Zwitterionic Vesicles

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pH-Switchable Vesicles from a Serine-Derived Guanidiniocarbonyl Pyrrole Carboxylate Zwitterion in DMSO**

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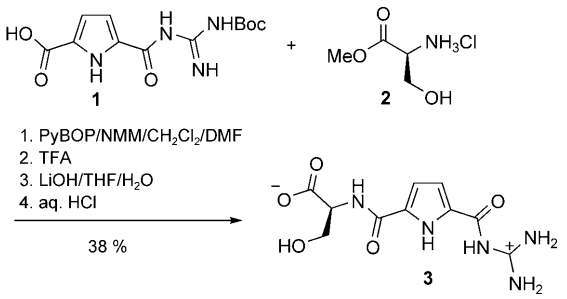
This work describes a new type of vesicles that can be reversibly opened and closed by changing the protonation state of a self-assembling zwitterion. Vesicles are interesting nanomaterials with potential applications such as delivery systems for drug targeting. For this purpose, however, external control of vesicle formation is required. To date, vesicle formation has often been based on the self-assembly of amphiphilic macromolecules such as lipid derivatives or block copolymers.[1,2] Recently, vesicle formation from other types of building blocks, such as shape-persistent macrocyles, cyclodextrins, or small peptides has also been reported.[3] Most of these molecules are still classical amphiphiles and their self-assembly is mainly driven by hydrophobic or aromatic interactions.

We have recently introduced a new type of vesicle forming-molecule, namely a self-complementary guanidiniocarbonyl pyrrole carboxylate zwitterion derived from alanine. We have shown by NOESY NMR studies in combination with TEM and AFM experiments that, in DMSO, the zwitterion forms ion-paired dimers that then further aggregate into hollow vesicles of approximately 40–50 nm diameter.[4] We have also used this special type of hydrogen-bond-assisted ion-pair formation between the guanidiniocarbonyl pyrrole cation[5] and a carboxylate unit for the construction of other types of nanostructures such as dimers,[6] loops,[7] and supramolecular polymers.[8] As the guanidiniocarbonyl pyrrole moiety has an approximate pK_a value of 6–7, ion-pair formation with a carboxylate (pK_a ca. 3–5) only occurs within a narrow pH range. This restriction offers the possibility to deliberately turn the ion-pair formation on or off by changing the pH from neutral to slightly acidic or basic.[9] We therefore wanted to explore whether we can also trigger vesicle formation by using pH as an external signal.[10] To test this hypothesis, we have synthesized a serine-derived guanidiniocarbonyl pyrrole carboxylate zwitterion **3** and studied its vesicle formation on surface with AFM as well as in solution by using dynamic light scattering (DLS) and a pulsed field gradient (PFG)-based NMR method. The latter method allows us to selectively detect the solvent molecules encapsulated in the vesicles and also to quantify the permeability of the vesicle membranes.[11] We show herein that vesicle formation of **3** can indeed be switched on and off in a controlled manner by reversible changes of its protonation state. Furthermore, we found that the vesicles are rather impermeable and the measured exchange rate of solvent molecules across the membrane is very small.

The synthesis of the serine-derived guanidiniocarbonyl pyrrole carboxylate zwitterion **3** is shown in Scheme 1. L-serine methyl ester hydrochloride **2** was coupled with the



Scheme 1. Synthesis of the serine derived zwitterion **3**. Boc = *tert*-butoxycarbonyl, DMF = *N*,*N*′-dimethylformamide, NMM = *N*-methylmorpholine, PyBOP = Benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate, TFA = trifluoroacetic acid, THF = tetrahydrofuran.

Boc-protected guanidinocarbonyl pyrrole carboxylic acid **1**[12] using PyBOP in a mixture of CH_2Cl_2 and DMF as the coupling reagent (67% yield). The Boc group was cleaved with TFA, and the methyl ester was subsequently hydrolyzed with LiOH to produce zwitterion **3** (57% yield).

