THE JOURNAL OF PHYSICAL CHEMISTRY B

Article

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Chain-Length-Dependent Autocatalytic Hydrolysis of Fatty Acid Anhydrides in Polyethylene Glycol

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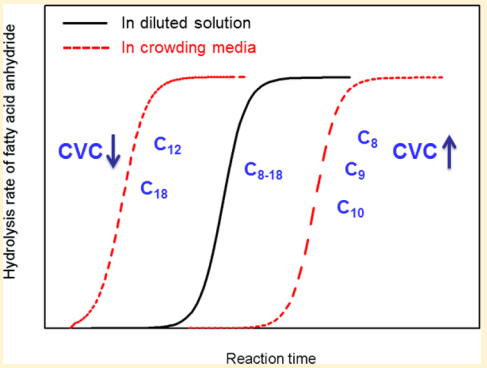
Supporting Information

ABSTRACT: Autocatalytic hydrolysis of fatty acid anhydrides induced by the spontaneously formed vesicles has been studied for years. However, whether the reaction autocatalyzed by vesicles formed in diluted solutions applies also to macromolecular crowded conditions remains unknown. The aim of this study is to characterize hydrolysis behavior of fatty acid anhydrides and formation of vesicles in crowded media. Inert macromolecular crowding agents such as polyethylene glycol (PEG) and Dextran were used to probe the impact of external crowding on the autocatalytic hydrolysis of fatty acid anhydrides with varied hydrophobic chain length. Under stringent conditions of crowding, hydrolysis rates of octanoic anhydride, nonanoic anhydride, and decanoic anhydride were found to decrease, but the rates of lauric anhydride and oleic anhydride increased. These results suggest that the effect of the crowding agent on the hydrolysis of fatty acid anhydrides was chain-length-dependent. Characterization of the size and polydispersity of vesicles formed from hydrolyzed fatty acid anhydrides in crowding revealed that long-chain fatty acids formed monodisperse vesicles easier at lower concentrations of PEG. Measurement of the critical aggregation concentration of ionized fatty acid in the presence of PEG showed that crowding media promoted vesicle formation from long-chain fatty acids but inhibited those from fatty acids with fewer carbon atoms. Further investigation of the diffusion property of ionized fatty acids in crowding agents suggested that PEG might create more hydrophobic areas for long-chain fatty acids anhydrides, which subsequently promoted the unreacted anhydride in the aqueous phase to be solubilized in the formed vesicles. This research provides information for understanding the autocatalytic reaction accompanied by self-producing aggregates and the behavior of fatty acids in crowding media.



INTRODUCTION

The molecular environment of biological systems is highly crowded because a significant fraction of the intracellular space is occupied by macromolecular species.[1] Macromolecular crowding can alter both molecular diffusion and equilibrium of bimolecular reactions and therefore likely affects the function of biochemical networks.[2] The data of the effect of macromolecular crowding on chemical or biochemical reactions are still inconsistent. For example, fibrillation of oligomeric proteins in a crowded environment was found to slow whereas fibrillation of monomeric natively unfolded proteins was accelerated.[3] In some catalytic reactions the rate increases in the presence of crowded macromolecules, whereas in other cases it decreases.[4] A traditional view may assume that viscous solutions cause the reaction rate to drop. Excluded volume proponents may conversely predict that reaction rates will increase because higher effective reactant concentrations will overcome slowed diffusion.[5] The net result of the crowding agent is the sum of several opposing effects and therefore difficult to predict. To date, the range of experimental substrates covered by these studies is not wide enough to fully understand the phenomenology of reaction kinetics in crowded media.

Hydrolysis of fatty acid anhydrides in basic solution forms vesicles from the generated ionized fatty acid, which will catalyze the reaction.[6,7] As fatty acid vesicles are excellent experimental models for protocell model,[8] biomimic membrane bioreactor,[9] and drug delivery carrier,[10] theoretical and experimental treatment of the kinetics involved in autocatalytic hydrolysis of anhydrides and self-reproduction of vesicles has been thoroughly studied for years.[11,12] Previous research on hydrolysis of fatty acid anhydrides is usually performed in diluted solution. Luisi's group recently reported crowding and overcrowding inside the vesicles as a model for the biological crowding of the biological cells.[13−15] However, to the best of our knowledge, no study has focused on the effects of an external crowding medium on the autocatalytic hydrolysis reaction or on vesicle formation. Hydrolysis of fatty acid anhydrides is always treated as a chemically irreversible surface process including vesicle formation, molecule distribution between the organic and water phases, solubilization of the hydrophobic compounds by the aggregates, and the acid−base reactions.[7] Considering that the dynamic viscosity of the

Received: December 21, 2013
Revised: February 25, 2014
Published: March 3, 2014

dx.doi.org/10.1021/jp4125233 | J. Phys. Chem. B 2014, 118, 3461−3468

solution, mobility of reactants, and binding affinity might be altered by the surrounding colloidal macromolecules, we focus on the effect of external crowding agents on the hydrolysis rate of fatty acid anhydrides.

Surface activity of amphiphilic sodium and potassium salts of fatty acids depends strongly on chain length. Several researchers have found that some physiological phenomena related to fatty acids are chain-length-dependent. For example, Mclaughlin et al.[16] found that chain length of fatty acid determines cholecystokinin secretion and its effect on human gastric motility. Plasma cholecystokinin concentration was consistently and similarly elevated by fatty acids with a chain of 12 carbon atoms or longer, whereas those of 11 or fewer carbon atoms failed to increase plasma cholecystokinin. The chain length dependence of biphasic behavior of free fatty acids was also found in protecting erythrocytes against hypotonic hemolysis[17] as well as its binding with human serum albumin.[18] These results suggest that investigating the behavior of fatty acid in crowding is also necessary for understanding its biphasic behavior in vivo. As a continuation of our interest in controllable formation and disassociation of vesicles,[19−21] we examine the autocatalytic hydrolysis of fatty acid anhydrides, which is accompanied by self-reproduction of fatty acid vesicles in crowding. Polyethylene glycol (PEG) and dextran were used as the crowding agents to probe the impact of external crowding on the autocatalytic hydrolysis of several fatty acid anhydrides with varied hydrophobic chain length. Various independent methods were used to find the mechanism behind the unexpected chain length dependence in the hydrolysis process. As systematic research on hydrolysis of fatty acid anhydrides with different carbon atoms numbers in crowding media has not been reported before, this research will provide useful information for understanding the autocatalytic reaction accompanied by self-producing aggregates and the behavior of fatty acids in macromolecular crowding media.

