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The Formation of Polymer Vesicles or "Peptosomes" by Polybutadiene-*block*-poly(L-glutamate)s in Dilute Aqueous Solution

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Abstract: Polybutadiene-*block*-poly(L-glutamate) copolymers were made by anionic polymerization and subsequent ring-opening polymerization of *N*-carboxyanhydrides and were characterized by NMR, IR, SEC, and circular dichroism. These polymers, when appropriately designed, form so-called "polymersomes" or "peptosomes", vesicles composed of modified protein units. The size and structure of the vesicles are determined by dynamic light scattering, small-angle neutron scattering, and freeze-fracture electron microscopy. It is also shown that the size of the peptosomes does not depend on the pH; that is, the solvating peptide units can perform a helix−coil transition without serious changes of the vesicle morphology.

Introduction

Amphiphilic block copolymers are unique building blocks in supramolecular polymer chemistry, both for the generation of highly organized, self-assembled structures and for the energetic and structural control of material interfaces.[1] This is due to their molecular structure, which consists of at least two different polymer components with very different polarity, polymer structure, and potential chemical functionality.

It was also pointed out that the notation "amphiphilic" can describe a variety of two-phase situations, but water-soluble polymers are still most relevant due to their omnipresence in biological systems and daily environments. In this context, the blocking of synthetic polymers with peptides or proteins is especially interesting, and here, the "amphiphile" connects the worlds of synthetic polymers and natural systems. The resulting molecular chimeras of polymer and polypeptide carry inherent promise to interface the technical world with biological systems; consequently, a number of experiments on these systems has already been described in the literature. The first work was published by Gallot et al.,[2−4] who synthesized either polystyrene or polybutadiene blocks and coupled those blocks to a variety of single-species polypeptides. Hayashi et al.[5] synthesized triblock copolymers consisting of a central polyisoprene block and two outer poly(γ-benzyl-L-glutamate) blocks for the incorporation into model membranes.[6] The group of Kataoka published a series of papers on block copolymers from poly(ethylene glycole) and different polypeptides, among them poly(L-aspartate), poly(L-glutamate), and the cationic poly(L-lysine).[7−10] The use of such polymers for drug delivery and gene therapy was also pioneered by Ringsdorf et al.[11] and was meanwhile specified and reviewed in a number of papers.[12−14]

The scope of the present paper is the analysis of the aggregate structure of such block copolymers. For that, we chose a soft hydrophobic block of a synthetic polymer, polybutadiene, since previous work has shown that soft blocks with a low glass transition temperature allow the characterization of equilibrium structures instead of frozen-in, metastable states.[15] As a peptide sequence, we selected poly(L-glutamate) due to the relative ease of synthesis as well as its ability to perform a pH-dependent helix−coil transition. After the synthesis of a small series of water-soluble polybutadiene-*block*-poly(L-glutamate) copolymers, their special aggregation behavior will be analyzed by dynamic light scattering, neutron scattering, and freeze-fracture electron microscopy.

Experimental Part

Chemicals. All reagents and solvents were purchased from either Aldrich or Fluka with the highest purity grade available and used as

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received unless otherwise noted. 1,3-Butadiene (99+%, Aldrich) was successively stirred at −40 °C and cryodistilled from *n*-Bu_2Mg and *n*-BuLi. Tetrahydrofuran (THF; BASF AG) was fractionally distilled, refluxed with Na/K alloy, distilled, degassed, and distilled from $LiAlH_4$ in high vacuum prior to use. Dimethylformamide (DMF; 99%, Aldrich) was stirred with CaH_2, degassed, and distilled in high vacuum. Argon (99.99%, Messer-Griesheim) was purified by passing successively over a 1 m column filled with P_2O_5 on silica and an Oxysorb catalyst (Messer-Griesheim). 1-(Chlorodimethylsilyl)-3-[*N*,*N*-bis(trimethylsilyl)-amino]propane was obtained from the hydrosilylation of 3-[*N*,*N*-bis-(trimethylsilyl)amino]-1-propene with dimethylchlorosilane;[16] the first of the reactants was synthesized from potassium *N*,*N*-bis(trimethylsilyl) amide and allyl bromide.[17] The γ-benzyl-L-glutamate-*N*-carboxyanhydride was prepared via the Fuchs−Farthing method from γ-benzyl-L-glutamic acid and triphosgene and was recrystallized several times from THF/petroleum ether (1:2 v/v) prior to use.[18]

Polymer Synthesis (General Procedure). All polymerization reactions were performed in flame-dried glassware under an argon atmosphere employing standard high-vacuum techniques.

