Final Draft
of the original manuscript:

Bolalipid fiber aggregation can be modulated by the introduction of sulfur atoms into the spacer chains

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Abstract

The aggregation behavior in aqueous suspension of two symmetrical single-chain bolaamphiphiles, namely 12,21-dithiadotriacontane-1,32-diyl-bis[2-(tri-methylammonio)ethylphosphate] (PC-C32SS-PC) and 12,21-dithiadotriacontane-1,32-diyl-bis[2-(dimethylammonio)ethylphosphate] (Me$_2$PE-C32SS-Me$_2$PE), containing sulfur as hetero atoms in the chains, was studied using differential scanning calorimetry (DSC), Fourier Transform infrared spectroscopy (FT-IR), small angle neutron scattering (SANS), and transmission electron microscopy (TEM). The rheological properties of hydrogels formed by the aggregation into nanofibers were studied by oscillatory rheology. Based on the well-characterized behavior of bolalipids with long alkyl chains which at room temperature can form a network of nanofibers leading to the formation of a hydrogel, we investigated whether the incorporation of two hetero atoms of sulfur into the spacer chain of the molecules has an influence on the aggregation properties. Compared to the analogues without sulfur, the fibrous aggregates formed by sulfur containing compounds are less stable and build weaker viscoelastic gels. This is due to a perturbation of the packing of the chains as the sulfur atoms change the bond angle in the chain compared to the molecules with pure alkyl chains leading to kinks in the chain. For the bolaamphiphile with the Me$_2$PE headgroups this effect is less pronounced due to the possibility of forming stabilizing hydrogen bonds between the headgroups.

Keywords

Introduction

Bolaamphiphiles are in the focus of an expanding area of research dealing with the properties of these molecules and their self-assembly into defined aggregates. The behavior and functionality of the resulting aggregates is of particular interest.[1-7] Bolaamphiphiles consist of a hydrophobic spacer chain (e.g., one or two alkyl chains) and two hydrophilic headgroups (e.g., phosphocholine, sugars) attached to each end of the spacer.[1] Recently, we reported on the synthesis and aggregation behavior of the two symmetrical single-chain bolaamphiphiles dotriacontane-1,32-diyl-bis[2-(trimethylammonio)ethylphosphate] (PC-C32-PC) and dotriacontane-1,32-diyl-bis[2-(dimethylammonio)ethylphosphate] (Me$_2$PE-C32-Me$_2$PE). Suspended in aqueous media, they self-assemble into helical nanofibers, which are able to gel water by forming a three-dimensional network of cross-linked and entangled nanofibers.[8-13] With increasing temperature of the suspensions these nanofibers transform into micellar aggregates resulting in the break-down of the gel structure.

Extensive characterization of the aggregation behavior of these bolaamphiphiles raised the question, how tolerant the fiber structure is to modifications of the chemical structure of the alkyl spacer chain. This structural modification was realized via the incorporation of hetero-atoms (e.g., oxygen or sulfur) into the spacer chain.[14] Modifications of bolaamphiphile spacer chains have been utilized before to influence the aggregation behavior of the molecules, e.g. to stabilize the aggregates by addition of mesogenic groups,[15, 16] or to attain functionalization of the aggregates,[17, 18] for instance, for interaction with metal surfaces or nanoparticles.[19]

In the alkyl chains of PC-C32-PC and Me$_2$PE-C32-Me$_2$PE two methylene units at the positions C12 and C21 were each replaced by one sulfur atom. The chemical structure of the two symmetrical bolaamphiphiles 12,21-dithiadotriacontane-1,32-diyl-bis[2-(tri-methylammonio)ethylphosphate] (PC-C32SS-PC) and 12,21-dithiadotriacontane-1,32-diyl-bis[2-(dimethylammonio)ethylphosphate] (Me$_2$PE-C32SS-Me$_2$PE) is shown in Figure 1.
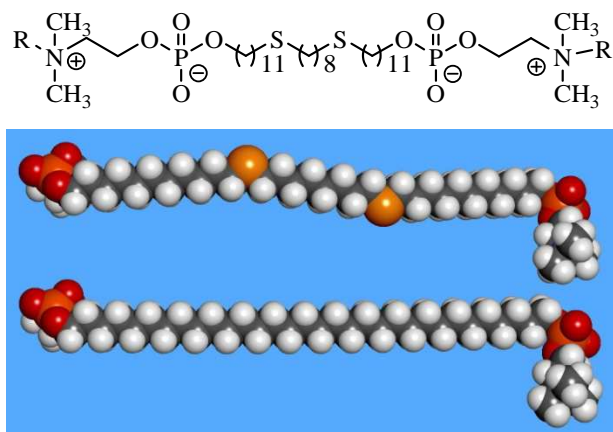
Figure 1: (Top) Chemical structure of PC-C32SS-PC with R = CH$_3$ and of Me$_2$PE-C32SS-Me$_2$PE with R = H and (bottom) CPK models of PC-C32SS-PC (top) and PC-C32-PC (bottom).

In comparison to the C-C-C bond angle of 111° the C-S-C angle is only approx. 97° leading to a chain conformation with two pronounced kinks. Based on preliminary experiments on the aggregation behavior of PC-C32SS-PC in aqueous suspension, we had concluded that the formation of the fiber structure is still possible, despite the changes in the chain conformation. However, the temperature stability of the fibers was reduced, as the fiber-micelle transition was shifted to lower temperature.[14]

The aim of this work was to study in detail the aggregation behavior of PC-C32SS-PC and to examine how the heteroatoms change the characteristics of the fiber structure and the rheological properties of the formed hydrogels in comparison to those formed by PC-C32-PC. In addition, we investigated suspensions of the new compound Me$_2$PE-C32SS-Me$_2$PE. Exchange of a methyl group in the headgroup of PC-C32-PC by a hydrogen atom usually leads to a stabilization of the fiber structures due to the possibility of additional interactions in the headgroup region via hydrogen bonds.[8-13] We will show that this is also the case for the bolalipids with sulfur atoms in the chain, where the destabilization of the chain packing can be partially compensated by the additional interactions in the headgroup region.

