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Controllable Self-Assembly of Sodium Caseinate with a Zwitterionic Vitamin-Derived Bolaamphiphile

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Supporting Information

ABSTRACT: The control of self-assembly of sodium caseinate (SC) including the formation of mixed layers, microspheres, or nanoparticles is highly relevant to the microstructure of food and the design of promising drug delivery systems. In this paper, we designed a structure-switchable zwitterionic bolaamphiphile, 1,12-diaminododecanediorotate (DDO), from orotic acid, which has special binding sites and can guide the self-assembly of SC. Complexation between SC and DDO was investigated using dynamic light scattering, transmission electron microscopy, differential scanning calorimetry, and fluorescence spectra measurements. Monomeric DDO was bound to the negatively charged sites on the SC micelle and made the structure of SC more compact with decreased electrostatic repulsion between the head groups. Vesicular DDO led to reassociation of vesicles with enlarged size via preferable hydrophobic interactions. Moreover, the aggregation between SC and DDO was found to be temperature-dependent and reversible. This research provides an effective way to control the reversible self-assembly of SC by the zwitterionic vitamin-derived bolaamphiphile.

KEYWORDS: *self-assembly, sodium caseinate, orotic acid, bolaamphiphile, vesicles*

INTRODUCTION

Sodium caseinate (SC), the soluble casein material, is derived from amphiphilic proteins that self-assemble into stable micellar structures in aqueous solutions.[1,2] Although numerous models have been proposed for describing casein micelles in the past decades,[3] the accepted structure of SC micelles as colloidal particles starts with a hydrophobic interior that is surrounded by a hydrophilic, charged, and diffuse surface layer. Intermicellar electrostatic and steric repulsion stabilize the micelle.[4] In addition to its use as a protein source, SC is often responsible for the structure, texture, and stability of food due to its amphiphilic properties. It is also a potential candidate for conventional and novel drug delivery systems.[5]

The function of SC comes from its adsorption at the interface and micellization in aqueous solutions. The amphipathic nature of caseins causes them to concentrate at the air−water or oil−water interfaces, thus reducing the energy required for the formation and stability of foams or emulsions. The micelles formed by this natural food protein can potentially encapsulate compounds of hydrophobic character. Both the self-assembled monolayers and the micelles in the protein networks can interact with a wide range of active compounds, thus offering a variety of possibilities for reversible binding and controllable release of active molecules. Therefore, the study on the forces and parameters to control the structure of self-assembled SC including the formation of microspheres or nanoparticles is highly relevant to the microstructure control of food and the design of promising drug delivery systems.

A small difference in protein primary structure can sometimes cause a major change in functionality.[6] However, because caseins exist in open structures and are not very sensitive to marked structural alterations, changing or modifying the structure of self-assembled SC remains a challenge. In our opinion, there are at least three factors that should be considered for the design of an effective molecule that could possibly induce a structural change of SC. First, it should be a molecule with amphiphilic character, which could interact with SC by its hydrophilic and hydrophobic groups. Actually, conventional surfactants[7,4] have been investigated to induce a change in SC self-assembly. The caseins were found to adopt more ordered conformations in the presence of both cationic surfactants such as CTAB[7] and DTAB[8] and anionic amphiphiles such as SDS.[4] Two different types of a "necklace and bead" model have been proposed for describing the structure of surfactant−casein complexes.[7] Second, because the presence of anionic phosphoserine and other anionic amino acid residues makes caseins available for cation binding,[9,10] molecules bearing charges will facilitate the binding process. Third, some specific binding sites on the molecule could monitor and help the complexation. In food formulation, polysaccharides are often used as ingredients to form mixed biopolymer systems, modifying the functionality of SC by binding with oppositely charged functional groups though important electrostatic interactions as well as other noncovalent interactions.[11] These macromolecular complexes, coacervates, and nano- or microparticles can be used as food ingredients, microencapsulation systems, or biomaterials.[12] However, whether such protein−polysaccharide complexation actually produces enhanced functionality depends on the origin, manufacturing, and polydispersity of the structure of polysaccharides. Therefore, designing a new amphiphilic

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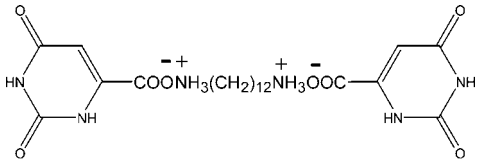
molecule with definite structure that binds optionally to SC using its functional groups could be an alternative way for modifying and controlling the structure change of SC self-assembly.

