Atomistic simulations of pH-dependent self-assembly of micelle and bilayer from fatty acids

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Detailed knowledge of the self-assembly and phase behavior of pH-sensitive surfactants has implications in areas such as targeted drug delivery. Here we present a study of the formation of micelle and bilayer from lauric acids using a state-of-the-art simulation technique, continuous constant pH molecular dynamics (CpHMD) with conformational sampling in explicit solvent and the pH-based replica-exchange protocol. We find that at high pH conditions a spherical micelle is formed, while at low pH conditions a bilayer is formed with a considerable degree of interdigitation. The mid-point of the phase transition is in good agreement with experiment. Preliminary investigation also reveals that the effect of counterions and salt screening shifts the transition mid-point and does not change the structure of the surfactant assembly. Based on these data we suggest that CpHMD simulations may be applied to computational design of surfactant-based nano devices in the future. © 2012 American Institute of Physics. [<http://dx.doi.org/10.1063/1.4766313>]

I. INTRODUCTION

Surfactants are amphiphilic molecules that can self-assemble in solution to form aggregates of various shapes and sizes. Recently, surfactants have received much attention as drug delivery vehicles. Liposomes containing pH-sensitive surfactants can be used to target drug release to areas of the body with different pH, such as tumors or infection sites.¹ Fatty acids, which contain a carboxylic acid headgroup and an unbranched hydrocarbon tail, are examples of pH-sensitive surfactants. Earlier studies show that fatty acids form micelles at a high pH, and vesicles at pH conditions around the pK_a where the concentrations of protonated and unprotonated molecules are similar.^{2,3} This presents a potential opportunity for the design of drug delivery vehicles, as the pK_a of fatty acid solutions can be adjusted by, for example, altering the tail length⁴ or adding substituents to the α -carbon of the carboxylic acid,⁵ and the pH range of stable vesicles can be adjusted lower or higher by adding anionic co-surfactants⁶ or alcohols.⁷

With the explosion of computing power, molecular dynamics simulations are becoming an increasingly valuable tool for elucidating microscopic mechanisms of surfactant assembly and phase behavior that are difficult or impossible to obtain using wet-lab experiments.^{8–10} Until now all simulations have been conducted based on fixed protonation states. The effect of solution pH has not been explored in a satisfactory manner. To mimic the pH condition, simulations can be conducted with surfactants being all protonated, all deprotonated, or by combining protonated and deprotonated forms in equal amounts.^{11,12} The latter simulation is unreliable because of the ambiguity in combining neutral and charged configurations. Also, the pK_a value of the surfactant assembly cannot be calculated. Thus, details of pH-dependent structure and conformational dynamics of surfactant assemblies remain elusive.

Here we present, to our best knowledge, the first atomistic simulation of the self-assembly of micelle and bilayer with simultaneous proton titration of individual surfactant molecules in aqueous solution. This study is enabled by a state-of-the-art molecular dynamics technique, continuous constant pH molecular dynamics (CpHMD),^{13,14} which propagates a set of titration coordinates that represent protonation states simultaneously with the atomic coordinates. We use the recent extension of the CpHMD technique which samples conformational dynamics in explicit solvent while driving protonation dynamics based on the generalized-Born implicit solvent model.¹⁵ Together with the pH-based replica-exchange protocol,¹⁵ the hybrid-solvent CpHMD titration allows the prediction of the pK_a value of a fatty acid solubilized in ionic and nonionic micelles,¹⁶ which is not possible using traditional pK_a calculation methods such as the Poisson-Boltzmann approach. In the current work we allow titration of all fatty acids in the simulation box. Remarkably, we observe the spontaneous formation of a bilayer at low pH conditions and a micelle at high pH conditions. We provide quantitative characterization of the pH-dependent surfactant structure and pH titration profile, both of which are in good agreement with experiment. We discuss the physical forces driving the phase transition and the effect of counterions and salt screening.

II. METHODS AND SIMULATION DETAILS

The current study utilizes the CpHMD method, which simultaneously propagates the spatial and titration coordinates.^{13,14,17} The latter are defined for all titratable sites and evolve with time by means of λ dynamics.¹⁸ The coupling between protonation/deprotonation and conformational dynamics is achieved by interpolating the partial charges on the titratable sites (between the protonated and deprotonated states) as well as the van der Waals interactions between

titratable protons and other atoms. The CpHMD method was initially developed for simulations using the generalized Born (GB) implicit-solvent model.^{13,14} The temperature based replica-exchange (REX) sampling protocol¹⁹ was applied to accelerate protonation-state sampling and hence convergence of the calculated pK_a values.¹⁷ Recently, a hybrid-solvent scheme was introduced which makes use of the GB model for calculating solvation forces on titration coordinates while propagating conformational dynamics in explicit solvent.¹⁵ The convergence of hybrid-solvent CpHMD is enabled by means of the pH-based REX sampling protocol.¹⁵ Both GB and hybrid-solvent based CpHMD methods were implemented in the PHMD module of the CHARMM program.²⁰ The temperature- and pH-based REX protocols were added to the REPDSTR module in CHARMM. More details of the CpHMD methods can be found elsewhere.^{13,14,17,21}

