Therefore, it does not suffer from the problem of low acceptance ratio when the system is highly aggregated. This acceptance ratio problem makes many other chain movement algorithms unsuitable for aggregated systems.

Interactions between segments are of the square-well type [6], and interaction is only allowed between a segment and one of its 8 nearest neighbours on the lattice. The strength of the interaction is measured in units of kT. Interactions between hydrophilic segments are set as athermal i.e. $E_{hp}/kT = 0$ per segment, and between hydrophobic segments as attractive with $E_{hb}/kT = -0.6$ per segment used in this work. This treats hydrophobic interactions as purely enthalpic (E_{hb} is equal to an internal energy U). In reality they are partly entropic in nature (i.e. E_{hb} is equal to the Helmholtz free energy, U-TS). To include the entropic nature of hydrophobic interactions would involve an explicit inclusion of the solvent (water) molecules. The entropy term for the Helmholtz free energy would then arise from disruption of the attractive interactions between the solvent. However, the net effect of this is the same as having a purely enthalpic interaction between hydrophobic groups, and it is easer to use this approach when developing the computer algorithm, and for this reason we choose the enthalpic description in this work.

The acceptance or rejection of the move is achieved by using importance sampling methods via the Metropolis form of a Monte Carlo algorithm [6]. In this, the internal energy of the system is calculated for the conformation before and after the move. If the energy decreases after chain movement ($\Delta E \leqslant 0$) the move is accepted. If $\Delta E > 0$ (energy increases) the move is accepted with a probability equal to the Boltzmann factor ($\exp(-\Delta E/kT)$) for that

move. This generates a Boltzmann distribution of energy states in the simulated conformations.

Simulations were started from an ordered conformation and were randomized as an athermal simulation (no interactions between the segments) for 4×10^9 Monte Carlo (MC) attempts. Interactions between segments were then turned on and the simulation was run for between 10^9 and 2×10^9 MC attempts to equilibrate the system. Equilibrium is achieved when the overall change in internal energy (the sum of ΔE over the whole simulation) converges to a constant value. This was found to occur during the equilibration period. The simulations were then run for a further period of 10^9 MC attempts during which time the chain conformations were sampled every 10^6 MC attempts.

Analysis of the simulated conformations involved the calculation of the mean size (number of chains) in each cluster and the proportion of each casein type in the clusters The cluster determination algorithm proposed by Stoddard [21], modified to account for the chain nature of the molecules, was used to calculate these.

3. RESULTS AND DISCUSSION

Figures 1–4 are simulations of model α_{s1}-, α_{s2}- and β-casein chains at an area fraction of 12%. In these simulations hydrophobic segments on the same chain are allowed to interact with each other. A consequence of this is that hydrophobic regions on the same chain fold onto themselves, and places a restriction on the way they pack in a micelle. Figure 1 is a snapshot conformation of model α_{s1}-casein. The chain clearly self-associates to form micelle-like aggregates. These are comprised of a dense core of hydrophobic blocks surrounded by a layer of hydrophilic blocks at the surface. This hydrophilic layer protects regions of the surface from further self-association. If we look closely at a typical α_{s1}-casein micelle (Fig. 2) we can see that the arrangement of the individual chains is similar to that in the putative flower-type micelle hypothesized for α_{s1}-casein. There is also some evidence in Figure 1 that α_{s1}-casein micelles form where a single α_{s1}-chain forms a bridge between two micelles, with separate hydrophobic blocks on the same chain participating in separate hydrophobic cores. From Table II we can see that the number average cluster size for α_{s1}-casein micelles is 2.44 with a maximum micelle size of 11 chains.

Simulated α_{s2}-casein micelles (Fig. 3) show a different behaviour to that observed for α_{s1}-casein. Relatively few micelles are formed, and those that do occur are small (mean cluster size = 1.26, maximum cluster size = 6, Tab. II). The tetra-block structure of α_{s2}-casein is such that there is a strong tendency for self-association within a single chain itself. This forms a structure which is micelle-like in its own right, with a small hydrophilic core and two surface hydrophilic blocks. This structure has a low tendency to self-associate with other α_{s2}-chains due to the relatively high coverage of the hydrophobic surface by hydrophilic segments.

Figure 4 is a snapshot of a system of simulated β-casein. Here the micelles are larger than for α_{s1}-casein (mean size = 3.74, maximum size = 18, Tab. II), but still have the same structure of a hydrophobic core covered by a "hairy" layer of hydrophilic blocks. The structure of this hairy layer is different to that observed in α_{s1}-casein micelles. In the later the hydrophilic blocks are attached to the hydrophobic core at both ends, and adopt a flattened conformation at the surface. In β-casein micelles the hydrophobic blocks are free at one end, and adopt an extended conformation. The large size of the β-casein micelle is most likely a result of the high proportion of hydrophilic segments (3/4 of

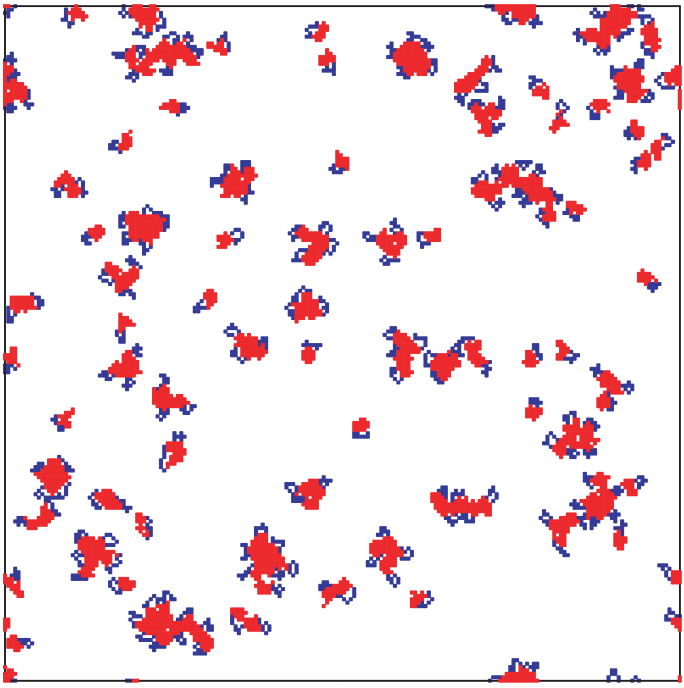

Figure 1. Snapshot conformation for model α_{s1}-casein with intra molecular hydrophobic interactions included (red = hydrophobic segments, blue = hydrophilic segments).

the chain) compared to α_{s1}-casein (2/3) and α_{s2}-casein (1/2).

The model casein chains in Figures 1–4 are highly folded. This restricts both the ability of the chains to self-associate, and the structure of the micelles that are formed. There are two current views as to the structure adopted by the caseins in solution. The rheomorphic hypothesis of Holt and Sawyer [11] puts forward the view that the caseins are essentially flexible proteins that alter their conformation according to the environment. Farrell et al. [8] take the view that caseins adopt a molten globule like conformation, which is not a true tertiary structure but is more compact than a random coil. We have also used a second model for our casein chains, one where interactions between hydrophobic segments on the same chain are ignored. This prevents prevent intra-molecular folding of the chains, and they behave like random coils when they are not part of an aggregate. The second model is closer to the block copolymer model for caseins proposed by Horne [12], since it implies a relatively high degree of block rigidity. It is likely that casein structure in solution is somewhere between these two ideal situations.

Figures 5–7 are simulated systems of α_{s1}-, α_{s2}- and β-casein using the same parameters as for Figures 1–4, but with intra-molecular hydrophobic interactions excluded. In Figure 5 it is clear that larger α_{s1}-casein micelles are formed (mean size = 18.10, maximum size = 79, Tab. II). Clearly, removing the intra-chain hydrophobic interactions allows for far more efficient packing in the micelle core, and larger micelle sizes. The micelles have a structure very different to those in Figure 1.