In a study on food−drug interactions, it was reported that casein showed higher binding affinity than whey protein with phenytoin.[13] This implies that SC might have some specific binding sites for phenytoin. Inspired by this, we used orotic acid, which has a similar pyrimidine group to that in the phenytoin molecule, as the starting material to synthesize a novel zwitterionic bolaamphiphile (1,12-diaminododecanediorotate, DDO).[14,15] We have proved that the hydrophilic groups of DDO, which cap the ends of a saturated, C12 linear hydrocarbon, could act as the host to recognize some molecules with potential hydrogen bond donors. The planar heterocyclic head groups made it form a stable vesicle spontaneously, which could provide nanosize aggregates conveniently. Moreover, we have shown that the structural transition of DDO could be controlled by anionic or cationic surfactants.[16] All of the results suggest that amphiphilic DDO has a switchable structure, from a linear zwitterionic surfactant to spherical vesicles, bearing charges and possible binding sites for SC. In this paper, we investigated the aggregation behavior of SC in the presence of DDO. We present data demonstrating that a marked change of the self-assembly of SC could be governed by monomeric and vesicular DDO. Moreover, the dissociation and re-formation of a complex of SC with DDO was found temperature-dependent and reversible. This research will provide the possibility to guide the self-assembly of protein by structure-controllable amphiphilic vitamins and shed light on the general mechanisms responsible for the structural control of SC.

MATERIALS AND METHODS

Materials. Sodium caseinate and pyrene (≥97%) were purchased from Sigma and were used without further purification. Synthesis of 1,12-diaminododecanediorotate was described in detail previously.[14] The structure of DDO is shown in Scheme 1. Ultrapure Millipore (B050091) water (18.2 MΩ) was used for the preparation of all of the solutions.

Scheme 1. Chemical structure of DDO.



SC and DDO powders were dispersed respectively in water by stirring at room temperature for 1 h. DDO was sonicated for an additional 15 min to ensure complete dissolution. Various concentrations of SC and DDO mixtures were obtained by diluting the stock dispersions (4 mg/mL of SC and 2 mmol/L of DDO) in water. The concentration of DDO varied from 0 to 1 mmol/L, which is within its critical aggregation concentration (CAC). The mixtures were incubated in a shaking bath at 30 °C before testing. Sample solutions were filtered through 0.45 μm Millipore filters before each measurement.

Particle Size and ζ-Potential Measurements. Particle size and ζ-potential of freshly prepared solutions were measured by dynamic light scattering (DLS) using a Zetasizer Nano-ZS (Malvern Instruments) with a He−Ne laser (633 nm, 4 mW). The instrument detected the scattered light at an angle of 173 °C. All measurements were performed in a temperature-controlled chamber. Equilibration time was set as 2 min, and each individual measurement was determined from the average of three readings or more made on the same sample.

Turbidity Measurements. Turbidity measurements were carried out with a UV-2550/vis spectrophotometer at a wavelength of 275 nm. A cuvette with a 1 cm pathway was used, and all of the measurements were conducted at 25 ± 0.1 °C. Each experiment was repeated three or more times.

Steady-State Fluorescence Spectral Measurements. Steady-state fluorescence experiments were performed with a Hitachi F-7000 fluorescence spectrophotometer. In the measurement of the intrinsic fluorescence of SC solution, the excitation and emission slits were fixed at 5.0 and 2.5 nm, respectively. The excitation wavelength was set at 295 nm, and the emission spectra were collected from 300 to 500 nm. The scan rate was set at 240 nm/min.

Pyrene (0.5 $\mu mol \cdot L^{-1}$) was used as the probe to determine the micropolarity of SC micelles. The fluorescence emission spectra of pyrene were measured as a function of temperature from 25 to 65 °C. Each spectrum was measured in the wavelength range 350−550 nm with the excitation wavelength being 335 nm. The intensities I_1 and I_3 were measured at the wavelengths corresponding to the first and third vibronic peaks in the fluorescence emission spectrum of pyrene.

Transmission Electron Microscopy (TEM). A Hitachi 7650 transmission electron microscope operating at a voltage of 120 kV was used for TEM measurements. Phosphortungstic acid (1% (w/v)) was used as the staining agent. A drop of the sample solution (10 μL) was placed onto a carbon Formvar-coated copper grid (200 mesh). Filter paper was employed to absorb the excess liquid. Each experiment was repeated two or more times. The process of adsorption was equilibrated in a thermostatic bath at the predetermined temperature.

Differential Scanning Calorimetry (DSC). The thermograms of SC with and without DDO were measured by VP-DSC (Microcal Inc., Northampton, MA, USA) in a temperature range of 10 °C to approximately 110 °C. The sample volume used was 0.52 mL. The samples were degassed before loading in the calorimeter cell and were kept at 10 °C for 30 min before the scan was started. All heating scans were recorded at a rate of 1.5 $°C \cdot min^{-1}$. Under the experimental conditions, the obtained thermal recordings were reproducible. The denaturation enthalpy change (ΔH) was obtained by fitting the data with a Non-2-State model (supplied by Microcal).

