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Structure and Dynamics of Highly PEG-ylated Sterically Stabilized Micelles in Aqueous Media

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Abstract

Molecular assemblies of highly PEG-ylated phospholipids are important in many biomedical applications. We study sterically stabilized micelles (SSM) of self-assembled DSPE-PEG₂₀₀₀ in pure water and isotonic HEPES buffered saline solution. The observed SSM sizes of 2 – 15 nm largely depend on the solvent and the lipid concentration used. The critical micelle concentration (CMC) of DSPE-PEG₂₀₀₀ is ≈ 10 times higher in water than in buffer and the viscosity of the dispersion dramatically increases with the lipid concentration. To explain the experimentally observed results, we perform atomistic molecular dynamics simulations of the solvated SSM. Our modeling reveal that the observed assemblies have very different aggregation numbers of $N_{agg} \approx 90$ (saline solution) and $N_{agg} < 8$ (water), due to very different screening of their charged $-PO_4^-$ groups. We also demonstrate that the micelle cores can inflate and their corona highly fluctuate, allowing thus storage and delivery of molecules with different chemistry.

1. Introduction

Lipids, surfactants, and amphiphilic block copolymers in aqueous media can assemble into numerous stable structures, such as micelles [1–4], disks [5], vesicles [6–8], and bilayers [9–11]. Poly(ethylene glycol) (PEG) lipid conjugates, assembled alone or with other lipids and cargo molecules, have broad applicability in novel biomedical formulations, due to favorable properties, such as low toxicity, biocompatibility, and ease of excretion [12]. For example, 1,2-distearoyl-sn-glycero-3-phosphatidylethanolamine-N-[Methoxy(polyethylene glycol) 2000] (DSPE-PEG₂₀₀₀) is a PEG-ylated phospholipid widely used in the preparation of various formulations, where the PEG layer often acts as a steric barrier stabilizing the molecular assemblies against uptake by the mononuclear phagocytic system (MPS) [13]. These formulations include micelles [14], large unilamellar vesicles [15, 16], lipid-protected particles, and immunoliposomes for gene delivery [17–19], coated carbon nanotubes for near-infrared imaging [20], biologically functional surfaces [21], and ultrasound contrast

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Supporting Information.

Complete reference 41, materials, experimental methods, computational methods. This material is available free of charge via the Internet at <http://pubs.acs.org>.

agents [22, 23]. Many DSPE-PEG₂₀₀₀-based nanostructures play an important role in the development of pharmaceutical drug delivery systems [13, 24–26]. For example, DSPE-PEG₂₀₀₀ is used as a component of the U.S. FDA approved pharmaceutical product Doxil.

Previously, we have studied sterically stabilized micelles (SSM) of self-assembled DSPE-PEG₂₀₀₀ as biocompatible and relatively nontoxic drug delivery nanocarriers [27]. SSM have a hydrophobic core, ionic interface, and a semi-polar palisade PEG layer that can all serve as platforms for association of hydrophobic and amphiphilic drugs and peptides [28, 29]. We have experimentally characterized simple and mixed SSM with respect to their shape, core polarity, and aggregation number, N_{agg} , in HEPES buffered saline (pH 7.4) [30, 31]. Understanding the structure and the dynamics of the self-assembled DSPE-PEG₂₀₀₀ monomers in different aqueous media is crucial for the optimization of SSM systems for drug delivery and other potential applications. Micellar aggregates are known to have different equilibrium sizes, CMC, and aggregation numbers [32] in solutions of different ionic strengths and monomer concentrations [33]. These parameters play a crucial role for the applicability of the PEG-ylated lipid micelles.

In this work, we experimentally characterize SSM in terms of their size, solution viscosity, and CMC, both in pure water and in isotonic salt concentration, represented by HEPES buffered saline. To understand the experimental results in molecular detail, we carry out large-scale atomistic molecular dynamics (MD) simulations of the DSPE-PEG₂₀₀₀ assemblies in water and saline solutions. We examine how the solvent type and lipid concentration affect the equilibrium structure and dynamics (fluctuations) of SSM, and physical properties of their solutions. Although various micelles have been modeled in the past [34–39], to the best of our knowledge, this is the first study that models highly PEG-ylated micelles using atomistic MD simulations and reveals their characteristics in atomistic details.