The pH-REX hybrid-solvent CpHMD simulations were performed using the CHARMM program (version c36b1). The all-atom CHARMM27 lipid force field²² was used to represent the hydrocarbon chain of lauric acid, while the CHARMM22 protein force field²³ was used to represent the carboxyl headgroup in both protonated and unprotonated forms. TIP3P²⁴ was used to represent water molecules. For calculation of solvation forces, the GBSW model²⁵ was applied with the atomic input radii for fatty acid taken from our previous work.²⁶ The potential of mean force for titrating the lauric acid in solution (model compound) was derived in our previous work.¹⁶ The model or solution pK_a value is 5.0.¹⁶

The pH-REX molecular dynamics was set up with 11 replicas placed at pH conditions ranging from 4 to 9 with 0.5 unit spacing. Each replica was subjected to constant NPT molecular dynamics at 300 K (controlled by a Hoover thermostat²⁷) and 1 atm pressure (controlled by Langevin piston pressure coupling²⁸). Every picosecond (500 molecular dynamics steps), an exchange of pH conditions between adjacent replicas was attempted according to the Metropolis criterion. The average and lowest exchange ratios are 35% and 30%, respectively. We note that the exchange ratio across the pH range is not even with the uniform distribution of the pH ladder. This is a topic discussed in an upcoming paper.⁴² The total length of the simulation for each replica was 50 ns, resulting in an aggregate time of 550 ns. If not otherwise noted, the first 15 ns of a replica were discarded in the analysis.

During the molecular dynamics run, the SHAKE algorithm²⁹ was applied to bonds and angles involving hydrogen atoms to allow a 2 fs timestep. Electrostatic interactions were calculated using the particle-mesh Ewald method^{30,31} with a charge neutralizing background plasma.³² A charge correction term³³ was applied to reduce pressure artifacts related to the background plasma when the net charge is small (for details see Ref. 15). The van der Waals interactions were calculated with a switching function starting at 10 Å and ending at 14 Å. In the CpHMD setup, the GB calculation and update of titration coordinates were executed every 10 dynamic steps (20 fs) as in our previous work.³⁴ All other molecular dynamics and GBSW related parameters were set to the default values as in our previous work.^{16,34}

At the start of the simulation, twenty lauric acid molecules were randomly distributed in a 40 Å cubic water box, with at least 5 Å between molecules. The system underwent 50 steps of steepest descent and 150 steps of adopted-basis Newton-Raphson minimization before being subjected to pH-REX CpHMD simulations. If not otherwise noted, explicit ions were not included in the simulation. Two additional sets of 30 ns test simulations were performed to explore the effect of including explicit ions: one with an ionic strength of 0.15 M in the Debye-Hückel term of the GB model and 20 sodium ions and another with the same screening but without sodium ions.

III. RESULTS AND DISCUSSION

As the initial condition, we randomly distributed twenty lauric acid molecules in a 40 Å simulation box filled with explicit water, which corresponds to an effective concentration of 0.5 M. While 20 is much lower than the micelle aggregation number of 55–70 found in the literature,³⁵ this proof-of-concept study can offer new and valuable insights into the pH-dependent mechanism of surfactant self-assembly. We note that additional simulations are underway to investigate more detailed aspects such as the effect of aggregation number, surfactant, and salt concentration.

We observed the formation of a single cluster of lauric acids after 5–7 ns at all solution pH conditions, on par with the aggregation time of surfactants of similar length found in other simulation studies.^{8,36} Shortly after 10 ns the distribution of angles between lauric acid molecules started to converge at all pH conditions (data not shown), indicating that the aggregate structure was no longer changing. Representative snapshots taken from three different pH conditions reveal remarkable differences in the aggregate structure (Figure 1). At pH 4 and 7, a bilayer was formed, with two layers of carboxyl headgroups extending out to solution. There is a considerable degree of interdigitation between the two layers, as the average thickness of the bilayer (defined as the C1–C1 distance, where C1 is the carboxylate carbon atom) is 16.4 and 16.9 Å at pH 4 and 7, respectively, while the hydrocarbon-chain length (defined as the C1–C12 distance) of an extended lauric acid is about 14 Å. This is consistent with small-angle neutron scattering data for vesicles of undecenoic acid (10-carbon fatty acid with a terminal double bond), which show a bilayer thickness of 17 Å compared to an extended tail length of about 13 Å.³⁷ At pH 9 a micelle was formed,

FIG. 1. pH-dependent self-assembly of lauric acids. Representative snapshots at pH 4 (left), pH 7 (middle), and pH 9 (right). The tails of lauric acids are rendered in cyan while the protonated and deprotonated carboxyl headgroups are shown as blue and red spheres, respectively.