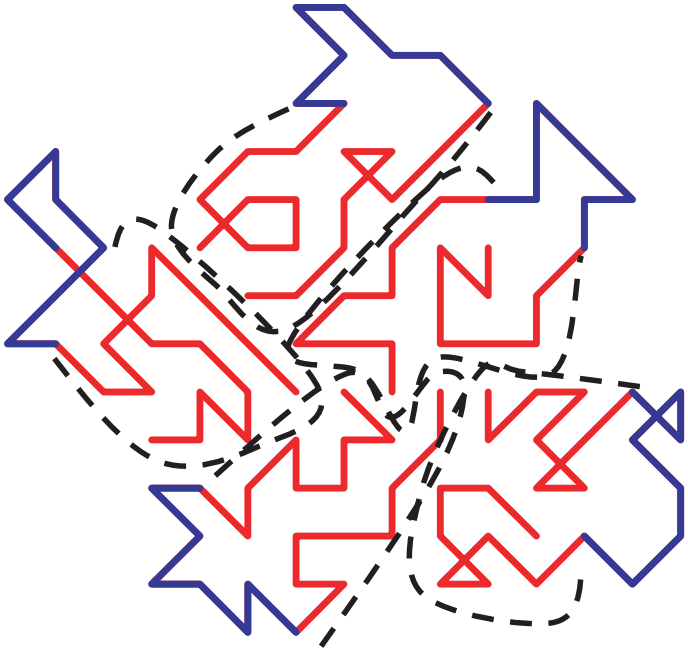

Figure 2. Simulated flower-like α_{s1}-casein micelle.

Table II. Mean cluster size for single chain and mixed chain systems.

System	Mean cluster size (number of monomers)	Largest cluster size	Mean percentage of monomers (%)
α_{s1}-casein (with intra-chain interactions)	2.44	11	18
α_{s2}-casein (with intra-chain interactions)	1.25	6	63
β-casein (with intra-chain interactions)	3.74	18	8
α_{s1}-casein (no intra-chain interactions)	18.10	79	0.6
α_{s2}-casein (no intra-chain interactions)	2.76	27	15
β-casein (no intra-chain interactions)	10.78	21	0.01
α_{s1} + α_{s2}	5.39	64	8
α_{s1} + β	13.58	58	0.2
α_{s2} + β	4.26	36	9
α_{s1} + α_{s2} + β (1:1:1)	6.17	55	6
α_{s1} + α_{s2} + β (4:1:4)	9.08	54	3

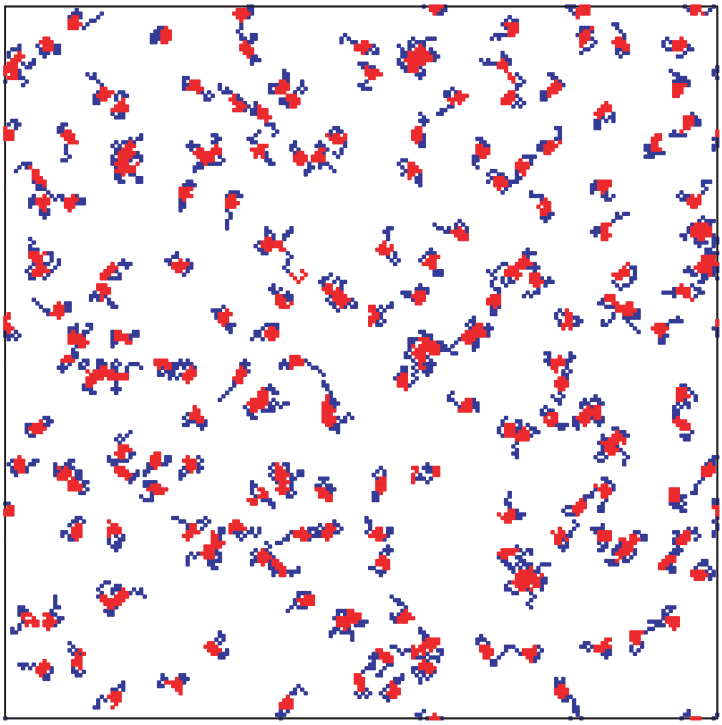

Figure 3. Snapshot conformation for model α_{s2}-casein with intra molecular hydrophobic interactions included (red = hydrophobic segments, blue = hydrophilic segments).

They consist of several hydrophobic cores, held together by hydrophilic block bridges. We can also see one elongated worm-like micelle where several chains have associated in an end to end arrangement (Fig. 5). If we compare these α_{s1}-micelles to β-casein micelles formed using the same model (Fig. 7) we can see that although the β-casein micelles are larger than those in Figure 1 (mean size = 10.78, maximum size = 21, Tab. II), they are smaller than the α_{s1}-micelles in Figure 5. It would appear that removing internal hydrophilic interactions favours α_{s1}-casein bridge formation and thus favour formation of larger micelles. The α_{s2}-casein micelles formed with the second model (Fig. 6) are also larger in size (mean size = 2.76, maximum size = 27, Tab. II). However, they are also very open and diffuse in structure. The open nature of the chains in this system allows them to occupy a large apparent area. This leads to a relatively large number of potential contacts with other chains, and probably leads to the large maximum size of the α_{s2} micelles compared to β-micelles. The large α_{s2} micelles although they form are probably only short-lived, and therefore do not contribute greatly to the mean cluster size. The size of the hydrophobic blocks and the relatively low proportion of hydrophobic segments make compact micelle formation difficult in this system. The size distribution of the micelles for each model casein type is shown in Figure 8. α_{s1}-casein (Fig. 8a) shows a broad range of micelle sizes. The number of monomers appears relatively high in the

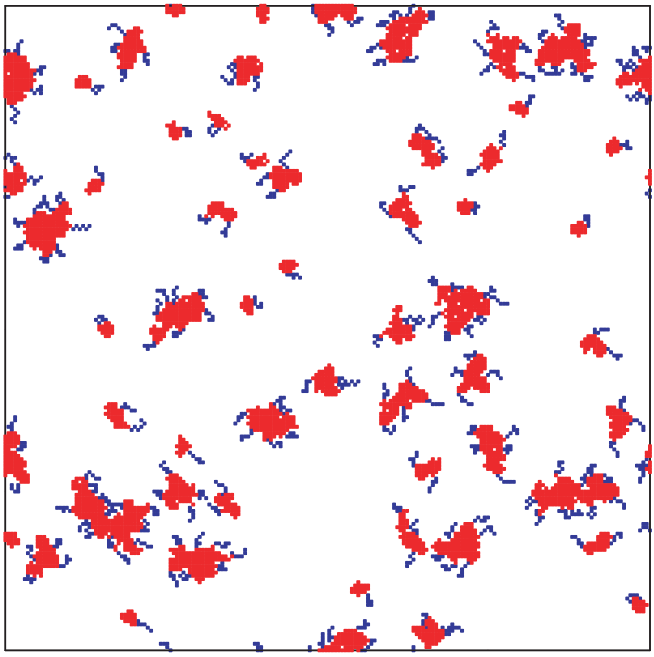

Figure 4. Snapshot conformation for model β-casein with intra molecular hydrophobic interactions included (red = hydrophobic segments, blue = hydrophilic segments).

system, although we should be aware that this is a number average size distribution and so it gives the small number of very large micelles a low weighting. In contrast α_{s2}-casein (Fig. 8b) shows a narrow range of micelle sizes with the frequency of a particular micelle size showing a smooth decrease as the size decreases. β-Casein (Fig. 8c) is the only one of the three casein types to show a maximum in the cluster size distribution which is not equivalent to the monomeric state. In fact the size distribution graph shows a very small probability of a β-casein chain being found in the monomeric state throughout the whole simulation (0.01%). There are also two peaks in the size distribution graph corresponding to micelle sizes of 4 and 11 aggregated monomer chains, suggesting a preference for a bidisperse system with micelle sizes centred on these two cluster sizes.