(i) Amino-Functional Polybutadiene. THF and 1,3-butadiene (∼2 wt %) were successively condensed into a two-neck round-bottom reactor capped with a septum. The polymerization was initiated at −78 °C by adding *sec*-butyllithium as a 1.3 M solution in hexane via a syringe, and the resulting bright yellow solution was stirred overnight at low temperature. After withdrawing a sample of the polybutadiene precursor for SEC analysis, a solution of 1-(chlorodimethylsilyl)-3-[*N*,*N*-bis(trimethylsilyl)amino]propane (1.2 equiv with respect to *sec*-BuLi) in THF was added, and the mixture was stirred overnight at room temperature. After the solvent was evaporated and the residue redissolved in petroleum ether 35/60, dilute aqueous HCl was added, and the mixture was vigorously stirred for 2 h at room temperature. The solution was then neutralized with NaOH, and the organic layer was isolated and dried over Na_2SO_4. The solvent was evaporated and the polymeric residue dried in a vacuum at +35 °C.

(ii) Polybutadiene-*block*-poly(γ-benzyl-L-glutamate). In separate two-neck flasks, the amino-functional polybutadiene and the γ-benzyl-L-glutamate-*N*-carboxyanhydride were dried for 1 h at room temperature in high vacuum and then dissolved in $CHCl_3$ and DMF, respectively. The ∼5 wt % solutions were combined via a transfer needle and stirred at +40 °C for 48 h under an argon atmosphere. The solvents were evaporated and residual DMF traces removed in high vacuum. The solid was redissolved in $CHCl_3$, precipitated from petroleum ether (to separate from polybutadiene residuals), and dried in vaccum at +35 °C to constant weight. As indicated by DLS analyses, the copolymer samples were not contaminated with homopolymer traces whatsoever.

(iii) Polybutadiene-*block*-poly(sodium L-glutamate). To a ∼10 wt % solution of polybutadiene-*block*-poly(γ-benzyl-L-glutamate) in DMF/AcOH/THF/H_2O (7:5:1:1 v/v/v/v) was added ammonium formiate (at least 5 equiv with respect to protecting ester units). After degassing of the solution and purging with argon, the palladium catalyst (10 wt % on charcoal) was added, and the mixture was stirred overnight. The reaction mixture was filtered through neutral Celite 500 to remove catalyst traces and then diluted with H_2O and dialyzed (molecular weight cutoff: 10^3 g/mol) for 24 h to remove any residuals of organic solvents. The polymer was then neutralized with NaOH, and the aqueous polymer solution was ultrafiltrated against bidistilled H_2O (molecular weight cutoff: 10^3 g/mol) and freeze-dried.

Sample Preparation for Structural Analysis. For structural analysis, the polybutadiene-*block*-poly(sodium L-glutamate) samples were dissolved in a 0.26 M (sample **1**) or 0.12 M (samples **2** and **3**) aqueous NaCl solution to yield 0.05 (CD), 0.1−0.5 (DLS), and 1.0−4.0 wt % (SANS) polymer solutions. For pH-dependent CD and DLS measurements, the solutions (pH ∼6) were titrated against 0.1 N NaOH or HCl employing a standard pH electrode. For DLS, solutions were passed through 0.45 μm filters (Schleicher-Schüll). Freeze-fractured specimens for TEM examination were prepared from 5.0 wt % aqueous polymer solutions without salt additives.