EXPERIMENTAL SECTION

Materials. Octanoic anhydride (C_8), nonanoic anhydride (C_9), decanoic anhydride (C_{10}), lauric anhydride (C_{12}), and oleic anhydride (C_{18}) were purchased from TCI-Shanghai. *N*,*N*-bis(2-hydroxyethyl)glycine (Bicine), NaH_2PO_4 (>98%), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (Hepes), and tris (hydroxymethyl) aminomethane (Tris) were from Sigma-Aldrich. PEG2000 and Dextran 20000 were from Aladdin-Shanghai. Ultrapure Millipore (B0500891) water (18.2 MΩ) was used for the preparation of all solutions.

Hydrolysis of Fatty Acid Anhydride in Crowding. The procedure was described in ref 22. The reaction was performed in a flat-bottom test tube of 2.5 cm diameter in an oil bath at a fixed temperature. PEG or dextran used as crowding agents in desirable concentration was dissolved by heating in 10 mL of buffer solution and then was added with fatty acid anhydride. The biphasic alkaline hydrolysis was carried out by vigorous mixing of the two liquid phases with a 15 × 6 mm^2 magnetic bar at about 1600 rpm. The system shows a distinct sensitivity toward the mixing intensity. Hence, the result depends on the stirring rate, the stirrer size, and the specific geometrical properties of the reaction vessel. The hydrolysis of fatty acid anhydride without crowding agents was also performed at the same conditions each time for comparison. The buffer used in this research for hydrolysis of octanoic anhydride (C_8), nonanoic anhydride (C_9), decanoic anhydride (C_{10}), lauric anhydride (C_{12}), and oleic anhydride (C_{18}) were phosphate (0.5 mol/L, pH 6.80), Hepes (0.5 mol/L, pH 7.50), Tricine (0.3 mol/L, pH 8.25), Bicine (0.2 mol/L, pH 8.45), and Bicine (0.2 mol/L, pH 8.50), respectively, which were selected according to the literature.[22,23]

Determination of the Concentration of Fatty Acid/Fatty Acid Salt during Hydrolysis. The total concentration of protonated and deprotonated fatty acid in vesicle suspensions was determined by Fourier transform infrared (FT-IR) spectroscopy using a Nicolet DTGS 380 FTIR spectrophotometer with a 0.02 cm CaF_2 cell. The typical procedure is the following: after fatty acid anhydride was added into buffers for reaction, 50 μL of the aqueous sample was taken from the reaction solution at intervals for the measurement of the concentration of fatty acid/fatty acid salt. To each sample were added 1 mL of HCl (1 mol/L) and 1.5 mL of isooctane, and then the mixture was vortexed for 2 min. After equilibration for 2 h at room temperature, the isooctane phase was subject to FT-IR analysis. The concentration of fatty acid anhydride was determined at 1715 cm^{-1}.

Dynamic Light-Scattering Measurements. The dynamic light scattering (DLS) measurements were determined by a Zetasizer Nano-ZS (Malvern Instruments Ltd., UK) using a laser-Doppler velocimetry technique. The instrument uses a laser at a wavelength of 632.8 nm and detects the scattered light at an angle of 173°. All measurements were performed in a temperature-controlled chamber. Experiment duration (equilibration time) was in the range of 2 min, and each test was repeated two or more times.

Determination of the Critical Aggregation Concentration of Ionized Fatty Acid. The critical aggregation concentration (CAC) values for sodium salt of fatty acid were obtained by monitoring the pyrene I_1/I_3 using the steady-state fluorescence measurements on a Hitachi F-7000 fluorescence spectrophotometer at 25 °C, which was reported in ref 21. See Supporting Information for details.

Micelle Diffusion Coefficient by Cyclic Voltammetry Measurements. Cyclic voltammetry (CV) measurements were carried out with a cyclic voltammeter CHI 1030 electrochemical system. (See Supporting Information for details). Samples of sodium octanoate (0.27 mol/L), sodium nonanoic (0.23 mol/L), sodium decanate (0.145 mol/L), sodium laurate (0.04 mol/L), and sodium oleate (1.2 mmol/L) were measured in buffer and in 10% PEG/buffer three times each before the data were averaged.

RESULTS AND DISCUSSION

Effect of Crowding Agents on the Rate of Autocatalytic Hydrolysis for Fatty Acid Anhydrides with Varied Hydrophobic Chain Length. Among several macromolecules, polyethylene glycol (PEG) can promote phase separation to a greater extent than other inert polymers because of its spherical conformation, which makes it appropriate for mimicking physiological crowding.[24,25] Besides its significant effect on the thermodynamic stability and folding−unfolding kinetics of proteins, PEG shows dominant influence on the folding process of RNA because of the excluded volume effect.[26] In the present research, we used PEG2000 as a crowding agent to evaluate the effect of crowding on the hydrolysis of fatty acid anhydride. In our preliminary essay, decanoic anhydride (C_{10}) was selected as typical fatty acid to screen the influence of PEG concentration on its hydrolysis and vesicle formation. When water-insoluble decanoic anhydride was layered on basic diluted solutions, an induction period was