Comparing our model results to experimental results is obviously difficult. We have made no explicit attempt to control environmental factors such as pH, ionic strength and temperature in our models, all parameters that can be varied experimentally and which have been shown to affect both protein structure and association. However, a general comparison with the available experimental data for one-component casein systems reveals some interesting features. α_{s1}-Casein is known to associate with a step-wise mechanism to form dimers, trimers, tetramers, etc., with the final micelle structure highly dependent on pH and ionic strength [19]. At 37 °C α_{s1}-casein micelles dissociate to form dimers [15]. Thurn et al. [23] report that at pH 6.7 and ionic strength 0.2 mol·L^{-1} α_{s1}-casein forms large worm like micelles with a contour length (roughly corresponding to a micelle end-to end length) of

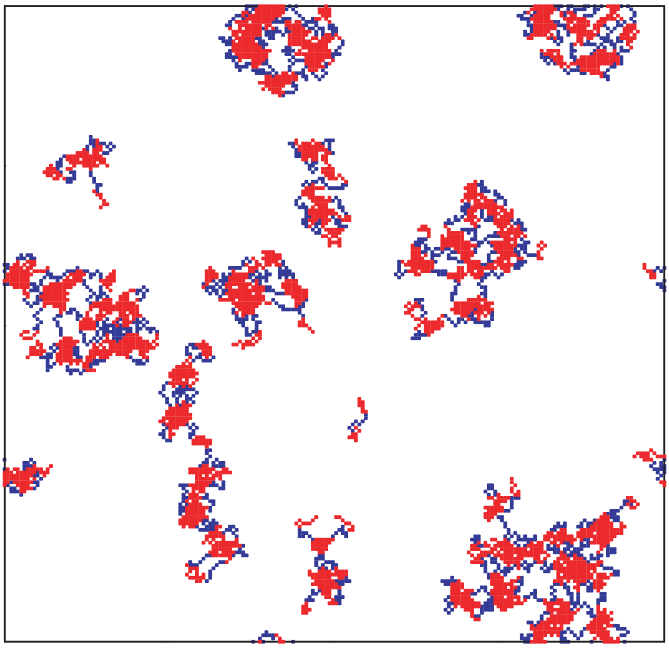

Figure 5. Snapshot conformation for model α_{s1}-casein without intra molecular hydrophobic interactions included (red = hydrophobic segments, blue = hydrophilic segments).

1600 nm, and a persistence length (mean distance the micelle propagates in one direction before changing direction) of 130 nm. Thus, these micelles are both long and non-linear. β-Casein micelles have been reported as having an average radius of 17 nm and an average aggregation number (number of monomers) of 23 at pH 7, ionic strength = 0.2 mol·L^{-1}, 21 °C [26], and a radius of 15.4 nm and aggregation number of 38 at 15 °C, pH 6.7, I = 0.2 mol·L^{-1} [22]. For α_{s2}-casein micelles, Snoeren et al. [20] report a mean radius of about 3.7 nm at pH 6.7, 0.005 mol·L^{-1} EDTA, i.e. far smaller than for α_{s1}-casein and β-casein micelles under similar conditions.

Although we do see some evidence of elongated micelles in our α_{s1}-casein simulations, the majority are roughly spherical or oblate spheres, and are closer to the flower-like micelles proposed by Horne [12]. Currently, our model does not appear to satisfactorily reproduce the worm-like micelles reported for α_{s1}-casein. We do see some worm-like micelles formed in α_{s1}-casein simulations, but they do not appear to be the predominant micelle type. If we compare our predictions for micelle size with the experimental results, however, our model does at least predict the correct qualitative order for increasing micelle size if we use the model with no intra-chain hydrophobic interactions. Here our model predicts that α_{s1}-chains form the largest micelles, with α_{s2}-chains the smallest. The prediction of the shape of β-casein micelles is closer to those of experiment, where an oblate spheroid structure has been predicted based on direct imaging using cryogenic TEM techniques [17].

In our simulations the structure of the hydrophilic surface layer partly controls

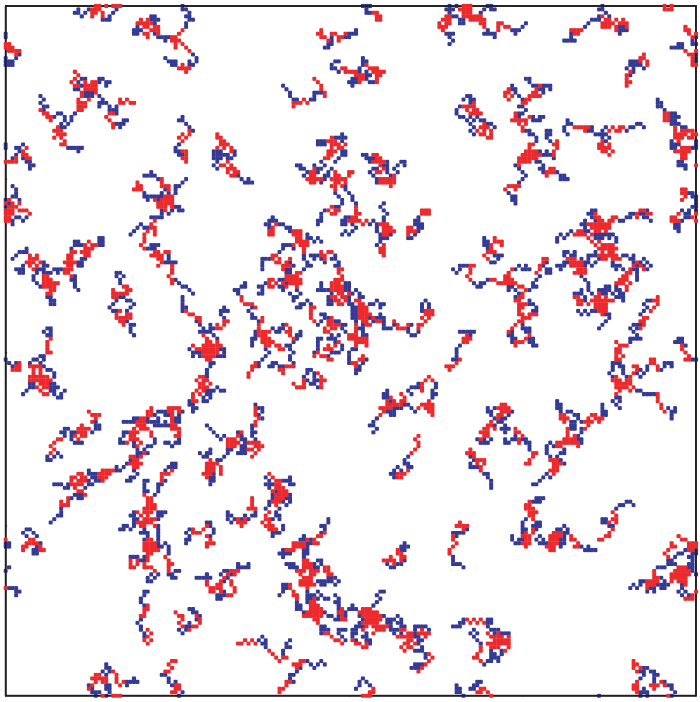

Figure 6. Snapshot conformation for model α_{s2}-casein without intra molecular hydrophobic interactions included (red = hydrophobic segments, blue = hydrophilic segments).

the size of the micelle, since this acts to prevent close approach of free chains or other micelles and stops them aggregating with the hydrophobic core. The structure and size of the micelle is also controlled by the flexibility of the hydrophobic block, with a more compact block giving relatively small micelle for all casein types. Flexible hydrophobic blocks promote bridging in α_{s1}-casein micelles, which leads to large micelles. This suggests that environmental factors that affect the conformation adopted by the caseins, and thus the flexibility of the backbone chain could affect the micelle structure. This prediction is broadly in line with the experimental results which suggest a strong dependence of α_{s1}-casein micelle structure and size on environmental conditions [19].

Binary 1:1 mixtures of α_{s1} + α_{s2}, α_{s1} + β and α_{s2} + β-caseins have been simulated using the model with no intra-chain hydrophobic interactions. Typical snapshot conformations of these systems are shown in Figures 9–11. For a 1:1 mixture of α_{s1} + α_{s2} the micelles consist mainly of α_{s1}chains to which α_{s2} chains have joined on to the surface (Fig. 9). Analysis of cluster composition statistics shows that the proportion of free monomers is low for α_{s1}, but high for α_{s2}. The structure of the micelles is midway between those of the individual components. They have dense cores bridged by hydrophilic blocks, which are surrounded by a layer of α_{s2} chains. There is only a small degree of intermixing of hydrophobic blocks from the two chain types. These micelles appear to be stabilised by the hydrophilic blocks from the α_{s2} chains.

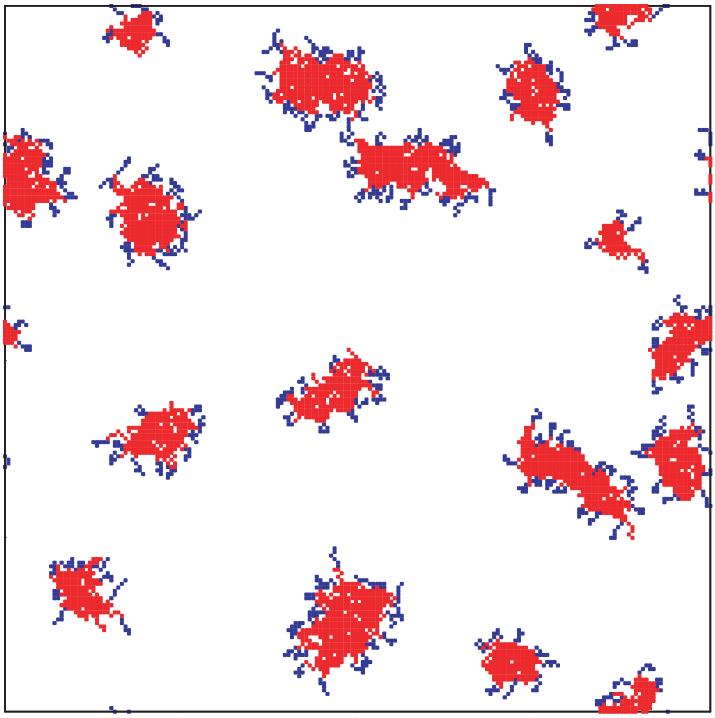

Figure 7. Snapshot conformation for model β-casein without intra molecular hydrophobic interactions included (red = hydrophobic segments, blue= hydrophilic segments).

