Insights into the role of biomineralizing peptide surfactants on making nanoemulsion-templated silica nanocapsules

*Yue Hui, David Wibowo, and Chun-Xia Zhao**

Australian Institute for Bioengineering and Nanotechnology, The University of Queensland, St.
Lucia QLD 4072, Australia

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ABSTRACT

We recently developed a novel approach for making oil-core silica-shell nanocapsules using designed bifunctional peptides (also called biomineralizing peptide surfactants) having both surface activity and biomineralization activity. Using the bifunctional peptides, oil-in-water nanoemulsion templates can be readily prepared, followed by the silicification directed exclusively onto the oil droplet surfaces thus the formation of silica shell. To explore their roles in the synthesis of silica nanocapsules, two bifunctional peptides AM1 and SurSi were systematically studied and compared. The peptide AM1, which was designed as a stimuli-responsive surfactant, demonstrated a quick adsorption kinetics with a rapid decrease of the oil–water interfacial tension, thus resulting in the formation of nanoemulsions with a droplet size as small as 38 nm. Additionally, the nanoemulsions showed good stability over four weeks because of the formation of a histidine–Zn²⁺ interfacial network. In comparison, the peptide SurSi which was designed by modularizing an AM1-like surface-active module with a highly cationic biosilicification-active module was unable to effectively reduce the oil–water interfacial tension due to its high molecular charge at neutral pH. The slow adsorption resulted in the formation of less stable nanoemulsions with a larger size (60 nm) than that of AM1. Besides, both AM1 and SurSi were found able to induce biomimetic silica formation. SurSi produced well dispersed and uniform silica nanospheres in the bulk solution, while AM1 only generated irregular silica aggregates. Consequently, well-defined silica nanocapsules were synthesized using SurSi nanoemulsion templates, whereas silica aggregates instead of nanocapsules predominated when templating AM1 nanoemulsions. This finding indicated that the capability of peptide surfactants to form isolated silica nanospheres might play a role in the successful fabrication of silica nanocapsules. This fundamental study provides insights into the design of bifunctional peptides for making silica nanocapsules.

INTRODUCTION

Nanomaterials having a core–shell architecture have been intensively studied in the past decades due to their unique properties such as a large inner cavity and a high loading-capacity.^{1–3} Among these emerging materials, silica-based nanocapsules are of great industrial and research interests because they synergistically combine the properties of both silica (e.g., biocompatibility,⁴ mechanical robustness⁵ and easy surface functionalization⁶) and the core–shell structure. Compared to solid and mesoporous silica nanoparticles, which can only accommodate active molecules either on the particle surface or inside the pores within the mesoporous structure, thus having limited loading capacities and uncontrolled burst release, silica nanocapsules have been used to encapsulate various active substances (drugs,^{1,5} pesticides,⁷ fluorescent dyes,⁸ magnetic nanoparticles,⁹ and semiconductors¹⁰) within their core domain to allow sustained release capability and a protective environment, which promises the potential applications of silica nanocapsules in bio-imaging, chemical sensing and targeted drug delivery.

Templated synthesis is the most commonly employed method for making silica nanocapsules.⁵ It generally involves four main steps: (1) preparation of either hard (e.g., polystyrene¹¹ and iron oxides¹²) or soft (e.g., bacteria,¹³ vesicles,¹⁴ gas bubbles¹⁵ and emulsion droplets⁷) templates; (2) functionalization of the template surfaces to facilitate the silicification (if applicable); (3) condensation of the silica exclusively on the template surfaces to form a compact shell; and (4) selective removal of the templates. Among these processes, step (4) is regarded as challenging and time-consuming for hard templates, because it always involves the employment of high-temperature calcination and/or toxic dissolving-agents, which can possibly result in the rupture of the silica shell.^{1,3,11} In contrast, soft template strategy offers less harsh ways of removing templates through evaporation or dissolution in mild solvents such as ethanol, thus has attracted considerable

attention in recent years.¹ Additionally, in order to promote the growth of silica shell at interfaces (step (3)), various approaches have been explored based on a sol–gel process, such as Stöber and reverse microemulsion methods.⁵ Although these methods are able to effectively facilitate the formation of silica shell, they often involve using toxic chemicals and harsh synthetic conditions (e.g., elevated temperature, extreme acidic/basic pH), which are usually harmful to our environment. Therefore, a more sustainable and biocompatible approach is urgently needed for making silica nanocapsules, especially for agricultural, cosmetic, pharmaceutical and medical applications.