FIG. 2. Proton titration of individual lauric acids in the aggregate. (a) The fraction of laurate ions vs. simulation time. (b) Simulated titration curve. Solid curve represents the fit to the Hill equation, which yields a pK_a value of 7.0 ± 0.2 . The fractions of laurate ions were calculated using the data of the last 35 ns. The model pK_a value is 5.0.

with the average radius (defined as the distance of C1 atoms to the center of mass) of $12 \text{ \AA}$, which is more compact than a pre-assembled 60-mer micelle, which has a corresponding radius of $17 \text{ \AA}$ in a reference simulation with the same setup (data not shown).

To begin exploring the pH-dependent mechanism of the self-assembly process, we first examine the degree of protonation of the aggregate at different pH conditions. The fraction of deprotonated lauric acids, hereafter referred to as laurate ions, converged after about 10 ns at all pH conditions (Figure 2(a)), in accord with the convergence time of the aggregate structure. At pH 4, the monomers are mostly protonated neutral lauric acids. At pH 7, lauric acids and laurate ions are present in almost equal amount, while at pH 9, 90% of the molecules are laurate ions. To obtain the apparent or bulk pK_a value of lauric acid in the aggregate, we fitted the fractions of laurate ions vs. pH to the Hill equation. The resulting pK_a is 7.0 ± 0.2 , about 2 units higher than the pK_a of an isolated lauric acid in solution. The resulting Hill coefficient is very small (0.4), which can be attributed to the negative cooperativity among monomers.

To quantify the pH-dependent change of the aggregate structure, we examine the relative orientation of lauric acids using the angle between the vectors joining the C12 (end of the hydrocarbon tail) and C1 (carboxylate carbon) atoms. The relative orientation of lauric acids is also characterized by the nematic order parameter P_2 following our previous work,³⁸

$$P_2 = \frac{1}{N} \sum_{i=1}^N (\vec{v}_i \cdot \vec{d})^2, \quad (1)$$

where $\vec{v}_i$ is a normalized vector connecting the C12 and C1 atoms of molecule i and $\vec{d}$ is the director, which is a normalized average vector of the unsigned orientations of all $\vec{v}_i$. P_2 is

FIG. 3. Relative orientation of lauric acids at different pH conditions. (a) Probability distribution of the angles between lauric acid monomers (defined in the main text). The distribution obtained from the simulation of a pre-constructed micelle of 60 laurate anions is shown as the dashed curve. (b) P_2 order parameter (defined in the main text) as a function of pH. Error bars represent the standard deviation. (c) Probability distribution of the carbon-chain length (defined as the C1-C12 distance).

bound by 0, when the aggregate is completely disordered, and 1, when all of the molecules are aligned exactly in a parallel or antiparallel fashion.

In the pH range 4 to 6, when the fraction of laurate ions is below 30%, P_2 remains fairly constant at about 0.75 (Figure 3(b)), and the angle distribution shows two sharp peaks in the vicinity of 0° and 180° (black, green, and yellow curves in Figure 3(a)), indicating that most monomers are aligned either parallel or antiparallel to each other. This corroborates the snapshots shown in Figure 1 and confirms the formation of a bilayer-like structure. As pH increases to 7, when the number of laurate ions becomes equal to that of acids, there is a drop in the P_2 value (to about 0.7) and the intensity of the two peaks in the angle distribution (blue curve). At the same time there is an increased number of molecules aligned with an angle of 45° – 135° towards each other, due to increased electrostatic repulsion between laurate ions. These changes indicate that the bilayer arrangement is still dominant but somewhat less ordered as compared to lower pH conditions. As pH further increases to 8, when the fraction of laurate ions is increased to 70%, P_2 drops further to 0.6, and the preferential parallel/antiparallel orientation is lost with angles between 0° and 175° occurring with equal probability (cyan curve). This suggests that when the ionic forms dominate the aggregate the electrostatic forces push the molecules further apart resulting in the formation of a distorted micelle. Finally, as pH reaches 9, when the fraction of laurate ions becomes

almost 90%, P_2 is about 0.5, and angles between 45° and 135° are more favored (red curve), making the distribution resemble that of a micelle with 60 monomers (dashed curve), which is in the range of the experimentally observed aggregation number.³⁵ The small deviation is likely due to the smaller number of surfactants in the aggregate.

