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A New Type of Soft Vesicle-Forming Molecule: An Amino Acid Derived Guanidiniocarbonyl Pyrrole Carboxylate Zwitterion

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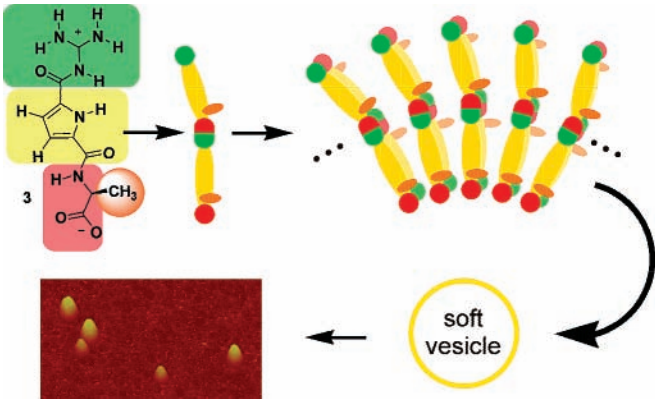
ABSTRACT



The self-assembly of the L-alanine derived zwitterion 3 leads to the formation of soft vesicles in solution even though this surprisingly small molecule does not possess the classical amphiphilic features of other vesicle-forming monomers.

The self-assembly of molecules can lead to the formation of large aggregates such as micelles, bilayers, or vesicles.[1] The size and structure of the aggregates formed are determined by the shape as well as kind of interactions between the monomers. The classical vesicle-forming molecules are amphiphilic lipids. Also other classes of amphiphiles, molecules with segmented hydrophilic and hydrophobic parts, form vesicles such as block-copolymers,[2] oligopeptides,[3] calixarenes, or cyclodextrins functionalized with lipophilic chains or hydrophobic shape persistent macrocycles with hydrophilic tails.[4] Bolaamphiphiles are another class of vesicle builders but also have the traditional amphiphilic

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(1) Reviews on vesicles: (a) Discher, D. E.; Eisenberg, A. *Science* **2002**, *297*, 967. (b) Antonietti, M.; Förster, S. *Adv. Mater* **2003**, *15*, 1323. (c) Morishima, Y. *Angew. Chem., Int. Ed.* **2007**, *46*, 1370.

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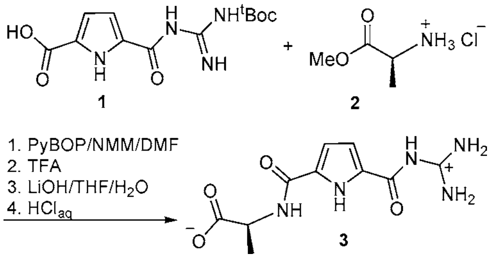
composition.[5] Dipeptides such as L-Phe-L-Phe can self-assemble into vesicles, although the kind of interaction and the aggregation mode leading to vesicle formation are unclear.[6] We report here that a molecule as small as zwitterion **3**, which does not have a classical amphiphilic structure with well-separated polar and unpolar segments, self-assembles into soft vesicles in DMSO.

The synthesis of zwitterion **3** is shown in Scheme 1: L-alanine methyl ester hydrochloride **2** was coupled with the Boc-protected guanidinocarbonyl pyrrole carboxylic acid **1**[7] using PyBOP in DMF as the coupling reagent (yield 63%). The Boc group was removed with TFA, and afterward, the methyl ester was cleaved with LiOH to yield zwitterion **3** (yield: 80%).

Scheme 1. Synthesis of Zwitterion **3**



Zwitterion **3** is self-complementary as the cationic guanidiniocarbonyl pyrrole[8] is an efficient binding site for carboxylates.[9] Intramolecular ion pairing of **3** is not possible for geometric reasons; therefore, an intermolecular ion pairing occurs as confirmed by the concentration-dependent shift changes in an NMR dilution study in DMSO-d_6 (Figure 1).[10] The rather small complexation induced shift change of the guanidinio amide NH proton *a* of $\Delta\delta = 0.47$ is indicative for the formation of a bidentate carboxylate-guanidinium ion pair as shown in Figure 1.[11] An NOE-contact between amide NH proton *b* and pyrrole CH proton *c* confirms that **3** is present in an extended conformation as shown with the amide NH of the alanine pointing to the back.