2. Materials and Methods

2.1. Sample Preparation

We have dissolved DSPE-PEG₂₀₀₀ in either pure water or 10 mM HEPES buffered saline (pH = 7.4) with the monomer concentration of $c = 5 - 40$ mM. All samples were vortexed, sonicated, and flushed with argon, and then equilibrated for 4 hours in the dark at room temperature ($T = 25^\circ\text{C}$).

2.2. Characterization of Experimental SSM Solutions

Aqueous solutions of SSM were analyzed for the mean particle diameter, solution viscosity, and CMC. Particle sizes in the SSM dispersions were measured by a dynamic light scattering (DLS) particle sizer (Agilent 7030 NICOMP DLS/ZLS), equipped with a 100 mW helium-neon laser at 632.8 nm and set up at the 90° angle, as well as by a Brookhaven instrument (with 3 major component: a BI-200SM Goniometer, BI-9000AT Digital Correlator from Brookhaven Instruments, and a Lexel 95 Argon Ion Laser from Lexel Inc.) equipped with a 3 W ion-argon laser at 514.5 nm, and set up at the 30° , 90° and 120° angles. Viscosities of SSM dispersions were measured using Brookfield DV-II+ Programmable Viscometer (Cone/Plate) with Spindle (cone) CPE-42 at the chamber temperature of $T = 25^\circ\text{C}$. The CMC of SSM in pure water was determined using 1,6-diphenyl-1,3,5-hexatriene [14]. Further experimental details and the sources of all the used chemicals are available in the Supporting Information.

2.3. Molecular Dynamics Simulations

We have performed atomistic MD simulations of micelles composed of DSPE-PEG₂₀₀₀ monomers with sodium counterions using the NAMD package [40] and the CHARMM27 force field (C35r revision for ethers) [41, 42]. Micelles with different aggregation numbers, $N_{agg} = 8 - 90$, were modeled in pure (TIP3P) water and in 0.166 M NaCl solutions. The ionic strength of the NaCl solution was matched to the ionic strength of the buffer solution used in experiments. The systems were equilibrated in NPT ensemble, at $P = 1$ bar and $T = 300$ K. Further simulation details are provided in the Supporting Information.

3. Results and Discussion

3.1. Experimentally Observed Micelle Sizes

In Fig. 1, we show the observed intensity weighted size distributions and average hydrodynamic diameters of the experimentally prepared DSPE-PEG₂₀₀₀ assemblies equilibrated in water (a–e) and ionic solution (f–j), as described above. As the monomer concentration increases from $c = 5$ mM to 40 mM, the hydrodynamic diameters of SSM in water observed by the NICOMP instrument at the 90° angle slowly increase from $d_h \approx 4.1$ nm to 5.6 nm, while the diameters observed by the Brookhaven instrument at the 90° angle increase from $d_h \approx 2.2$ nm to 4.0 nm. The differences in the results obtained by the two instruments at low concentrations ($c = 5 - 20$ mM) may be due to the DLS measurements being less reliable when the particles are smaller than 5 nm in diameter (instrument documentations). At these extremely small sizes, the Brookhaven instrument might average over the peaks of the 4–5 nm micelles and the 1–2 nm monomers (and impurities), seen in the NICOMP data. Nevertheless, we expect that the trend (observed by both instruments) of the slowly increasing SSM sizes in water with the increasing monomer concentrations to hold. This size dependence may be caused by the fact that the effective ionic strength of the solutions (micelle screening) increases with the monomer concentration, as the monomer counterions become crowded in the limited space between the micelles (see inset in Fig. 4).

In ionic solutions, the observed micelles always have narrow size distributions, whose peaks shift from the diameter of $d_h \approx 15$ nm at $c = 5$ mM to $d_h = 8$ nm at $c = 40$ mM (Fig. 1 (f–j)). Previously, Johnsson, *et al.* [43] have studied DSPE-PEG₂₀₀₀ aggregates in 0.15 M NaCl aqueous solutions at lipid concentrations of $c = 0.4 - 7$ mM. The aggregation numbers and hydrodynamic radii of their DSPE-PEG₂₀₀₀ micelles in this media are similar to our observations in HEPES buffered saline at monomer concentration of $c = 5$ mM [14]. This indicates that the presence of ions is of large importance for the micelle sizes and aggregation numbers.