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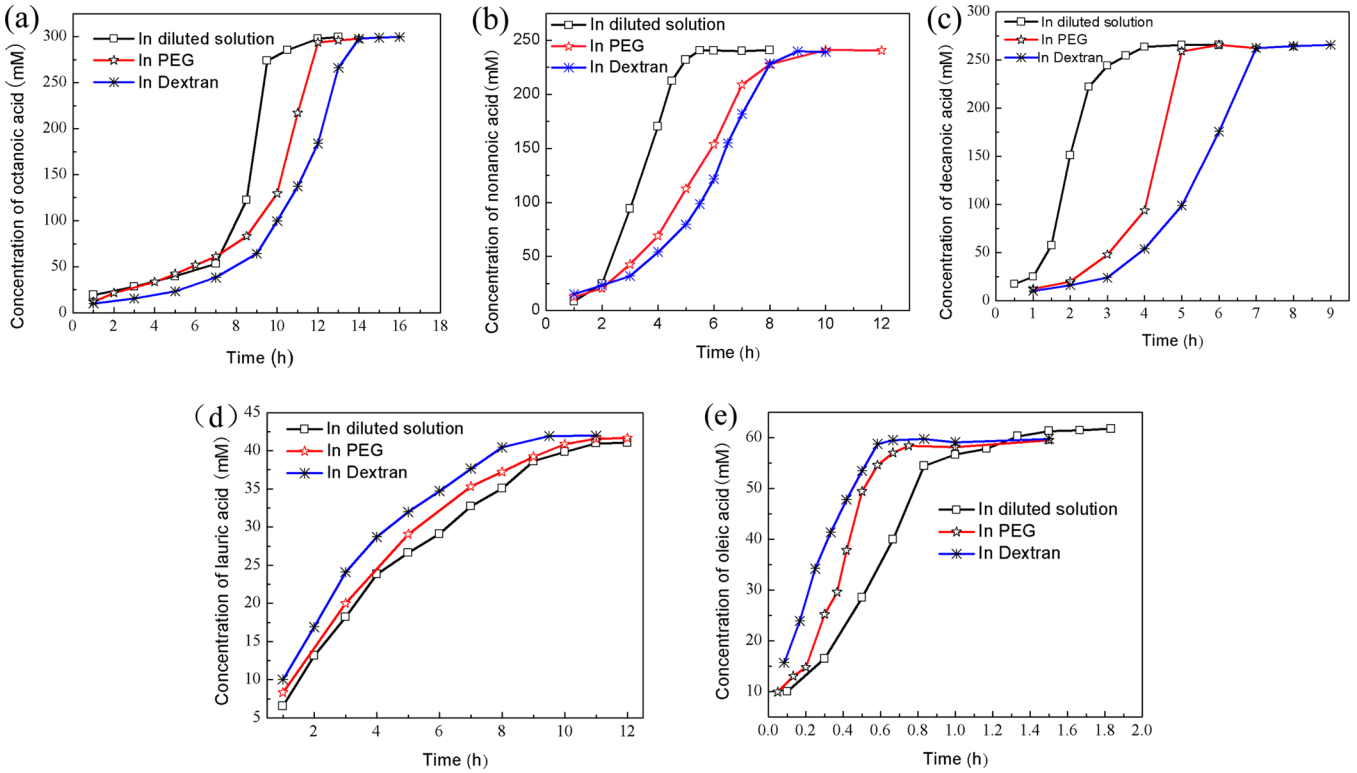

Figure 1. Autocatalytic hydrolysis curves of fatty acid anhydride in diluted solutions and in crowding: (a) octanoic anhydride, (b) nonanoic anhydride, (c) decanoic anhydride, (d) lauric anhydride, and (e) oleic anhydride. Octanoic anhydride, nonanoic anhydride, and decanoic anhydride were hydrolyzed at 60 °C; lauric anhydride and oleic anhydride were hydrolyzed at 50 °C and 40 °C, respectively. PEG (10 wt %) and Dextran (10 wt %) were used as the crowding agents.

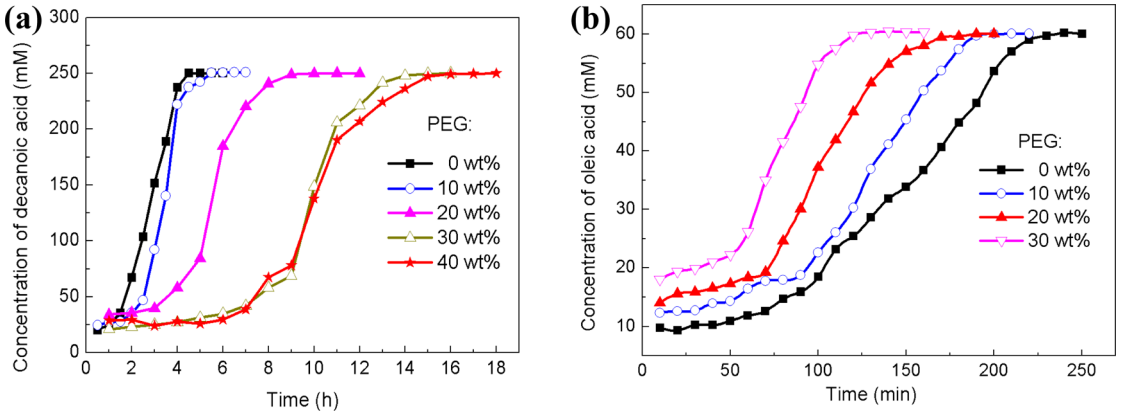

Figure 2. Autocatalytic hydrolysis curves of (a) decanoic anhydride and (b) oleic anhydride in PEG at different concentrations.

observed during which the rate of hydrolysis was very slow. However, after this induction period, rapid hydrolysis occurred. When decanoic anhydride was completely hydrolyzed, the solution was optically clear. The rate of hydrolysis decreased strongly with an increased level of PEG. The time for complete hydrolysis of decanoic anhydride without PEG was about 5 h. When the use of PEG increased, the time for reaction became longer. The presence of 40 wt % PEG resulted in 12 h for hydrolysis to complete. At the same time, the induction time (the lag time) became prolonged with increased concentration of PEG. Considering the effects of macromolecular crowding on the intrinsic hydrolysis activity of fatty acid anhydride without the interference from macromolecular association, PEG at 10 wt % was used for hydrolysis of all of the fatty acid anhydrides for comparison.

To elucidate even more clearly the effect of the crowding agent on hydrolysis of fatty acid anhydride with varied hydrophobic chain length, we then studied hydrolysis of other fatty anhydride such as octanoic anhydride (C_8), nonanoic anhydride (C_9), lauric anhydride (C_{12}), and oleic anhydride (C_{18}). Dextran 20000 was also used as the crowding agent for comparison. The reaction started by preparing a two-phase reaction system which was then exposed to a mildly diluted buffer solution. Under these conditions, the lipid layer gradually disappeared, and in the meantime, the liquid phase became opalescent. Figure 1 shows the time courses of the autocatalytic hydrolysis curves for five fatty acid anhydrides in diluted solutions and in crowding. In all cases, the fatty acid anhydride hydrolyzed initially at a very low rate, but as soon as sufficient ionized species formed, which then aggregated into