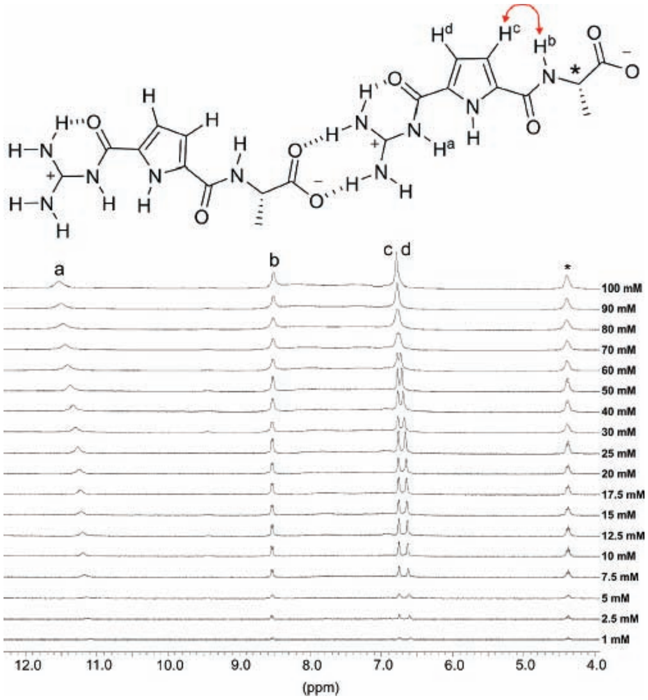


Figure 1. Self-association of zwitterion **3** (the red arrow indicates the NOE contact); parts of the ^{1}H NMR spectrum showing the concentration dependent shift changes in DMSO-d_6 (1−100 mM from bottom to top).

Unexpectedly, all NMR samples of higher concentration ($c > 10$ mM) showed a strong *Tyndall* effect, indicating the formation of even larger aggregates than just ion-paired dimers or smaller oligomers. Therefore, atomic force microscopy (AFM), dynamic light scattering (DLS), and transmission electron microscopy (TEM) were performed to further investigate the self-assembly of **3**. For AFM analysis, a 5 mM solution of **3** in DMSO was spin coated onto silica wafers and analyzed in the tapping mode. Figure 2 shows spherical particles with a mean diameter of ca. 25 nm (measured at half-height of the particles) and a height of ca. 4 nm, about six times smaller than the average diameter. The mean diameter of 25 nm of the particles is significantly larger than the molecular dimension of zwitterion **3** (*ca.* 1.4 nm estimated from molecular modeling; see Supporting Information). Therefore, it is very unlikely that the particles

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(4) Recent examples for vesicle formation from non-polymers: (a) Xie, D.; Jiang, M.; Zhang, G.; Chen, D. *Chem.−Eur. J.* **2007**, *13*, 3346. (b) Dong, D.; Baigl, D.; Cui, Y.; Sinay, P.; Sollogoub, M.; Zhang, Y. *Tetrahedron* **2007**, *63*, 2973. (c) Schmuck, C.; Rehm, T.; Klein, K.; Gröhn, F. *Angew. Chem., Int. Ed.* **2007**, *46*, 1693. (d) Seo, H. S.; Chang, J. Y.; Tew, G. N. *Angew. Chem., Int. Ed.* **2006**, *118*, 7688; (e) Balakrishnan, K.; Datar, A.; Zhang, W.; Yang, X.; Naddo, T.; Huang, J.; Zuo, J.; Yen, M.; Moore, J. S.; Zang, L. *J. Am. Chem. Soc.* **2006**, *128*, 6576. (f) Hoeben, F. J. M.; Shklyarevskiy, I. O.; Pouderoijen, M. J.; Engelkamp, H.; Schenning, A. P. H. J.; Christianen, P. C. M.; Maan, J. C.; Meijer, E. W. *Angew. Chem., Int. Ed.* **2006**, *45*, 1232. (g) Holowka, E. P.; Pochan, D. J.; Deming, T. J. *J. Am. Chem. Soc.* **2005**, *127*, 12423. (h) Lee, H.-K.; Park, K. M.; Jeon, Y. J.; Kom, D.; Oh, D. Y.; Kim, H. S.; Park, C. K.; Kim, K. *J. Am. Chem. Soc.* **2005**, *127*, 5006. (i) Lee, M.; Lee, S.-J.; Jiang, L.-H. *J. Am. Chem. Soc.* **2004**, *126*, 12724. (j) Zhou, Y.; Yan, D. *Angew. Chem., Int. Ed.* **2004**, *43*, 4896.