Next we examine the shape of the individual lauric acids at different pH. Figure 3(c) gives the distribution of the hydrocarbon chain length, defined as the distance between C1 and C12 atoms. At all pH conditions, the distribution is bimodal with the first mode being sharply peaked at $14 \text{ \AA}$, corresponding to a fully extended chain, and the second mode being broad with the maximum at $13 \text{ \AA}$. The latter shortening by $1 \text{ \AA}$ is due to tilting of either C1 or C12 atom towards the carbon chain. With decreasing pH, the intensity of the first mode increases relative to the second one, indicating that more molecules become extended at low pH when the aggregate is in a bilayer arrangement. If we use $12.5 \text{ \AA}$ as a lower bound for defining extended chains, the probability of having contracted configurations is 0.48 at pH 9 and decreases to 0.29 at pH 4. The presence of a significant fraction of contracted states at pH 4 indicates that the bilayer is fluid (see later discussions).

To further characterize the aggregate structure, we calculated the average density of the C1 atoms in the xy -plane (Figure 4). For each configuration, the center of mass of the aggregate was placed at the origin and the aggregate was rotated such that the average orientation vector $\vec{d}$ was aligned with the y -axis. At pH 4 (top), there is a high density of C1 atoms at $y \approx \pm 8$ with almost no C1 atoms appearing between these areas of high density, indicating a well-defined bilayer. When the pH is raised to 7 (middle) the bilayer is still present, although the C1 density is slightly more evenly distributed, indicating that the aggregate is less ordered and more mobile in accord with the angle distribution data shown in Figure 3. Consistent with greater disorder and mobility, it is evident that the thickness of the bilayer is also increased at pH 7. As pH is further raised to 9 (bottom), the C1 density in the xy -plane is much more evenly distributed, with the highest density occurring in a ring centered at the origin, indicating the formation of a micelle.

The pH-dependence of the aggregate structure can be explained by the balance between electrostatic repulsion among the ionized headgroups and hydrophobic attraction among the carbon tails. At high pH, lauric acids are deprotonated, resulting in a strong electrostatic repulsion between the headgroups and consequently a spherical micelle. At low pH, lauric acids are protonated, resulting in the absence of repulsion between the headgroups and consequently the formation of a bilayer. In an early work it was proposed that hydrogen bond formation between a pair of fatty acids with one protonated and one deprotonated headgroup provides the driving force for bilayer formation.³⁹ Our data do not support this hypothesis. In our simulation, the number of hydrogen bonds among 20 molecules at different pH conditions varies between 1 and 2.5 (Figure 5). The maximum occurs, as expected, at pH 6.5 and 7, where there is an equal number of carboxylic acids and carboxylate groups. However, the number of hydrogen bonds decreases as pH decreases. Together with the small number of

FIG. 4. Average density (atoms/ $\text{\AA}^2$) of C1 atoms in the xy -plane at pH 4 (top), 7 (middle), and 9 (bottom). Aggregate has been centered around the origin and rotated such that the average orientation vector $\vec{d}$ is aligned with the y -axis.

hydrogen bonds at pH 7, it suggests that the bilayer formation is not stabilized by hydrogen bonds, in support of the conclusion reached in the experimental work.³ The small number of hydrogen bonds is also consistent with data from an earlier simulation study using fixed protonation states.¹¹

Finally, we consider the effect of including explicit ions. Since the total net charge of the solute varies at different pH conditions and fluctuates during titration, inclusion of an exact number of counterions cannot be accommodated in the hybrid-solvent CpHMD.¹⁵ Rather, a background plasma is applied to maintain charge neutrality of the system. A test simulation using a highly charged protein shows that the effect of including explicit counterions is negligible on the calculated pK_a 's.¹⁵ However, the effect of dielectric screening by salt ions is significant and is implicitly taken into account by a Debye-Hückel term in the GB model.¹⁵ The limitation of the implicit treatment is that effects due to binding of

FIG. 5. Number of hydrogen bonds between lauric acid headgroups, averaged over the final 35 ns of simulation. Error bars represent standard deviation.