Material and methods

Materials. PC-C32-PC,[20] PC-C32SS-PC,[14] and Me$_2$PE-C32-Me$_2$PE[12] were synthesized as described recently. The synthesis of Me$_2$PE-C32SS-Me$_2$PE is described in the Supporting Information. Solvents and buffer components (sodium acetate and acetic acid) were purchased from Carl Roth GmbH + Co. KG (Karlsruhe, Germany) and used as received. Ultra-pure water was used from a Milipore Milli-Q A10 system (Millipore GmbH, Schwalbach, Germany)

Sample preparation. The appropriate amount of bolalipid was suspended in water (PC-C32SS-PC) or acetate buffer at pH 5 (Me$_2$PE-C32SS-ME$_2$PE). To achieve a homogeneous suspension the samples were heated to 70 °C three times and vortexed.

Differential Scanning Calorimetry (DSC). DSC measurements were carried out with a MicroCal VP-DSC (MicroCal Inc., Northampton, USA). The bolalipids were suspended in water or acetate buffer (pH 5) at a concentration c = 1 mg ml^{-1}. Water or buffer was used as reference. Measurements were carried out using heating rates of 20 K min^{-1} in the temperature interval from 2 to 95 °C. Three consecutive heating and cooling scans of each sample were recorded to check the reproducibility. The water-water or buffer-buffer baseline was subtracted from the thermograms of the samples, and the DSC scans were evaluated using the Origin 8.0 software.

Fourier Transform Infrared Spectroscopy (FT-IR). FT-IR-spectra as a function of temperature were recorded with a Bruker Vector 22 Fourier transform spectrometer (Bruker Optik GmbH, Karlsruhe, Germany) operating at 2 cm^{-1} resolution. The bolalipid suspension (c = 50 mg ml^{-1}) was spread between CaF$_2$ windows with a 12 μm Teflon spacer and equilibrated for 2 h at 5 °C prior to the measurement. 64 scans each were recorded in the temperature range from 5 to 95 °C with an equilibration time of 8 minutes at each temperature. Spectra of water or buffer were measured with an identical setup and were subtracted from the sample spectra using the OPUS software supplied by Bruker Optik GmbH.

Transmission Electron Microscopy (TEM). TEM images of negatively stained samples were recorded with a Zeiss EM 900 transmission electron microscope (Carl Zeiss NTS GmbH, Oberkochen, Germany). The samples were prepared and stored (24 h prior to the preparation) below ambient temperature in a cold room (5 °C). 5 μl of the suspension (c = 0.1 - 0.03 mg ml^{-1}) were spread on a copper grid coated with Formvar film. After 1 minute the excess solution was blotted off with filter paper. The samples were negatively stained with 1% uranyl acetate (20 μl) solution, which was drained off after 1 minute. They were dried for

2 days at 5 °C and were kept in a desiccator at ambient temperature until the images were recorded.

The cryo-TEM images were recorded with a Zeiss 902A instrument (Carl Zeiss NTS GmbH, Oberkochen, Germany). The samples were prepared in a chamber with controlled temperature and humidity. 2 μl of the bolaamphiphile suspension (c = 1 mg ml^{-1}) was placed on a grid coated with a perforated polymer film. Excess solution was removed after 1 minute with filter paper. Vitrification of the remaining thin film was achieved by rapid plunging of the grid into liquid ethane, held just above the freezing point. The vitrified sample was kept below -165 °C after this process and while recording the images.

Dynamic Light Scattering (DLS). DLS experiments were carried out with an ALV-NIBS-HPPS particle sizer (ALV-Laser Vertriebsgesellschaft m.b.H., Langen, Germany). A 3 mW HeNe laser with a wavelength of 632.8 nm and a scattering angle of 173° was used. All samples (c = 1 mg ml^{-1}) were filtered through a membrane filter of 0.45 μm pore size at 80 °C into quartz cuvettes (path length 10 mm). Before starting the measurement, each sample was equilibrated for 30 minutes at the required temperature. Three individual measurements were performed for each sample to test the reproducibility with one measurement consisting of 3 runs of 30 seconds each. The experimental data were analyzed with the aid of the ALV-correlator software taking into account the temperature correction of viscosity.

Small Angle Neutron Scattering (SANS). SANS experiments were carried out with the SANS-1 instrument in the FRG 1 research reactor, Helmholtz Zentrum Geesthacht, Germany. The bolaamphiphiles were suspended in deuterated water or in deuterated acetate puffer at pH 5 (10 mM) at a concentration of 1 mg ml^{-1} and the suspensions were filtered through a membrane filter of 0.45 μm pore size at 80 °C. The samples were placed in a thermostated sample holder for isothermal conditions ($\Delta T = 0.5$ °C) in quartz cuvettes with a path length of 5 mm. Four sample-to-detector distances were employed to cover the range of scattering vectors q from 0.05 to 2.5 nm^{-1}. Transmission of the samples was approx. 70 % and the contribution of multiple scattering can be considered negligible. The raw scattering data were corrected for the background from the solvent, sample cell, and other sources using conventional procedures. Subsequently, the scattering data were fitted by using the Indirect Fourier Transform Method. A detailed description of this process was given by Meister et al.[11, 12]

Oscillatory Rheology. The rheological measurements were carried out with an Anton Paar MCR 301 rheometer (Anton Paar GmbH, Graz, Austria) controlled by the software Rheoplus 3.0. For the measurements a cone-plate shear system (2 °, 50 mm) with a thermostatting unit

(Peltier system: -40 °C to 200 °C) and a thermostatted hood to improve temperature stability were used. The bolaamphiphile suspensions had a concentration of 1 mg ml^{-1}. The sample was left to equilibrate for 30 minutes at 2 °C prior to starting the heating and cooling cycle with a heating rate of 20 K h^{-1}. To prevent the evaporation of water, cone and gap were covered with small amounts of low viscosity silicone oil.