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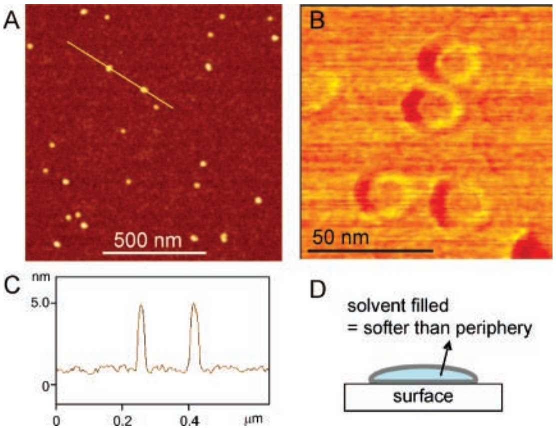

Figure 2. AFM height image (A) of **3** on silica wafer (Z scale is 12 nm) shows spherical aggregates. The cross section analysis along the yellow line (C) provides a mean diameter of ca. 25 nm and a height of 4 nm. The appearance of the vesicles in the phase image (B) is typical for deformed soft hollow vesicles on a surface (D).

are micelle-like aggregates, which generally have a diameter about twice as large the molecular dimension. Instead, the profile of the particles in the phase image (Figure 2B) clearly identifies these particles as soft, hollow vesicles, which typically show a lower central part surrounded by a higher periphery in AFM experiments.[2f,4a]

DLS experiments in solution ([**3**] = 5.55 mM in DMSO) also confirm the presence of vesicles in solution with a hydrodynamic radius of ca. $r_H = 20-25$ nm, resulting in a particle diameter of 40−50 nm.[12] The larger particle size seen in DLS is most likely due to the drying occurring during sample preparation in AFM, which causes shrinking of the particles. Also, the surface of the silica wafer can have an effect on the aggregate size in AFM experiments, whereas DLS measurements detect the real and original state of the aggregates in solution.

The vesicular nature of the aggregates was further confirmed by TEM. The TEM image (Figure 3) clearly shows a vesicle with a diameter of ca. 60 nm and an estimated membrane thickness of ca. 3 nm. In contrast to hard vesicles, the electron contrast is relatively weak as typically observed for soft vesicles in TEM studies.[2f,4a] Collapsed soft vesicles have an almost constant thickness from the periphery to the center of the particle (as seen in the height profile of the AFM image). However, as the particles were prepared from DMSO solution and not water, the hollow, solvent-filled inner part of the vesicle is still loaded with DMSO molecules. DMSO is a strongly coordinating ligand for the uranyl acetate staining agent giving rise to the even darker inner area compared to the membrane itself. The slightly larger size of the aggregates observed in the TEM images compared to the DSL studies is most likely caused by a swelling due to water from the uranyl acetate staining solution entering the DMSO filled vesicles. Therefore, all experimental data (DLS, AFM, and TEM) consistently confirm that zwitterion **3** forms soft vesicles in solution that however collapse upon interaction with a surface as typical for soft particles. The differences in the particle sizes obtained from these experiments are surprisingly small for this type of soft vesicle.

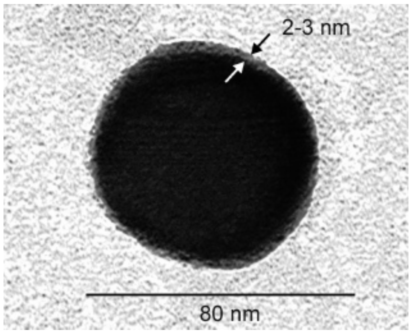

Figure 3. TEM image of **3** prepared from DMSO solution and stained with uranyl acetate.

Vesicle formation by zwitterion **3** is most likely due to a hierarchical self-assembly as shown in Figure 4. In solution, **3** forms ion-paired dimers that have a kink of ca. 38° according to molecular modeling studies (Macromodel V 8.0, amber* force field, GB/SA water solvation model, MC conformational search with 50 000 steps). A similar dimerization has been observed before for a more simple rigid zwitterion.[11] These dimers then aggregate into membranes. As the dimers are tilted, a curvature is induced that gives rise to the formation of soft vesicles.