Similar to the previously reported alanine zwitterion,[4] zwitterion **3** also forms vesicles, as confirmed by AFM after deposition on a mica surface. A 5 mM solution of **3** in DMSO was spin-coated onto mica and analyzed in the tapping mode. Deformed vesicles with a mean diameter of approximately 50 nm (measured at half the height of the particles) and a height of approximately 1.75 nm were observed (Figure 3). DLS studies showed that these vesicles are also present in solution and have a diameter of approximately 57 nm (5 mM). A more detailed insight into the properties of these vesicles was obtained from PFG NMR experiments.[11] This method allows different molecules to be distinguished according to their diffusion properties. It is evident from the overall plot in Figure 1 a that two different fractions of DMSO molecules are

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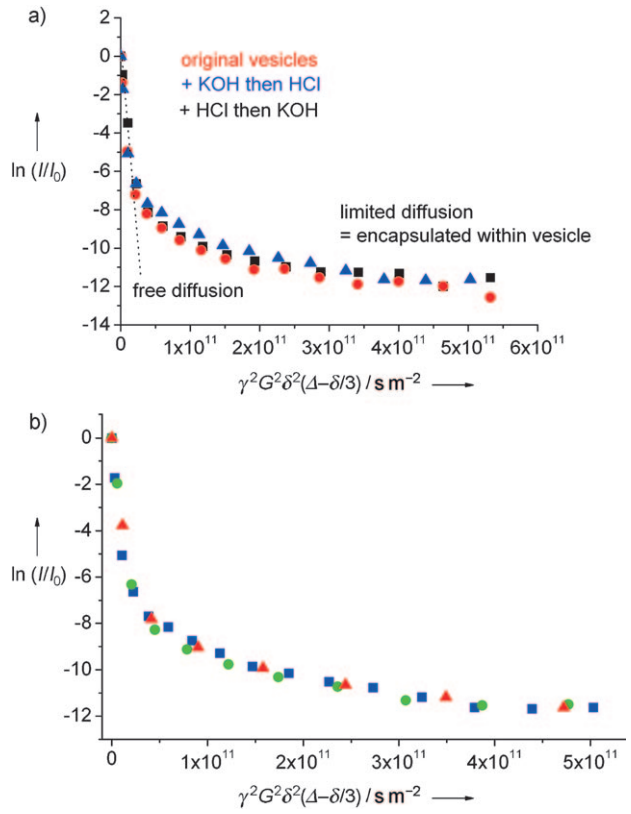

Figure 1. a) Logarithmic plot of the relative echo signal intensities against a function of the gradient strength G and the pulse spacing Δ. b) Logarithmic plot of the relative echo signal intensity of DMSO (with the vesicles) as a function of the pulse spacing Δ (■ 100 ms, ● 200 ms, ▲ 300 ms).

present in solution: the freely diffusing molecules in the bulk solution and a smaller fraction that has limited self-diffusion and represents the fraction of solvent molecules encapsulated inside the vesicles. For free diffusion, a logarithmic plot of the relative echo intensity I/I_0 versus the parameter $\gamma^2G^2\delta^2(\Delta-\delta/3)$ gives a straight line with a slope equal to the negative self-diffusion coefficient. As Figure 1a shows for a 70 mM solution of **3** in [D_6]DMSO, this behavior is observed for the majority of the DMSO molecules down to a value of $\ln(I/I_0)$ of -7. However, another relatively flat section of the plot is observed for $-7 > \ln(I/I_0) > -13$, which represents the DMSO molecules that are inside the vesicles and undergo limited self-diffusion. According to the data shown in Figure 1a, approximately 0.1% of the overall DMSO molecules are in the encapsulated state.[13] The slope for the encapsulated species levels off at a very small value of approximately $5\times10^{-12}\ m^2s^{-1}$, which is compatible with the Brownian motion of typical vesicles. From this value of the self-diffusion coefficient, and the viscosity constant of $0.002\ N s m^{-2}$ for DMSO, the average vesicle radius can be calculated to be $r =$ 20–25 nm. This value is in good agreement with the data obtained from both DLS and AFM experiments.

The PFG NMR experiments also allow us to study the permeability of the vesicle membrane in more detail. When the spacing Δ between the two gradient pulses is increased and comes into the same range as the exchange rate of the DMSO molecules, the echo signal for the encapsulated species vanishes.[11] The data on the vesicles show no dependence on Δ in the range between 100 ms and 400 ms (Figure 1b), thus we can conclude that the molecular exchange of DMSO through the vesicle membranes is negligible within this time span. Compared to other systems, the vesicles of zwitterion **3** appear to be relatively impermeable for the solvent. For example, in a similar study that was recently carried out on different block-copolymer vesicles, the average residence times of the solvent (water) in the encapsulated state were between 68 and 250 ms.[14] The corresponding residence time of DMSO in this case is at least one order of magnitude larger, thus indicating a very slow molecular exchange for vesicle membranes formed from zwitterion **3**.