Results and Discussion

Aggregation behavior of PC-C32SS-PC and Me₂PE-C32SS-Me₂PE in aqueous suspension

TEM. For PC-C32SS-PC suspensions, previous TEM images with negative staining of samples prepared at 5 °C have proven the presence of a fiber structure. However, the fibers of PC-C32SS-PC seemed to have an irregular structure with diameters ranging from 5 to 10 nm (Figure 2B).[14] As images prepared with the negative staining method are not free from artifacts caused by the drying process we investigated the same suspensions now using cryo-TEM. The cryo-TEM images presented here of a PC-C32SS-PC suspension reveal long regular fibers with a uniform diameter of 5 to 6 nm (Figure 2A). Also for the suspension of the bolalipid Me₂PE-C32SS-Me₂PE the cryo-TEM image shows regular fibers (Figure 2C). This indicates that the observed irregularities in the negatively stained samples originated probably from the preparation and drying process of the TEM samples (Figure 2B,D).
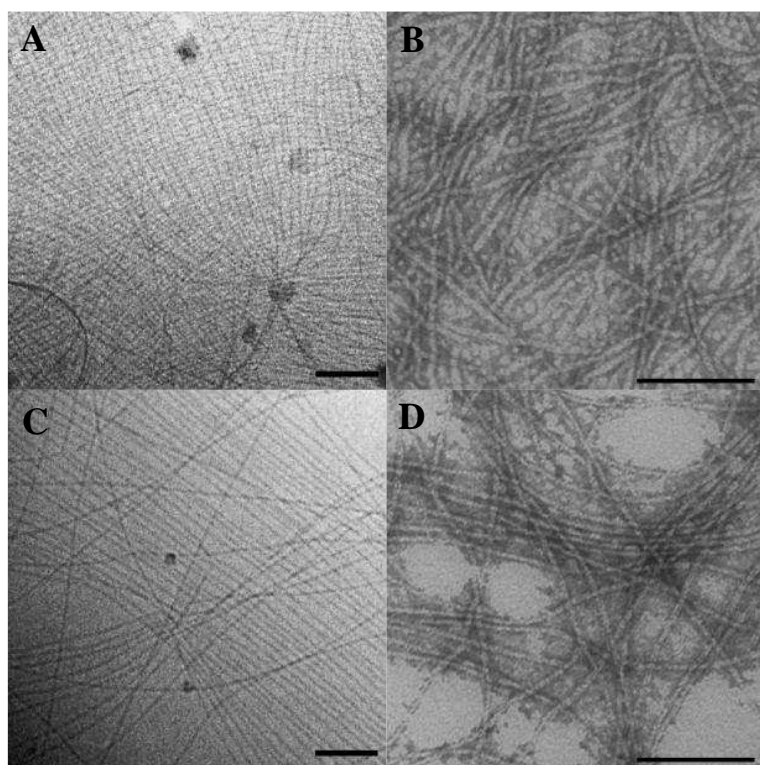

Figure 2: Cryo-TEM images of suspensions of (A) PC-C32SS-PC (water) and (C) Me₂PE-C32SS-Me₂PE (acetate buffer, pH 5) at 7 and 15 °C, respectively. TEM images obtained with negative staining of suspensions of (B) PC-C32SS-PC (water) and (D) Me₂PE-C32SS-Me₂PE (acetate buffer, pH 5) with uranyl acetate were prepared at 5 °C. The bars correspond to 100 nm.

DSC. To examine the temperature dependent aggregation behavior of the bolaamphiphile suspensions we used DSC measurements. For PC-C32-PC, the analogue without sulfur atoms in the chains, we observed before a major transition where the fibers convert into micelles and a second transition at higher temperature between two different types of micellar aggregates.[8, 9] PC-C32SS-PC suspended in water shows only one transition at 14 °C, as reported previously (Figure 3).[4] For the bolalipid Me$_2$PE-C32-Me$_2$PE without sulfur atoms, the thermotropic behavior is characterized by three transitions, a first one between two different types of fiber aggregates, a second one at higher temperature, where the fibers break apart into micelles and a third high temperature transition between two different types of micellar aggregates. The DSC heating scan of a Me$_2$PE-C32SS-Me$_2$PE suspension in acetate buffer at pH 5 shows again only one transition, but this is shifted to 24.5 °C, i.e. a higher temperature compared to the transition of PC-C32SS-PC (Figure 3). The reason for the higher transition temperature is the stabilization of the fiber structure by intermolecular hydrogen bonds in the headgroup region. The transition enthalpies for the transitions of the two compounds are ~56 (PC-C32SS-PC) and ~66 kJ mol^{-1} (Me$_2$PE-C32SS-Me$_2$PE). Compared to the bolalipids without sulfur atoms in the chains, the thermotropic behavior is distinctly different, as only one transition is observed. This transition is caused by the break-down of the fiber structure into micellar aggregates. A micelle I to micelle II transition seen for the analogues without sulfur cannot be observed.[8, 9] The transition enthalpies for the two bolalipids are much higher than the total transition enthalpy for all transitions observed for PC-C32-PC (34 kJ mol^{-1})[8] and for Me$_2$PE-C32-Me$_2$PE (43 kJ mol^{-1}).[9] The DSC cooling scans (not shown) show a broader peak than observed in the heating scan and a hysteresis for the reformation of the fiber structure for both bolaamphiphiles as it was also observed for the other bolalipid fiber systems.[7-10]

FT-IR. FT-IR spectroscopy shows that the wavenumber of the symmetric stretching vibrational band of the methylene groups are at 2850.2 cm^{-1} for Me$_2$PE-C32SS-Me$_2$PE and 2850.4 cm^{-1} for PC-C32SS-PC at a temperature of 5 °C (Figure 3). These values are higher by 1 cm^{-1} compared to the analogues without sulfur.[7, 8] The wavenumber of the methylene stretching vibrations is a measure of the order within the alkyl chain.[21] It increases with a higher amount of *gauche*-conformers within the chain. However, in the present case the shift to higher wavenumber at lower temperature is caused by the reduction of the maximal length of a segment with all-*trans* conformers in the alkyl chain by the replacement of methylene groups by sulfur atoms. The number of the methylene groups in the all-*trans* segments is only

11 on both sides and 8 in the middle part, respectively, compared to 32 in the chains not interrupted by sulfur atoms.