specific ions, which in some instances are large, cannot be reproduced.¹⁶

The simulations discussed so far do not include ions or salt screening. To qualitatively explore the possible effects, we compare our data without explicit ions or screening with two additional sets of test simulations each lasting 30 ns per replica. One simulation used a Debye-Hückel term with an ionic strength of 0.15 M to account for salt screening but no explicit ions were included. The other simulation used the same Debye-Hückel screening but also included 20 sodium ions. We note that these sodium ions only offset the total net charge of the solute at high pH conditions when lauric acids are fully unprotonated. Thus, the simulation with the explicit counterions is somewhat unphysical at a low pH, while the two simulations without the ions are somewhat unphysical at a high pH. The titration curves for these simulations are shown in Figure 6. Compared to the simulation without ions or screening, the pK_a decreases by 0.5 unit to 6.5 with the implicit salt screening of 0.15 M, and it further decreases to 6.2 when 20 sodium ions were added. The former decrease in the pK_a shift (relative to the model value of 5.0) is expected because salt screening reduces the magnitude of electrostatic repulsion. The latter decrease with the addition of positive ions further enhances the screening effect. This can also be seen from a slight increase in the Hill coefficient, indicating a small reduction in the anti-cooperativity among lauric acids.

Although the pK_a value depends on the amount of screening, the bilayer-to-micelle transition near the pK_a was

FIG. 6. Simulated titration curve with three different setups to model ionic screening. Red: No Debye-Hückel screening, no explicit ions. Filled black: Debye-Hückel term with ionic strength of 0.15 M, no explicit ions. Open black: Debye-Hückel term with ionic strength of 0.15 M, 20 sodium ions. Solid curves represent fits to the Hill equation.

FIG. 7. Effect of including explicit ions. Probability distribution of the angles between lauric acids with two different setups to model ionic screening. (a) Debye-Hückel term with ionic strength of 0.15 M, no explicit ions. (b) Debye-Hückel term with ionic strength of 0.15 M, 20 sodium ions. The color scheme for pH conditions is the same as in Figure 3.

clearly seen in all these simulations. The angle distributions (Figure 7) show the same trend as the simulation without salt-screening (Figure 3(a)) with the parallel/anti-parallel arrangement favored at low pH and the peaks near 0° and 180° vanishing near the pK_a , although with inclusion of the explicit ions (Figure 7(b)) the peaks are lower than the other two simulations, suggesting that the bilayer is less ordered at pH 4 and 5 as compared to the simulations without explicit ions. There are two possible reasons. First, due to the shift in pK_a , the fraction of laurate ions may be higher in the simulation with explicit ions. However, this is not the case. At pH 4 the fraction of laurate ions is negligible (6%). We suggest that the less-ordered bilayer arises from having a neutral surfactant aggregate surrounded by only positive ions, with no compensating negative ions, an artifact of the simulation with the fixed number of ions at all pH conditions. Thus, the effect of ions and dielectric screening shifts the bilayer-to-micelle transition mid-point and does not change the structure of the surfactant assembly.

IV. CONCLUSION

In summary, our simulation shows that lauric acids self-assemble in aqueous solution in a pH-dependent manner. At pH 7 or below, when the number of laurate ions is less than or equal to the number of lauric acids, a bilayer structure is formed. At pH above 7, an aggregate is formed that increasingly resembles a spherical micelle with an increasing pH. We compare these results with experiment. First, the calculated apparent pK_a value (7.0 ± 0.2) for lauric acid with an effective concentration of 0.5 M is in agreement with the value of 7.5 measured for a 1 mM to 1 M solution of lauric acid.^{4,40} Our data are also in agreement with the main experimental findings of Cistola *et al.*, i.e., formation of micelles at pH greater than 9 and bilayers at pH 7 to 9.³ Nevertheless, our simulation has certain limitations. The small number of surfactants

does not allow us to capture the coexistence of bilayer and micelle at pH 9 as observed in experiment.³ The force field used is not optimized to reproduce the temperature dependence of phase transitions, which may explain why the bilayer seen in the simulation is more fluid than the one observed by Cistola *et al.*³ Finally, the current version of the CpHMD technique does not allow explicit ions or salt-screening to be treated rigorously. While the net charge is compensated for by a background plasma, an approximate Debye-Hückel term is included in the GB calculation to account for dielectric screening due to salt. Consequently, the effects of specific ion types (e.g., instability of vesicles in the presence of divalent cations⁴¹) cannot be studied. Despite these limitations, the data presented here are promising and demonstrate that CpHMD can be applied for unveiling atomically detailed mechanisms of pH-coupled global conformational changes of surfactant assemblies. With the most recent development that completely removes the dependence on implicit-solvent models (Shen lab, unpublished data), we envision that CpHMD simulations will be applied to aid in the design of pH-sensitive drug carriers or other surfactant-based nano devices in the future.

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