In these micelles it may be that the α_{s2} chains have the effect of either slowing micelle growth or terminating it. Figure 13a is a graph of the composition of mixed α_{s1} + α_{s2} micelles. The size distribution for mixed α_{s1} + α_{s2} micelles (Fig. 12a) resembles that for a 1-component α_{s2} system (Fig. 8b), again suggesting that α_{s2} chains dominate the micellization behaviour of the system. If we look at the composition of the micelles in this system we see a preference for micelles that contain a high proportion of α_{s1}-casein, and for a mixture of about 45%:55% α_{s1}:α_{s2}, although this is not a particularly strong preference.

For α_{s1} + β-casein 1:1 mixtures (Fig. 10) mixed micelles are formed with a high degree of mixing of the hydrophobic blocks from the two chains. The structure is possibly closer to that of the pure β-casein micelles, although in some micelles the α_{s1} chains can be seen to bridge between two or more micelles. The formation of α_{s1} bridges is less obvious than in the 1-component α_{s1}-casein system. The mean micelle size is relatively high at 13.58 chains per micelle (Tab. II) which is between that for the one-component α_{s1} and β systems. The largest micelle formed in the system is also high (58 chains per micelle, Tab. II), and the mean number of monomers low (0.48 chains per micelle). The micelle size distribution (Fig. 12b) bears a greater resemblance to that of the 1-component β-casein (Fig. 8c) rather than the 1-component α_{s1}-casein (Fig. 8a), with micelle sizes in the range 7–18 chains being preferred. There are also two peaks in the size distribution graph (Fig. 12b) suggesting some tendency

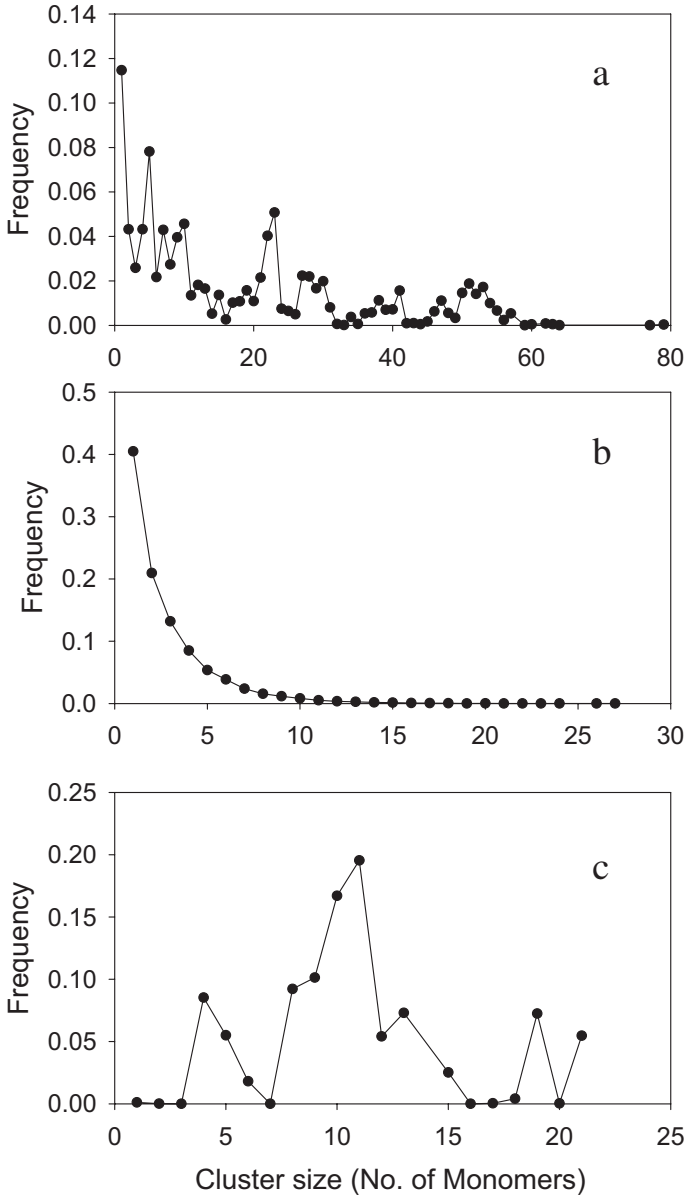

Figure 8. Micelle size distribution (number of micelles) for 1-component model casein systems with no intra molecular hydrophobic interactions. (a) α_{s1}-Casein; (b) α_{s2}-casein; (c) β-casein.

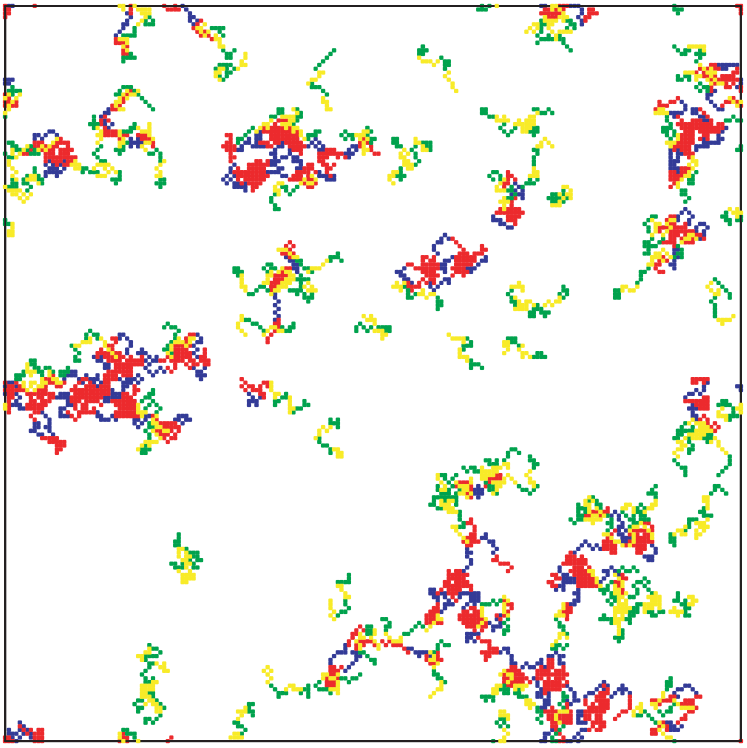

Figure 9. Snapshot conformation for mixed α_{s1}-casein + α_{s2}-casein (1:1 ratio) model system without intra molecular hydrophobic interactions included (red = α_{s1} hydrophobic segments, blue = α_{s1} hydrophilic, yellow = α_{s2} hydrophobic segments, green = α_{s2} hydrophilic).

towards a bi-disperse system. The system shows a strong tendency to form mixed micelles composed of about 45% α_{s1} chains and 55% β chains, as seen in the peaks in the composition frequency distribution plot of Figure 13b.

The snapshot conformation for a 1:1 mixture of α_{s2} + β-casein is shown in Figure 11. The micelles are a mixture of large clusters similar to those formed in the one-component β-casein system (Fig. 5) and more open small micelles and monomers. The large micelles are composed mainly of β chains with a relatively small proportion of α_{s2} chains. In some cases the two micelle types join together, although there appears to be only limited mixing of the hydrophobic blocks from the two casein types. The mean cluster size is relatively small (4.26 chains per micelle, Tab. II) although the largest cluster size is actually bigger than for both the 1-component α_{s2} and β systems (Tab. II). The micelle size distribution (Fig. 12c) resemble that for the 1-component α_{s2}-system, and the mixed α_{s1} + α_{s2} binary system, although for the α_{s2} + β there is a secondary peak in the distribution that suggests a small number of larger micelles. The composition of the micelles (Fig. 13c) shows no strong preference for a particular micelle composition, other than for the formation of micelles that contain very high proportions (or low proportions) of the two caseins. This suggests that phase separation may be occurring in the mixture. The micelles that