The discovery of biosilicification in marine organisms has provided an alternative environmentally-friendly strategy of silica formation. Specifically, diatoms^{16,17} and sponges^{18,19} were found able to generate silica-based exoskeletons and spicules under benign conditions, and proteins silaffin and silicatein isolated from their cells were proved to play important roles in the formation of these structures.^{16,19} Study on these proteins revealed their distinct sequence feature, such as densely packed with hydroxyl amino acids (e.g., serine, tyrosine) and/or cationic amino acids (e.g., lysine, arginine, histidine), and further studies confirmed that these amino acid residues can interact with silica precursors through either hydrogen bond formation or electrostatic attraction, thus promoting the nucleation and subsequent growth of silica.^{16,17,20} In this regard, a number of amino acid-based catalysts, such as peptide R5,²¹ poly-lysine²² and block copolypeptides²³ have been employed to induce silica formation at ambient temperature and neutral pH, which offered a new approach for making silica-based nanomaterials. Graf et al.²⁴ reported the successful formation of silver-core silica-shell nanoparticles by modifying the silver nanoparticles with a designed tripeptide, cysteine–lysine–lysine. Park et al.¹³ designed an arginine-

and cysteine-rich peptide that can adsorb to the surface of yeast cells and facilitate the formation of cytoprotective silica shells.

We recently developed a novel and facile method, namely, a nanoemulsion and biosilicification dual-templating technology, for making silica nanocapsules.⁷ This technology is based on the design of bifunctional peptides by modularizing a surface active peptide with a biomineralizing amino acid sequence. The preparation of oil-in-water nanoemulsions facilitated by the surface active domain along with the formation of silica shell at the oil–water interface induced by the catalytic peptide sequence led to the formation of oil-core silica-shell nanocapsules at room temperature and near-neutral pH without using any toxic reagents. Furthermore, hydrophobic active components can be loaded by directly dissolving in the oil phase prior to the formation of nanoemulsions and silica shells, allowing a high-capacity pre-loading strategy. Additionally, the silica shell thickness can be tuned by controlling the reaction time and silica precursor concentration, endowing a shell-thickness dependent sustained-release profile.⁷

The development of this dual-templating technology provides a unique opportunity to expand molecular and engineering knowledge across the fields of sol–gel chemistry, interface science, biomolecular engineering and biomimetic synthesis. By taking advantage of our capability in precisely tuning the molecular properties and functions of the designed peptides, we attempted to discover the fundamental links between the sequence design of bifunctional peptides and their interfacial properties as well as biosilicification activities, aiming to gain better understanding and control over the fabrication of silica-based nanocapsules. Therefore, in this study, two different peptide designs (i.e., AM1 and SurSi) were studied and compared systematically, including their surface activities, their abilities in making nanoemulsions and their stability, as well as their biomimetic silification activities both in bulk and at oil-water. The findings in this study will

generate a deeper understanding of the fundamental mechanisms of this dual-templating method we developed, which will in turn provide a theoretical guidance for the further design of bifunctional peptides and the synthesis of other core-shell materials with desirable properties.

EXPERIMENTAL SECTION

Materials. Peptides AM1 (*MW* 2473) and SurSi (*MW* 3632) were custom-synthesized by GenScript Corporation (Piscataway, NJ). The purity of each peptide was $\geq 95\%$ by reversed-phase high-performance liquid chromatography (RP-HPLC). Miglyol 812 oil was purchased from Caesar & Loretz GmbH (Hilden, Germany) and was passed through heat-activated silica gel (Sigma-Aldrich, USA) prior to use. Water having a resistivity of $>18.2 \text{ M}\Omega \cdot \text{cm}$ was obtained from a Milli-Q system (Millipore, Australia) equipped with a $0.22 \mu\text{m}$ filter. Other reagents and chemicals were of analytical grade, purchased from Sigma-Aldrich and used as received unless otherwise stated.

