Communication

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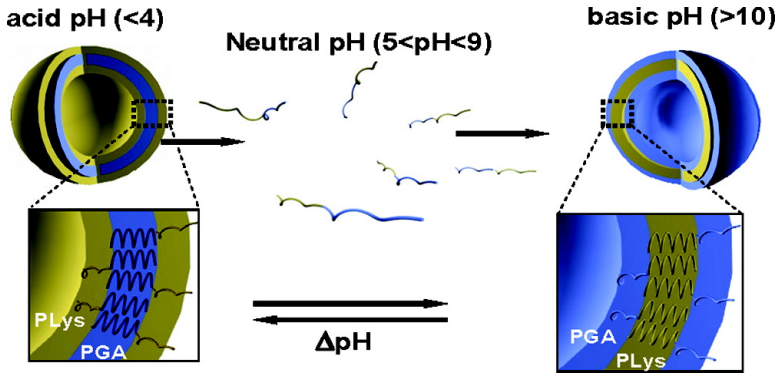

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Reversible Inside−Out Micellization of pH-responsive and Water-Soluble Vesicles Based on Polypeptide Diblock Copolymers

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Block copolymers with an amphiphilic character can self-assemble into a variety of micellar structures, including spherical micelles, rodlike micelles, or vesicles, at the mesoscopic level.[1] Due to their macromolecular nature, an increase in stability can generally be reached with polymer micelles as compared to low molecular weight surfactants. Among the structures obtained by self-assembly of amphiphilic diblock copolymers in aqueous media, vesicles ("polymersomes")[2] have been demonstrated to be particularly interesting for a variety of applications such as biological vectors,[3] protective shells for sensitive enzymes,[4] or as containers where chemical reactions can be performed at the molecular level.[5] As the domain size that can be reached (10−200 nm) is similar to that of viruses or lipoproteins, progressive interest is presently raised in the use and application of these block copolymer nanoparticles as novel carriers in the field of drug targeting or drug delivery and more generally in nanobiotechnologies.[6] Such systems that can also respond to a stimulus such as pH are particularly attractive for biological applications due to the large number of pH variations that can be found in normal or infected tissues.[7] A peculiar class of polyampholyte copolymers has been recently developed by Armes et al.[8] and presents very interesting reversible self-assembly properties from free chains to micelles, depending on the pH: these systems have been called "schizophrenic" micelles. Reversibility of self-assembled vesicles with pH has also been recently reported by Eisenberg et al.[9] from triblock copolymers in DMF/THF/water mixtures.

In this communication, we report for the first time the formation of schizophrenic vesicles that can be reversibly produced in moderate acidic or basic aqueous solutions from polypeptide diblock copolymers (Figure 1). Even if some block copolypeptides have been already synthesized, especially by Deming,[10] the self-assembly of such systems that combine two different polypeptide segments to form a zwitterionic diblock copolymer poly(L-glutamic acid)-*b*-poly(L-lysine) (PGA-*b*-PLys) has never been studied. As an attempt to prepare performing vectors, our group recently focused on the preparation of water-soluble vesicles that exhibit stimuli-responsive properties. Polybutadiene-*b*-poly(L-glutamic acid) (PB-*b*-PGA) and polyisoprene-*b*-poly(L-lysine) (PI-*b*-PLys) diblock copolymer micelles and vesicles[11] have been demonstrated to respond to pH or to ionic strength with a change in the hydrodynamic radius. The observed size variations have been assigned to the neutralization of the polypeptide block that changes from a random coil conformation (charged form) into a neutral and compact α-helical structure ("rod") and was demonstrated to be reversible.

