

## Impact of the Stereochemical Structure on the Thermal Phase Behavior of a Cationic Gemini Surfactant

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Differential scanning calorimetry (DSC) revealed the stereochemical structure of the headgroup to be an important determinant for the thermal phase behavior of aqueous dispersions of a cationic gemini surfactant. More specifically, the meso form (2S,3R)-2,3-dimethoxy-1,4-bis(N-hexadecyl-*N,N*-dimethylammonium)butane dibromide (abbreviated SR-1) exhibited complex pattern of multi-peak endotherms with two more pronounced peaks at approximately 29 °C and 39 °C. Different behaviors were evident for the stereochemically pure enantiomers (2S,3S)-2,3-dimethoxy-1,4-bis(N-hexadecyl-*N,N*-dimethylammonium)butane dibromide (SS-1) and (2R,3R)-2,3-dimethoxy-1,4-bis(N-hexadecyl-*N,N*-dimethylammonium)butane dibromide (RR-1), which both had major endotherms at approximately 36 °C and 41 °C. The heating scans recorded for both RR-1 and SS-1 immediately after a heating–cooling cycle were smooth while SR-1 demonstrated a simple endotherm at 29 °C. These results suggest that the geometry of the headgroup of the above gemini surfactants is an important determinant for their lateral packing and organization in an aqueous solution reflected as pronounced effects on both thermal phase behavior and relaxation kinetics. In keeping with this interpretation, including either SS-1 or RR-1 at a mole fraction of 0.10 into SR-1 suspensions resulted in lower transition temperatures and enthalpies, thus demonstrating perturbation of the packing of SR-1 by the presence of the stereoisomeric “impurities”.

### Introduction

Lately, gemini surfactants, characterized by two conventional surfactant molecules whose polar headgroups are connected by a spacer, have received a lot of attention in colloid and surface chemistry.<sup>1</sup> A plethora of structurally different gemini surfactants with academically and industrially interesting properties have been synthesized during the past decade. When compared to conventional surfactants, gemini surfactants have almost 100-fold lower critical micellar concentrations (CMCs)<sup>1</sup> and even 1000-fold higher surface activities.<sup>2</sup> They have also been shown to be of practical value in, for example, disinfection<sup>1,3</sup> and solubilization processes.<sup>4</sup> Of particular interest is the potential of their use in transfection,<sup>5,6</sup> conveying foreign genetic material into living cells.

Thorough understanding of the behavior of gemini surfactants is required to employ their full potential in different applications. The great structural variation made possible by organic synthesis further emphasizes the importance of studies concentrating on the fundamental qualities of gemini surfactants. The impact of systemically altered structures on the physical properties of supramolecular assemblies involving gemini surfactants has been

characterized by techniques such as differential scanning calorimetry (DSC),<sup>5,7</sup> Langmuir balance,<sup>7,8,9</sup> and microscopy<sup>7,10,11</sup>. Yet, despite empirical<sup>5,7,10</sup> and theoretical efforts<sup>12</sup> more information is needed to understand the phase behavior of gemini surfactants. Decades of comparable research on structurally similar biomolecules, phospholipids, have revealed a wide spectrum of different patterns of thermal phase behavior with important functional and structural consequences.<sup>13</sup> Analogously, knowledge on the phase behavior of gemini surfactants can be expected to be crucially important in developing their future applications.

In the course of our studies on the gemini surfactant, (2S,-3R)-2,3-dimethoxy-1,4-bis(N-hexadecyl-*N,N*-dimethylammonium)butane dibromide (abbreviated SR-1), (Figure 1), we observed that the DSC traces of pure compound had peculiar reproducibility and exhibited complex multi-peak patterns. This lead us to investigate the thermal phase behavior of SR-1 in more detail. Comparison with the stereoisomers of SR-1, (2S,-3S)-2,3-dimethoxy-1,4-bis(N-hexadecyl-*N,N*-dimethylammonium)butane dibromide (SS-1) and (2R,3R)-2,3-dimethoxy-1,4-bis(N-hexadecyl-*N,N*-dimethylammonium)butane dibromide (RR-1), revealed that the configuration of the chiral carbons in the spacer had a pronounced impact on thermal phase behavior of this gemini surfactant.