Characterization Methods. (i) NMR. ^{1}H NMR spectra were recorded with a Bruker DPX-400 spectrometer in $CDCl_3$ (99.8% D, Aldrich) at room temperature. The signals were referenced to that of residual nondeuterated solvent at $\delta = 7.24$ ppm.

(ii) Fourier Transform Infrared Spectroscopy. FT-IR spectra of solid samples were recorded at room temperature with a Bio-Rad 6000 FT-IR spectrometer equipped with a single reflection diamond ATR.

(iii) Size Exclusion Chromatography. Thermo Separation Products SEC setups were used equipped with UV (TSP UV1000) and RI (Shodex RI-71) detectors. The column set employed was 30 × 0.8 cm, 5 μm MZ-SD*plus*: 10^3, 10^5, and 10^6 Å. Analyses were performed at 30 °C with THF as the eluent at a flow rate of 1.0 mL/min. Poly(1,2-butadiene) standards from PSS GmbH, Mainz, Germany, were used for calibration.

(iv) Differential Scanning Calorimetry. DSC measurements were carried out with a Netzsch DSC 200 instrument at −50 to 20 °C. Glass transition temperatures were determined from the second heating curve at a heating rate of 10 K/min.

(v) Circular Dichroism. CD analyses were performed at room temperature with a JASCO J 715 spectrometer employing quartz cells with 0.5 mm optical path length.

(vi) Dynamic Light Scattering. DLS measurements were carried out at 20 °C with a spectrometer consisting of an argon laser ($\lambda = 488$ nm, 500 mW; Coherent Innova 300), a self-constructed goniometer, a single-photon detector (ALV SO-SIPD), and a multiple-τ digital correlator (ALV 5000/FAST). From the measured time-correlation functions, intensity-weighted particle size distributions were calculated according to ref 19.

(vii) Small-Angle Neutron Scattering. SANS measurements were performed at the Hahn-Meitner-Institut (HMI), Berlin, Germany. Neutrons were derived from a liquid hydrogen cold source and monochromated by a mechanical velocity selector; the mean de Broglie wavelength was set to $\lambda = 0.60$ nm with a wavelength distribution of $\Delta\lambda/\lambda_0 = 0.1$. The two-dimensional ^{3}He detector with 64 × 64 elements of 10 × 10 mm^2 was positioned at sample-to-detector distances of 1, 4, and 16 m. Quartz cells with a path length of 1 mm were used as sample containers which were inserted into aluminum sample holders. All measurements were carried out at 25 °C. The scattered intensity was put on absolute scale by calibration with water ($I_{H_2O}^{d=1mm} = 0.857$ cm^{-1}). The scattered intensity was fitted to $I(q) = I_0P(q) + I_{inc}$, where $P(q)$ is the form factor of a vesicle and I_{inc} the contribution from the incoherent scattering of the polymer. The scattering vector is defined as $q = (4\pi/\lambda)\sin(\theta/2)$, where θ is the scattering angle. $P(q)$ for polydisperse vesicles with outer radius R_0, inner radius $R_i = pR_0$, and relative standard deviation of the radii σ is given by[20]

$$P_z(q) = \int_0^\infty P(q,R)h(R)\,\mathrm{d}R$$

$$P(q,R) = \frac{9\pi}{2(qR)^3}[J_{3/2}(qR) - p^2J_{3/2}(pqR)]^2$$

where $R = R_0$ and $J_{3/2}(x)$ denotes the Bessel function. If $h(R)$ is taken to be the Schulz−Zimm distribution $h(R) = [(z+1)^{z+1}/\bar{R}^{z+1}\Gamma(z+1)]\exp[-(z+1)R/\bar{R}]$, the integral can be solved analytically in terms of hypergeometric functions.[21] The distribution function $h(R)$ is characterized by an average particle radius $\bar{R}$ and a relative standard deviation $\sigma = (z+1)^{-1/2}$. The parameters R_0, R_i, and σ are obtained from a

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Scheme 1. Synthesis of Polybutadiene-*block*-poly(sodium L-glutamate) Copolymers