In Fig. 1, we also show the average hydrodynamic diameters for SSM in all the studied solutions, obtained by the NICOMP instrument (N) at the 90° angle and the Brookhaven instrument (B) at the 30°, 90° and 120° angles. The average diameters obtained by the Brookhaven instrument at different angles are similar, since the angular dependence of (632.8 nm) light scattering on particles with $d_h < 25$ nm is very small (documentation).

3.2. Simulations of DSPE-PEG₂₀₀₀ Monomer Self-assembly in Water

In order to understand the observed behavior of DSPE-PEG₂₀₀₀ in water, we first simulate the SSM formation from hydrated DSPE-PEG₂₀₀₀ monomers. Initially, randomized monomers with the concentration of $c = 40$ mM are hydrated at $T = 300$ K, as shown in Fig. 2 (left). From this configuration, the system develops within 30 ns into an assembly of small micelles, with $N_{agg} < 11$, as shown in Fig. 2 (right). At $c = 40$ mM, neighboring SSM often come in contact through their extended PEG coronas. Although the system is not yet

equilibrated at 30 ns, these results showing the stabilization of small micelles support the data observed in Fig. 1 (left).

3.3. Molecular Dynamics Simulations of Equilibrated Micelles

In order to better understand the experimentally observed results, we simulate individual equilibrated molecular aggregates formed by a different number of DSPE-PEG₂₀₀₀ monomers in water and ionic solution. In Fig. 3 (a–e), we show micelles formed by 8, 10, 15, 20, and 50 DSPE-PEG₂₀₀₀ monomers equilibrated in water for 5 – 16 ns. In our analysis below, we focus on the 10-monomer SSM in water, whose size is in a rough agreement with the SSM sizes observed in water by both DLS instruments at the monomer concentrations of $c > 30$ mM. For comparison, we show in Fig. 3 (f) a 90-monomer micelle, equilibrated in the 0.166 M NaCl solution for 10 ns. The 90-monomer estimate used in the preparation of this micelle is based on our SANS measurements of SSM in HEPES buffered saline at monomer concentration of $c = 5$ mM [14, 30].

3.3.1 Structure and Dynamics of the Micelle Core—In all the equilibrated micelles, we can recognize three unique regions: core, ionic interface, and PEG corona. The micelle cores in Fig. 3 (a–f) are formed by aggregated alkane blocks, shown in Fig. 3 (g) (same order). At smaller aggregation numbers, the micelles reorganize in sub-nanosecond timescales, and their cores gain a spherical shape. Larger micelles ($N_{agg} > 20$) reorganize in several nanoseconds and their cores become more ellipsoidal. This is particularly clear in the case of the 90-monomer micelle formed in ionic solution, whose core has an oblate shape (with the aspect ratio of ≈ 1.7), in agreement with our previous measurements of SSM with the aggregation number of $N_{agg} \approx 93$, obtained by small-angle neutron scattering using a low-Q diffractometer [30].

It is of large importance to understand the dynamical reorganization of the SSM core. In Fig. 3 (h), we show two snapshots of the 90-monomer SSM core, as it relaxes from the initial spherical shape with the vacancy in the center (hollow) to the oblate shape (splashed). The hollow core might carry drugs and other molecular or nanoparticle cargo, as observed in experiments [28, 31]. The filled SSM with more spherical core might become more stable.

3.3.2 Structure and Dynamics of the Ionic Interface—The cores of all the SSM are covered by the charged phosphate groups ($-\text{PO}_4^-$) from the DSPE-PEG₂₀₀₀ monomers, which are screened by the Na^+ counterions freely present in our (neutral) systems. Screening of the ionic interface is of large importance for the micelle stabilization, as discussed in detail later. In Fig. 4, we show the molarity of the ionic species present in the 90-monomer SSM in 0.166 M NaCl solution and the 10-monomer SSM in water (inset) as a function of the radial distance from the micelle center. In the 10-monomer SSM, the distribution of the $-\text{PO}_4^-$ groups has a single peak localized at $r \approx 1.7 - 1.8$ nm (spherical shape of the core). In the 90-monomer SSM, the core has an oblate shape, which results in two peaks of the $-\text{PO}_4^-$ groups, localized at $r \approx 2.2$ and $r \approx 3.1$ nm. In both cases, the $-\text{PO}_4^-$ groups are largely screened by the Na^+ counterions in the Stern layer, located in the region of the $-\text{PO}_4^-$ groups. The following diffuse ionic atmosphere [32] maintains the neutrality of the whole molecular complex. In Fig. 4, we can see that in water the neutrality is achieved within $\approx 4 - 6$ nm from the $-\text{PO}_4^-$ groups, while in the ionic solution, it is achieved within $\approx 2 - 3$ nm, due to the short screening length of $\lambda \approx 0.75$ nm. The Na^+ concentration approaches the bulk value of either zero (water) or 0.166 M, where in the latter case the Cl^- concentration also increases to the same bulk value. The Stern and diffuse ion layers complete the charge double layer region, permeating the PEG corona.