RESULTS AND DISCUSSION

Self-Assembly of SC upon Addition of DDO at Ambient Temperature. At neutral pH, κ-caseins on the surface of SC micelles have an extended conformation, which can provide strong steric stability. In SC dispersions, the caseins are organized into soft, fragile aggregates that are formed through weak attractive interactions.[17] The average hydrodynamic diameter (D_H) is one of the basic parameters to describe the assembly in solution. Figure 1(a) shows the aggregate size of the mixed system as a function of C_{DDO} determined by DLS. The D_H in 0.2 wt % SC solution is about 175 nm, which is consistent with the reported results[18] but was smaller than the theoretical value of SC micelles of 300 nm and bigger than the submicelles of 10−20 nm.[19] Upon addition of DDO, a continuous decrease in the average micelle size from 175 to 148 nm was observed until the concentration of DDO reached 0.5 mM; then the size of the SC micelles showed an increase at the higher concentration of DDO. Previous results reported that the apparent particle diameter of the pure SC solution dropped from 250 nm to 45 nm after the first addition of positively charged lactoferrin.[20] The value of D_H of the SC solution, however, slightly increased from 201.2 nm to 232.9 nm with the increase of xanthan gum concentration.[21] We confirmed that vesicles were the main aggregates when the DDO concentration was higher than 0.47 mM.[14] Therefore, we speculate that the DDO vesicles formed in SC solution could

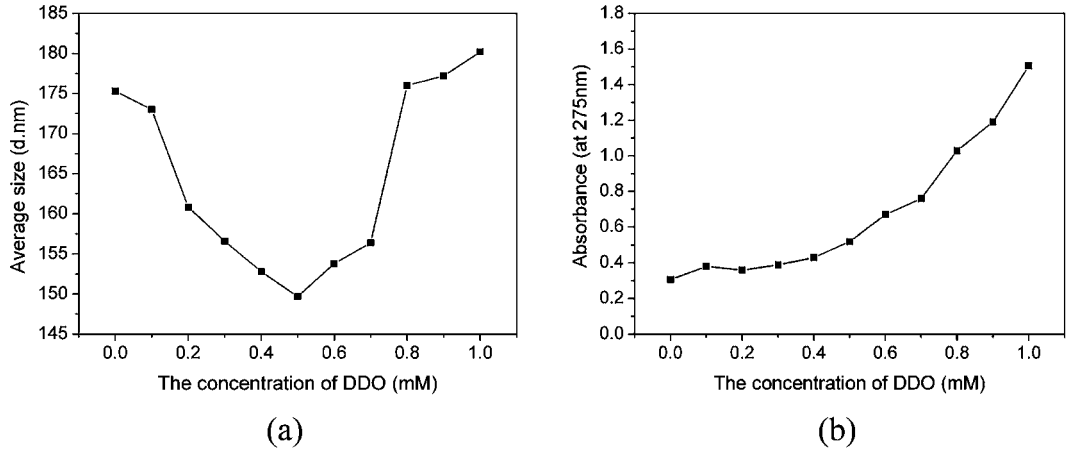


Figure 1. Change of the aggregates of SC (0.2 wt %) with varing DDO concentration at 25 °C. (a) Average size of a mixed SC−DDO solution. (b) Turbidity of a mixed SC−DDO solution.

account for the break point in Figure 1(a) and make the size of the aggregates return to the initial value.

Figure 1(b) shows the turbidity profiles of the mixtures. The turbidity of mixtures remained almost constant when C_{DDO} was lower than 0.5 mM and then increased with varying concentration of DDO. These trends in turbidity are consistent with the change in particle size. Because the change in turbidity arises mainly from the change of mass and size of aggregates in the solution,[8] the above changes of turbidity might come from the formation of casein−DDO complexes. The results in Figure 1 indicate that binding of DDO monomers (at concentrations less than 0.5 mM) to SC micelles makes its structure more compact. The slight increase in D_H might come from the complexation of SC with self-assembled vesicular aggregates. Interestingly, a pure solution of DDO at 1 mM determined by DLS was found centered at 295 nm,[14] while the size of the SC−DDO complex herein was less than 190 nm. Moreover, the mixed solution turned from colorless to transparent blue when the concentration of DDO was higher than 0.5 mM, which is a typical indicator of the formation of vesicles. Further increasing the addition of DDO (higher than 0.9 mM) led to coagulation.

It is well accepted that the hydrophobic interactions are mutually exclusive when the concentration of hydrophobic groups in casein solution is lower than a critical concentration, which will give a destabilizing contribution to the native structure of the protein.[22] Therefore, addition of amphiphilic DDO should lead to the attenuation of these interactions and favor the clustering of hydrophobic groups and more compact structures. In addition to hydrophobic forces the electrostatic attraction also contributes to the size change of the mixtures because DDO bears both negative and positive charges. Therefore, measuring the surface charge density around the new formed complexes could be an efficient way to quantify the electrostatic interactions between SC and DDO molecules. As shown in Figure 2, the ζ-potential decreased gradually with the addition of DDO and then reached a plateau. At lower concentration, the positive sites of DDO monomers might bind to the negative sites on the SC micelle, which made it bear less negative charge, but the remaining net charge was still enough to stabilize the micelle. This observation primarily points out the prevailing contribution of the electrostatic attraction between the positively charged groups of DDO and negatively charged groups of SC to their interactions at lower concentration. Such charge neutralization could lead to a