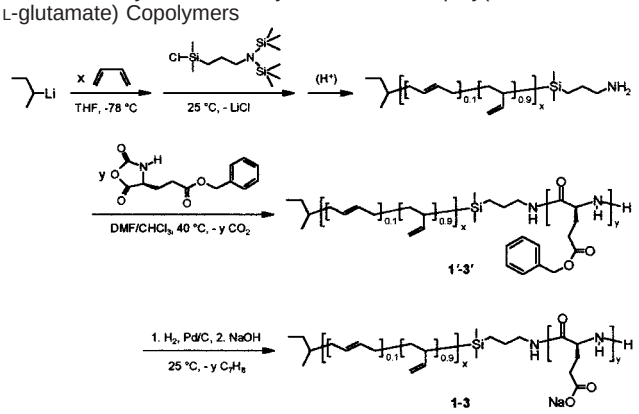

Table 1. Molecular Characteristics of the Prepared Polybutadiene-*block*-poly(sodium L-glutamate)s PB_x-*b*-PG_y[a]

PB_x-*b*-PG_y	precursor	x[b]	y[c]	M_n (g/mol)	f_G
1	**1′**	27	64	11 100	0.70
2	**2′**	85	55	12 900	0.39
3	**3′**	119	24	10 100	0.17

[a] x, y, number of butadiene and L-glutamate repeating units, respectively; M_n, number-average molecular weight; f_G, mole fraction of L-glutamate in the copolymer. [b] Determined by SEC analysis of the PB precursors. [c] Determined by ^{1}H NMR on the protected copolymers **1′**−**3′** (see text).

nonlinear least-squares fit to the measured scattering curve using the Evenberg−Marquardt algorithm. The relative errors of the fit parameters as calculated from the covariance matrix are <10%.

(viii) Transmission Electron Microscopy. TEM micrographs were taken with a Zeiss Omega 912 electron microscope operating at an acceleration voltage of 120 kV. Freeze-fractured specimens were prepared with a Balzers BAF 400 as follows: after the cyrofixation of the aqueous polymer solution with liquid propane, the sample was fractured with a microtome knife and the water partially removed by sublimation for 60 s at −100 °C in a vacuum (10^{-6} Torr). The surface of the replica was then successively coated with Pt/Ir/C at an angle of 45° and with carbon at 90°.

Results and Discussion

Synthesis and Molecular Characterization of the Block Copolymers. Three samples of linear polybutadiene-*block*-poly(sodium L-glutamate)s, PB_x-*b*-PG_y, with similar molecular weights but vastly different chemical compositions (see Table 1), were synthesized in a three-step procedure as outlined in Scheme 1.

First, ω-amino-functional polybutadiene samples were prepared via anionic polymerization of 1,3-butadiene initiated by *sec*-butyllithium in THF at −78 °C and subsequent quenching with 1-(chlorodimethylsilyl)-3-[*N*,*N*-bis(trimethylsilyl)amino]-propane; the primary amino group was released by HCl-catalyzed hydrolysis of the trimethylsilyl protecting groups and neutralization with NaOH. ^{1}H NMR analysis revealed the expected microstructure for polybutadienes obtained under such polymerization conditions, i.e., 90% of 1,2- and 10% of 1,4-trans structures (cf. Figure 1).[22] Accordingly, the glass transition temperature of the samples is −14 °C (DSC). The number-average molecular weights were determined by SEC on the H-terminated precursors to be 1520, 4650, and 6480 g/mol, corresponding to 27, 85, and 119 butadiene repeating units, respectively. The molecular weight distributions of the polybutadiene samples are narrow, with polydispersity indices in the range of 1.08−1.10. The amino functionality of either polymer is, according to ^{1}H NMR end group analyses, greater than 90%.