3.3.3 Structure and Dynamics of the Micelle Corona—The local density and conformations of PEG chains forming the SSM corona also depend on the aggregation number, N_{agg} . As seen in Fig. 3 (a–f), the PEG chains in all the studied SSM can transiently form clumps or remain isolated. We analyze the chain dynamics in more detail for the 10-monomer SSM in water and for the 90-monomer SSM in 0.166 M NaCl solution, shown in Fig. 3 (b) and (f), respectively.

In Fig. 5, we show equilibrium fluctuations of the local PEG thickness in the micellar PEG corona, d_{PEG} , in dependence of the inclination angle, θ and the azimuthal angle ϕ (spherical coordinates with the origin at the SSM center of mass). In Fig. 5 (a–d), we observe that in the 10-monomer SSM a large fraction ($\approx 30\%$) of the core is always fully exposed to water. We can see at the four snapshots taken at 4, 6, 10 and 16 ns that most of the PEG chains fluctuate but remain folded at the core, while one or two chains can occasionally protrude away from the core ($d_{PEG} > 5$ nm).

In Fig. 5 (e–h), we show the same in the 90-monomer SSM in 0.166 M NaCl during a 3 ns trajectory. The large SSM has a more homogeneous PEG corona, which occasionally exposes the core to the aqueous solution. Here, only $< 10\%$ of the hydrophobic core is always exposed to water. The corona fluctuations create pockets of different chemistry which might be able to carry molecular cargo, such as drugs and peptides.

In Fig. 6, we show the average density distributions, $\rho(r)$, for the hydrophobic core groups, the PEG corona groups, and water for (top) the 10-monomer SSM in water and (bottom) the 90-monomer SSM in 0.166 M NaCl solution. The densities are calculated from

$$\rho(r) = \frac{1}{N_t} \sum_{t=1}^{N_t} \sum_{i=1}^{N_a} \frac{m_i}{V}, \quad (1)$$

where m_i is the mass of each of the N_a atoms found in the bin of the volume V (each bin is a spherical shell of the thickness of $\Delta r = 1$ Å centered at the SSM center). The averaging is performed over $N_t = 2,000$ frames during 4 ns simulations for the core and PEG, and over $N_t = 5$ –10 frames during 0.1 – 0.2 ns of simulations for water.

In the 10-monomer SSM, shown in Fig. 6 (top), the hydrophobic core has a relatively sharp boundary at $r \approx 1.7 - 1.8$ nm. It is followed by the $-\text{PO}_4^-$ groups and a narrow PEG layer of the thickness of $\Delta_{PEG} \approx 0.5$ nm. The individual PEG chains are highly coiled and they have “mushroom-like” conformations, observed on flat surfaces (membranes) at low PEG densities [44]. Water also fills the space between the PEG chains, as seen from its distribution.

The 90-monomer SSM, shown in Fig. 6 (bottom), has a rather different structure of its layers, due to the oblate core terminated at $r \approx 2.5$ –4 nm. The onset of the PEG layer is broader than in the 10-monomer SSM, due to the core ellipticity. The congested PEG chains have more “brush-like” conformations, as seen on flat surfaces in the limit of high surface coverage [44]. The average thickness of the PEG corona, $\Delta_{PEG} \approx 3.8$ nm, is in good agreement with the value of $\Delta_{PEG} \approx 3.5$ nm [43], found in DSPE-PEG₂₀₀₀ micelles in 0.15 M NaCl solution. Water again fills the space between the PEG chains.