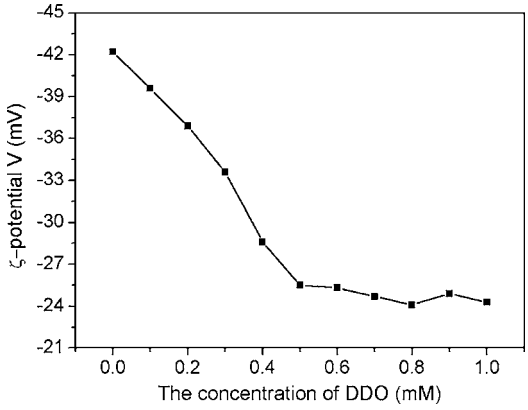


Figure 2. ζ-Potential of SC micelles (0.2 wt %) in the presence of DDO at 25 °C.

decrease in electrostatic repulsion between casein molecules, which in turn leads to the more compact structure of SC micelles and the contraction of the hydrodynamic diameter. When C_{DDO} reached 0.5 mM, a blue transparent solution was observed, indicating that vesicles formed. The positively charged DDO accommodated in vesicles is not easily accessible for SC; therefore, increased DDO could not induce significant change of the ζ-potential of SC micelles. In the meantime, the reduced electrostatic stability was accompanied by aggregation of caseinate molecules, which resulted in unstable colloid solution (less than ±30 mV). It is possible that the hydrophobic interactions between the hydrocarbon chains of the DDO molecules and the nonpolar groups of protein make the hydrocarbon chains of the surfactant molecules transfer from an aqueous medium into the hydrophobic interior of the protein. As a result, the mixture returns to the initial size of SC micelles. In the interaction between casein and other oppositely charged surfactant such as DTAB, at very low surfactant concentrations, D_H of the SC micelles was found to decrease to a minimum, then increase sharply at higher concentration followed by flocculation.[8] Semenova et al.[23] pointed out that the dissociation of the original protein particles as a result of complex formation with dextran sulfate was correlated with size reduction of SC. They also found that the increased neutralization of both the protein and the polysaccharide net molecular charges also contributed to the contraction in size of the complex particles. However, our results suggested that the

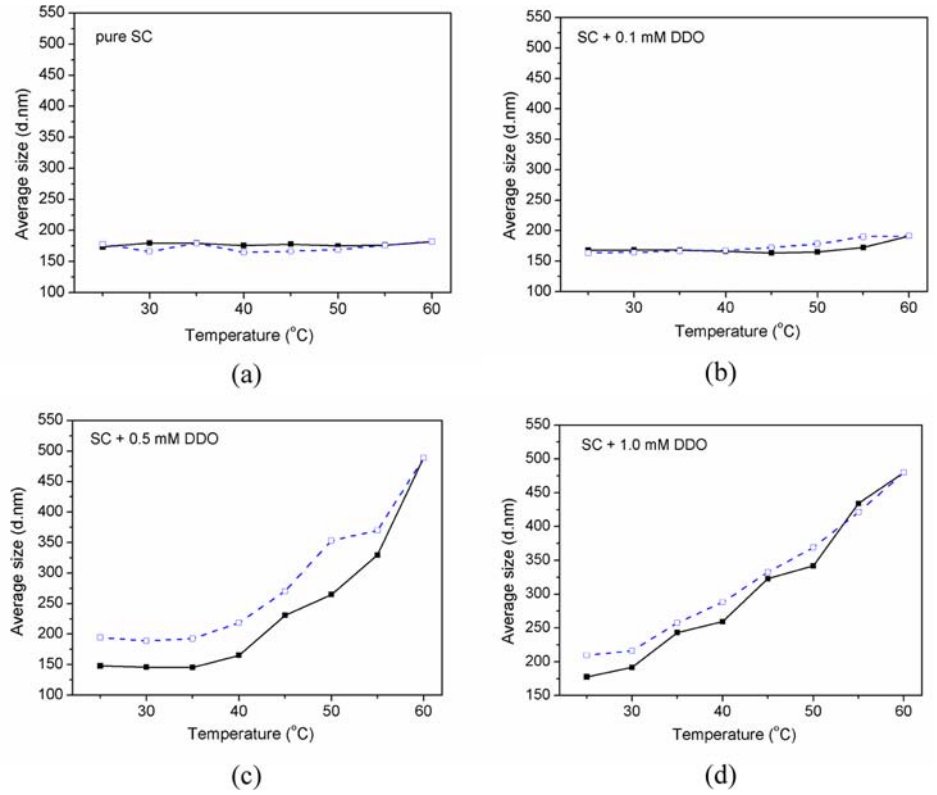


Figure 3. Change of the average size of the mixture of SC (0.2 wt %) and DDO upon heating and cooling. Concentration of DDO: (a) 0 mM, (b) 0.1 mM, (c) 0.5 mM, (d) 1.0 mM. The solid line represents the heating process, and the dash line is for the cooling process.