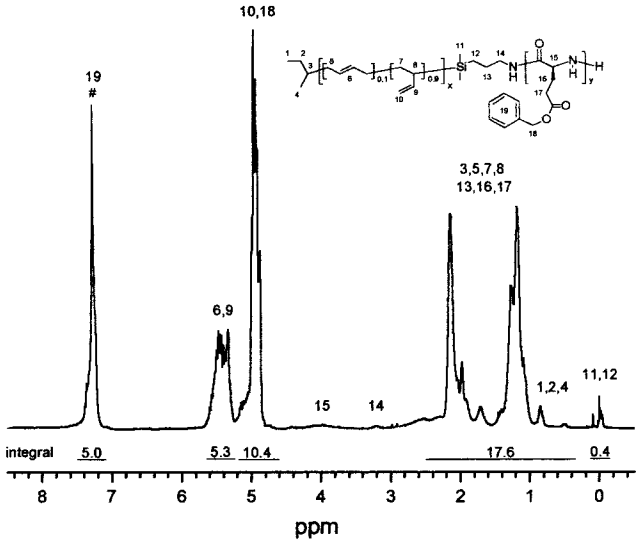

Figure 1. ^{1}H NMR spectrum (400 MHz) of the copolymer sample **3′** in $CDCl_3$. Signals were assigned with the aid of ref 9; signals of amide protons are not observed. The signal of $CHCl_3$ residuals in the solvent is denoted as #.

Second, the amino-functional polybutadienes were used to initiate the ring-opening polymerization of γ-benzyl-L-glutamate-*N*-carboxyanhydride in DMF/$CHCl_3$ (1:1 v/v) at +40 °C to yield the amphiphilic polybutadiene-*block*-poly(γ-benzyl-L-glutamate) samples **1′**−**3′**.[3,5] The copolymers were separated from any macroinitiator residuals by the precipitation from petroleum ether. ^{1}H NMR spectra were found to be in accord with the expected chemical structure of the copolymers (cf. Figure 1),[9] and the number of γ-benzyl-L-glutamate repeating units was calculated from the signals at δ/ppm = 0.0−0.2 (11 + 12, 8H, $-Si(CH_3)_2CH_2-$) and 7.2−7.4 (19, 5H, =CH−) to be 64, 55, and 24, respectively. So far, it was not possible to determine the molecular weight distributions of these block copolymers with SEC or any other classical method due to the vastly different solubilities of the two block segments and thus the high tendency to form aggregates in any kind of organic solvent. However, it is known that the primary amine-initiated polymerization of *N*-carboxyanhydrides usually yields products with broadened molecular weight distributions (PDI > 1.2),[23] which is as well assumed for the copolymers **1′**−**3′**.

Third, the γ-benzyl protecting groups of **1′**−**3′** were removed by palladium-catalyzed hydrogenation at room temperature using ammonium formiate as the hydrogen source;[24] neutralization of the carboxylic functional groups with NaOH yields the polybutadiene-*block*-poly(sodium L-glutamate) copolymers **1**−**3** which are readily soluble in water. FT-IR analyses on the final products prove the successful deprotection of **1′**−**3′** by the lack of the characteristic ester vibrational bands at $\tilde{\nu}$ = 1731 and 1165 cm^{-1} and appearance of a carboxylate band at 1395 cm^{-1} (cf. Figure 2). Unlike the alternate hydrolysis in a strongly alkaline (NaOH) or acidic environment (HBr/AcOH), hydro-

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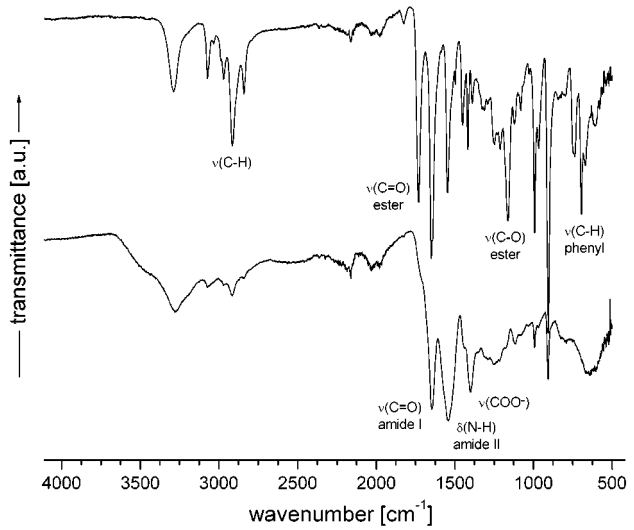

Figure 2. FT-IR spectra of PB_{119}-*b*-PG_{24} (sample **3**, bottom) and the corresponding protected precursor copolymer (sample **3′**, top). Absorption bands were assigned with the aid of ref 23; ν and δ denote valance and deformation vibrations, respectively.