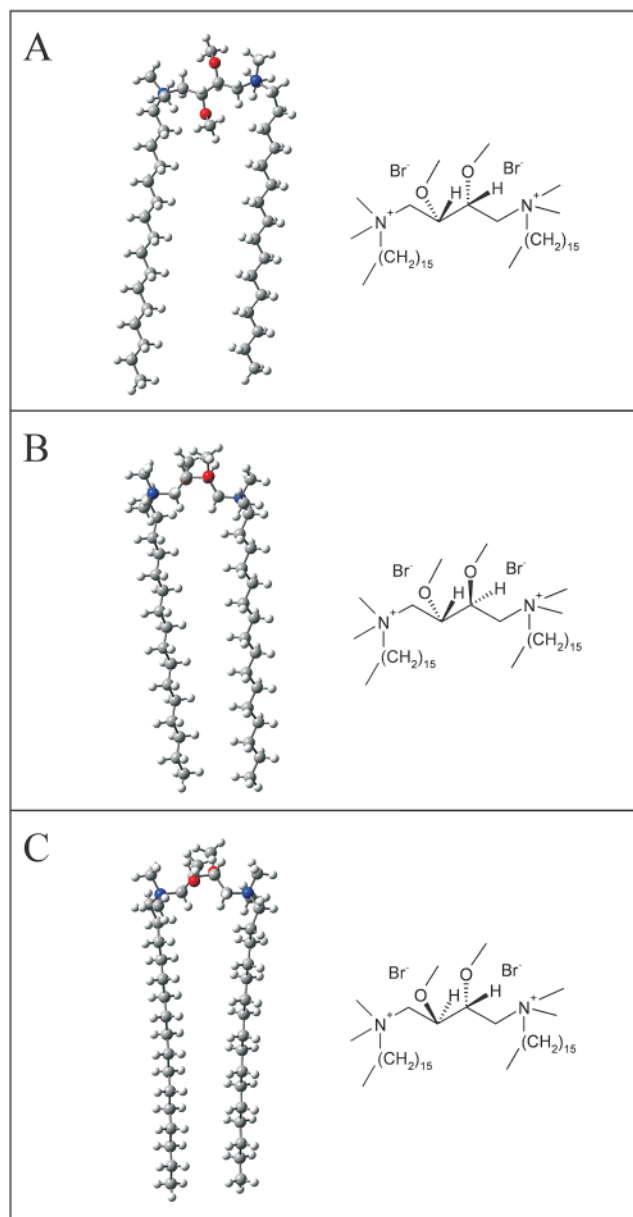
### Experimental Section

**Materials.** Hepes and EDTA were from Sigma. The *meso* form of gemini (2S,3R)-2,3-dimethoxy-1,4-bis(N-hexadecyl-*N,N*-dimethylammonium)butane dibromide (SR-1), and its ste-

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**Figure 1.** Chemical structures of SR-1 (A) and its stereoisomers SS-1 (B) and RR-1 (C) are shown on the right side of the panels. On the left side of the panels are shown the geometry optimized 3-D illustrations of the gemini surfactants.

reoisomers (2*S*,3*S*)-2,3-dimethoxy-1,4-bis(*N*-hexadecyl-*N,N*-dimethylammonium)butane dibromide (SS-1) and (2*R*,3*R*)-2,3-dimethoxy-1,4-bis(*N*-hexadecyl-*N,N*-dimethylammonium)butane dibromide (RR-1) were synthesized as described previously.<sup>14</sup> The structure of the surfactants was verified by NMR and their optical activities (in ethanol) were  $[\alpha] = -19.4$  and  $20.3$  for SS-1 and RR-1, respectively. The concentrations of amphiphiles were determined gravimetrically using a high precision electrobalance (Cahn, Cerritos, CA). Freshly deionized filtered water (Milli RO/Milli Q, Millipore Inc., Jaffrey, NH) was used in all experiments.

**Molecular Modeling.** Gaussian 98 (Gaussian Inc., Carnegie, PA) was used to optimize the geometry of different optical isomers of cationic gemini surfactants. Ab initio calculations were performed without any explicit or implicit solvent at the level HF/6-31G\* with SGI Origin 2000 (Silicon Graphics Inc., Mountain View, CA) and IBM RS/6000 SP (IBM Inc., Armonk, NY). Input files were generated using MSI Cerius2 (Molecular

Simulations Inc., San Diego, CA). Results were visualized with GaussView 2.1 (Gaussian Inc., Carnegie, PA).

**Preparation of Surfactant Dispersions.** Aqueous suspensions of the gemini surfactants were prepared by mixing appropriate amounts of their stock solutions in dry chloroform to obtain the desired compositions. Thereafter, the solvent was removed by evaporation under a stream of nitrogen. For removal of residual amounts of solvent, the samples were further maintained under high vacuum for at least 2 h. The resulting dry surfactant films were then hydrated with 5 mM Hepes, 0.1 mM EDTA, pH 7.4 and thereafter incubated for 30 min at approximately 60 °C, i.e., above the temperatures of the transition endotherms of the surfactants and their mixtures.