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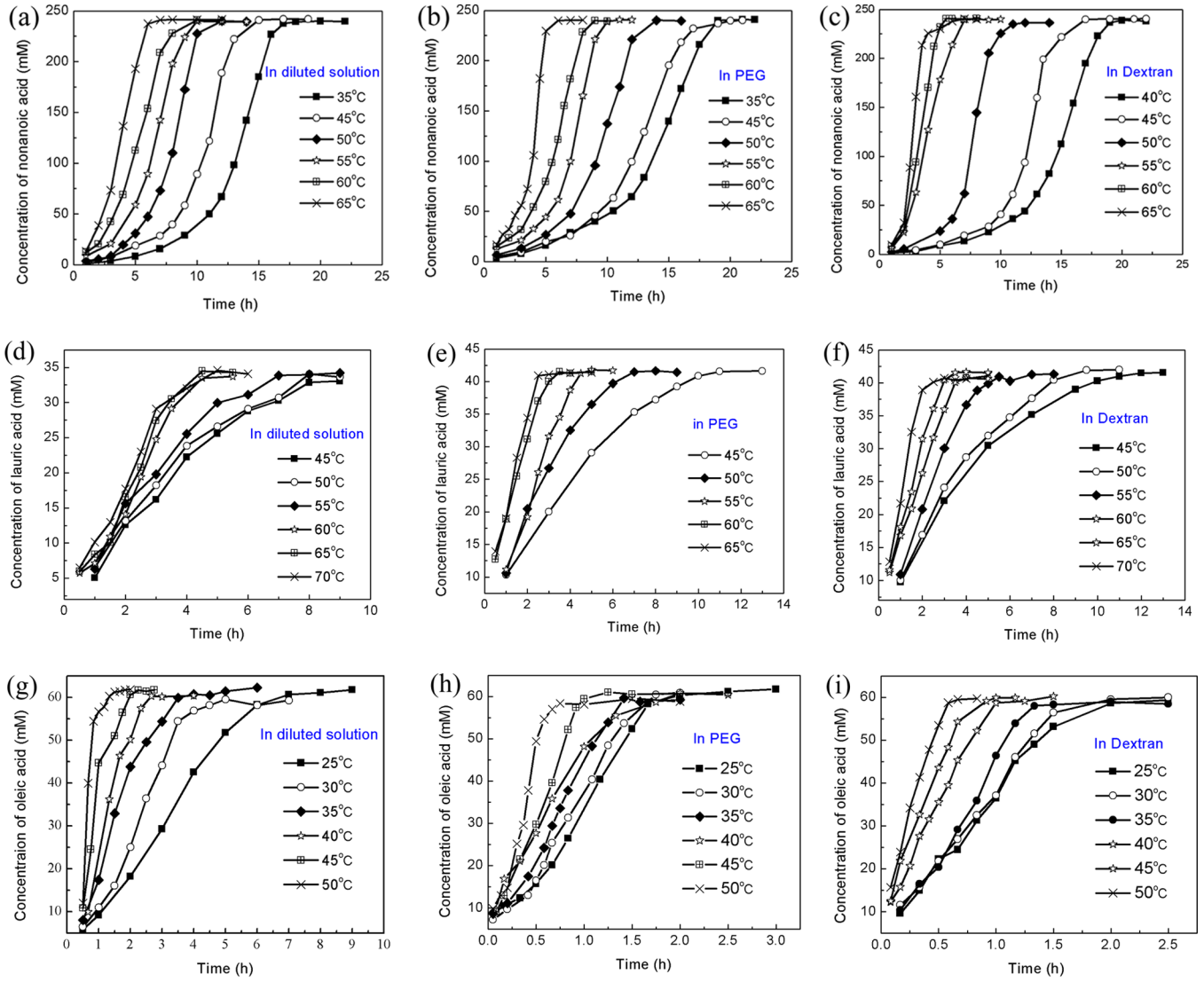

Figure 3. Autocatalytic hydrolysis curves of fatty acid anhydrides in diluted solutions; 10 wt % PEG and 10 wt % Dextran at different temperatures. (a)−(c), nonanoic anhydride; (d)−(f), lauric anhydride; and (g)−(i), oleic anhydride.

vesicles, there was an exponential increase in reaction rate because of vesicular catalysis. The increase in rate finally reached a plateau, which corresponded to the total consumption of fatty acid anhydride. Interestingly, compared with the hydrolysis in diluted solutions, the presence of crowding agents slowed down the hydrolysis rate of octanoic anhydride (C_8), nonanoic anhydride (C_9), and decanoic anhydride (C_{10}). However, it led to increased hydrolysis rates in the case of lauric anhydride (C_{12}) and oleic anhydride (C_{18}). Obviously, the effect of PEG on the hydrolysis of fatty acid anhydride was carbon chain-length-dependent. We changed the additive to inert Dextran 20000, which is widely used as another polysaccharide-type crowding agent, and got similar results. Dextran decreased the hydrolysis rate of octanoic anhydride, nonanoic anhydride, and decanoic anhydride, but increased that of lauric anhydride and oleic anhydride, suggesting that fatty acid anhydride with varied hydrocarbon chain length (i.e., more than or less than 10 carbons) showed opposite hydrolysis behavior in the presence of the crowding agent. Furthermore, it can be seen from Figure 2 that the more concentrated the crowding (PEG), the slower the hydrolysis for decanoic anhydride (C_{10}). For fatty acid anhydride whose hydrolysis can be accelerated by crowding agent (i.e., oleic anhydride), increased addition of PEG made the reaction progress faster and faster.

To clarify if the chain-length-dependent effect in hydrolysis of fatty acid anhydride still exists at elevated temperature, nonanoic anhydride (C_9), lauric anhydride (C_{12}), and oleic anhydride (C_{18}) were subjected to hydrolysis at different temperatures. As shown in Figure 3, the influence of temperature is very significant. For a single fatty acid anhydride, the higher the reaction temperature, the shorter the lag time. For fatty acid anhydrides with different carbon atoms, the lag time before the onset of the autocatalytic hydrolysis for C_{12} and C_{18} fatty acid anhydrides was considerably shorter than that of C_8, C_9, and C_{10}. Therefore, hydrolysis of fatty acid anhydrides in macromolecular crowding agents is still chain-length-dependent at elevated temperatures. In characterizing the effect of chain length of fatty acid on the rate of arylester hydrolysis by various albumins, Wolfbeis and Giirakar[27] demonstrated that protein-catalyzed and uncatalyzed hydrolysis strongly depends on the chain length. Very recently, Mitrinova et al.[28] found that C8−C10 fatty acids transform concentrated surfactant solutions into viscoelastic fluids with very high apparent viscosity,