Interfacial Tension Study. A drop shape analysis (DSA) system DSA-10 (Krüss GmbH, Germany) was employed to measure oil-water interfacial tension. To measure interfacial tension kinetics, a quartz cuvette (Hellma GmbH, Germany) was filled with a peptide solution sample (8 mL) of either AM1 or SurSi in 4-(2-hydroxyethyl)-1-piperazine ethanesulfonate acid (HEPES) buffer (25 mM, pH 7). A Miglyol 812 oil droplet was formed in the peptide solution through an inverted stainless steel needle with a diameter of 1.507 mm connected to a glass syringe. The interfacial tension was measured automatically over 400 s after the initial formation of an oil droplet. Prior to the measurement, the instrument was calibrated by forming a pure oil droplet (Miglyol 812) in the buffer, confirming a constant interfacial tension of approximately 29 mN/m and remained unchanged for 10 min. To observe the formation of interfacial network, contraction

experiments were carried out. An oil droplet was formed in the peptide solution (20 μM) and aged for 30 min, then a sudden contraction in its volume was imposed and the process was recorded using a camera attached to the instrument.

Nanoemulsion Preparation and Stability Study. To make nanoemulsions, a certain amount of lyophilized AM1 or SurSi was dissolved in HEPES buffer (25 mM, pH 7) and diluted into five different concentrations (50, 100, 200, 400 and 800 μM), followed by the addition of ZnCl_2 (at twice the concentration of either AM1 or SurSi). Miglyol 812 oil (2% v/v) was then added to the peptide solutions (800 μL), and the mixtures were sonicated using a Sonifier 450 ultrasonicator (Branson, U.S.A) for four 30 s bursts at 40 W and interspersed in an ice bath for 60 s. To study the effect of storage temperature on the nanoemulsion stability, every nanoemulsion sample was divided equally and stored separately at room temperature and 4 $^{\circ}\text{C}$. Number-averaged diameter and zeta potential of the samples were measured at certain time points (day 1, 2, 3, 7, 14 and 28) over 28 days.

Biomimetic Silicification in Bulk. The AM1 or SurSi peptide solution (400 μM) was prepared by dissolving lyophilized peptide in HEPES buffer (25 μM , pH 7.5). Then a 400 μL aliquot was transferred into a 4-mL glass vial containing 45 μmol tetraethoxysilane (TEOS). Hydrolysis and condensation of TEOS were conducted under magnetic stirring at room temperature for 24 h. A negative control reaction of TEOS (45 μmol) in HEPES buffer (25 μM , pH 7.5) was conducted in parallel.

Oil-Core Silica-Shell Nanocapsules. Nanoemulsion with a peptide concentration of 400 μM (either AM1 or SurSi) was prepared following the procedures aforementioned, and then was dialyzed in HEPES buffer (25 mM, pH 7.5) with a molecular weight cut-off of 10 kDa (SnakeSkin Dialysis Tubing, Thermo Fisher Scientific, Australia) for 20 h to remove the residual peptide in

the bulk solution. An aliquot of dialyzed nanoemulsion (400 μ L) was then transferred into a 4-mL glass vial containing 32 μ mol TEOS. Hydrolysis and condensation of TEOS were conducted in the presence of the nanoemulsion under magnetic stirring at room temperature for 30 h.

Characterization. *Dynamic Light Scattering (DLS).* Size distribution and zeta potential of the nanoemulsion were measured by DLS using a Malvern Zetasizer Nano ZS (Malvern Instrument, UK) at a scattering angle of 173° and a temperature of 25 °C. Samples were diluted by a factor of 50 for measurement to avoid multiple scattering effects.

Transmission Electron Microscopy (TEM). Morphology of the silicification products was observed by TEM using a JEOL 1010 transmission electron microscope (JEOL, Japan) operated at 100 kV. To prepare TEM specimens, suspensions of silicification products (2 μ L) were placed onto Formvar-coated copper grids (ProSciTech, Australia) and the grids were then dried in air. The size of the nanocapsules was analyzed by using iTEM software (version 3.2, Soft Imaging System GmbH).