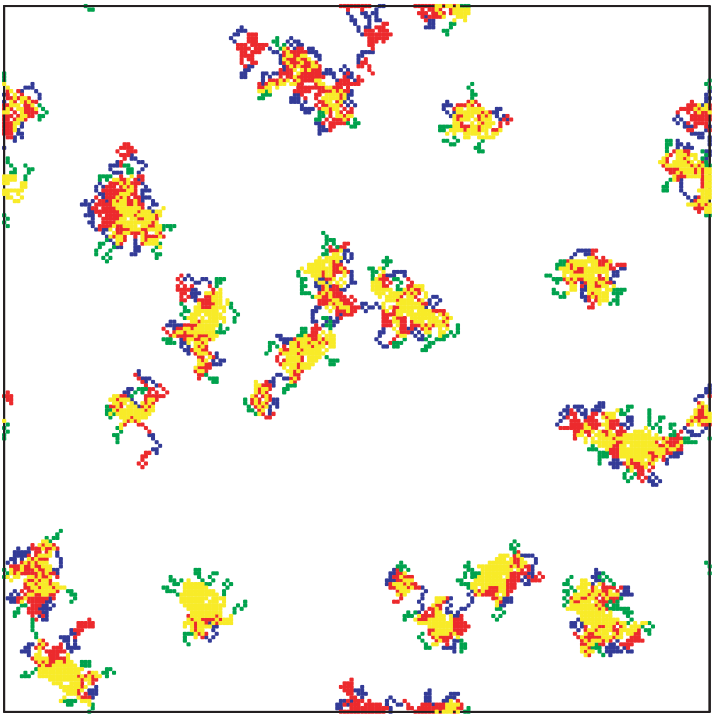

Figure 10. Snapshot conformation for mixed α_{s1}-casein + β-casein (1:1 ratio) model system without intra molecular hydrophobic interactions included (red = α_{s1} hydrophobic segments, blue = α_{s1} hydrophilic, yellow = β hydrophobic segments, green = β hydrophilic).

form with a high proportion of α_{s2}-chains are most likely dimers.

In the binary mixtures two general types of behaviour are observed. For α_{s1} + β mixtures mixed micelles are formed where hydrophobic blocks from the two chains become intermixed in the hydrophobic core of the micelle. When α_{s2} is present, either in combination with α_{s1} or β-casein, inter-mixing of hydrophobic blocks from the two casein types is far less pronounced, although mixed micelles are still formed. Distinct α_{s2} rich and α_{s1}/β rich regions are observed in the mixed micelles (Figs. 10 and 11), and it is as if microphase separation of the two chain types occurs. It is also possible of course that this is purely a kinetic effect and that given a long enough simulation, intermixing of the hydrophobic blocks would occur.

Three-component mixtures of the model caseins add a further level of complexity to our model, and we have simulated these for systems with no intra-chain hydrogen bonding. The snapshot conformations for α_{s1} + α_{s2} + β 1:1:1 and 4:1:4 mixtures are shown in Figures 14 and 15. For the 1:1:1 mixture we can see that the system is composed of a range of micelle sizes (see Fig. 16a for the number average size distribution). The large micelles are composed of mixed α_{s1} and β chains, with α_{s2} sitting close to the outer edge of the micelle and not penetrating too much into the hydrophobic core. This is similar to the binary α_{s1} + α_{s2} and α_{s2} + β mixtures in Figures 9 and 11. The mean micelle size

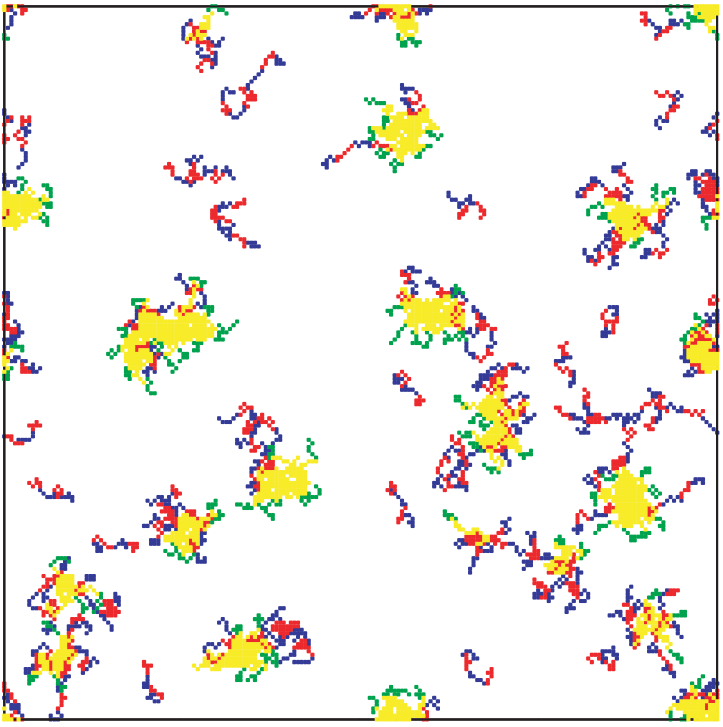

Figure 11. Snapshot conformation for mixed α_{s2}-casein + β-casein (1:1 ratio) model system without intra molecular hydrophobic interactions included (red = α_{s2} hydrophobic segments, blue = α_{s2} hydrophilic, yellow = β hydrophobic segments, green = β hydrophilic).

is smaller than that of the binary mixture of the α_{s2} + β casein and smaller than the one-component α_{s1} and β casein systems. Comparison of the micelle size distribution for the 1:1:1 three-component mixture (Fig. 16a) and those for the binary mixtures (Fig. 12) we can see that the size distribution has more of a resemblance to that for the mixed α_{s2} + β system than to any of the other size distributions for binary mixtures or one-component systems. There is a high proportion of monomer present (probably α_{s2}-chains) and a second peak in the distribution for a micelle of about 8 chains. Larger micelle sizes do occur, but with a lower frequency. A similar observation is seen in the 4:1:4 mixture (Fig. 15). The mean micelle size is greater than for the 1:1:1 mixture (although still less than for the α_{s1} + β mixture and the α_{s1} and β one-component systems), and there is a lower average number of monomers present in the system. The latter reflects the lower proportion of α_{s1} which has a higher tendency to exist as a monomer. The size distribution (Fig. 16b) also has a peak around a micelle size of 8 chains, although this has a higher probability than for the 1:1:1 mixture. The size distribution still, however, resembles that for the α_{s2} + β binary mixture (Fig. 12c). If we look at the composition of the micelles for the three-component mixture (Fig. 17), we can see that for the 1:1:1 mixture (Fig. 17a), there are a relatively high proportion of the micelles that

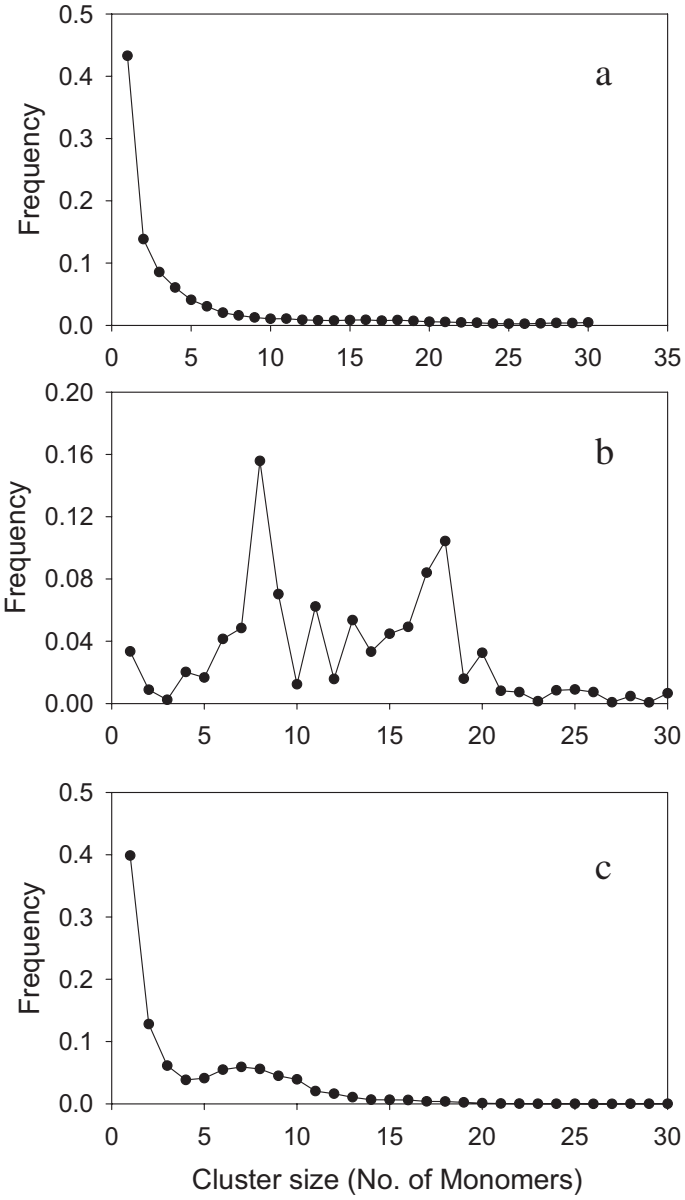

Figure 12. Micelle size distribution (number of micelles) for binary mixtures of model caseins with no intra molecular hydrophobic interactions. (a) α_{s1} + α_{s2}-casein; (b) α_{s1} + β-casein; (c) α_{s2} + β-casein.