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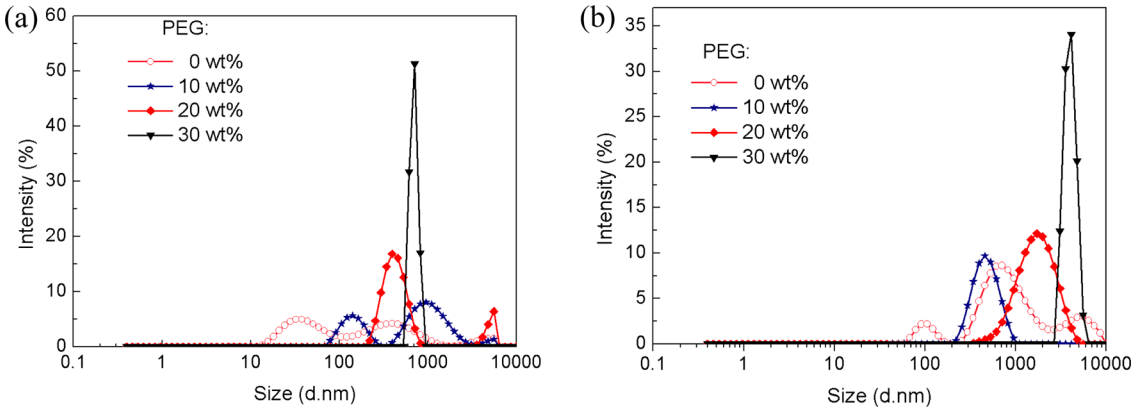

Figure 4. Size distributions of hydrolysis of (a) decanoic anhydride and (b) oleic anhydride at different concentrations of PEG2000. The temperature is 60 °C for decanoic anhydride and 40 °C for oleic anhydride.

whereas C14−C18 fatty acids have a small effect on the viscosity of the concentrated solutions. The biphasic behavior of free fatty acids in protecting erythrocytes against hypotonic hemolysis is also chain-length-dependent. Octanoic acid (C8) and fatty acids with a shorter chain length do not have any effect on the osmotic resistance of erythrocytes. Decanoic acid (C10) decreases the extent of hypo-osmotic hemolysis and does not become hemolytic at higher concentrations. Dodecanoic acid (C12) represents the minimum chain length for the typical concentration-dependent biphasic behavior with protection against hypo-osmotic hemolysis at a certain low concentration range and subsequent hemolysis at higher concentrations.[17] These results clearly demonstrate that the effect of the hydrophobic chain length on the property of fatty acid is complicated, and fatty acids with short chains (<C10) behave differently from those with longer chains (>C10).

Characterization of the Size and Polydispersity of Vesicles Formed from the Hydrolyzed Fatty Acid Anhydrides in Crowding. When fatty acid anhydrides hydrolyzed in aqueous solution, dispersed fatty acids formed different types of aggregates depending on the ionization degree of the carboxyl group.[29,30] As vesicle formation is closely related to autocatalytic hydrolysis, DLS was used to determine the particle size and distribution. We found that anhydride hydrolysis was very slow at the beginning, but as soon as the first vesicles formed, it sharply accelerated and a dramatic increase of vesicle number was observed. It is accepted that the first vesicles can solubilize water-insoluble precursor, which is then hydrolyzed efficiently by the vesicles themselves, a process that brings about the formation of more vesicles in a typical autocatalytic fashion.

As shown in Figure 4, vesicles formed both in PEG solution and in diluted buffer. Increased levels of PEG induced vesicles to form some large aggregates both for C10 and for C18 fatty acid anhydride. When PEG goes from 0 to 30 wt %, both average size and polydispersity increase. The suspension turned transparent when PEG concentration was at 50 wt % (data not shown). For decanoic anhydride, the resulting vesicle size distribution was multimodal before PEG reached 30%. Whereas for oleic anhydride, a remarkable narrow size distribution was obtained when 10 wt % PEG was used. Note that a "matrix effect" has been reported to explain the narrow size distributions in self-reproducing fatty acid vesicles;[31] we speculate that a similar mechanism might operate here. In the presence of PEG, vesicles formed for long-chain fatty acids could be very active "catalysts" and self-reproduce efficiently so that the final population is actually monodisperse and the hydrolysis could be accelerated; vesicles formed for short-chain fatty acids could not be so efficient, and they aggregate to give the large particles, slowing the reaction. However, the fact was that both short-chain and long-chain fatty acid vesicles became larger and tended to display monodispersity when more PEG was added. The only difference was that long-chain fatty acids formed monodisperse vesicles easier at lower concentrations of PEG. Although the crowding conditions and chain length were related and might codetermine the size and polydispersity of vesicles, there was not enough evidence to explain the observed alteration of the hydrolysis rate.

Measurement of the Critical Aggregation Concentration of Ionized Fatty Acid with or without PEG. Several scientists have attempted to describe the mechanism of the autocatalized hydrolysis of fatty acid anhydride and have proposed different kinetic models. The process includes vesicle formation, solubilization of the hydrophobic compounds by the aggregates, surface hydrolysis, and so on.[12,23] Luisi's group has done pioneering work on fatty acid vesicles. They found that a long lag period of the self-formation of fatty acid vesicles could be eliminated by addition of preformed vesicles.[31,32] The vesicles were found to be present in solution from the beginning of the process in the oleic-buffered system, whereas in the caprylic hydroxide system they would appear only when the conditions for their stability were reached.[22] Kinetic descriptions of two examples of autopoietic vesicle chemical systems, i.e., caprylic hydroxide system and the oleic-buffered system, have demonstrated that they were different under the same theoretical approach.[11] Actually, the critical aggregation concentration is related to the number of $-CH_2$ groups for simple *n*-alkyl amphiphiles. CAC will decrease with the increase of the chain length of fatty acid salts. As the aggregation behavior of amphiphilic molecules greatly depends on the length of the hydrocarbon chain, which strongly affects the vesicular catalysis, we speculate that PEG might affect the stability of vesicles formed from fatty acid anhydrides. Figure 1 and Figure 3 revealed that at same conditions, for fatty acid anhydride with longer hydrocarbon chains, lower CAC facilitated the hydrolysis process and resulted in shorter lag time. Increased surface area of vesicles also contributed to the shortened induction time. These facts suggested that the number of vesicles present in the aqueous phase and the stability of the self-assembly played key roles in affecting the