RESULTS AND DISCUSSION

Design of Two Different Peptides. Two peptides AM1 and SurSi were designed in our lab based on distinct principles. The peptide AM1 (Ac-MKQLADS LHQLARQ VSRLEHA-CONH₂) was designed²⁵ as a stimuli-responsive surfactant, based on a well-studied amphipathic peptide Lac21 (Ac-MKQLADS LMQLARQ VSRLESA-CONH₂). Like its parent sequence Lac21, AM1 shows excellent surface activity as it can adsorb quickly at the interface and decrease the interfacial tension dramatically. Moreover, the two metal-binding histidine residues located at positions 9 and 20 allow the formation of a cohesive interfacial network at the interface in the presence of metal ions such as Zn²⁺, which not only enhances the stability of foams and emulsions, but also provides

them with a switching mechanism either by adding chelating agents or by pH.²⁶ At the same time, as demonstrated by previous studies, peptides and proteins bearing hydrogen bond forming residues (e.g., glutamine, serine) and/or cationic residues (e.g., arginine, histidine, lysine), which are present in the AM1 sequence, were able to induce silicification through interactions with silica precursors.^{16,17,27-29} Additionally, a pair of serine–histidine has also been proved critical in biomimetic silicification.^{18,20} This is consistent with an earlier study that the serine–histidine pair in AM1 were believed to catalyze the TiO₂ biomineralization.³⁰ Based on these considerations, AM1 is expected to be able to facilitate the biomimetic formation of silica.

The peptide SurSi (Ac-MKQLAHS VSRLEHA RKKRKKRKKRKKGGGY-CONH₂)⁷ adopted a dual-module design by modularizing a sequence consisting two heptads (MKQLAHS VSRLEHA) of AM1 for surface activity (i.e., Sur module), with a highly cationic peptide sequence (RKKRKKRKKRKKGGGY) for biosilicification activity (i.e., Si module). This modular design allows facile synthesis of silica nanocapsules in two sequential steps, i.e., formation of oil-in-water nanoemulsion templates followed by the interfacial biosilicification.

Surface Activity. It is of importance to investigate the surface activity of peptides AM1 and SurSi, as it strongly affects the size and size distribution of the prepared nanoemulsion templates, which will further determine the corresponding properties of the synthesized silica nanocapsules. The interfacial tension kinetics of Miglyol 812 oil droplets in the peptide solutions were studied and compared for AM1 and SurSi. Figure 1a shows a rapid drop of the interfacial tension of the oil-in-AM1 aqueous solutions. At a concentration of 5 μ M AM1, the interfacial tension started from 29 mN/m, similar to that of the pure Miglyol 812 oil in the HEPES buffer (25 mM, pH 7), and dropped to 17.2 mN/m by 100 s before reaching an equilibrium of 16.6 ± 0.1 mN/m. Increasing the AM1 concentration to 10 and 20 μ M dramatically accelerated its adsorption to the oil–water

interface, resulting in a more rapid decrease of the interfacial tension. This is consistent with previous finding that the initially rapid interfacial tension reduction shortens as the surfactant concentration increases.^{31,32}

Figure 1. Dynamic interfacial tension of Miglyol 812 oil droplets in peptide solutions at pH 7. (a) AM1 and (b) SurSi in HEPES buffer (25 mM, pH 7).

Compared to AM1, the interfacial tension profiles of Miglyol 812 droplets in the SurSi show a slower adsorption kinetics at pH 7 (Figure 1b). When the SurSi concentrations are 5, 10 and 20 μM , the oil–water interfacial tension do not experience an initially rapid dropping, but decrease slowly from 29 mN/m to 28.4, 28.3 and 28.1 mN/m, respectively, in a time period of 400 s. Then,