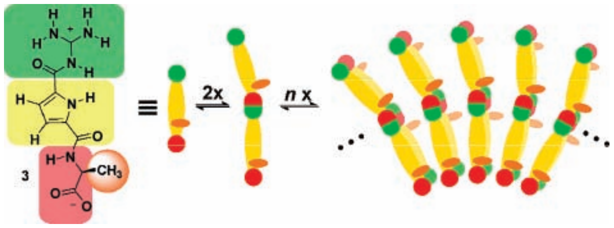

Figure 4. Suggested membrane formation via the self-assembly of **3**.

To test this aggregation model, we have performed a molecular mechanics calculation of a membrane segment composed of 32 such dimers. For the membrane construction, dimers of **3** in the conformation obtained from the Monte Carlo conformational search were used. Structure minimization then provided a stable curved membrane (Figure 5) with a thickness of 2.7 nm in perfect agreement with the value of ca. 3 nm for the membrane thickness extracted from the TEM

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(12) Static light scattering to further characterize the particles in solution was not possible due to the presence of a small amount of larger aggregates ($r_h > 100$ nm), which limits the analysis to the DLS part. Also, SANS measurements to further characterize the internal structure of the vesicles in more detail were not successful due to an insufficient scattering contrast between sample and solvent.

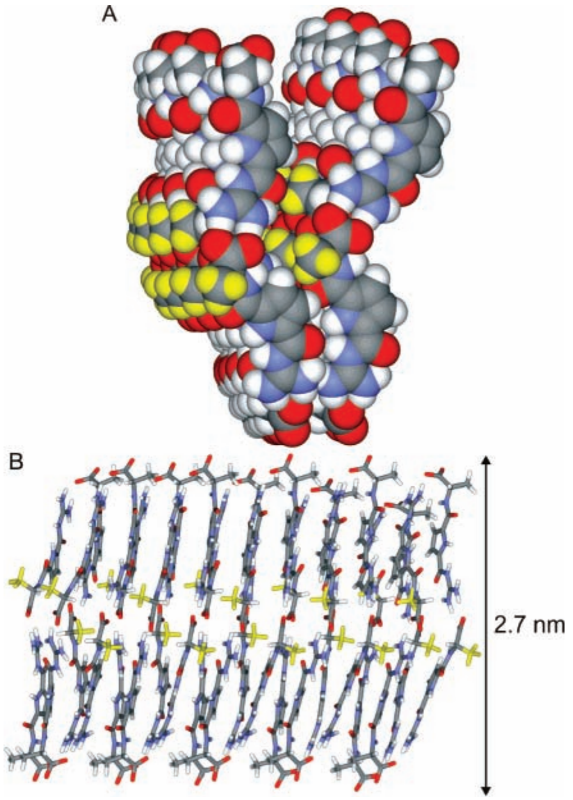

Figure 5. Calculated structure of a membrane segment (the alanine methyl groups needed for vesicle formation are shown in yellow): (A) view along the rows of stacked dimers showing the vesicle curvature; (B) side view of the stacked dimers showing their alternating antiparallel orientation.

image. The membrane is composed of dimers which stack next to each other in an antiparallel orientation. This allows for further electrostatic interactions of the oppositely charged groups between the dimers and most likely π-stacking or hydrophobic interactions between the aromatic moieties. At least in the energy minimized structure the planar aromatic parts of the molecules are in close contact with each other. All methyl groups are presented at the same side, which results in a steric bulk especially in the middle part of the membrane. This together with the tilt of each dimer induces the curvature. The important role of the alanine methyl group is also supported by the observation that an analogous glycine derived zwitterion, which lacks this methyl group, does not form vesicles but just small linear oligomers.[13] Hence, the alanine methyl group in **3** is crucial for vesicle formation.

In conclusion, we have shown here that a hierarchical self-assembly of zwitterion **3** causes the formation of soft vesicles even in polar solutions. Further studies with similar zwitterions derived from other amino acids with other polar and nonpolar side chains are currently underway to further explore the potential of this new class of vesicle-forming molecules.

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Supporting Information Available: Full synthesis and characterization of compound **3**, including experimental data of the NMR, AFM, DLS, and TEM measurements. This material is available free of charge via the Internet at http://pubs.acs.org.

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