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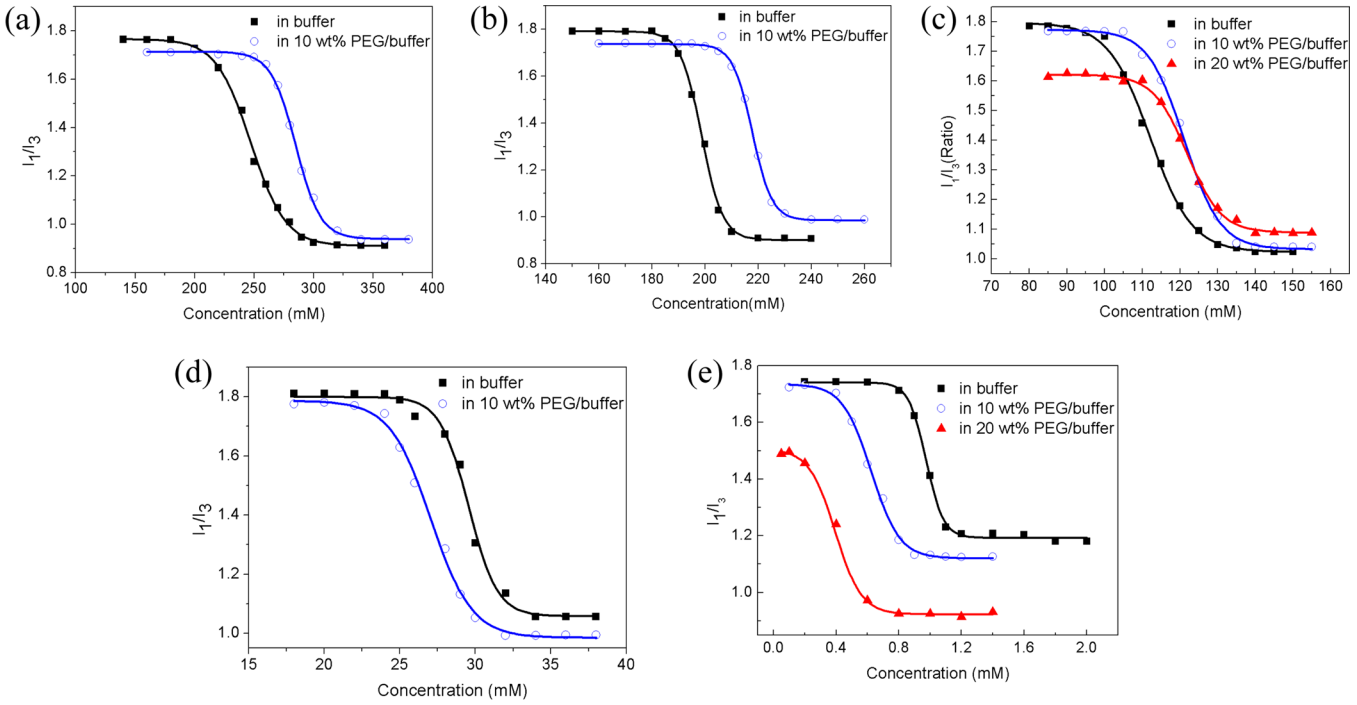

Figure 5. Plot of I_1/I_3 versus the concentration of sodium salts of fatty acids in buffer solutions with or without PEG: (a) octanoic anhydride, (b) nonanoic anhydride, (c) decanoic anhydride, (d) lauric anhydride, and (e) oleic anhydride. The intensities of I_1 and I_3 were measured at wavelengths corresponding to the first and third vibronic peaks of pyrene located at ca. 373 and 384 nm, respectively.

hydrolysis rate of anhydrides. Therefore, it is desirable to find the variation of CAC caused by PEG.

As an ionized fatty acid was generated during the hydrolysis of fatty acid anhydride, we used sodium of the fatty acid to determine its CAC by measuring the conductivity of solution. When we performed the experiment in water, the CAC of each fatty acid really changed, but the results were unexpected. It was found that PEG favored the aggregation of fatty acids with shorter hydrophobic chains, i.e., making the CAC sodium salt of octanoic acid, nonanoic acid, and decanoic acid lower than that without PEG. However, PEG seemed to have a negative impact on the aggregation of the sodium salt of lauric acid and oleic acid, resulting in a CAC higher than that in noncrowding solution (see Supporting Information). These unexpected results did not support the observations in Figures 1−3.

However, when we used buffer solution to keep pH the same as that for the hydrolysis of each fatty acid anhydride, different results were obtained. Because ions from the buffer interfere with the measurement of conductivity, fluorescence measurements were performed by using pyrene as a probe molecule to determine the CAC. As shown in Figure 5, the intensities of I_1 and I_3 were measured at wavelengths corresponding to the first and the third vibronic peaks of pyrene located at ca. 373 and 384 nm, respectively. The ratios of I_1/I_3 were plotted as a function of the total surfactant concentration. The CAC was taken as a point of intersection by fitting the prevesicular and postvesicular data in linear equations. The results showed that PEG increased the CACs of C8, C9, and C10 fatty acids, whereas it decreased those of C12 and C18 fatty acids. More PEG resulted in a lower CAC for C10 fatty acids (Figure 5c) but a higher CAC for C18 fatty acids (Figure 5e). These results are in agreement with the observation that in the presence of PEG, the lag phase increased for C8, C9, and C10 but decreased for C12 and C18 fatty acids. Therefore, it is concluded that the main role of PEG (or dextran) is related to the variation of CAC of ionized fatty acid.

Investigation of the Diffusion of Ionized Fatty Acids with or without Crowding Agents. To further clarify the effect of crowding agents on the diffusion nature of fatty acids with different hydrophobic chains, we used a cyclic voltammety technique to measure the diffusion changes induced by the crowding agents. Ferrocene is often used as the electroactive probe because it does not perturb the micelle and its rates of entrance into and exit from the aggregate with fast and reversible electron transfer are at least comparable to those of the surfactant monomers. Therefore, it is possible to compute the diffusion coefficient D of electroactive species by using a reported equation.[33] As shown in Figure 6, the diffusion coefficient of almost all of the fatty acids decreases in the

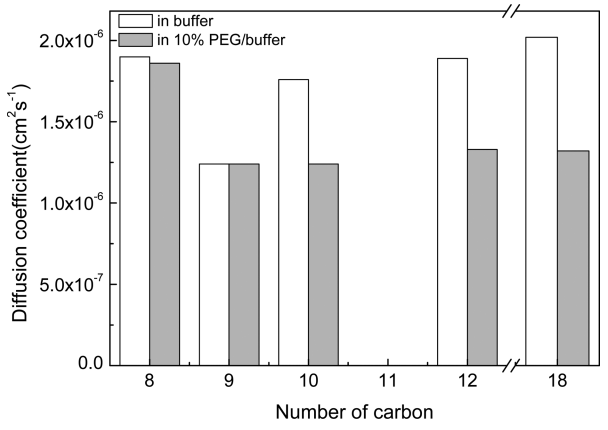

Figure 6. Diffusion coefficients of C8, C9, C10, C12, and C18 ionized fatty acid in buffer with or without 10 wt % PEG. The buffer is the same as that used for hydrolysis.