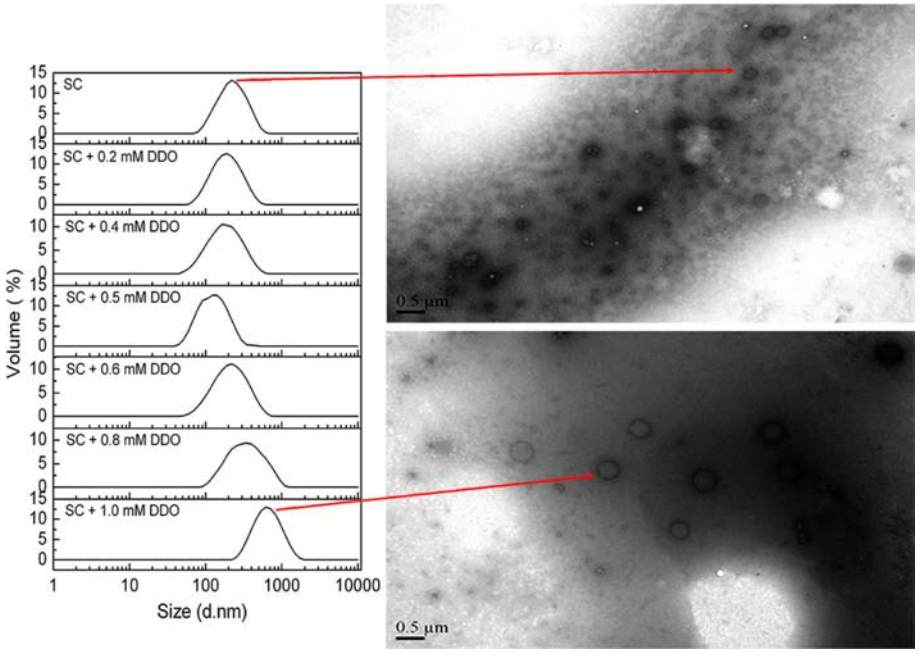


Figure 4. Change of hydrodynamic diameter distribution and TEM images of the mixed SC micelles (0.2 wt %) with different concentrations of DDO at 45 °C.

steric-induced expansion of SC−DDO complex particles tends to work in the opposite direction. The dissociation of the SC micelles upon the initial addition of DDO monomers seems to show itself in the lower size as compared with the original SC particles. On the other hand, the significant role of the steric expansion from vesicular DDO in SC−DDO complex particles seems to manifest itself in the similar size compared with pure SC micelles. Unlike the complex of SC with dextran sulfate, whose size growth came from the electrostatic repulsion between sulfate groups on the polysaccharide due to interaction with the protein,[23] we exclude the contribution of electrostatic repulsion to the size growth because increased DDO could not change the ζ-potential of the SC micelle. Overall, it seems that monomeric or vesiclular DDO binding to SC micelles showed

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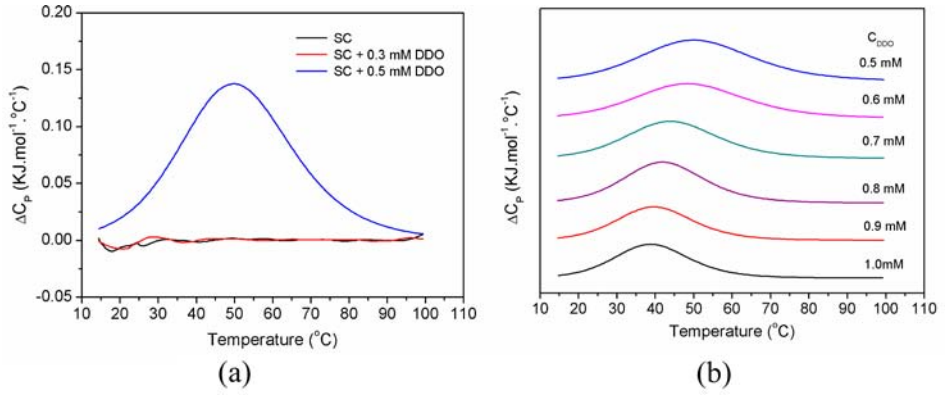

Figure 5. DSC thermographs for SC (0.2 wt %) at varying DDO concentrations. (a) Change of ΔC_p versus T for heating an SC solution with or without DDO vesicles. (b) ΔC_p traces have been shifted vertically for clarity.

Table 1. Thermodynamical Parameters Derived from DSC Thermograms of SC (0.2 wt %) at various DDO Concentrations[a]

C_{DDO}/mM	T_m/°C	ΔH/kcal·mol^{-1}	ΔH_V/kcal·mol^{-1}	ΔS/cal·K^{-1}·mol^{-1}	CU
0.5	51.11	5.39	21.28	16.63	3.95
0.6	49.41	4.37	21.81	13.55	4.99
0.7	44.63	3.91	25.35	12.31	6.48
0.8	42.42	3.79	28.36	12.02	7.48
0.9	40.10	2.95	29.44	9.42	9.99
1	39.27	2.91	29.89	9.32	10.29

[a] T_m is peak temperature during heating, ΔH is the calorimetric enthalpy change, and ΔS is the entropy change on heating SC with various DDO concentrations. Cooperative unit: CU = $\Delta H_v/\Delta H$.[32]

different behavior, but there was no unlimited growth for the aggregates at ambient temperature.