a much higher concentration of 400 μM SurSi was tested, and the interfacial tension declined relatively faster at the beginning, reaching around 27 mN/m at 30 s, then a final equilibrium (25.9 ± 0.1 mN/m) by 400 s. So in comparison with AM1, the surface activity of SurSi at neutral pH is much weaker, suggesting that the adsorption of SurSi molecules from the bulk solution to the interface is a very slow process. Considering that the Si module of SurSi is densely packed with positively charged amino acid residues, i.e., arginine (R) and lysine (K), the electrostatic repulsion between the peptide molecules might play an important role in retarding their adsorption. The theoretical peptide net charge can be calculated through a derivation of the Henderson-Hasselbalch equation,³³

$$Q_{\text{peptide}} = \sum_i \frac{(+1)}{1+10^{+(\text{pH}-\text{pKa}_i)}} + \sum_j \frac{(-1)}{1+10^{-(\text{pH}-\text{pKa}_j)}} \quad (1)$$

where pKa_i are the pKa values of the *N*-terminus and the side chains of arginine (R), lysine (K) and histidine (H), pKa_j are the pKa values of the *C*-terminus and the aspartic acid (D), glutamic acid (E), cysteine (C) and tyrosine (Y). The theoretical net charges of SurSi and AM1 molecules at pH 7 calculated using Eq 1 are 13.44 and 1.23, respectively. Therefore, in contrast to AM1, the electrostatic repulsion between SurSi molecules is much stronger, leading to their slow adsorption from the bulk solution to the oil–water interface, hence the slow dropping of the interfacial tension. Moreover, when 400 μM SurSi was used, the equilibrium of interfacial tension was still relatively high, which was assumed to be the result of a low surface coverage of SurSi on the interface.³¹

As shown in a previous study, the highly negatively charged peptide Lac21E (pI 3.95) was less surface active at neutral pH, but became very surface active with a lower interfacial tension at the same concentration when the pH was dropped to 3, which is close to its pI .³⁴ The corresponding neutron reflectometry experiment demonstrated a high surface coverage at pH 3 and depopulation of the peptide molecules from the interface at neutral pH, which in turn explained the significant

drop of the interfacial tension from neutral pH to pH 3. Based on the finding of Lac21E, the interfacial tension of SurSi at higher pH conditions (pH 10.3 and 11.8 at which the theoretical net charges of SurSi are 8.8 and 0, respectively) was investigated (Figure 2). Although the interfacial tension did not experience an initially rapid reduction, it decreased much faster compared to that at pH 7 i.e., from a starting point of 26.0 mN/m to a very low level of only 9.9 and 6.9 mN/m by 200 s at pH 10.3 and 11.8, respectively. This result confirmed the presence of the strong electrostatic repulsion between SurSi molecules at neutral pH and showed that reducing the molecular net charge by simply adjusting the pH can result in faster adsorption and higher surface coverage of surfactant molecules at the interface. In fact, the Sur module of SurSi was designed by using only two of the three heptads of AM1 in order to promote fast adsorption, as the square of interfacial adsorption rate is inversely proportional to the molecular weight of peptide.⁷ However, compared to molecular weight, the intermolecular electrostatic interaction of SurSi turns out to be much more prominent in affecting its surface activity (Figure 2).

Figure 2. Dynamic interfacial tension of Miglyol 812 oil droplets in 20 μ M SurSi solution (25 mM HEPES buffer) at pH 7, 10.3 and 11.8.

Contraction experiments were further carried out using the peptide solutions (20 μM) in the presence of Zn^{2+} to visually observe the formation of interfacial network (Figure 3). For AM1+ Zn^{2+} at pH 7, the oil droplet shape remained almost unchanged after 30-min of aging (Figure 3a), but when the droplet shrunk rapidly by sucking out the oil through a needle, the droplet surface became wrinkled which, to some extent, confirmed the presence of a viscoelastic interfacial film because of the interaction between histidine (H) and Zn^{2+} .^{25,26} In contrast, for SurSi + Zn^{2+} at pH 7, no wrinkle was observed as the droplet shrunk, suggesting that no substantial film was formed at the droplet surface (Figure 3b). This was mainly due to the low surface coverage of SurSi at pH 7, as previously explained, thus the adjacent molecules were too distant to be connected by the histidine– Zn^{2+} interaction.