Poly(γ-benzyl-L-glutamate)-*b*-poly(N$^{\epsilon}$-trifluoroacetyl-L-lysine) (PBLG-*b*-PTFALys) diblock copolymer was synthesized by sequential ring-opening polymerization of the corresponding α-amino acid *N*-carboxyanhydrides. The poly(trifluoroacetyl-L-lysine) block was prepared first using *n*-hexylamine as the initiator. This led to ω-amino PTFALys homopolymer from which the PBLG block was grown.[12] Both polymerizations were performed in dried DMF at room temperature during 5 days. Copolymers were precipitated in diethyl ether. Monomodal distributions obtained by gel permeation chromatography (GPC) analysis with polydispersities of ∼1.4 typical for this kind of polymerization confirmed the quantitative incorporation of the second monomer onto the preformed homopolymer. The length of both blocks was targeted to be 15 units via monomer/initiator ratio. Chemical composition was determined by ^{1}H NMR in DMSO-*d*.[6] Benzyl (Bn-) and trifluoroacetyl (TFA) protective groups of L-glutamic acid and L-lysine are both base labile protective groups[13] and can be completely removed after treatment with KOH (1.5 equiv) in THF at room temperature during 3 days. The zwitterionic diblock copolymer PGA_{15}-*b*-$PLys_{15}$ was then obtained with positive charges of the protonated lysine and negative charges of the deprotonated glutamic acid at neutral pH. The self-assembly behavior in acid or basic conditions was studied by means of fluorescence spectroscopy, ^{1}H NMR, light scattering (SLS and DLS), and small-angle neutron scattering (SANS).

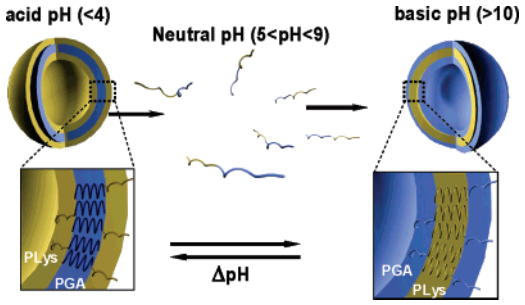

Figure 1. Schematic representation of the self-assembly into vesicles of the diblock copolymer PGA_{15}-*b*-$PLys_{15}$.

Upon dissolution of the diblock copolymer in aqueous basic and acid solutions, self-assembly occurred spontaneously as demonstrated by ^{1}H NMR and fluorescence spectroscopy. NMR spectra of the diblock copolypeptide obtained at both acid (pH ≈ 3) and basic pH (pH ≈ 12) values (see Figure 2a) were compared with standard homopolymers. At acidic pH, the poly(L-glutamic acid) block is neutralized, and its secondary conformation changes from a charged coil to a neutral and more compact α-helical structure. The structure variation is accompanied with a decrease in solubility; this hydrophobicity is the driving force for self-assembly, and insoluble PGA is forming the core of the aggregates while PLys block forms the shell. At this pH, ^{1}H NMR signals of the PGA block at 2.0−2.4 ppm have almost completely disappeared, indicating a relatively compact and nonpolar environment, while the PLys segment remains fully solvated. Under basic conditions the protonated poly(L-lysine) block ($-NH_3^+$) is transformed into neutral and insoluble $-NH_2$ groups, forming the core of the aggregates. This fact is supported by the observation of several chemical shifts: the previous signal at 3.0 ppm ($-C\underline{H}_2-NH_2$)

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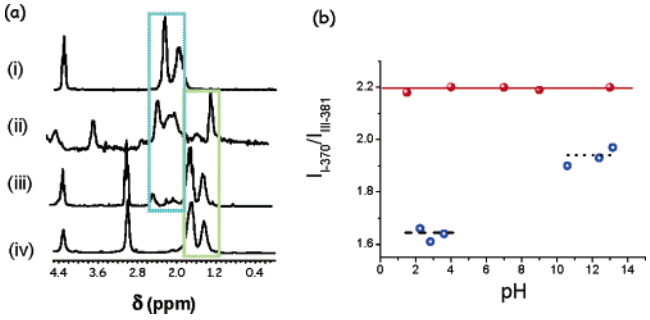

Figure 2. (a) ^{1}H NMR spectra recorded in D_2O: (i) poly(L-glutamic acid) at pH 12, (ii) PGA-*b*-PLys at pH 12, (iii) PGA-*b*-PLys at pH 3.5, and (iv) poly(L-lysine) at pH 3.5. (b) Fluorescence spectroscopy experiments. Red dots: pyrene in water; blue circles: pyrene with block copolymer in water at acid pH (left) and basic pH (right).