The lower temperature stability of the fibers is caused by steric effects due to the presence of kinks in the chains due to the sulfur atoms. In addition, the sulfur atoms are less hydrophobic due to their higher polarity reducing the gain in free energy in the aggregation process in water.
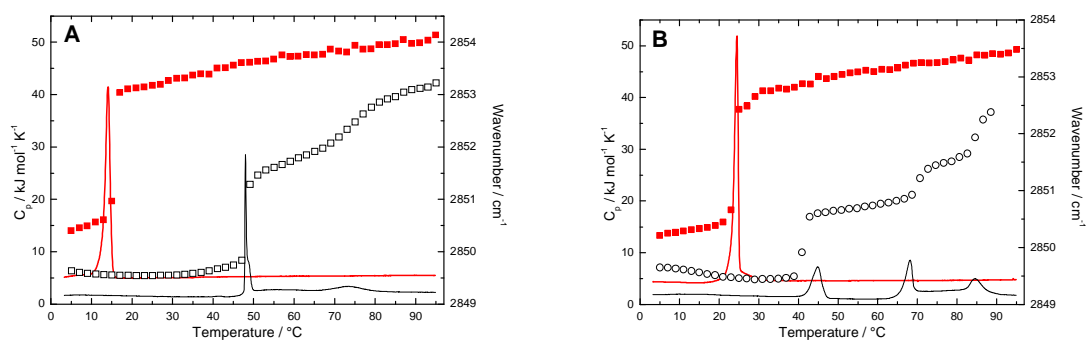

Figure 3: DSC heating curves (solid lines, $c = 1$ mg ml^{-1}) and FT-IR spectroscopic data (scattered data, $c = 50$ mg ml^{-1}, wavenumber of the symmetric methylene stretching vibrational band) of aqueous suspensions of (A) PC-C32SS-PC (red) and PC-C32-PC (black) in water and of (B) Me$_2$PE-C32SS-Me$_2$PE (red) and Me$_2$PE-C32-Me$_2$PE (black) in acetate buffer at pH 5. The DSC curves are shifted for clarity. The DSC heating rate was 20 K h^{-1}.

The large increase in wavenumber for the symmetric CH$_2$-vibrational band observed for both bolalipids to 2853.0 cm^{-1} for PC-C32SS-PC (14 °C) and to 2852.5 cm^{-1} for Me$_2$PE-C32SS-Me$_2$PE (24 °C), respectively, at a defined temperature correlates with the endothermic transition seen by DSC. As mentioned above, this transition is linked to the collapse of the fiber structure into micelles and the transformation of the all-*trans* alkyl chain into a more flexible chain with a higher amount of *gauche*-conformers. The results of FT-IR measurements with PC-C32-PC and Me$_2$PE-C32-Me$_2$PE suspensions showed that in these systems the wavenumber of the symmetric methylene stretching vibrational band reaches these high values only after the micelle I – micelle II transition above 70 °C.[9, 10] This might explain the higher transition enthalpies observed for the two sulfur compounds by DSC (see above) as the micelles with a higher degree of disorder are immediately formed when the fibers break apart. As also observed by DSC, FT-IR measurements with the sulfur modified bolalipids show no micelle I to micelle II transition as observed for PC-C32-PC and Me$_2$PE-C32-Me$_2$PE.[8, 9]

DLS. Using DLS we determined the size of the micelles above the fiber-micelle transition. At 25 °C, the PC-C32SS-PC micelles have a hydrodynamic radius of 2.7 ± 0.1 nm. The micelles of Me$_2$PE-C32SS-Me$_2$PE have a slightly larger hydrodynamic radius of r = 3.1 ± 0.1 nm at 35 °C (see Figure S1, supplementary data). The slightly higher hydrodynamic radius for the Me$_2$PE-C32SS-Me$_2$PE micelles might be due to a more rod-like shape caused by the interactions between the headgroups. However, overall the micellar size is not much different from the size obtained for the micelles of the analogues without sulfur.

SANS. In addition, SANS measurements were carried out to identify the aggregate structure and to determine the structural parameters for both bolalipids at temperatures below and above the fiber-micelle transition. In Figure 4 the scattering data of PC-C32SS-PC and Me$_2$PE-C32SS-Me$_2$PE suspensions are shown. The data were analyzed by the Indirect Fourier Transformation (IFT) method, which requires only limited input information about the geometry of the aggregates.[22] A detailed description of the IFT fit procedure was given previously.[11, 12] For the low temperature measurements we used the approximation of stiff infinite long cylinders and for the high temperature measurements the approximation of a spherical-like object.
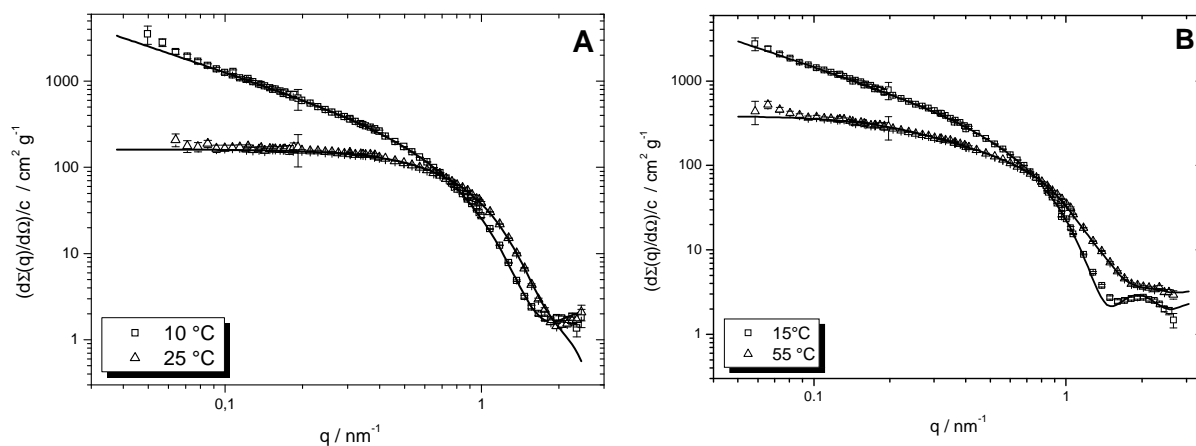

Figure 4: SANS data of suspensions of (A) PC-C32SS-PC in D$_2$O and (B) Me$_2$PE-C32SS-Me$_2$PE in 10 mM deuterated acetate buffer at pH 5 with c = 1 mg ml^{-1} (scattering data) and IFT fits (solid lines) at different temperatures.