Figure 3. Photographs of a 30 min-old oil droplet formed from an inverted needle in (a) 20 μM AM1 solution at pH 7, (b) 20 μM SurSi solution at pH 7, in the presence of 40 μM ZnCl_2 . The droplets were subjected to a sudden contraction in volume, and the images were acquired using the drop shape analysis system.

Nanoemulsion Properties. The successful fabrication of silica nanocapsules depends on the formation of nanoemulsions having good size distribution and stability during the biosilicification

process, hence it is important to understand the basic properties of the nanoemulsions (i.e., size and zeta potential) and their stability over a period of time.

Nanoemulsion preparation vs. peptide concentration. To study the effect of peptide concentration on the properties of the produced nanoemulsions, Miglyol 812 oil-in-water nanoemulsions were prepared using a series of peptide concentrations (50, 100, 200, 400 and 800 μM). The number-averaged diameter (d_n) and zeta potential data for the nanoemulsions prepared using AM1 (Figure 4a) and SurSi (Figure 4b) are presented in Figure 4. At a concentration of 50 μM AM1, the d_n of the nanoemulsion is approximately 160 nm, and it decreases monotonously as the concentration increases, reaching 38 nm at 800 μM of AM1. SurSi is also capable of facilitating the formation of nanoemulsions despite its slow adsorption and the high interfacial tension at neutral pH, but the sizes of nanoemulsions are larger than that of AM1 at the same concentrations. In addition, similar to AM1, the d_n of SurSi nanoemulsions also decreases monotonously with the SurSi concentration, from 180 nm at 50 μM to 60 nm at 800 μM .

Figure 4. Droplet size and zeta potential of nanoemulsions prepared using (a) AM1 or (b) SurSi at various concentrations (50, 100, 200, 400 and 800 μM) in the presence of ZnCl_2 in HEPES buffer (25 mM, pH 7). ZnCl_2 was added at twice the concentration of the peptides.

The droplet size of an emulsion depends on various factors, such as energy input, adsorption kinetics and surfactant concentration.^{35,36} At the same surfactant, when the energy input is fixed, the droplet size solely relies on the surfactant concentration until the droplet interface is saturated. Therefore, the decrease of droplet size with increasing concentration was observed for both AM1 and SurSi, and this decreasing trend became less pronounced at a high concentration region (after 400 μM). This finding is similar to an earlier report by Tcholakova et al.³⁵ that the effect of emulsifier concentration on the mean diameter of emulsion droplets became negligible after it exceeded a certain value. In the meantime, as the adsorption rate of SurSi is much slower than that

of AM1, the droplet sizes of SurSi nanoemulsions were found bigger than that of AM1 at the same concentrations.

The zeta potentials of both AM1 and SurSi nanoemulsions showed an overall rising trend as the concentration of peptides increased. Nanoemulsion prepared using 50 μM AM1 already displayed a quite high zeta potential (+44 mV), which then grew to +63 mV at 400 μM AM1 and remained relatively stable at around +60 mV when further increased the AM1 concentration to 800 μM . In comparison, the zeta potentials of nanoemulsions made by 50 and 100 μM of SurSi were only +21 mV, and increased steadily to +33 mV at 800 μM of SurSi. The SurSi nanoemulsions gave lower zeta potentials than that of AM1 at the same concentrations (Figure 4b), even though SurSi is more positively charged, which we believe is mainly due to the higher surface coverage of AM1 molecules on the droplet surface. The contribution of chelated Zn^{2+} is negligible as the nanoemulsions using AM1 and AM1 + Zn^{2+} (at an AM1 concentration of 400 μM) have the same zeta potential values at 64 ± 1 mV.