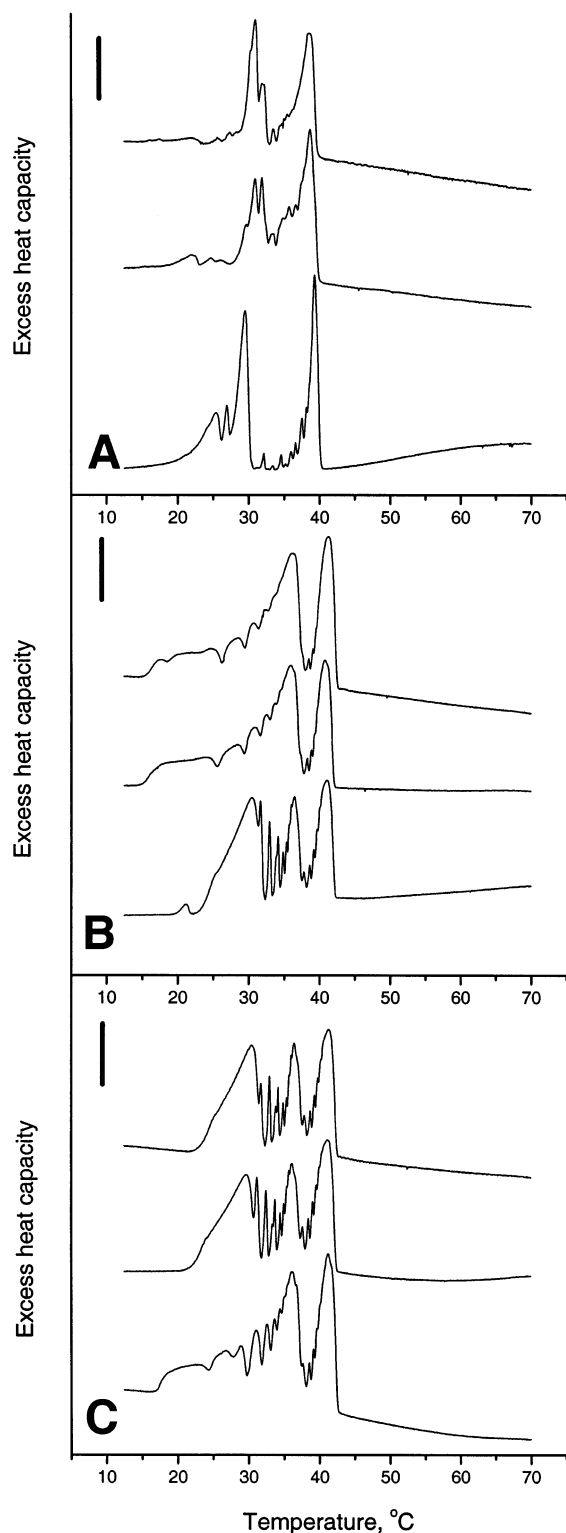
**Differential Scanning Calorimetry.** Surfactant suspensions were vortexed and maintained on an ice water bath for at least 12 h before loading into the calorimeter cuvette (final concentration one mM). A VP-DSC microcalorimeter (Microcal Inc., Northampton, MA) was operated at a heating rate of 0.5 °C per min. When multiple scans were recorded for the same sample (e.g., first upscan and then downscan) the sample was allowed to equilibrate at the starting temperature of the scan for 1 h. The instrument was interfaced to a 486 PC, and the data were analyzed using the routines of the software provided by the instrument manufacturer.

## Results and Discussion

The DSC traces of heating scans from 10 to 70 °C for SR-1 dispersion incubated at approximately 4 °C for 12 h exhibited a pattern of multiple endotherms (Figure 2A). Total enthalpy contained in these peaks is  $57.4 \pm 3.9$  kJ/mol (average enthalpy calculated from the three traces shown, S. D.) In addition to the several smaller peaks, two more pronounced endotherms were evident at approximately 29 and 39 °C. When the sample of SR-1 was cooled from 70 to 10 °C only a simple exotherm was observed at 25 °C (Figure 3A). Upon subsequent immediate heating of this sample to 70 °C an endotherm at 29 °C and with an enthalpy of 29.6 kJ/mol was evident, with the array of small peaks being absent.