Temperature-Dependent Reversible Self-Assembly of SC with DDO. Due to limited secondary structure and high content of hydrophobic amino acids, the interactive force for the self-assembly of casein mainly comes from endothermic hydrophobic bonding and thus is markedly temperature dependent.[24] The particle sizes of SC with DDO determined by DLS upon heating and cooling are shown in Figure 3. In the absence of DDO, an SC solution at 0.2 wt % displayed a low D_H of less than 200 nm and remained almost constant at temperatures from 25 to 60 °C. Initial addition of DDO at less than 0.1 mM did not induce significant change in the micelle size of SC. Our previous results showed that the particle size of DDO solutions does not change at elevated temperature either. However, heating the mixture of SC with 0.5 mM DDO at 35 °C resulted in the formation of larger particles. In the presence of DDO at 1.0 mM, the average diameter increased dramatically. In addition, the enlarged aggregates at high temperatures decreased to their initial size upon cooling to ambient temperature. Hence, the change in particle size and the color of the suspension was temperature-dependent and reversible. Compared with the plot of particle size of the mixture at 25 °C (Figure 1), a more pronounced increase of particle size from 150 nm to 340 nm with increasing DDO concentration was found at 45 °C (Figure 4). TEM images of SC with or without DDO also confirmed vesicle formation.

It was found that the size distribution of pure caseinate is unaffected by the heating treatment, but when it was loaded with calcium, a bimodal size distribution was observed at ambient temperature and evolved into a monomodal distribution with a diameter of about 430 nm at higher temperatures.[25] The disappearance of the smaller peak was explained with the model that smaller micelles incorporated into a bigger micelle. To our knowledge, only a few self-assemblies of SC were found to be temperature-dependently reversible to date. For example, a reversible particle size transition between SC and polymer gum arabic at different temperatures has been reported.[26] Herein, the dissociation and re-formation of complexed SC aggregates was realized reversibly in the presence of zwitterionic DDO upon heating and cooling. Increasing the temperature is known to increase the strength of hydrophobic interactions and is likely to shift the balance of electrostatic repulsion and hydrophobic attraction in favor of hydrophobic attraction.[26] Huang's group has investigated the nature of temperature-induced vesicle aggregation in some catanionic surfactant systems systematically.[27−29] They found that the main driving force for this phenomenon was the intervesicular hydrophobic interaction among the exposed hydrophobic part of the surfactants. In this case, when DDO interacted with SC at lower temperature, strong electrostatic interactions predominate over hydrophobic attractions. Upon heating, the hydrophobic attraction between the nonpolar segments of DDO and SC became the driving force for aggregation. We have found previously that heating showed a repairing effect on the solubilized DDO vesicles in the presence of a common surfactant.[16] Here it works similarly during its binding to the protein surfactant of SC.

Figure 5 is the thermographs for SC determined by DSC during its complexation with DDO. A pure SC aqueous solution showed smooth C_p at 10−110 °C. The addition of DDO at less than 0.5 mM did not change the smooth line. Similar results were also found in the interaction of norbixin with SC, where the addition of norbixin did not lead to obvious changes of the DSC curve of SC.[30] However, when the concentration of DDO reached 0.5 mM, a single endothermic peak centered at 51.1 °C was observed (Figure 5(a)). The peak temperature defined as the main phase transition temperature

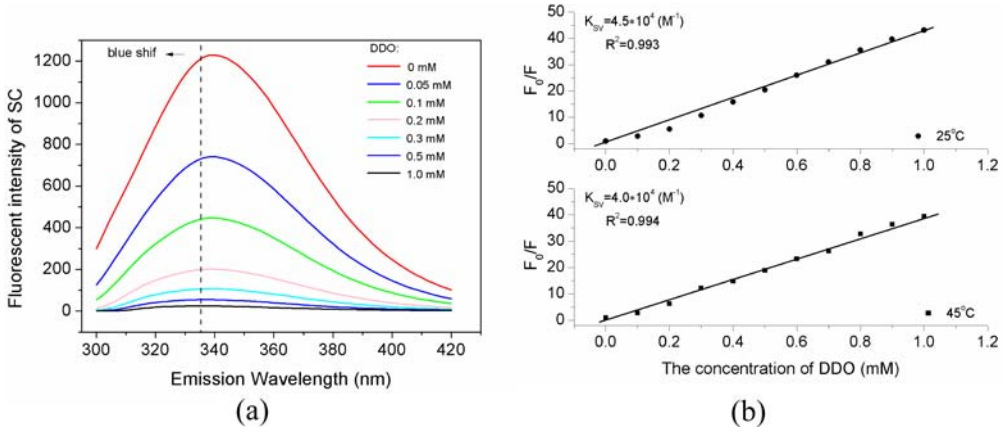

Figure 6. Fluorimetric detection of the interaction of SC (0.2 wt %) with DDO. (a) Change of the fluorescene emission spectra of SC with different concentrations of DDO. (b) Stern−Volmer plots of fluorescence quenching of SC by DDO at 25 and 45 °C.