Nanoemulsion Stability. Droplet size and zeta potential of AM1 and SurSi nanoemulsions were monitored for 28 days at either room temperature or 4 °C to investigate their stabilities. Figure 5 demonstrates the size and zeta potential evolutions of AM1 nanoemulsions as a function of time. d_n of the 50 μM AM1 nanoemulsion grew dramatically from 160 to 340 nm (at room temperature, Figure 5a) and to 270 nm (at 4°C, Figure 5b) in the 28 days, indicating that this concentration was insufficient to effectively stabilize the nanoemulsion. In comparison, AM1 nanoemulsions at a concentration of 100 μM or higher experienced a much smaller size increase when stored at either room temperature or 4 °C, reflecting better emulsion stabilities. This is due to the effective formation of AM1– Zn^{2+} interfacial network on the droplet surface at these higher concentrations, which is capable of slowing down droplet coalescence.²⁵ Zeta potential evolution profiles were

also observed for the AM1 nanoemulsions stored at either room temperature (Figure 5c) or 4 °C (Figure 5d). Corresponding to its notable size variation, zeta potential of the nanoemulsion prepared with 50 μM AM1 also experienced a significant change, increasing from +45 to around +63 mV at both storage temperatures. As the AM1 concentration increased, the zeta potential increments of the nanoemulsions became less significant, until a stable value of $+62 \pm 2$ mV was recorded during the aging when the concentration reached 400 μM or higher (800 μM).

Figure 5. Droplet size (a, b) and zeta potential (c, d) of the AM1 nanoemulsions stored at room temperature (a, c) and 4 °C (b, d) in four weeks.

Figure 6 illustrates the variations of droplet sizes and zeta potentials of SurSi nanoemulsions in four weeks. Because of the relatively high polydispersity indexes (Đs) for the nanoemulsions prepared using 50 and 100 μM of SurSi (Đs were above 0.3 for the freshly prepared samples and grew to over 0.4 in the second day), their results are not presented. When stored at room

temperature, d_n of the nanoemulsions prepared with 200 and 800 μM of SurSi increased noticeably on the third day and then remained relatively stable, while at 400 μM of SurSi the droplet size of the prepared nanoemulsions grew gradually in the first week before it reached a stable d_n (Figure 6a). At 4 °C, profiles of the 200 and 800 μM samples again showed a similar trend, with their droplet sizes increasing from the fourth day to the end of the first week and remaining stable afterwards (Figure 6b). Interestingly, the nanoemulsion prepared with 400 μM SurSi experienced no notable changes in the first two weeks, showing a better stability compared to the other two samples. Therefore, 400 μM SurSi was used for the preparation of silica nanocapsules. As for the zeta potential, an overall rising trend was observed for all of the three samples at room temperature, which might be caused by the continuing adsorption of the excess SurSi molecules from bulk to the droplet surfaces (Figure 6c), while this adsorption seemed very slow at 4 °C as the zeta potentials only experienced little variations (Figure 6d). Overall, both AM1 and SurSi nanoemulsions demonstrated better stability at 4 °C than those at room temperature.

Figure 6. Droplet size (a, b) and zeta potential (c, d) of the SurSi nanoemulsions stored at room temperature (a, c) and 4 °C (b, d) in four weeks.

Biomimetic Silicification in Bulk. The silicification activity of AM1 and SurSi was investigated by reacting TEOS with the peptide in aqueous solutions for 24 h. Representative TEM images of the products after 12 and 24 h are shown in Figure 7. Silica precipitates with distinct morphologies were obtained for AM1 and SurSi, with the negative control group (TEOS mixed with only HEPES buffer) showing no silica formation after 24 h (data not shown). In the AM1 solution, silica aggregates with irregular shape dominated after 12 h, whereas in the SurSi solution, small silica particles with a uniform size of less than 50 nm in diameter were observed to be well-dispersed in the solution. As the reaction continued to 24 h, the morphological difference between the products in AM1 and SurSi solutions became more pronounced. Larger aggregates were

observed for the AM1 (Figure 7c), and silica nanoparticles (80 nm in diameter) formed in the SurSi solution had a regular spherical morphology and high dispersity (Figure 7d).

The distinct morphological features of the silicification products indicated that AM1- and SurSi-mediated biosilicification involved different mechanisms as a result of their different sequence designs. The Si module of SurSi was designed to be densely packed with lysine (K) and arginine (R) residues, which were believed to be involved in the catalysis of silicification at benign conditions through electrostatic interactions with anionic silica precursors.^{16,17,27,28} The negatively charged monomeric and/or oligomeric silicate species can be attracted into close proximity with the positively charged Si molecule and favor their polycondensation forming silica nanoparticles with high uniformity and dispersity (Figure 7b, d). In contrast, the cationic and hydrogen-bond forming residues^{5,16,29} and the serine–histidine pairs^{18,20} necessitate for inducing silica precipitation which located at random positions within AM1 sequence were unable to form well-dispersed silica nanoparticles (Figure 7a, c).