3.4. Comparison of the Experimental and Theoretical Micelle Sizes

In Table I, we show the effective diameters of the equilibrated micelles and their cores, d_{tot} and d_c , respectively. These values are obtained by angular averaging (2 – 5 ns) of the

maximum radial extensions, r_{max} , of the PEG chains and core groups with respect to the SSM center of mass,

$$d_{tot/c} = \frac{2}{N_t N_\theta N_\phi} \sum_{t=1}^{N_t} \sum_{\theta=1}^{N_\theta} \sum_{\phi=1}^{N_\phi} r_{max}(\theta, \phi, t). \quad (2)$$

Here, the averaging is performed on the spherical grid with $N_\theta = 10$ bins for the inclination angle, θ , and $N_\phi = 20$ bins for the azimuthal angle ϕ , over the selected number of frames $N_t = 1,000 - 2,500$ for 2 – 5 ns trajectories.

Comparison of these theoretical effective sizes, d_{tot} , with the experimental hydrodynamic sizes, d_h , shown in Fig. 1, indicates that micelles observed in pure water with the diameters of $d_h \approx 4 - 5$ nm should contain $N_{agg} < 8$ monomers. We should keep in mind that the two definitions of micelle sizes are different, which can make this comparison unreliable, especially at small sizes of the aggregates with distantly protruding individual chains (see Fig. 3 a). The SSM with $N_{agg} = 8$ or less are also observed in our simulations of 40 mM DSPE-PEG₂₀₀₀ solution, shown in Fig. 2 (right). On the other hand, in the buffered solutions the experimental SSM sizes are always $d_h > 8$ nm, as seen in Fig. 1 (bottom), indicating $N_{agg} > 20$. At the DSPE-PEG₂₀₀₀ monomer concentration of $c = 5$ mM in buffer [14, 30], the experimentally observed SSM have the aggregation number of $N_{agg} \approx 90$ and the hydrodynamic diameter of $d_h \approx 15$ nm. This diameter is in close agreement with the value of $d_{tot} = 13.9$ nm obtained for the modeled 90-monomer SSM (Fig. 3 (f) and Table I).

In Table I (last two columns), we also present the calculated sizes of the 10- and 15-monomer micelles in the 0.166 M NaCl solution. This ionic strength matches the buffer solution in which stable micelles of $N_{agg} = 90$ are formed at the monomer concentration of $c = 5$ mM. The simulated small micelles (10–15 monomers) acquire very similar sizes in water and in the NaCl solution. This indicates that the experimental SSM sizes are determined by their aggregation numbers, N_{agg} , rather than different arrangement of the monomers.

3.5. Effect of the Ionic Concentration on the Micelle Sizes

When ionic lipid and surfactant micelles are assembled in ionic solutions, the overall large number of counterions provides better screening of the charged headgroups. This results in a reduced repulsion of the headgroups, eventually leading to stabilization of more monomers in each assembled structure [45, 46]. On the other hand, in micelles assembled from non-ionic (neutral) monoalkyl-PEGs the aggregation number increases only slightly when the salt concentration is increased (up to $c = 1.3$ M) [47].

The micelle morphology can be also influenced by the nature of the headgroups. Ionic (DSPE-PEG₂₀₀₀) and non-ionic PEG-ylated monomers (monoalkyl-PEGs, DS-PEG₂₀₀₀ and DSG-PEG₂₀₀₀) all assemble into globular micelles [47, 48]. But when the PEG-ylated polymers have headgroups with attractive interactions, the monomers can form aggregates of different morphologies. For example, lipids with one to four 16-carbon acyl chains connected by amide groups to PEG₂₀₀₀ tend to form fibrous structures in water [49], due to hydrogen bonding between amide groups.

The fact that the aggregation numbers, N_{agg} , in our SSM are larger in ionic solution than in pure water is likely related to the enhanced screening of the charged and aggregated $-\text{PO}_4^-$ headgroups by abundant free counterions from the ionic solutions [45, 46]. To clarify this possibility, we theoretically examine how 10-monomer PEG-ylated micelles in water and

0.166 M NaCl solution are screened by the ion distributions formed around the ionic $-\text{PO}_4^-$ groups.

In Fig. 7 (top), we show the average number of Na^+ and Cl^- ions, N , at the distance r from the phosphorus (P) atoms in DSPE-PEG₂₀₀₀ monomers. These data are obtained by integrating the P- Na^+ and P- Cl^- radial distribution functions (RDFs) using Visual Molecular Dynamics (VMD) [50]. In these calculations, we found that Na^+ counterions can assume metastable configurations in PEG pockets around the $-\text{PO}_4^-$ groups, as shown in the inset of Fig. 7 (top left). Since these configurations transiently occur only in some of the trajectories, we separately show the plots in Fig. 7 for the trapped (top left) and free (top right) Na^+ configurations. In both Na^+ configurations, the integrated RDFs always show more positive counterions surrounding the negative $-\text{PO}_4^-$ group in ionic solution than in pure water. Moreover, at short distances from phosphorus atoms ($r < 0.7$ nm), the average number of Na^+ ions is observed to be larger when the ions are trapped.