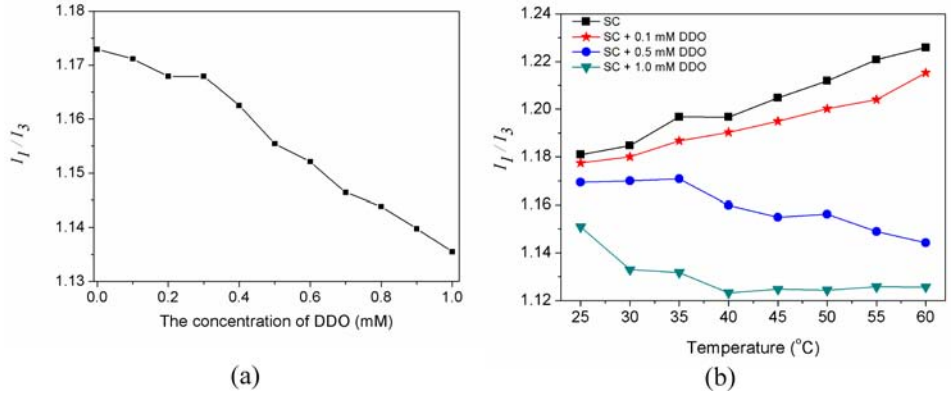

Figure 7. Change of I_1/I_3 band ratio in the fluorescence spectra of pyrene (a) dissolved in SC solution (0.2 wt %) with varying concentrations of DDO at 25 °C and (b) in a mixed DDO solution with SC (0.2 wt %) as a function of temperature.

(T_m) for the mixture solution in the heating process decreased from 51.1 °C to 39.3 °C, indicating that the mixed aggregates became more thermally unstable due to DDO binding (Figure 5(b)).

In addition to identifying the temperatures where phase transitions occur, DSC thermograms provide the enthalpy change associated with the transition, which is quantified as the area under the curves. The width at half-height of the transition temperature ($\Delta T_{1/2}$) and the shape of the peaks also contain useful information and the cooperative nature of the transition. The van't Hoff enthalpy change (ΔH_v) can be calculated from $\Delta T_{1/2}$,[31] and the cooperative unit (CU) can be described as the ratio ΔH_v and ΔH.[32] CU gives valuable information on lipid organization, which represents the number of molecules going through the gel−liquid crystalline phase transition simultaneously.[33] Table 1 lists the thermodynamic parameters of the mixture estimated from DSC data. Both T_m and ΔH underwent a decrease with increasing addition of DDO. The decrease of ΔH can be attributed to the formation of a complex between SC and DDO, which resulted in a looser structure with enlarged size, and therefore required less heat to compensate for the structure transformation during DSC scans. The increased CU was observed when a higher DDO concentration was added into the mixture, suggesting more molecules had cooperatively participated in the phase transition process,[15] which is also consistent with the decreased entropy change during the association.

Characterization of the Interaction of SC with DDO by Fluorescence Technique. *Intrinsic Fluorescence Studies.* Among the amino acid residues in proteins, tryptophan (Trp) has by far the highest fluorescence yield, and its fluorescence is very sensitive to solvent polarity. Earlier studies showed that in a hydrophobic environment the emission maximum (λ_{max}) of the Trp residue occurs at 331 nm and then shifts to about 351 nm when exposed to an aqueous phase where the hydrophobic domain is disrupted completely.[34] Therefore, the Trp residue is sensitive enough to monitor the changes in the micellar structure.[35] Figure 6(a) shows the fluorescence intensity and λ_{max} of SC in the presence of DDO. Obviously, the fluorescence of SC was quenched by DDO but without a change in peak shape. Instead, a blue shift in λ_{max} was observed with increasing concentration of DDO, indicating a strong interaction between DDO and SC. This result was different from the fluorescence emission of casein as the temperature increases, in which the intensity decreases with a shift toward the red of the spectrum.[25] The data indicate that the fluorescent amino acids are facing a more polar microenvironment.

The fluorescence data were analyzed by the Stern−Volmer equation:

$$F_0/F = 1 + K_{SV}[Q]$$

where F_0 and F are the fluorescence intensities in the absence and presence of quencher, respectively, K_{SV} is the Stern−Volmer quenching constant, and [Q] is the concentration of DDO. A plot of F_0/F versus [Q] yields a slope equal to K_{SV},

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which is shown in Figure 6(b). A linear Stern−Volmer plot is generally indicative of a single class of fluorophores all equally accessible to quencher. As both static and dynamic quenching can result in a linear Stern−Volmer plot, measurement of the change of K_{SV} with temperature is a good way to distinguish whether static or dynamic quenching is involved. For dynamic quenching, the value of K_{SV} will increase with elevated temperature because the accelerated rate of molecule motion can promote collisions between the quencher and excited molecule. On the other hand, for static quenching, the K_{SV} will decrease in an increase in temperature, which causes the instability of the complex. As shown in Figure 6(b), a decreasing of K_{SV} with increasing temperature was observed. Moreover, fluorophore and quencher complexes typically exhibit different absorption behavior in comparison with free fluorophore due to intermolecular interaction in the ground state.[36] Thus, the results suggest a static quenching mechanism and complex formation between SC and DDO.