The controlled opening and reformation of the vesicles triggered by acid or base can be demonstrated by focusing the NMR experiments on the encapsulated solvent. If the gradient strength G is adjusted such that the value for $\gamma^2G^2\delta^2(\Delta-\delta/3)$ is $1.51\times10^{11}\ s m^{-2}$, the corresponding NMR spectrum shows exclusively the signals that arise from vesicle-encapsulated molecules, as all the other contributions vanished because of their free self-diffusion. Such a spectrum for the original vesicles obtained from zwitterion **3** is shown in Figure 2a. The signal at $\delta = 2.5$ ppm arises from the DMSO

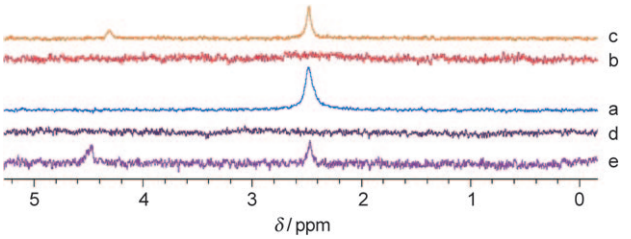

Figure 2. Sections of the NMR spectra of a solution of zwitterion **3** in DMSO. The solvent molecules encapsulated within the vesicles are monitored (gradient strength $\gamma^2G^2\delta^2(\Delta-\delta/3) = 1.51\times10^{11}\ s m^{-2}$). The signal for the encapsulated DMSO (a) disappears upon addition of 1 equivalent of HCl (b) and reforms again after addition of 1 equivalent of NaOH (c). The same behavior is observed when first equivalent of NaOH is added to the original vesicles (d) and then the solution is neutralized again with 1 equivalent of HCl (e).

molecules within the vesicles. Upon addition of one equivalent of HCl to the solution (in form of 0.1M aqueous HCl), the signal for the encapsulated DMSO immediately disappears (Figure 2b). Hence, the vesicles are destroyed upon protonation of the carboxylate unit. The resulting cation is no longer self-complementary, and obviously does not form vesicles. This behavior confirms that ion-pair formation is crucial for self-assembly. Upon addition of one equivalent of KOH (0.1M in H_2O), the vesicles reassemble, as seen by the reappearance of the NMR signal for the encapsulated DMSO at $\delta = 2.5$ ppm (Figure 2c). Upon addition of the base, the zwitterion of **3** is reformed, thus re-enabling self-assembly and therefore vesicle formation. A signal for encapsulated water (from the addition of the aqueous acid and base) at $\delta =$ 4.5 ppm was also observed.

The same observation is made when the vesicles are switched in the opposite direction: after addition of one

equivalent of KOH (0.1 M in H_2O) to the original solution, the signal for the encapsulated DMSO (and hence the vesicles) disappeared (Figure 2d). Upon addition of one equivalent of HCl (0.1 M in H_2O) the vesicles reappear, as indicated by the reappearance of the NMR signal for encapsulated DMSO and water (Figure 2e). Furthermore, the corresponding echo decay plots in Figure 1b prove that the diffusion behavior of the encapsulated DMSO after the reformation of the vesicles is exactly the same as that of the original vesicles. Hence, vesicle formation is completely reversible upon changing the protonation state of the zwitterion.

This pH-triggered vesicle formation was also confirmed by AFM experiments. The initial solution (5 mM) contains the original L-serine-derived zwitterions, which aggregate in DMSO to form hollow soft vesicles with a mean diameter of approximately 50 nm and a height of approximately 1.75 nm (Figure 3a), which are in good agreement with the DLS data and the vesicle size extrapolated from their diffusion properties. After addition of one equivalent HCl (1 M in H_2O), disappearance of the particles was observed (Figure 3b). After addition of one equivalent of NaOH (1 M in H_2O) the reformation of the vesicles could be observed (Figure 3c).

In conclusion, we have shown that a small zwitterion **3** forms vesicles in DMSO. The vesicles can be opened and closed both fully and reversibly by the addition of either acid or base. Vesicle formation is dependent on the zwitterionic protonation state of **3**, and either protonation (addition of acid) or deprotonation (addition of base) leads to molecules that no longer self-assemble. The reformed vesicles are completely identical to the original vesicles with respect to their size and diffusion behavior. Furthermore, compared to classical vesicles that are derived from amphiphilic copolymers, the permeability of the vesicles formed from zwitterion **3** is surprisingly low, as shown by PFG-NMR experiments. This new type of vesicle-forming molecule with unique properties may allow the development of stimuli-responsive nanomaterials as carrier systems.