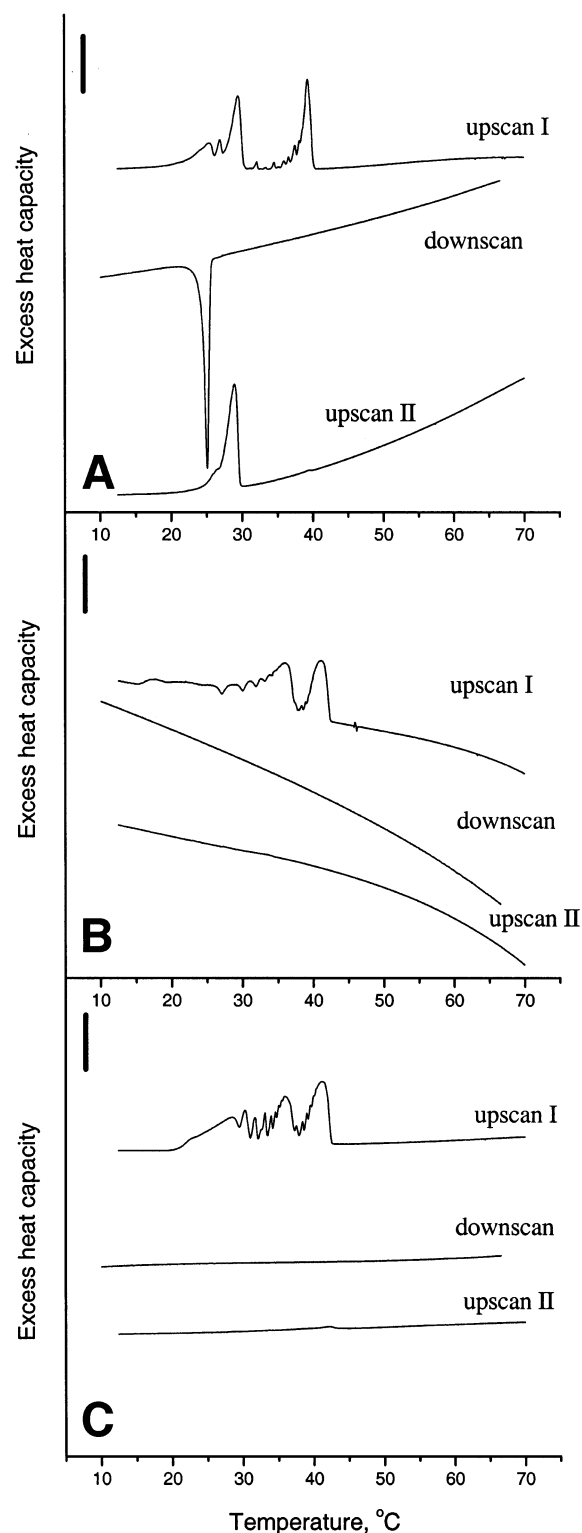
The enantiomers SS-1 and RR-1 are readily expected to have identical DSC profiles. Aqueous suspensions of these gemini surfactants indeed revealed highly similar thermal phase behaviors (Figure 2, B and C, respectively), and for both SS-1 and RR-1 two major endotherms were evident at approximately 36 and 41 °C. However, two distinct patterns could be observed, one being more prevalent for SS-1 and the other for RR-1. In three out of four scans recorded for SS-1, a broad and smooth endotherm beginning at ~15 °C was evident, followed by four smooth, smaller peaks at 28, 31, 32, and 34 °C, as depicted by the two uppermost DSC traces in Figure 2B. In contrast, the remaining scans recorded for SS-1 showed a wide endotherm at approximately 30 °C and with an enthalpy of ~76.3 kJ/mol, followed by multiple sharp and reproducible peaks, illustrated by the lowermost DSC trace in Figure 2B. With RR-1 same general patterns of thermal behavior were evident, but with the opposite prevalences (Figure 2C). It is possible that some unknown factor that we were unable to take into account in sample preparation caused the variation in DSC traces. Yet, the sample preparation procedure used (see the Experimental Section for details) is generally accepted in DSC studies on amphiphiles. Thus, the variation in patterns of thermal phase behavior between the enantiomers is interesting, and can be regarded to reflect the differences in their stereochemical structures.

In contrast to SR-1 neither SS-1 nor RR-1 showed exothermic peaks upon cooling (from 70 to 10 °C) after the heating scan



**Figure 2.** DSC traces recorded in three separate experiments for aqueous suspensions of SR-1 (A), or its stereoisomers SS-1 (B) and RR-1 (C). Total concentration of surfactant was one mM in 5 mM Hepes, 0.1 mM EDTA, pH 7.4. The heating rate was 0.5 °C/min. The calibration bars correspond to 2 mJ \* °C<sup>-1</sup>.