Micropolarity of the Mixed Aggregates. To further explore the interaction between SC and DDO, pyrene solubilized in the interiors of the micelles was used to probe the micropolarity change. The ratio of the intensity of the first and the third vibronic peak in the fluorescence spectra of pyrene (I_1/I_3) is very sensitive to solvent polarity and therefore has been widely used as a measurement of the micropolarity of a membrane. Figure 7(a) shows the change of I_1/I_3 dissolved in SC in the presence of DDO at 25 °C. The intensity ratio decreased with the addition of DDO, which implied that a more hydrophobic region was formed. The compact structure of SC micelles promoted pyrene to locate at a more hydrophobic domain and in turn led to a smooth decrease of I_1/I_3. With increasing addition of DDO, the value of I_1/I_3 decreased more sharply, suggesting a stronger hydrophobic interaction between aggregated DDO and SC. Upon heating, the value of I_1/I_3 increased slightly for DDO at lower concentration but decreased for vesicular DDO. As discussed above, the electrostatic interaction predominates over hydrophobic attraction during the binding of monomeric DDO with SC; therefore elevated temperatures will cause the acceleration of the molecular motion rate and hamper the aggregation of molecules, resulting in an increased I_1/I_3. In the interaction of vesicular DDO with SC, the hydrophobic interactions between the nonpolar segments of the aggregated DDO and the hydrophobic patches of SC seem to be more preferable, thus forming mixed aggregates with less micropolarity. The decreased I_1/I_3 confirmed this preferable hydrophobic interaction of DDO vesicles with SC at higher temperatures.

In the control experiment, we mixed a conventional surfactant such as anionic SDS or cationic CTAB with SC to see if conventional surfactant micelles could induce the self-assembly change of SC. SC was found to adopt a more ordered conformation in the presence of both SDS and CTAB, which was consistent with the results reported by Chakraborty et al.[7] However, heating the mixture did not yield enlarged SC aggregates. We further examined the cationic vesicles made from mixed oppositely charged surfactants[27,28] to see if the regular vesicular surfactants affect the self-assembly of SC. We found that the size of SC grew with the vesicles, but heating did not show an effect (see Supporting Information). Taken together, the results suggested that the special hydrogen-bond donor in the head group, the ability of spontaneously formation of vesicles, and the repairing effect of heating might enable DDO to guide the reversible self-assembly of SC. Our preliminary results also showed that the stability of the emulsion made from these mixed protein−vitamin bolaamphiphiles has been enhanced. The narrow size distribution with higher viscosity of the emulsions suggested that DDO modified the microstructure of the adsorption layer (see Supporting Information).

In summary, although the flexible, more open, and irregular particle structure of SC makes it insensitive to marked structural alterations as in other proteins, we found that the zwitterionic bolaamphiphile DDO could guide the association of SC in different states due to the special structure and the property of spontaneous transition from small molecule to nanoscale vesicle. At lower concentration, the monomeric DDO attached to the SC micelles through its positive sites, which made the micelle bear fewer negative charges and lead to a more compact structure with decreased hydrodynamic diameter due to less electrostatic repulsion, while vesicular DDO tended to interact with SC via preferable hydrophobic interactions. At ambient temperature, though monomeric or vesicular DDO interacted with SC micelles in different ways, the complexes formed were stable and the size changes were not significant. However, upon heating, the solubilized SC micelles reassembled with DDO vesicles to form larger aggregates. Moreover, the whole process was temperature-dependent and reversible. Characterization of the intrinsic fluorescence and the micropolarity of the mixed aggregates supported the mechanisms proposed for the interaction of SC with DDO. It is worth noting that current research on the additives that could affect the structure of SC micelles and cause changes of the functional properties of SC focuses either on small species or on large biopolymers. This research provides a potential way to use a structure-switchable zwitterionic bolaamphiphile (DDO) for guiding the self-assembly of SC. The located DDO vesicles in SC networks will modify the properties of the adsorption layers and provide more hydrophilic and hydrophobic areas for the solubilization of bioactive molecules. Systematic research on the functional properties induced by the these structural changes, including emulsification, gelation, foaming, dispersing, and water binding capacity as well as their applications in drug delivery systems, is in progress.

■ ASSOCIATED CONTENT

Supporting Information

The results of the control experiments for the change of the average size of a mixture of SC (0.2 wt %) in the presence of CTAB, SDS, and cationic vesicles as a function of temperature and the preliminary results on the emulsions stabilized by mixed protein−vitamin vesicles. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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