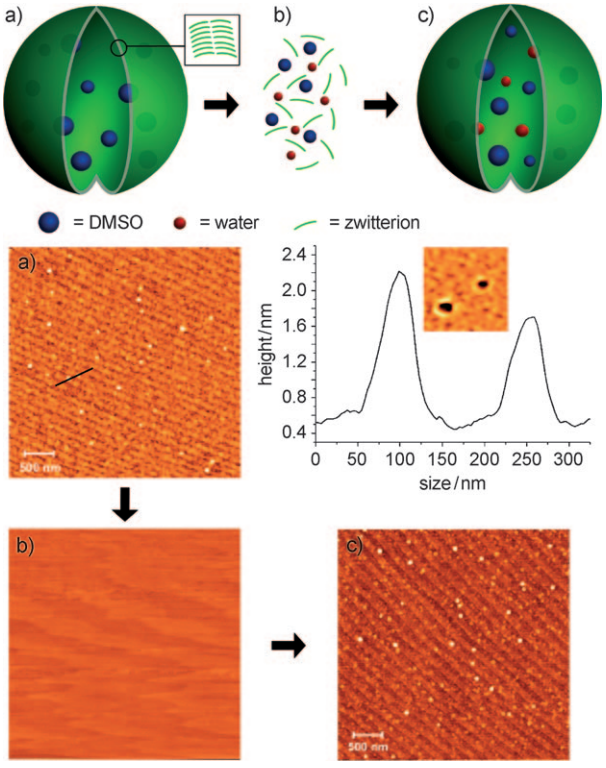

Figure 3. AFM height image of **3** on mica (a). The cross-section analysis along the black line provides a mean diameter of ca. 50 nm and a height of ca. 1.75 nm. Vesicles disappear after addition of 1 equivalent of HCl (b) and reform after addition of 1 equivalent of NaOH (c).

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[1] For reviews on vesicles, see: a) D. E. Discher, A. Eisenberg, *Science* **2002**, *297*, 967; b) M. Antonietti, S. Förster, *Adv. Mater.* **2003**, *15*, 1323; c) Y. Morishima, *Angew. Chem.* **2007**, *119*, 1392; *Angew. Chem. Int. Ed.* **2007**, *46*, 1370.

[2] For selected representative examples, see: a) H. Shen, A. Eisenberg, *Angew. Chem.* **2000**, *112*, 3448; *Angew. Chem. Int. Ed.* **2000**, *39*, 3310; b) F. Chécot, S. Lecommandoux, Y. Gnanou, H.-A. Klok, *Angew. Chem.* **2002**, *114*, 1395; *Angew. Chem. Int. Ed.* **2002**, *41*, 1339; c) A. Kros, W. Jesse, G. Metselaar, J. J. L. M. Cornelissen, *Angew. Chem.* **2005**, *117*, 4423; *Angew. Chem. Int. Ed.* **2005**, *44*, 4349; d) E. P. Holowka, D. J. Pochan T. J. Deming, *J. Am. Chem. Soc.* **2005**, *127*, 12423; e) D. M. Vriezema, J. Hoogboom, K. Velonia, K. Takazawa, P. C. M. Christianen, J. C. Maan, A. E. Rowan, R. J. M. Nolte, *Angew. Chem.* **2003**, *115*, 796; *Angew. Chem. Int. Ed.* **2003**, *42*, 772; f) M. Yang, W. Wang, F. Yuan, X. Zhang, J. Li, F. Liang, B. He, B. Minch, G. Wegner, *J. Am. Chem. Soc.* **2005**, *127*, 15107.

[3] For recent examples of vesicle formation from nonpolymers, see: a) D. Xie, M. Jiang, G. Zhang, D. Chen, *Chem. Eur. J.* **2007**, *13*, 3346; b) D. Dong, D. Baigl, Y. Cui, P. Sinay, M. Sollogoub, Y. Zhang, *Tetrahedron* **2007**, *63*, 2973; c) H. S. Seo, J. Y. Chang, G. N. Tew, *Angew. Chem.* **2006**, *118*, 7688; *Angew. Chem. Int. Ed. Engl.* **2006**, *45*, 7526; d) K. Balakrishnan, A. Datar, W. Zhang, X. Yang, T. Naddo, J. Huang, J. Zuo, M. Yen, J. S. Moore, L. Zang, *J. Am. Chem. Soc.* **2006**, *128*, 6576; e) F. J. M. Hoeben, I. O. Shklyarevskiy, M. J. Pouderoijen, H. Engelkamp, A. P. H. H. Schenning, P. C. M. Christianen, J. C. Maan, E. W. Meijer, *Angew. Chem.* **2006**, *118*, 1254; *Angew. Chem. Int. Ed.* **2006**, *45*, 1232; f) M. Reches, E. Gazit, *Science* **2003**, *300*, 625; g) X. Yan, Q. He, K. Wang, L. Duan, Y. Cui, J. Li, *Angew. Chem.* **2007**, *119*, 2483; *Angew. Chem. Int. Ed.* **2007**, *46*, 2431; h) F. Garcia, L. Sanchez, *Chem. Eur. J.* **2010**, *16*, 3138; i) B. J. Ravoo, R. Darcy, *Angew. Chem.* **2000**, *112*, 4494; *Angew. Chem. Int. Ed.* **2000**, *39*, 4324.