In Fig. 7 (bottom), we show the normalized RDFs, $g(r)$, of phosphorus atoms from different DSPE-PEG₂₀₀₀ monomers for the 10-monomer micelles equilibrated in water and ionic solution. In both cases, trajectories with trapped Na^+ conformations are used. The first peaks in $g(r)$, observed at $r < 1$ nm, are eight times smaller in water than in the ionic solution. In the inset of Fig. 7 (bottom), we also plot the average number of phosphorus atoms, N , within the distance r of another phosphorus atom, obtained by integration of the RDFs. These plots clearly show that the first neighbor phosphate groups are much closer to each other in the ionic solution than in water. Although neutralizing Na^+ counterions are present around SSM also in water, their screening ability decreases at their smaller concentrations.

The results in Fig. 7 confirm the hypothesis that the presence of electrolytes provides better stabilization of the PEG-ylated SSM by decreasing the Coulombic repulsion of the $-\text{PO}_4^-$ groups and allowing more monomers to be accommodated in the SSM. Our MD simulations also show that PEG can further stabilize the SSM by forming stable counterion configurations close to the $-\text{PO}_4^-$ groups. While in our relatively short simulations we only observe single Na^+ ions locked by PEG chains close to the $-\text{PO}_4^-$ groups (inset in Fig. 7 (left)), these Na^+ configurations may be more common in real systems.

3.6. Effect of the Ionic Concentration on the Micelle Morphology

The sizes and morphologies of self-assembled molecular aggregates are controlled by the monomer [51, 52] and solvent properties [3, 53, 54]. The morphology is determined by the packing of alkane blocks within the aggregate core, characterized by the packing parameter, $p = v/a_0l_c$. Here, v is the volume of the alkane block, a_0 is the effective area per headgroup at which the interaction energy per monomer is minimized, and l_c is the critical length of the alkane block [32]. If the effective size of the headgroup decreases, p grows and the aggregate becomes less spherical [32]. In the SSM formed by solvated DSPE-PEG₂₀₀₀ monomers, a_0 decreases with better screening of the $-\text{PO}_4^-$ groups and the SSM morphology changes from spherical (pure water - small p) to oblate (ionic solution - larger p). Notice that as p increases, the SSM grows and the micelle core develops a cavity (see Fig. 3 (h)) that can be potentially filled by other molecules. The SSM morphology thus also depends on the filling of its potentially hollow core.

When the solution is varied in a more significant manner, the aggregates can undergo dramatic changes. For example, adding acids or salts to aqueous solutions of asymmetric polystyrene and poly(acrylic acid) diblock copolymers can result in the change of the aggregate morphologies from spherical micelles to rods and vesicles [3]. The presence of

acids or ions can either reduce the number of ionic groups in the corona or screen the electrostatic repulsions between these ionic groups, both of which results in increased N_{agg} .

3.7. Effect of the Lipid Concentration on the Micelle Size

In Fig. 1, we have seen that the average micelle sizes change in buffer as $d_h = 15 - 8$ nm at monomer concentrations of $c = 5 - 40$ mM. At the same time, SSM sizes slightly increase in water for the monomer concentrations of $c = 5 - 40$ mM in the approximate range of $d_h \approx 3 - 6$ nm. The sizes of micelles formed in dilute monomer solutions are usually relatively independent of the monomer concentration [32]. However, in the 40 mM DSPE-PEG₂₀₀₀ solution the average distances between PEG coronas of two neighboring 10- and 90-monomer SSM are both ≈ 2 nm. These small intermicellar distances are directly visible in the 40 mM monomer solution in water, shown in Fig. 2 (right).