The results of the fits of the scattering data are provided in Table 1. The radius of the fibers agrees with the one determined by cryo-TEM. The higher aggregation number per nanometer (N$_{agg}$) of the Me$_2$PE analogue compared to the PC counterpart is due to the smaller size of the headgroup leading to a slightly denser arrangement of the molecules within the fiber aggregates. The size of the micelles of PC-C32SS-PC is in very good agreement with the results of the DLS measurements at 25 °C (see Figure S1, supplementary data). In the case of

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the Me$_2$PE-C32SS-Me$_2$PE suspension measured at 55 °C the analysis of the fit becomes difficult as the pair distance distribution function p(r) shows two maxima around 3 nm and 10 nm indicating the presence of a multicomponent system of short fiber segments and micelles (see Figure S2, supplementary data).

Table 1: Results of the IFT fits of the SANS data for suspensions of PC-C32SS-PC in D$_2$O and Me$_2$PE-C32SS-Me$_2$PE in deuterated acetate buffer at pH 5 at different temperatures.[a]

	T [°C]	aggregate shape	D$_{max}$ [nm]	M$_L$ [g cm^{-1}] M [g]	N$_{agg}$ [nm^{-1}] N$_{agg}$ per micelle	R$_{CS,g}$ or R$_g$ [nm]	R$_{CS}$ or R [nm]
PC-C32SS-PC	10	fibers	6.0	1.59·10^{-13}	11 ± 1	1.80 ± 0.01	2.55 ± 0.01
	25	micelles	6.0	6.06·10^{-20}	43 ± 3	2.04 ± 0.01	2.63 ± 0.01
Me$_2$PE-C32SS-Me$_2$PE	15	fibers	5.5	1.84·10^{-13}	14 ± 1	1.84 ± 0.01	2.60 ± 0.01
	55	fiber segments and micelles	20.0	-	-	5.2 ± 0.2 [b]	≈ 3 and ≈ 10 nm

[a] D$_{max}$: maximal size or cross-section of aggregate, M: mass, M$_L$: mass per unit length, N$_{agg}$: aggregation number, R$_g$: radius of gyration, R$_{CS,g}$: radius of gyration of cross-section, R: radius, R$_{CS}$: radius of cross-section.

[b] average value

In comparison with the scattering data of PC-C32-PC at 25 °C and Me$_2$PE-C32-Me$_2$PE at 20 °C[11, 12] the fiber radii are slightly higher for the PC-C32SS-PC fibers and the Me$_2$PE-C32SS-Me$_2$PE fibers. The additional stabilizing hydrogen bonds between the headgroups of the Me$_2$PE-C32SS-Me$_2$PE molecules seem to be able to partially compensate the disorder induced by the sulfur atoms in the spacer chain. This leads to an almost identical fiber structure to the one observed for Me$_2$PE-C32-Me$_2$PE despite the reduced temperature stability. In the case of PC-C32SS-PC the larger radius of the fiber structure might be caused by irregularities in the ordering of the molecules inside the fibers that cannot be compensated by stabilizing H- bond interactions between the zwitterionic headgroups.

The number of bolaamphiphile molecules in the micelles (N$_{agg}$) is 43 ± 3 for PC-C32SS-PC micelles at 25 °C. For PC-C32-PC suspensions at 60 °C, which is below the micelle I – micelle II transition, the corresponding value of N$_{agg}$ is 77 ± 5 (micelles I) showing again the difference in the two micellar aggregates.[11] There are no data available on the aggregation

number of PC-C32-PC micelles II above the micelle-micelle transition, because of the high temperature needed to reach this state and the difficulties of obtaining reliable SANS scattering at this high temperature. For analogue bolalipids with shorter alkyl chain lengths the transitions are shifted to lower temperature so that a value for N_{agg} for the high temperature micelles (micelles II) could be obtained.[12] For PC-C26-PC micelles the aggregation number per micelle is 44 ± 3 at 35 °C (micelles I) and 28 ± 3 at 75 °C (micelles II). This suggests that the micelles in the PC-C32SS-PC suspensions have, even at low temperature, more similarities to the high temperature micellar aggregates (micelles II) with lower aggregation numbers. This is also indicated by the higher degree of disorder within the spacer chain of the bolalipids as indicated by the FT-IR measurements and the high transition enthalpy of the fiber-micelle transition found for PC-C32SS-PC or Me$_2$PE-C32SS-Me$_2$PE compared to PC-C32-PC and Me$_2$PE-C32-Me$_2$PE.[9, 10]