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presence of PEG. Among five fatty acids, the difference of D value induced by PEG is trivial for C8 and C9 fatty acids but most significant for C18 and C12 fatty acids. A large decrease in D value could be related to the stronger hydrophobic environment and the obstruction effects faced by the electroactive probe in a predominantly hydrophobic environment.[34] On the other hand, simplified hypotheses proposed by Chen and Szostak[12] assumed that the surface hydrolysis was the slowest reaction of all the processes occurring in the system. We speculate that binding of monomeric fatty acid anhydride to the self-assembled vesicles also affects the hydrolysis rate. Kragh-Hansena et al.[18] found that association constants of fatty acid anions to human serum albumin and the number of high-affinity sites increased with the chain length of the fatty acid, reflecting the importance of hydrophobic effects for binding. Therefore, our findings indicated that the presence of PEG might bring more hydrophobic areas for C18 and C12 fatty acids, which subsequently promoted the unreacted anhydride in the aqueous phase to be solubilized in the formed vesicles. In the case of C8 and C9 fatty acids, addition of PEG did not change much for the hydrophobic membrane of vesicles and the obstacle of diffusion along with the inhibition of forming vesicles contributed to the slowing of the hydrolysis rate. The intermediate chain C10 fatty acid combined some of the properties seen with fatty acids with fewer or more carbon atoms. Note that the autocatalytic stage of the reaction might start before the concentration of substrate has reached the CMC. Modeling indicated that salting in and solvent effects caused by the alkanoate anions and ethanol determined the autocatalytic kinetics in the hydrolysis of C-4 ethyl ester where no aggregation occurred.[35] The kinetic behavior of the biphasic alkaline hydrolysis of C-4 to C-8 ethyl alkanoates is always autocatalytic whatever the chain length, i.e., even if micellization does not occur within the experimental concentration range.[36] These findings suggest that in addition to the stably formed aggregates which play the key role in autocatalytic hydrolysis for fatty acid related systems, some other factors might also exist that codetermine the reaction rate.

CONCLUSIONS

In summary, autocatalized hydrolysis of several fatty acid anhydrides in diluted solutions and in crowding agents induced by vesicle formation was investigated. It was found that under stringent conditions of crowding, hydrolysis rates of octanoic anhydride, nonanoic anhydride, and decanoic anhydride decreased, whereas those of lauric anhydride and oleic anhydride increased, suggesting that the effect of crowding agent on the hydrolysis of fatty acid anhydrides is chain-length-dependent. Characterization of the size and polydispersity of vesicles formed from the hydrolyzed fatty acid anhydrides in crowding revealed that both short-chain and long-chain fatty acid vesicles became larger and tended to display monodispersity with the addition of PEG. Measurements of the CACs of ionized fatty acids in the presence of PEG showed that the crowding media promoted vesicle formation from long-chain fatty acids but inhibited those from the fatty acids with fewer carbon atoms; these results were in agreement with the observation of unusual chain-length-dependent effect. Further investigation of the diffusion property of ionized fatty acids in crowding agents suggested that the difference of D values induced by PEG was significant for C18 and C12 fatty acids. These results implied that the presence of PEG might create more hydrophobic areas for long-chain fatty acids anhydrides, which promoted the unreacted anhydride in the aqueous phase to be solubilized in the formed vesicles.

It is noteworthy that the external macromolecular crowding does affect the autocatalytic reaction of the hydrolysis whereby the influence efficacy and the underlying mechanisms might markedly differ because of the length of the hydrocarbon chains. This preliminary research mainly deals with the critical concentration of vesicle formation and diffusion properties for understanding the role of macromolecular crowding in autocatalytic reactions accompanied by self-producing aggregates; some other factors might deserve theoretical and experimental attention.

ASSOCIATED CONTENT

Supporting Information

Results of conductivity of sodium of fatty acids in PEG, detailed determination of the critical aggregation concentration (CAC) of ionized fatty acid, and micelle diffusion coefficient by cyclic voltammetry measurements. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The authors are grateful to the National Natural Science Foundation of China for financial support (NSFC, 20973155). This work was partly supported by the Natural Science Foundation of Zhejiang province (LY13C200001).

REFERENCES

(1) Ellis, R.; Minton, A. Cell biology: Join the crowd. *Nature* **2003**, *425*, 27−28.
(2) Morelli, M. J.; Allen, R. J.; Wolde, P. R. Effects of macromolecular crowding on genetic networks. *Biophys. J.* **2011**, *101*, 2882−2891.
(3) Munishkina, L. A.; Ahmad, A.; Fink, A. L.; Uversky, V. N. Guiding protein aggregation with macromolecular crowding. *Biochemistry* **2008**, *47*, 8993−9006.
(4) Pastor, I.; Vilaseca, E.; Madurga, S.; Garcés, J. L.; Cascante, M.; Mas, F. Effect of Crowding by Dextrans on the Hydrolysis of *N*-Succinyl-L-phenyl-Ala-*p*-nitroanilide Catalyzed by α-Chymotrypsin. *J. Phys. Chem. B* **2011**, *115*, 1115−1121.
(5) Wenner, J. R.; Bloomfield, V. A. Crowding effects on EcoRV kinetics and binding. *Biophys. J.* **1999**, *77*, 3234−3241.
(6) Bachmann, P. A.; Luisi, P. L.; Lang, J. Autocatalytic self-replicating micelles as models for prebiotic structures. *Nature* **1992**, *357*, 57−59.
(7) Morigaki, K.; Dallavalle, S.; Walde, P.; Colonna, S.; Luisi, P. L. Autopoietic self-peproduction of chiral fatty acid vesicles. *J. Am. Chem. Soc.* **1997**, *119*, 292−301.
(8) Chen, I. A.; Walde, P. From self-assembled vesicles to protocells. *Perspect. Biol.* **2010**, *2*, 1−13.
(9) Noireaux, V.; Libchaber, A. A vesicle bioreactor as a step toward an artificial cell assembly. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 17669−17674.
(10) Torchilin, V. P. Structure and design of polymeric surfactant-based drug delivery systems. *J. Controlled Release* **2001**, *73*, 137−172.
(11) Mavelli, F.; Luisi, P. L. Autopoietic self-reproducing vesicles: A simplified kinetic model. *J. Phys. Chem.* **1996**, *100*, 16600−16607.
(12) Chen, I. A.; Szostak, J. W. A kinetic study of the growth of fatty acid vesicles. *Biophys. J.* **2004**, *87*, 988−998.