In buffer solutions, intermicellar interactions are well screened at small monomer concentrations. At increased monomer concentrations, screening of intermicellar repulsions become less effective due to reduced number of counterions per negative headgroup. At the same time, intermicellar distances become smaller and ionic clouds of neighboring micelles overlap, leading to intermicellar coupling and partial micelle destabilization, where the micellar sizes might change. This might explain why at $c = 40$ mM, micelle sizes in water ($d_h \approx 5$ nm, $N_{agg} < 8$) and in ionic solution ($d_h \approx 8$ nm, $N_{agg} \approx 15 - 20$) are similar, as shown in Fig. 1 (d) and (h), and in Table I.

3.8. Effect of the Solvent on CMC

In Fig. 8, we also show the observed DPH probe fluorescence intensity in dependence of the lipid concentration in water. The concentration at which the fluorescence intensity starts to grow signals the presence of micelles and gives the CMC. The obtained CMC $\approx 10 - 20$ μ M for DSPE-PEG₂₀₀₀ in water is in excellent agreement with the previously measured CMC of $\approx 10 - 25$ μ M [55], and it is approximately ten times higher than that obtained in HEPES buffered saline, $c = 0.5 - 1.0$ μ M [14]. The observed CMC decreases with the increasing ionic strength of the solvent in agreement with previous studies [26]. This CMC drop is predominantly caused by the increased screening of the charged headgroups, leading to better stabilization of the micelles formed [56]. Another factor that may lower the CMC of DSPE-PEG₂₀₀₀ in the ionic solution could be the smaller number of water molecules available to solubilize DSPE-PEG₂₀₀₀, when the water is shared between phospholipids and ions [57].

3.9. Effect of the Lipid Concentration and Solvent on the Solution Viscosity

Our measurements of DSPE-PEG₂₀₀₀ dispersions in pure water and HEPES buffered saline, presented in Fig. 8 (inset), show that their viscosity increases with the increasing lipid concentration. In addition, viscosity of pure water solutions is higher at every DSPE-PEG₂₀₀₀ monomer concentration and it increases at a higher rate than in the buffer solutions. These trends can be understood from Fig. 2, where we have shown that in 40 mM DSPE-PEG₂₀₀₀ solutions, neighboring SSM can easily come in contact through their PEG coronas. Therefore, the probability of SSM interactions is rapidly increasing with the lipid concentration, which explains the largely growing viscosity. The slower increase of viscosity in buffer can be explained by the presence of fewer, larger and more separated micelles.

4. Conclusion

We have experimentally and theoretically characterized DSPE-PEG₂₀₀₀ assemblies in pure water and HEPES buffered saline. We found that the structural and dynamical properties of

SSM strongly depend on the concentration of lipids and the solvent medium. The SSM sizes (aggregation numbers) increase and CMC decreases with increasing ionic strength (in buffer), as the repulsions between negatively charged phosphate groups are better stabilized by counterion screening in buffered solutions. Our simulations reveal that the inflatable SSM core, complex ionic interface and highly fluctuating corona form suitable nesting sites for the drugs and other carried molecules. The observed behavior of DSPE-PEG₂₀₀₀ in aqueous solutions is of crucial importance for the design of new nanomedicines and other nanocon-structs with versatile applications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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FIG. 1.

Experimental intensity weighted size distributions of SSM (left) in pure water at concentrations: a) 5 mM, b) 10 mM, c) 20 mM, d) 30 mM, e) 40 mM and (right) in HEPES buffered saline at concentrations: f) 5 mM, g) 10 mM, h) 20 mM, i) 30 mM, j) 40 mM. Line histograms show data obtained by the NICOMP instrument at the 90° angle, and shadow histograms show data obtained by the Brookhaven instrument at the 90° angle. Average hydrodynamic diameters, d_h , obtained by the NICOMP instrument (N) at the 90° angle, and by the Brookhaven instrument (B) at 30°, 90° and 120° angles, are shown in each plot.

FIG. 2.

Formation of DSPE-PEG₂₀₀₀ micelles in water at the monomer concentration of $c = 40$ mM (left) at the beginning of the assembly and (right) after 30 ns of equilibration at $T = 300$ K. The average first neighbor SSM core-core distance is $d_{C-C} \approx 7$ nm. The projected images show slices of the aqueous solutions, 18.7 nm in width.

FIG. 3.