Rheology. In contrast to suspensions of PC-C32-PC and Me$_2$PE-C32-Me$_2$PE, the formation of fibers observed for sulfur containing molecules does not lead to the formation of a hydrogel when the concentration is only 1 mg ml^{-1}. However, when the concentration is raised to 4 mg ml^{-1}, gelation is observed in both systems at temperatures below the fiber-micelle transition temperature observed by DSC. The temperature dependence of the rheological properties of the bolalipid suspensions of PC-C32SS-PC and Me$_2$PE-C32SS-Me$_2$PE inside the linear viscoelastic (LVE) region is shown in Figure 5. The limits of the LVE region were determined using an amplitude- and a frequency-sweep (see Figures S3 and S4, supplementary data).
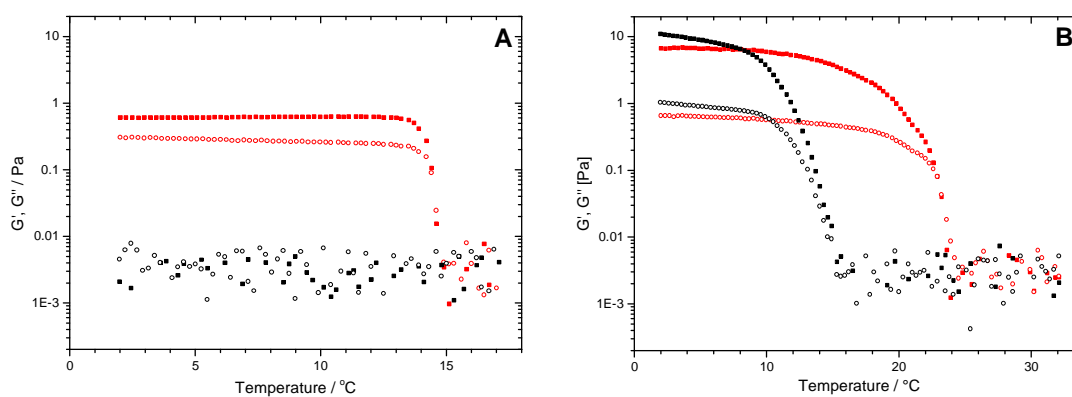

Figure 5: Temperature dependent rheological data of aqueous suspensions (c = 4 mg ml^{-1}) of (A) PC-C32SS-PC in water and (B) Me$_2$PE-C32SS-Me$_2$PE in acetate buffer at pH 5 with ω = 1 rad s^{-1} and γ = 1%. G': filled squares, G'': open circles. Heating: red and cooling: black.

Even though the concentration (c = 4 mg ml^{-1}) is four times higher, the PC-C32SS-PC suspension shows smaller storage and loss moduli than the related PC-C32-PC suspension at a concentration of 1 mg ml^{-1}.[23] Both moduli are independent of temperature with G' being larger than G'' up to the DSC transition at 14 °C (Figure 5A). The loss factor tan δ is 0.51 indicating a high degree of viscosity in the viscoelastic gel and little elastic behavior. Above the transition G' and G'' have about the same value and show the break-down of the viscoelastic gel into a viscous fluid. However, upon cooling of the sample no immediate reformation of the gel structure can be monitored by rheometry.

The rheological data for the Me$_2$PE-C32SS-Me$_2$PE suspension exhibit a similar trend with gradual decreasing G' and G'' prior to the DSC transition at 25 °C (Figure 5B). However, G' and G'' are significantly higher compared to the PC-C32SS-PC sample. Also, the loss factor tan δ is only 0.10 and implies a higher elasticity in the gel structure than for the PC analogue. During the cooling scan G' and G'' are starting to increase at 13 °C, indicating that for Me$_2$PE-C32SS-Me$_2$PE the reaggregation of the micelles into fibers and the reestablishment of the cross-links between the fibers starts already during the cooling process in the rheometer. This correlates well with the peak observed in the DSC cooling scan that shows a hysteresis of approximately 10 °C for the fiber reformation (data not shown). At 2 °C similar G'' values are reached as initially observed.

By comparing the behavior of both bolaamphiphiles during a 12 hour period at constant temperature (2 °C) after the cooling cycle, the different properties of the gels become more apparent (see Figure S5, supplementary data). The moduli for the Me$_2$PE-C32SS-Me$_2$PE sample, where fibers have already been reformed, show merely further a small increase and the loss factor reaches again a value of 0.10. In the case of the PC-C32SS-PC sample the transformation from the micellar solution to the hydrogel can only be observed after a waiting time of at least 5 hours at 2 °C. The higher loss factor (tan δ = 0.64) reveals the very weak viscoelasticity of the gel network and the initial values of G' and G'' are not reached even after 12 hours of incubation.

This shows that the disordering influence of the two sulfur atoms in the chain is more pronounced for the bolaamphiphile PC-C32SS-PC resulting in a gel structure that is weaker by a factor of 10 compared to Me$_2$PE-C32SS-Me$_2$PE. The Me$_2$PE headgroups increase the stability of the fiber structure via additional hydrogen bonds and therefore minimize the effect of the disorder induced by the sulfur containing chains. The higher gel strength of the suspensions of PC-C32-PC and Me$_2$PE-C32-Me$_2$PE, the analogues without sulfur, indicates that the three-dimensional network of the fibers formed by the sulfur containing bolalipids is

not as strong.[23, 24] The cross-linking of the fibers via hydrophobic interactions between parts of the chain structure of different fiber strands that are exposed at the fiber surface is weakened due to the disorder induced by the thioether moieties.

Summary and Conclusions

The modification of the bolalipid spacer chains of PC-C32-PC and Me₂PE-C32-Me₂PE by exchange of two methylene groups at the positions C12 and C21 for sulfur atoms altered the aggregation behavior in aqueous suspensions. The order of the spacer chains is disturbed by the heteroatoms caused by a decreased bond angle for the C-S-C bond compared to the C-C-C bond, but the aggregation into fibers is still possible. However, the stability range of the fibers is reduced and the temperature where a break-down of the fibers into micellar aggregates is observed is shifted to lower temperature. In the case of Me₂PE-C32SS-Me₂PE, no transition between two different types of fiber aggregates can be observed as seen for the analogue bolalipid without sulfur.[10] The properties of the micelles formed by the sulfur containing bolaamphiphiles in suspensions at temperatures above the fiber-micelle transition resemble the highly unordered micellar aggregates (micelles II) formed by the analogue bolaamphiphiles without sulfur at high temperature. Gelation of the aqueous suspensions when fiber aggregates are present can only be observed at a concentration significantly higher than the concentration necessary for the gelation of bolalipid suspensions of molecules without sulfur containing chains. The viscoelasticity of the PC-C32SS-PC suspension is significantly decreased compared to the gels formed by PC-C32-PC. In the case of Me₂PE-C32SS-Me₂PE this effect is less pronounced, which is due to the possibility of forming stabilizing hydrogen bonds between the headgroups. The results of this study show that the aggregation of symmetrical bolalipids into fiber structures and the cross-linking of the fibers leading to hydrogels are critically dependent on steric effects. Not only the ratio of the cross-sectional area of the headgroup in relation to the chain is important, but also slight perturbations of the all-*trans* conformation due to the incorporation of sulfur atoms have a large influence.