[4] T. Rehm, V. Stepanenko, X. Zhang, F. Würthner, F. Gröhn, K. Klein, C. Schmuck, *Org. Lett.* **2008**, *10*, 1469.

[5] C. Schmuck, *Coord. Chem. Rev.* **2006**, *250*, 3053.

[6] C. Schmuck, W. Wienand, *J. Am. Chem. Soc.* **2003**, *125*, 452.

[7] a) C. Schmuck, T. Rehm, F. Gröhn, K. Klein, F. Reinhold, *J. Am. Chem. Soc.* **2006**, *128*, 1430; b) C. Schmuck, T. Rehm, K. Klein, F. Gröhn, *Angew. Chem.* **2007**, *119*, 1723; *Angew. Chem. Int. Ed.* **2007**, *46*, 1693.

[8] G. Gröger, V. Stepanenko, F. Würthner, C. Schmuck, *Chem. Commun.* **2009**, 698.

[9] J. Voskuhl, T. Fenske, M. C. A. Stuart, B. Wibbeling, C. Schmuck, B. J. Ravoo, *Chem. Eur. J.* **2010**, *16*, 8300.

[10] For examples of other pH-triggered vesicles, see: a) J. Du, Y. Tang, A. L. Lewis, S. P. Armes, *J. Am. Chem. Soc.* **2005**, *127*, 17982; b) J. Du, R. K. O'Reilly, *Macromol. Chem. Phys.* **2010**, *211*, 1530; c) Z. Shi, Y. Zhou, D. Yan, *Macromol. Rapid Commun.* **2008**, *29*, 412; d) F. Versluis, I. Tomatsu, S. Kehr, C. Fregonese, A. W. J. W. Tepper, M. C. A. Stuart, B. J. Ravoo, R. I. Koning, A. Kros, *J. Am. Chem. Soc.* **2009**, *131*, 13186; e) S. Yu, T. Azzam, I. Rouiller, A. Eisenberg, *J. Am. Chem. Soc.* **2009**, *131*, 10557; f) S. Ghosh, M. Reches, E. Gazit, S. Verma, *Angew. Chem.* **2007**, *119*, 2048; *Angew. Chem. Int. Ed.* **2007**, *46*, 2002.

[11] This method is based on the combination of a stimulated echo sequence with a set of two field gradient pulses. The gradient strength G as well as the spacing Δ between the gradient pulses is systematically varied, leading to a set of echo decay patterns. The latter are analyzed by a numeric analysis based on a molecular exchange process between two domains with different self-diffusion characteristics: a) A. Rumplecker, S. Förster, M. Zähres, C. Mayer, *J. Chem. Phys.* **2004**, *120*, 8740; b) A. Bauer, S. Hauschild, M. Stolzenburg, S. Förster, C. Mayer, *Chem. Phys. Lett.* **2006**, *419*, 430.

[12] C. Schmuck, V. Bickert, M. Merschky, L. Geiger, D. Rupprecht, J. Dudaczek, P. Wich, T. Rehm, U. Machon, *Eur. J. Org. Chem.* **2008**, 324.

[13] The volume fraction of the encapsulated DMSO can be estimated from an extrapolation of the flat part of the plot in Figure 1. In the given case, its intersection with the ordinate axis near $\ln(I/I_0) \approx -7$ means that the decay plot for this echo contribution would start around $I/I_0 \approx e^{-7} \approx 0.001$. In contrast, the starting point of the DMSO fraction which undergoes free diffusion is at $I/I_0 \approx \exp(0) \approx 1$, hence the relative fraction of the encapsulated volume is approximately 0.001 or 0.1 %.

[14] A. Leson, V. Filiz, S. Förster, C. Mayer, *Chem. Phys. Lett.* **2007**, *444*, 268.