Snapshots of equilibrated micelles ($T = 300$ K) containing the following number of monomers N_{agg} : (a) 8, (b) 10, (c) 15, (d) 20, (e) 50 - water, and (f) 90 - 0.166 M NaCl solution. (g) Equilibrated alkyl cores of the micelles in (a-f) are shown in the same order. Water molecules are not shown for clarity. Images (a-g) are shown at the same scale (scale bar shown in (g)). (h) Relaxation of the 90-monomer SSM core in 0.166 M NaCl solution, shown on a 1 nm thick cross-section.

FIG. 4.

Molarity of P atom ($-\text{PO}_4^-$ group on DSPE-PEG₂₀₀₀ monomers), Na^+ and Cl^- as a function of the radial coordinate, r , with respect to the SSM center of mass for 90-monomer SSM in 0.166 M NaCl solution. (inset) Molarity of P and Na^+ for 10-monomer micelle in water.

FIG. 5. Local thickness of the PEG corona, d_{PEG} , for SSM with $N_{agg} = 10$ in water, at (a) 3 ns, (b) 6 ns, (c) 10 ns, (d) 16 ns, and for SSM with $N_{agg} = 90$ in 0.166 M NaCl solution, at (e) 7 ns, (f) 8 ns, (g) 9 ns, and (h) 10 ns, as a function of the inclination angle, θ , and the azimuthal angle, ϕ .

FIG. 6. Density distribution as a function of the radial co-ordinate, r , with respect to the SSM center of mass for 10-monomer SSM in pure water (top) and 90-monomer SSM in 0.166 M NaCl solution (bottom). Core and PEG distributions are averaged over last 4 ns of the simulations, while the water distribution is averaged over last 0.1 – 0.2 ns.

FIG. 7. (top) The average number of ions (Na^+ or Cl^-), N , at the distance r from phosphorus atoms on DSPE-PEG₂₀₀₀ monomers in 10-monomer micelles in water and 0.166 M NaCl solution. The data are obtained by integration of the radial distribution function, $g(r)$, between P and Na^+ (Cl^-). Two plots are obtained from (left) the trajectory where one of the Na^+ ions is trapped by a PEG chain close to the $-\text{PO}_4^-$ group (shown in the inset) and (right) the trajectory where all the ions are free. (bottom) Normalized RDFs, $g(r)$, of phosphorus atoms on different DSPE-PEG₂₀₀₀ monomers in 10-monomer micelles in water and in 0.166 M

NaCl solution. Plots of the average number of phosphorus atoms, N , within distance r of another phosphorus atom are shown in the inset.

FIG. 8.

Fluorescence intensity of DPH probe versus DSPE-PEG₂₀₀₀ concentration in pure water. Solid lines represent logarithmic fits to the data points, $I = I_0 \ln(c_{red}) + I_1$, where I is the fluorescence intensity, $c_{red} = c/1 \text{ mM}$ is the unitless lipid concentration, and I_0 and I_1 give the slope and the intercept. The fitting parameters are $I_0 = 233$, $I_1 = 2,051$ and $I_0 = 6,282$, $I_1 = 26,999$ for the first ($c < 0.01 \text{ mM}$) and the second ($c > 0.05 \text{ mM}$) concentration regimes, respectively. Two fitting lines are connected by a guiding dashed line. (inset) Viscosity of DSPE-PEG₂₀₀₀ solution versus lipid concentration in pure water (diamonds) and HEPES buffered saline (circles). Exponential fits to the data, $\eta = \eta_0 e^{k c}$, are represented by two lines. The obtained fitted parameters are $\eta_0 = 0.76 \text{ cP}$, $k = 0.067 \text{ mM}^{-1}$ and $\eta_0 = 0.84 \text{ cP}$, $k = 0.052 \text{ mM}^{-1}$ for water and buffer solutions, respectively.

TABLE I

Dependence of the micelle (d_{tot}) and core (d_c) diameters on the aggregation number N_{agg} . Labels (a–f) correspond to those in Fig. 3. The last two systems are simulated in the 0.166 M NaCl solution, used also in the case (f).

N_{agg}	8(a)	10(b)	15(c)	20(d)	50(e)	90(f)	10	15
d_{tot} [nm]	5.9	6.0	7.3	7.7	11.8	13.9	6.3	6.9
d_c [nm]	2.3	2.5	2.9	3.4	4.6	6.3	2.5	2.9