Supplementary data

Synthesis of Me_2PE-$C32SS$-Me_2PE, DLS and SANS data, rheological measurements.

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Bolalipid fiber aggregation can be modulated by the introduction of sulfur atoms into the spacer chains

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Synthesis of Me$_2$PE-C32SS-Me$_2$PE

The synthesis of Me$_2$PE-C32SS-Me$_2$PE was carried out applying the general phosphorylation and quarternisation procedure of long-chain 1,ω-diols as described previously [S1] using 0.5 mmol of the sulfur-modified diol (0.26 g).[S2]

12,21-Dithiadotriacontane-1,32-diyl-bis[2-(dimethylammonio)ethylphosphate].

Yield: 0.32 g (77%); R_f = 0.47 (CHCl$_3$/MeOH/NH$_3$ = 13/7/1); ^{1}H-NMR (400 MHz, CDCl$_3$/CD$_3$OD, 27 °C): δ = 1.25–1.39 (m, 36 H, 2× −O(CH$_2$)$_2$(CH$_2$)$_7$(CH$_2$)$_2$S(CH$_2$)$_2$(CH$_2$)$_2$−), 1.52–1.63 (m, 12 H, 2× −OCH$_2$CH$_2$(CH$_2$)$_7$CH$_2$CH$_2$SCH$_2$CH$_2$−), 2.48 (t, ^3J(H,H) = 7.5 Hz, 8 H, 2× −CH$_2$SCH$_2$−), 2.85 (s, 12 H, 4× −CH$_3$), 3.15–3.18 (m, 4 H, 2× NCH$_2$CH$_2$O−), 3.88 („quar", J = 6.6 Hz, 4 H, 2× −OCH$_2$(CH$_2$)$_{10}$S−), 4.23–4.28 ppm (m, 4 H, 2× NCH$_2$CH$_2$O−); ^{13}C-NMR (100 MHz, CDCl$_3$/CD$_3$OD, 27 °C): δ = 25.66 (−O(CH$_2$)$_2$CH$_2$(CH$_2$)$_8$S−), 28.68, 28.83, 28.93, 29.13, 29.22, 29.41, 29.46, 29.57, and 29.65 (−O(CH$_2$)$_3$(CH$_2$)$_7$CH$_2$SCH$_2$(CH$_2$)$_3$−), 30.62 (d, ^3J(C,P) = 7.4 Hz, −OCH$_2$CH$_2$(CH$_2$)$_9$S−), 32.08 and 32.11 (−CH$_2$SCH$_2$−), 43.44 (−CH$_3$), 58.84–59.20 (NCH$_2$CH$_2$O−), 66.13 ppm (d, ^2J(C,P) = 5.9 Hz, −OCH$_2$(CH$_2$)$_{10}$S−); MS (ESI): m/z: 820.0 [M − H]$^-$, 822.2 [M + H]$^+$, 844.1 [M + Na]$^+$; elemental analysis calcd. (%) for C$_{38}$H$_{82}$N$_2$O$_8$P$_2$S$_2$ × H$_2$O: C 54.39, H 10.09, N 3.34, S 7.64; found: 54.33, H 9.77, N 3.38, S 7.14.

Aggregation behavior of PC-C32SS-PC and Me$_2$PE-C32SS-Me$_2$PE in aqueous suspension
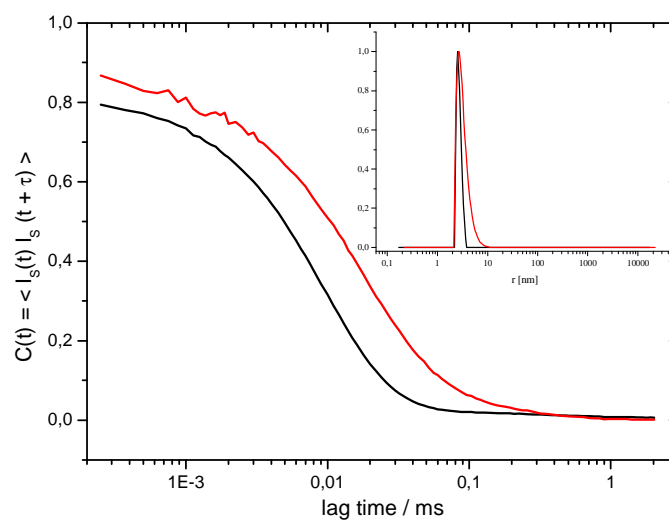
DLS.

Figure S1: DLS autocorrelation functions for aqueous suspensions (c = 1 mg ml^{-1}) of PC-C32SS-PC (black) and of Me$_2$PE-C32SS-Me$_2$PE in acetate buffer at pH 5 (red) at 25 and 35 °C, respectively, i.e. at temperatures where micelles exist. The inset shows the number-weighted size distribution.
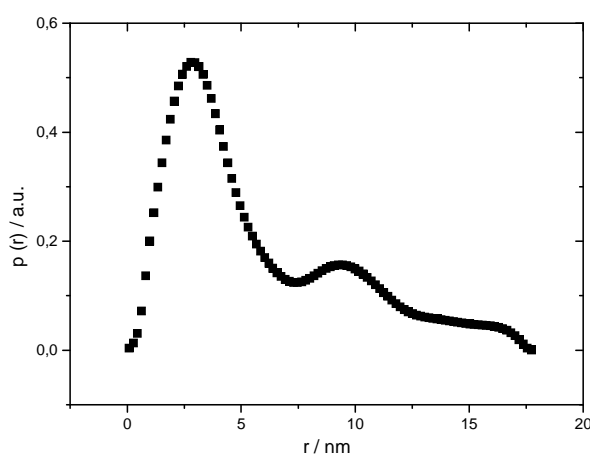
SANS.