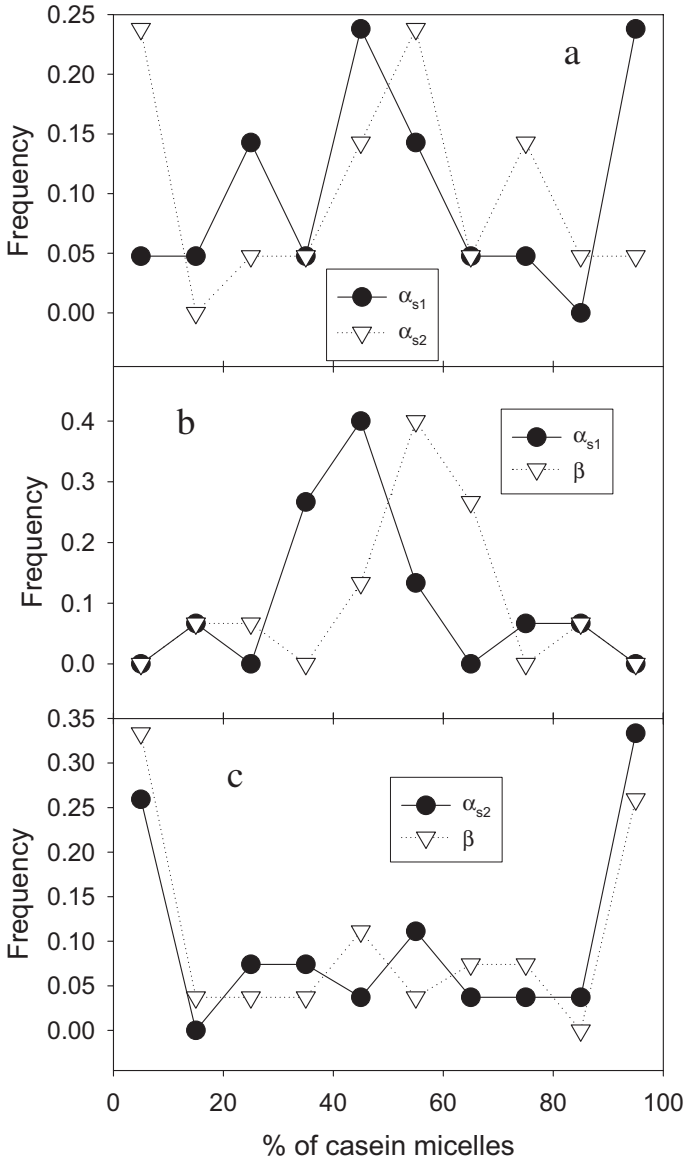

Figure 13. Composition of micelles found in binary casein systems. The percentage of the micelle that is α_{s1}, α_{s2} or β-casein is plotted against the frequency with which that composition occurs. (a) α_{s1} + α_{s2}-casein; (b) α_{s1} + β-casein; (c) α_{s2} + β-casein.

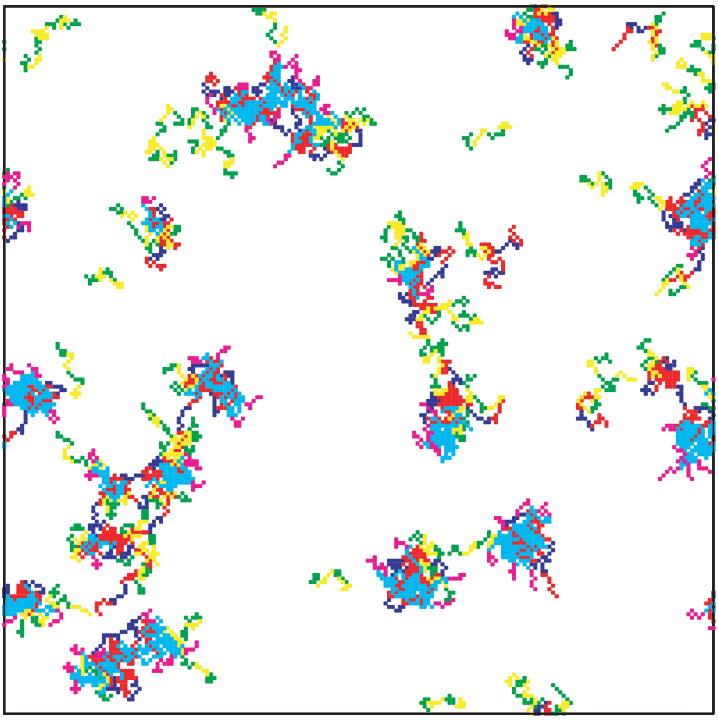

Figure 14. Snapshot conformation for mixed model α_{s1}-casein + α_{s2}-casein + β-casein (1:1:1 ratio) without intra molecular hydrophobic interactions included (red = α_{s1} hydrophobic segments, blue = α_{s1} hydrophilic, yellow = α_{s2} hydrophobic segments, green = α_{s2} hydrophilic, light blue = β hydrophobic, purple = β hydrophilic).

contain no α_{s1} or β, reflecting the tendency for α_{s2}-chains to self-associate with other α_{s2}-chains to from small micelles. The proportion of α_{s1}-casein shows a maximum around 35%, whilst for β-casein there is a peak around 45%. The α_{s2}-chain shows a broad range of most probable compositions between 15–35%. For the 4:1:4 mixture (Fig. 17b) the proportion of α_{s2} in each micelle is lower (below 15% is favoured) due to the reduced proportion of the chains. For α_{s1} chains 45% is till the most probable composition, whilst for β-casein compositions in the range 45–55% is most probable. We can compare these most probable micelle compositions with the compositions for the binary mixtures (Fig. 13). For the binary mixture, only the α_{s1} + β mixture showed any great tendency towards a preferred micelle composition of 45%:55% α_{s1}:β. For the other two binary mixtures there is a far wider spread of micelle compositions. From this perspective the three-component mixtures adopt micelle compositions between those found in the binary and three-component mixtures. There is some preference for particular chain type compositions, but this is not as pronounced as for the α_{s1} + β binary mixture.