Figure 7. Representative transmission electron microscopy (TEM) images of biomineralized silica nanoparticles formed in the AM1 and SurSi bulk solutions. Sample was taken 12 h and 24 h after the initiation of the biomineralizing reaction.

Silica Nanocapsules. As demonstrated in the above experiments, AM1 and SurSi exhibited different surface activity and biosilicification activity due to their distinct amino acid sequence designs. Therefore, to further study the effect of different module designs on the synthesis of oil-core silica-shell nanocapsules, nanoemulsions prepared using either AM1 or SurSi at 400 μ M were mixed with a silica precursor TEOS and reacted for 30 h under vigorous stirring. Figure 8 shows the representative TEM images of the products. When AM1 nanoemulsion was used as the template, massive aggregation of silica dominated (Figure 8a), which was consistent with the silicification products observed in the AM1 bulk solution (Figures 7a, c), indicating that there was no intrinsic difference between the biosilicification induced in bulk and at the interface. However, silica growth was directed exclusively at the surfaces of the SurSi nanoemulsion droplets, resulting in the formation of uniform silica nanocapsules with well-defined core-shell structure (Figure 8b).

Despite having both emulsifying ability and biosilicification-catalyzing ability, AM1 failed to facilitate the formation of nanocapsules because of the aggregated structure of the silica produced. However, peptide SurSi, which allowed the formation of isolated silica nanoparticles (Figure 7b, d), was able to facilitate the formation of well-defined silica nanocapsules (Figure 8b), which demonstrated the critical and distinct roles played by the two modules in SurSi (surface-active module and biosilicification-active module).

Figure 8. Representative transmission electron microscopy (TEM) images of silica nanocapsules formed using (a) AM1 and (b) SurSi nanoemulsions.

CONCLUSIONS

Two peptides SurSi and AM1 with different design principles were studied and compared systematically for their properties, including surface activity, emulsifying capacity and biosilicification activity. As a stimuli-responsive peptide surfactant, AM1 showed excellent surface activity, i.e., fast adsorption, very low interface tension and the formation of viscoelastic interfacial network. Therefore very stable nanoemulsions were formed using AM1. In comparison, SurSi having a highly cationic Si module showed a retarded adsorption kinetics and high interfacial tension at neutral pH due to the strong electrostatic repulsion. As a result, SurSi generated nanoemulsions with larger sizes and lower zeta potentials, and are less stable than that prepared by AM1. In terms of biomimetic silicification, AM1 and SurSi showed distinct results both in bulk

solutions and at droplet interfaces. AM1 was only able to induce the formation of silica aggregates with irregular morphologies, thus no silica nanocapsules were made using AM1 nanoemulsion template. However, SurSi produced well-dispersed spherical silica nanoparticles in its bulk solution, and hence well-defined silica nanocapsules. The comparison between SurSi and AM1 leads us to better understand the role of the peptide sequence in affecting their surface activity, biosilicification activity and the formation of silica nanocapsules, which is of great importance for the future design of the bifunctional peptides for making core–shell nanomaterials having desirable properties.

AUTHOR INFORMATION

Corresponding Authors

*E-mail: z.chunxia@uq.edu.au.

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

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ABBREVIATIONS

A, alanine; D, aspartic acid; E, glutamic acid; G, glycine; H, histidine; K, lysine; L, leucine; M, methionine; P, proline; Q, glutamine; R, arginine; S, serine; V, valine; Y, tyrosine; DLS, dynamic light scattering; DSA, drop shape analysis; d_n , number-averaged diameter; HEPES, sodium 4-(2-hydrox-yethyl)-1-piperazine ethanesulfonate; MW , molecular weight; $\mathcal{D}$, polydispersity index; pI , isoelectric point; RP-HPLC, reversed-phase high-performance liquid chromatography; TEM, transmission electron microscopy; TEOS, tetraethoxysilane.

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