Figure S2: Pair distance distribution function p(r) for a Me$_2$PE-C32SS-Me$_2$PE suspension in deuterated acetate buffer at pH 5 (c = 1 mg ml^{-1}) at 55 °C.

Rheology. Amplitude sweeps were recorded following the 12 hour period of incubation of the sample at 2 °C and the results are provided in Figure S3. In this experiment, the reformation of the gel was not fully completed prior to starting the amplitude sweep. However, the general behavior of the hydrogel is not affected.
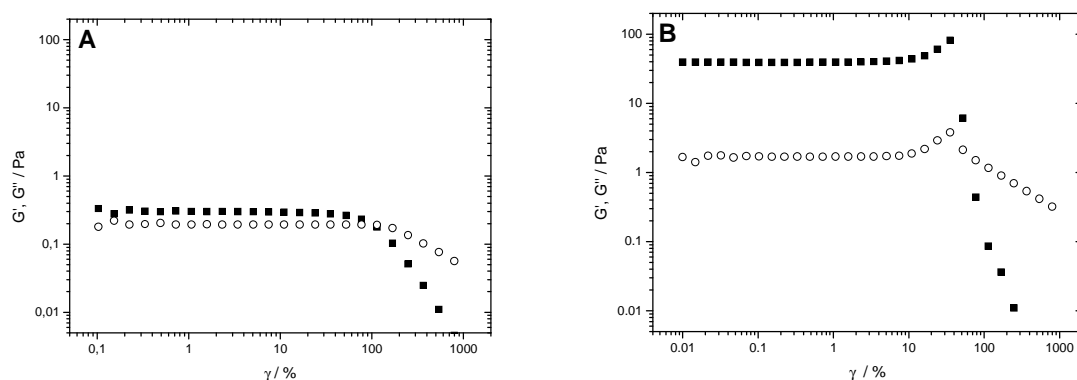

Figure S3: Amplitude sweeps of aqueous suspensions of (A) PC-C32SS-PC and (B) Me$_2$PE-C32SS-Me$_2$PE (buffer at pH 5) with c = 4 mg ml^{-1} at 2 °C with ω = 1 rad s^{-1}. G': filled squares, G'': open circles.

Despite the low G' and G'' values of the PC-C32SS-PC suspension, the straining limit is above 100 %. This is also the case for the Me$_2$PE-C32SS-Me$_2$PE suspension. This value is similar to the ones determined for the bolalipid suspensions of PC-C32-PC and Me$_2$PE-C32-Me$_2$PE.[S3] A significant difference in the amplitude sweeps can be noted prior to the crossover of storage and loss modulus indicating the transition from hydrogel to micellar solution. The Me$_2$PE-C32SS-Me$_2$PE suspension exhibits strain stiffening whereas the PC-C32SS-PC suspension shows a continual decrease of G' and G''. The absence of this increase of the moduli in the case of the PC analogue might be due to the low values of G' and G'' as well as the high loss factor at the beginning of the amplitude sweep. However, this strain stiffening effect was observed in numerous measurements with different bolaamphiphiles (e.g. Me$_2$PE-C32-Me$_2$PE[S3]) and the level of strain stiffening generally seems to be a good indication of strength and viscoelasticity in the specific sample. Frequency sweeps were recorded with a deformation of γ = 1% (Figure S4).
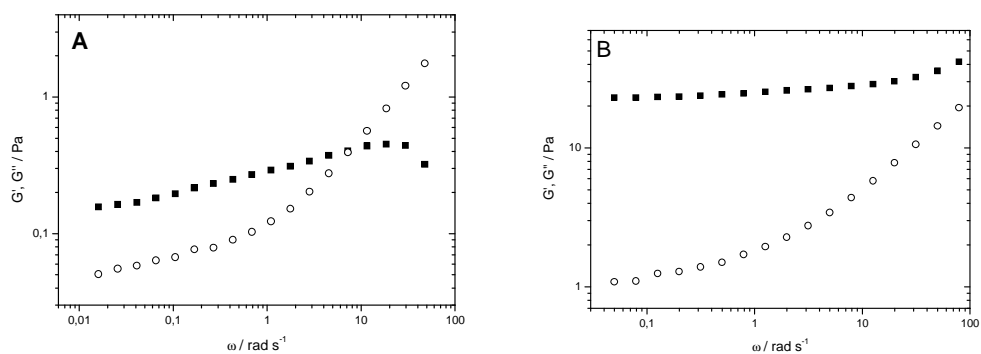
Figure S4: Frequency sweep of a (A) PC-C32SS-PC suspension in water and a (B) Me_2PE-C32SS-Me_2PE suspension in buffer at pH 5 at 2 °C (c = 4 mg ml^{-1}) with γ = 1%.
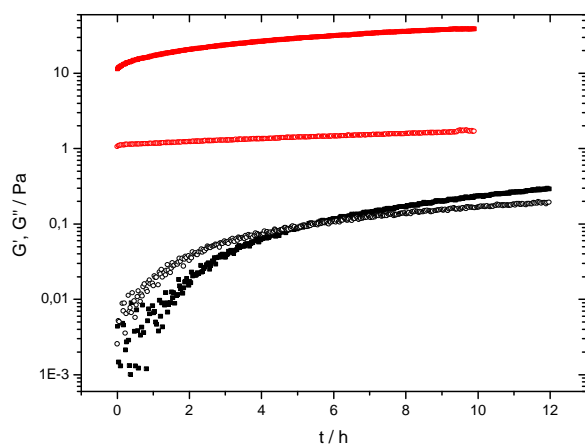

Figure S5: Time dependent rheological data at 2 °C of aqueous suspensions of PC-C32SS-PC (black) and Me_2PE-C32SS-Me_2PE in acetate buffer at pH 5 (red) with c = 4 mg ml^{-1}. The sample was heated to temperatures above the transition temperature. and After cooling to 2 °C the time dependent measurements were started with ω = 1 rad s^{-1} and γ = 1%. G': filled squares, G'': open circles.

References for Supplementary data

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