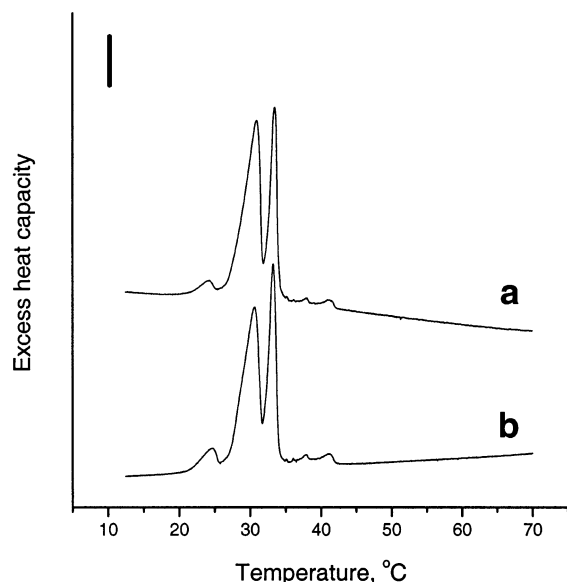
(Figure 3, B and C). For both SS-1 and RR-1 the heating scans recorded immediately following a heating–cooling cycle lacked any endotherms (Figure 3, B and C), thus revealing that the low temperature incubation preceding the first heating scan was required for relaxation of the surfactant assembly into a lower energy phase state. Accordingly, the transition kinetics of the phase formed upon heating back to this lower energy state are



**Figure 3.** DSC thermograms obtained for aqueous suspensions of SR-1 (A), SS-1 (B), or RR-1 (C) by first heating the sample from 10 °C to 70 °C (upscan I), then cooling it to 10 °C (downscan), and finally heating it again to 70 °C (upscan II). Total surfactant concentration was one mM in 5 mM Hepes, 0.1 mM EDTA, pH 7.4. The heating rate was 0.5 °C/min. The calibration bars correspond to 4 mJ \* °C<sup>-1</sup>.

slow and after cooling the surfactant aggregates remain in a metastable state.

As noted above, the structural differences between SR-1, SS-1, and RR-1 are restricted to the spacer connecting the two polar headgroups (Figure 1). The configuration of the headgroup is thus an important determinant for the thermal phase behavior



**Figure 4.** DSC traces for aqueous suspensions of SR-1 containing either SS-1 (a), or RR-1 (b). The mole fraction  $X$  of the latter two surfactants was 0.10. Total concentration of surfactant was one mM in 5 mM Hepes, 0.1 mM EDTA, pH 7.4. The heating rate was 0.5 °C/min. The calibration bar corresponds to 2 mJ \* °C<sup>-1</sup>.

of these gemini surfactants. In keeping with the above SR-1, which is the meso form, exhibited a very different thermal phase behavior compared to the stereochemically pure compounds (Figure 2A-C). A likely explanation for the observed behavior is that the stereochemically pure compounds, i.e., SS-1 and RR-1, as well as the meso form, arrange upon prolonged cooling into regular semicrystalline structures whose melting caused the observed array of endotherms. The lower transition temperatures and enthalpies observed for SR-1 compared to SS-1 and RR-1 suggest that the organization of semicrystalline lattice adopted by the meso form is easier to disturb by thermal energy. If the lateral packing and organization are indeed the key determinants of the observed complex thermal behavior, then including a small amount of either SS-1 or RR-1 into a suspension of SR-1 should disturb the lateral organization required to create the most stable semicrystalline lattices. Consequently, the transition temperatures should decrease significantly. This is in fact the case, and the presence of either SS-1 or RR-1 ( $X = 0.10$ ) in SR-1 resulted in two endotherms being observed at 31 and 33 °C (Figure 4). Also significant is the fact that the array of multiple smaller peaks is nearly absent. To this end, similar

observations have been made for the mixtures of *sn*-1 and *sn*-3 enantiomers of 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC). More specifically, mixing the two stereoisomers lowered the so-called pretransition temperature of this lipid.<sup>17</sup>

A likely reason for the above differences between SR-1 and the pure enantiomers is the orientation of the dipoles associated with the moieties linked to the chiral atoms. Accordingly, different lattice structures should form upon prolonged cooling. The structures appear to have rather similar free energies, thus allowing for stochastic variation. The latter would thus be reflected as variation in the patterns of thermal phase behavior for the SS-1 and RR-1.

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