There are fewer experimental results for binary and three-component mixtures than for one-component casein systems. The results that are available, however, bring out some interesting comparisons with the simulations. Farrell et al. [9] have

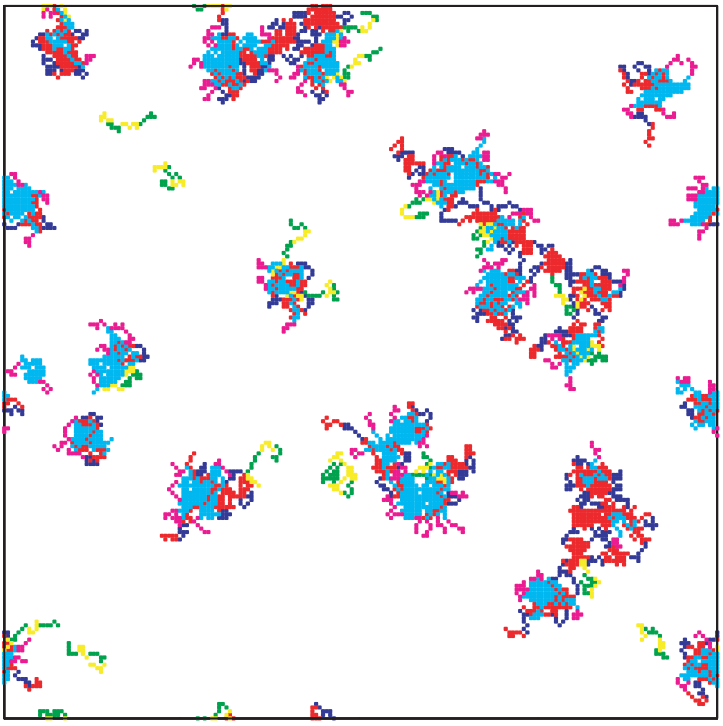

Figure 15. Snapshot conformation for mixed model α_{s1}-casein + α_{s2}-casein + β-casein (4:1:4 ratio) without intra molecular hydrophobic interactions included (red = α_{s1} hydrophobic segments, blue = α_{s1} hydrophilic, yellow = α_{s2} hydrophobic segments, green = α_{s2} hydrophilic, light blue = β hydrophobic, purple = β hydrophilic).

measured the weight average molecular weight of mixtures of α_{s1} + β casein. At 37 °C this was found to be 213 000 (a nonomer) for the α_{s1} + β mixture compared to 1 250 000 (a 52-mer) for β-casein and 56 000 (a dimer) for α_{s1}-casein. This confirms the earlier finding of Malin et al. [15] that α_{s1}-casein depolymerises at 37 °C to form the dimer. This they attribute to a loss of the ability of the N-terminal hydrophobic block to participate in self-association interactions. Thus, in the mixed α_{s1} + β casein micelles at 37 °C the α_{s1} casein is unable to propagate the micelles, and acts to limit their size. It has been proposed that this effect has a physiological significance during casein micelle synthesis in the mammary gland [2]. The caseins are synthesised in the endoplasmic reticulum of the mammary gland secretory cells. To escape from this it is believed that any casein aggregates must be below a certain size or they are recycled by the endoplasmic reticulum associated degradation system. It is hypothesized that α_{s1}-casein reduces the size of, in particular β-casein micelles so that they can escape from the endoplasmic reticulum. We do not see this in our simulations when α_{s1} casein is present in the mixtures. However, we should remember that our model α_{s1} chains have two potentially interacting hydrophobic blocks at all times in the simulations. If we were to modify the systems and substitute one of the hydrophobic blocks in model α_{s1}-casein

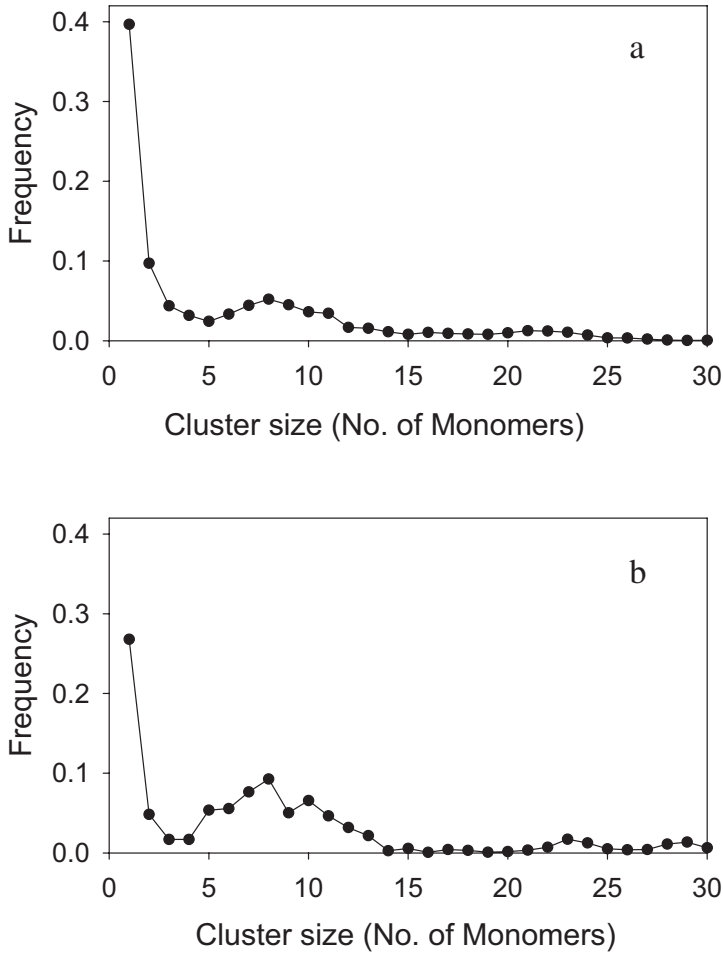

Figure 16. Micelle size distribution (no of micelles) for three-component mixtures of model caseins with no intra molecular hydrophobic interactions. (a) α_{s1}:α_{s2}:β-casein (1:1:1); (b) α_{s1}:α_{s2}:β-casein (4:1:4).

with a non-interacting hydrophilic block, a similar effect to that observed in the experiments might well be observed. In our simulations, it appears that α_{s2}-chains are taking on the role that α_{s1}-casein has in vivo. This is not surprising as simulated α_{s2}-chains show a relatively low tendency to self-associate due to their block structure. Although our α_{s2}-chains have two hydrophobic blocks, they are relatively small (1/4 of the chain) and one is attached at both ends to a hydrophilic block and is therefore relatively "protected" against self-association. It will thus behave in similar way to a chain that has a larger hydrophilic block at one end, albeit with a small but finite probability that the second block will form aggregate with other blocks. Thus, we can envisage the α_{s2}-chains as adsorbing to the surface of mixed micelles via their N-terminal hydrophobic block, and then the central hydrophobic block (which is

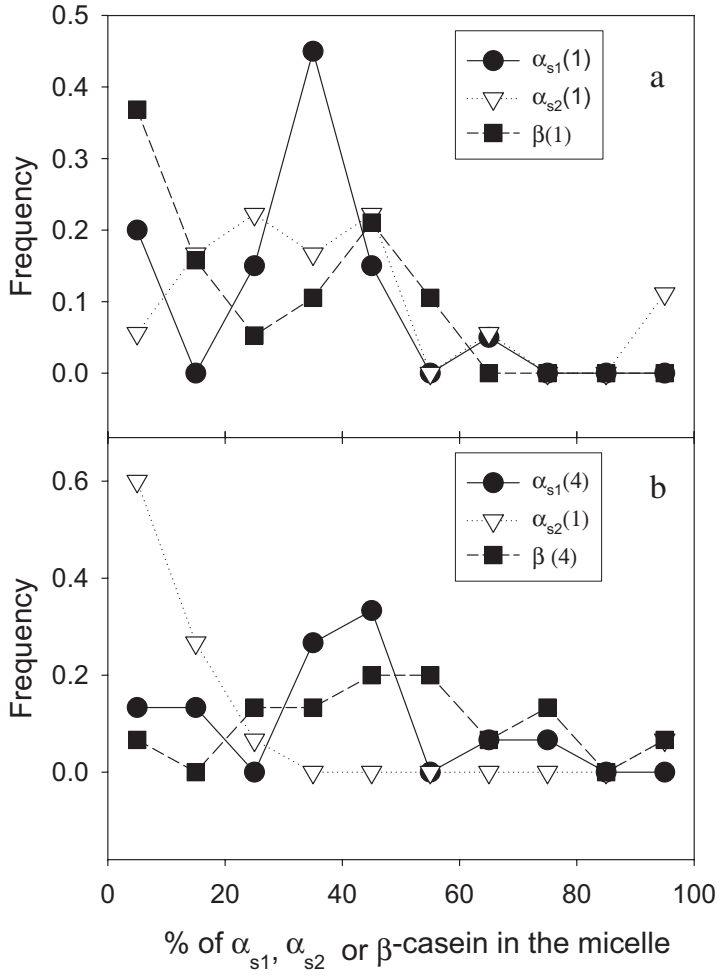

Figure 17. Composition of micelles found in three-component casein systems. The percentage of the micelle that is α_{s1}, α_{s2} or β-casein is plotted against the frequency with which that composition occurs. (a) α_{s1}:α_{s2}:β-casein (1:1:1); (b) α_{s1}:α_{s2}:β-casein (4:1:4).

surrounded by hydrophilic blocks) finds it difficult to propagate the micelle by forming associations with other hydrophilic blocks. According to the Horne's dual-binding model [12] α_{s2}-casein would be able to propagate the micelle through calcium phosphate nanocluster formation, but that option is not offered in our simulations. So in these "calcium-free" simulations, the α_{s2}-chains are acting as terminators of micelle growth in a similar way to that proposed for κ-casein in the dual binding model, and for α_{s1}-casein in vivo.