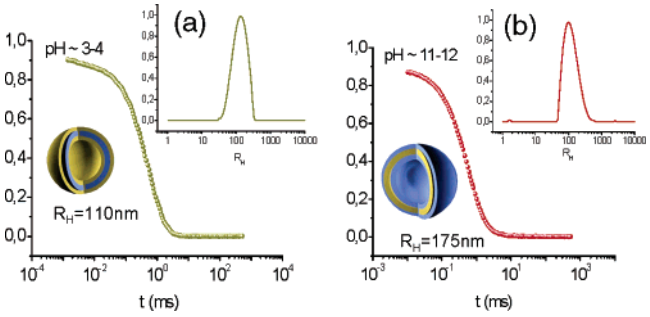

Figure 3. Autocorrelation functions (90°) at pH = 3 (a) and at pH = 12 (b) of PGA_{15}-*b*-$PLys_{15}$. Inserted are the corresponding R_H distributions after CONTIN analysis.

appears now at 3.6 ppm, and the two signals between 1.3 and 1.8 ppm ($[-C\underline{H}_2-]_3$) appear now as a single peak at 1.2 ppm. At intermediate pH values both segments are charged, the assembled structures are disassembled, and eventually precipitation occurs. However, the precipitate can be easily redissolved as cationic or anionic polyelectrolyte aggregates by adding acid or base even at high salt concentration. In addition to NMR, fluorescence spectroscopy was measured using pyrene as a probe (see Figure 2b). Environmental changes of pyrene can be directly correlated to modifications of its absorption and fluorescence spectra, and therefore the micellization process can be addressed. For this purpose, the relation in intensity of the signals at 370 and 381 nm was plotted at different pH values. I_{370}/I_{381} remains constant when pyrene is dissolved in water at about 2.2 at all ranges of pH. On the other side, when the diblock copolymer is added to acid and basic pyrene/water solutions, the intensity ratio decreases to values of 1.7−1.9, confirming the formation of supramolecular objects with a hydrophobic environment.

Once the formation of aggregates at both extremes of pH has been demonstrated, further analysis by dynamic light scattering (DLS) focused on the evaluation of the size distribution and morphology of the aggregates (Figure 3). Solution of the dipeptide in water at acid and basic pH values revealed hydrodynamic radius R_H = 110 and 175 nm, respectively, both showing a narrow size distribution. The angular dependence obtained is linear and confirms the spherical shape of the aggregates. Comparison of the small molecular dimensions with the large R_H values suggests the formation of a vesicular structure. DLS experiments were accompanied with additional SLS measurements. The radii of gyration (R_G) thus obtained were 123 nm for acid and 174 nm basic pH. From the ratio R_G/R_H which is sensitive to the particle morphology,[14] values close to 1 were obtained in both cases indicating a vesicular structure in acid as well as in basic conditions. The presence of hollow spheres or vesicles was further supported by neutron scattering experiments performed in a *q*-range between 0.004 and 0.03 Å^{-1} where a typical q^{-2} slope confirms the presence of the vesicle membrane with a flat interface.

In summary, new zwitterionic copolypeptides were synthesized, and their original self-assembly behavior in water as a function of pH was analyzed in detail. We evidenced here for the first time the formation of schizophrenic vesicles that can be reversibly produced as a function of pH in pure water. We believe that the vesicle formation is related to the systematic presence of the polypeptide in a rodlike conformation in the hydrophobic part of the membrane, inducing a low interfacial curvature and as a result a hollow structure. These pH-sensitive nanoparticles are expected to be very promising candidates in macromolecular nanobiotechnologies. Future work will focus on the development of encapsulation strategies of different sensitive drugs and proteins and the evaluation of the delivery properties as a function of pH.

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Note Added after ASAP Publication. After this communication was published ASAP on January 28, 2005, a correction was made in the Figure 2b caption. The corrected version was published ASAP on February 8, 2005.

Supporting Information Available: Polymerization and deprotection procedures, NMR, SANS and light scattering measurements. This material is available free of charge via the Internet at http://pubs.acs.org.

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