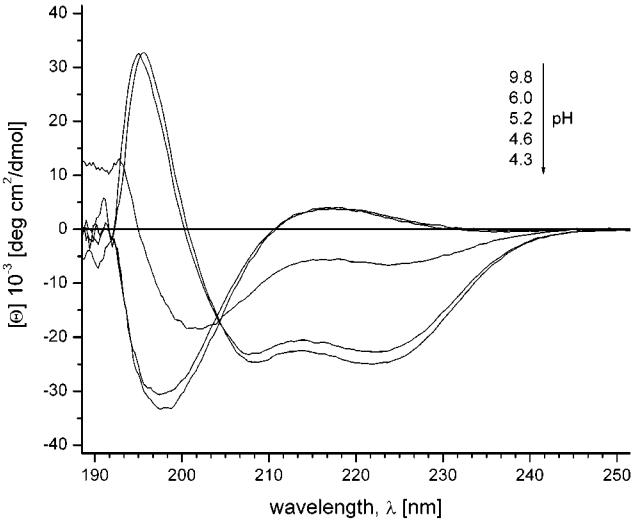

Figure 3. CD spectra of PB_{85}-*b*-PG_{55} (sample **2**) in a 0.12 M aqueous NaCl solution at pH 4.3−9.0.

genolysis proceeds at very mild reaction conditions without risking considerable racemization or degradation of the polypeptide segment.[25] The average number of L-glutamate repeating units of **1**−**3** is therefore considered to be identical to that of the protected precursor polymer.

Characterization of the Poly(L-glutamate) Secondary Structure. It is well known that poly(L-glutamate)s are strong α-helix formers but take a random coil conformation when charged.[26] Accordingly, the CD spectra of the copolymers **1**−**3** in aqueous solutions show the characteristic curve of a random coil at pH > 6 and that of an α-helix at lower pH; the coil−helix transition occurs at pH ∼5.2 (cf. Figure 3). The appearance of a single isodichroistic point at $\lambda = 204$ nm suggests that no other than these two conformations are present in solution. From the absolute value of molar ellipticity [Θ], one can estimate the percentage of coil to be close to 100% at pH >6 and ∼20% at pH 4.3.[27] Further lowering of the pH (the pK_a value of glutamic acid is 4.32) results in precipitation of the copolymers from solution. Sample **1**, with the highest mole fraction of glutamate units ($f_G = 0.7$), however, already precipitates from solution at pH 4.6. Hence, 80% is the maximum percentage of α-helix which could be achieved for the copolymers **1**−**3** under preservation of solubility.

Table 2. Hydrodynamic Radii (R_h) of Aggregates Formed by the Block Copolymers **1**−**3** in Dilute Aqueous Solution, Dependent on the Conformation of the Poly(L-glutamate) Segment

	R_h (nm)[a]		
sample	pH 4.6 70% α-helix[b]	pH 5.2 20% α-helix[b]	pH 6.0 0% α-helix[b]
PB_{27}-*b*-PG_{64} (**1**)	*c*	16	17
PB_{85}-*b*-PG_{55} (**2**)	81	70	90
PB_{119}-*b*-PG_{24} (**3**)	84	86	90

[a] Determined by DLS. [b] Percentage of α-helix as estimated from CD molar ellipticities according to ref 27. [c] Slow precipitation of the copolymer.