(13) Luisi, P. L.; Allegretti, M.; de Souza, T. P.; Steiniger, F.; Fahr, A.; Stano, P. Spontaneous protein crowding in liposomes: A new vista for the origin of cellular metabolism. *ChemBioChem* **2010**, *11*, 1989−1992.
(14) de Souza, T. P.; Steiniger, F.; Stano, P.; Fahr, A.; Luisi, P. L. Spontaneous crowding of ribosomes and proteins inside vesicles: A possible mechanism for the origin of cell metabolism. *ChemBioChem* **2011**, *12*, 2325−2330.
(15) Stano, P.; Luisi, P. L. Semi-synthetic minimal cells: Origin and recent developments. *Curr. Opin. Biotechnol.* **2013**, *24*, 633−638.
(16) Mclaughlin, J.; Luca, M. G.; Jones, M. N.; D'Amato, M.; Dockray, G. J.; Thompson, D. G. Fatty acid chain length determines cholecystokinin secretion and effect on human gastric motility. *Gastroenterology* **1999**, *116*, 46−53.
(17) Rybczynska, M.; Csordas, A. Chain length-dependent interaction of free fatty acids with the erythrocyte membrane. *Life Sci.* **1989**, *44*, 625−632.
(18) Kragh-Hansena, U.; Watanabe, H.; Nakajou, K.; Iwao, Y.; Otagiri, M. Chain length-dependent binding of fatty acid anions to human serum albumin studied by site-directed mutagenesis. *J. Mol. Biol.* **2006**, *363*, 702−712.
(19) Sun, Y.-L.; Wang, S.-S.; Han, X.; Chen, Z.-X. Realization of the reversible vesicle−micelle transition of Vitamin-derived bolaamphiphiles by heat change monitoring. *J. Phys. Chem. B* **2012**, *116*, 12372−12380.
(20) Chen, Z.-X.; Cao, C.; Deng, S.-P. Chaotrope-assisted color visualization mechanism and thermodynamics involved in molecular recognition of melamine by bolaamphiphiles embedded in polydiacetylene vesicles. *Acta Phys.-Chim. Sin.* **2012**, *28*, 1320−1328.
(21) Chen, Z.-X.; Su, X.-X.; Deng, S.-P. Molecular recognition of melamine by vesicles spontaneously formed from orotic acid derived bolaamphiphiles. *J. Phys. Chem. B* **2011**, *115*, 1798−1806.
(22) Walde, P.; Wick, R.; Fresta, M.; Mangone, A.; Luisi, P. L. Autopoietic Self-Reproduction of Fatty Acid Vesicles. *J. Am. Chem. Soc.* **1994**, *116*, 11649−11654.
(23) Morigaki, K.; Walde, P. Fatty acid vesicles. *Curr. Opin. Colloid Interface Sci.* **2007**, *12*, 75−80.
(24) Long, M. S.; Jones, C. D.; Helfrich, M. R.; Mangeney-Slavin, L. K.; Keating, C. D. Dynamic microcompartmentation in synthetic cells. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 5920−5925.
(25) Christiansen, A.; Wang, Q.; Samiotakis, A.; Cheung, M. S.; Wittung-Stafshede, P. Factors defining effects of macromolecular crowding on protein stability: An in Vitro/in Silico case study using Cytochrome *c*. *Biochemistry* **2010**, *49*, 6519−6530.
(26) Kilburn, D.; Roh, J. H.; Guo, L.; Briber, R. M.; Woodson, S. A. Molecular crowding stabilizes folded RNA structure by the excluded volume effect. *J. Am. Chem. Soc.* **2010**, *132*, 8690−8696.
(27) Wolfbeis, O. S.; Giirakar, A. The effect of fatty acid chain length on the rate of arylester hydrolysis by various albumins. *Clin. Chim. Acta* **1987**, *164*, 329−337.
(28) Mitrinova, Z.; Tcholakova, S.; Popova, Z.; Denkov, N.; Dasgupta, B. R.; Ananthapadmanabhan, K. P. Efficient control of the rheological and surface properties of surfactant solutions containing C8−C18 fatty acids as cosurfactants. *Langmuir* **2013**, *29*, 8255−8265.
(29) Gebicki, J. M.; Hicks, M. Ufasomes are stable particles surrounded by unsaturated fatty acid membranes. *Nature* **1973**, *243*, 232−234.
(30) Haines, T. H. Anionic lipid headgroups as a proton-conducting pathway along the surface of membranes: A hypothesis. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 160−164.
(31) Blochliger, E.; Blocher, M.; Walde, P.; Luisi, P. L. Matrix effect in the size distribution of fatty acid vesicles. *J. Phys. Chem. B* **1998**, *102*, 10383−10390.
(32) Berclaz, N.; Muller, M.; Walde, P.; Luisi, P. L. Growth and transformation of vesicles studied by ferritin labeling and cryotransmission electron microscopy. *J. Phys. Chem. B* **2001**, *105*, 1056−1064.
(33) James, J.; Ramalechume, C.; Mandal, A. B. Self-diffusion studies on PEO−PPO−PEO triblock copolymer micelles in SDS micelles and vice versa using cyclic voltammetry. *Chem. Phys. Lett.* **2005**, *405*, 84−89.
(34) Mahajan, R. K.; Chawla, J.; Bakshi, M. S. Effects of monomeric and polymeric glycol additives on micellar properties of Tween nonionic surfactants as studied by cyclic voltammetry. *Colloids Surf., A* **2004**, *237*, 119−124.
(35) Buhse, T.; Nagarajan, R.; Lavabre, D.; Micheau, J. C. Phase-transfer model for the dynamics of "micellar autocatalysis". *J. Phys. Chem. A* **1997**, *101*, 3910−3917.
(36) Buhse, T.; Lavabre, D.; Nagarajan, R.; Micheau, J. C. Origin of autocatalysis in the biphasic alkaline hydrolysis of C-4 to C-8 ethyl alkanoates. *J. Phys. Chem. A* **1998**, *102*, 10552−10559.