4. CONCLUSION

We have presented results for a very simple model for the self-association of

1-component and mixed model casein solutions. Even though this is a very simple model that does not begin to address the true structural complexity of the caseins, we are still able to reproduce some of the features we would expect of casein micelles. In particular our results would suggest that the micelle size differences observed between the caseins may be due in large part to the constraints of the block-copolymer like structure they have. Clearly, α_{s2}-casein has difficulty forming micelles due to the relative inability of its central hydrophobic block in forming self-association structures. This is because it is "protected" by the presence of surrounding hydrophilic blocks. This also explains why we see α_{s2}-casein acting as a controller of micelle growth in our binary and three-component systems. The same role has been postulated for α_{s1}-casein in vivo, and the fact that we do not see the same behaviour for our model α_{s1}-casein is due to our model allowing hydrophobic character in both hydrophobic blocks of α_{s2}-casein under all conditions. One of the blocks appears to loose its ability to self-associate at 37 °C both in vivo and in vitro and this then allows it to act as a micelle growth terminator. Of course, in casein micelles both α_{s1}- and α_{s2}-casein are able to form calcium phosphate bridges to allow micelle propagation, a situation we have chosen to leave out in our simulations.

As they stand the simulations throw up some interesting results that may or may not be relevant to casein micelle structure and formation, but are relevant to artificial micelle formation. We model the formation of our micelles as a random association process. It is still unclear whether casein micelle formation in the mammary gland is such a random process, or whether the process is under the control of cellular processes. Certainly the results of Chanet et al. [2] on the role of α_{s1}-casein on controlling micelle size in the endoplasmic reticulum suggest that the structure of the initial submicelles may not be random after all. If this is the case, then to advance our model we would require more information on both the composition of caseins and order of addition of casein to the micelle within the secretory cells. It is still a useful exercise, however to extend our modelling studies to a full 4-component α_{s1}:α_{s2}:β:κ (4:1:4:1) mixture and to include simulation of calcium phosphate bridges. This may give us some insight as to whether it is possible to form a casein-micelle-like structure from a random association process. In turn this may be useful in helping to decide whether casein micelle biosynthesis is a random process or not. In addition, a general study of casein self-association is useful from the viewpoint of understanding artificial self-association structures. The ability to form artificial nanostructures in foods depends on our ability to control the interactions between them. Modelling studies of this type could be one approach to the understanding and rational design of protein nanostructures.

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REFERENCES

[1] Bates F.S., Fredrickson G.H., Block copolymers – Designer soft materials, Phys. Today 52 (1999) 32–38.

[2] Chanet E., Martin P., Olivier-Bosquet M., α_{s1}-casein is required for the efficient transport of β- and κ-casein from the endoplasmic

reticulum to the Golgi apparatus of mammary gland cells, J. Cell Sci. 112 (1999) 3399–3412.

[3] Dickinson E., Euston S.R., Monte Carlo Simulation of colloidal systems, Adv. Colloid Int. Sci. 42 (1992) 89–148.

[4] Ding J., Carver T.J., Windle A.H., Self-assembled structures of block copolymers in selective solvents reproduced by lattice Monte Carlo simulation, Comp. Theoret. Polym. Sci. 11 (2001) 483–490.

[5] Euston S.R., Computer simulation of proteins: adsorption at surfaces, gelation and self-association, Curr. Opin. Colloid Int. Sci. 9 (2004) 321–327.

[6] Euston S.R., Modelling and computer simulation of food structures, in: McClements D.J. (Ed.), Understanding and controlling the microstructure of complex foods, CRC Press, Cambridge, in press.

[7] Euston S.R., Horne D.S., Simulating the self-association of caseins, Food Hydrocoll. 19 (2005) 379–386.

[8] Farrell H.M. Jr, Qi P.X., Brown E.M., Cooke P.H., Tunick M.H., Wickham E.D., Unruh J.J., Molten globule structures in milk proteins: implications for potential new structure-function relationships, J. Dairy Sci. 85 (2002) 459–471.

[9] Farrell H.M. Jr., Malin E.L., Brown E.M., Qi P.X., Casein micelle structure: What can be learned from milk synthesis and structural biology? Curr. Opin. Coll. Int. Sci. 11 (2006) 135–147.

[10] Haire K.R., Carver T.J., Windle A.H., A Monte Carlo lattice model for chain diffusion in dense polymer systems and its interlocking with molecular dynamics simulation, Comp. Theoret. Polym. Sci. 11 (2001) 17–28.

[11] Holt C., Sawyer L., Caseins as rheomorphic proteins, J. Chem. Soc. Faraday Trans. 89 (1993) 2683–2690.

[12] Horne D.S., Casein interactions: casting light on the black boxes, the structure in dairy products, Int. Dairy J. 8 (1998) 171–177.

[13] Horne D.S., Casein structure, self-assembly and gelation, Curr. Opin. Colloid Int. Sci. 7 (2002) 456–461.

[14] Kajiwara K, Niki R., Urakawa H., Hiragi Y., Donkai N., Najura M., Micellar structure of β-casein observed by small angle X-ray scattering, Biochim. Biophys. Acta 955 (1998) 128–134.

[15] Malin E.L., Brown E.M., Wickham E.D., Farrell H.M. Jr, Contributions of terminal peptides to the associative behaviour of α_{s1}-casein, J. Dairy Sci. 88 (2005) 2318–2328.

[16] Payens T.A.J., Schmidt D.G., Boundary spreading of rapidly polymerizing α_{s1}-casein B and C during sedimentation. Numerical solutions of the Lamm-Gilbert-Fujita equation, Arch. Biochem. Biophys. 115 (1966) 136–145.

[17] Portnaya I., Cogan U., Livney Y.D., Ramon O., Shimoni K., Rosenberg M., Danino D., Micellization of bovine β-casein studied by isothermal titration microcalorimetry and cryogenic transmission electron microscopy, J. Agric. Food Chem. 54 (2006) 5555–5561.

[18] Schmidt D.G., Differences between the association of the genetic variants B, C and D of α_{s1}-casein, Biochim. Biophys. Acta 221 (1970) 140–142.

[19] Schmidt D.G., Association of caseins and casein micelle structure, in: Fox P.F. (Ed.), Developments in dairy chemistry, Vol. 1: Proteins, Applied Science Publishers, London, 1982, pp. 61–85.

[20] Snoeren T.H.M, van Markwijk B., van Montfoort R., Some physicochemical properties of bovine α_{s2}-casein, Biochim. Biophys. Acta 622 (1980) 268–276.

[21] Stoddard S.D., Identifying clusters in computer experiments on systems of particles, J. Computational Phys. 27 (1978) 291–293.

[22] Thurn A., Burchard W., Niki R., Structure of casein micelles 1. Small angle neutron scattering and light scattering from β-casein and κ-casein, Coll. Polym. Sci. 265 (1987) 653–666.

[23] Thurn A., Burchard W., Niki R., Structure of casein micelles. 2. α_{s1}-casein, Coll. Polym. Sci. 265 (1987) 897–902.

[24] Vreeman H.J., The association of bovine SH κ-casein at pH 7.0, J. Dairy Sci. 46 (1976) 271–276.

[25] Vreeman H.J., Both P., Brinkhuis J.A., van der Spek C., Purification and some physicochemical properties of bovine κ-casein, Biochim. Biophys. Acta 491 (1977) 93–103.

[26] Vreeman H.J., Brinkhuis J.A., van der Spek C., Some association properties of bovine SH-κ-casein, Biophys. Chem. 14 (1981) 185–193.