Characterization of the Copolymer Aggregates. Aqueous solutions of **1**−**3** (0.1−0.5 wt %) were analyzed by dynamic light scattering (DLS) at pH 4.6−6.0 to yield the hydrodynamic radii R_h of aggregates dependent on the conformation of the poly(L-glutamate) segment (Table 2). Measurements were carried out in the presence of NaCl ([NaCl]/[L-glutamate] > 6) in order to suppress electrostatic interactions between the polyelectrolyte molecules. It should be pointed out that virtually identical DLS results were obtained whether or not the solutions had been treated with ultrasound and/or heat, and the samples were analyzed once again after several days or weeks of storing. It is found that PB_{27}-*b*-PG_{64} (**1**) forms small aggregates with an average hydrodynamic radius of ∼16 nm, while the aggregates of PB_{85}-*b*-PG_{55} (**2**) and PB_{119}-*b*-PG_{24} (**3**) are about 5 times larger, with $R_h = 70-90$ nm. In all cases, the polydispersity is low, indicating well-equilibrated structures without impurities from the synthesis. The size of aggregates remains virtually the same regardless of whether the conformation of the poly(L-glutamate) segment is 100% coil (at pH 6.0) or 70% α-helix (at pH 4.6). Depolarized scattering of light was not observed for any of the samples, which suggests the exclusive presence of spherical aggregates.

The aggregates formed by PB_{27}-*b*-PG_{64} at pH 6.0 and pH 5.2 (20% α-helix) supposedly are spherical micelles consisting of a polybutadiene core and a poly(L-glutamate) solvating corona. The diameter is in good agreement with the typical diameters of other amphiphilic block copolymers of similar molecular weight, see refs 28 and 29. Estimating the core radius to be 5 nm (the contour length of the polybutadiene chains is 7.7 nm), the thickness of the corona is 11 nm, which is well between the limiting end-to-end distances of a randomly coiled (2.8 nm) and a fully streched polypeptide chain (24.3 nm). It is interesting to note that a PG_{64} α-helix would have a very similar dimension, i.e., 9.6 nm.[3] This is why a change of the conformation of the poly(L-glutamate) segment might not be reflected in the size of the micelle: stretched corona chains and helices simply have very similar dimensions. The determination of the aggregation number by static light scattering (SLS) is heavily

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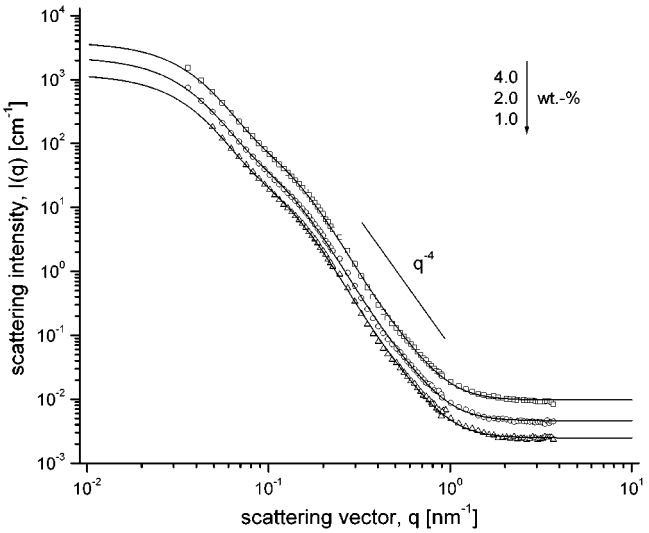

Figure 4. SANS curves of 1−4 wt % solutions of PB_{85}-*b*-PG_{55} (sample **2**) in 0.12 M NaCl in D_2O (pH ∼6).

complicated by the fact that polycarboxylates are weak polyelectrolytes, which complicates data evaluation; these examinations are therefore out of the scope of the present paper.

In the case of the copolymers PB_{85}-*b*-PG_{55} and PB_{119}-*b*-PG_{24}, the hydrodynamic radius of aggregates, although spherical, exceeds the contour length of the polymer chains (∼45 nm) by a factor of 1.5−2. The aggregates are therefore too large to be simple micelles, and one may suppose that these are rather vesicular entities or "polymersomes" [30,31] or even "peptosomes",[32] as vesicles made of polymer- or peptide-containing units have been recently called. To prove the vesicular structure of the aggregates, we investigated the aqueous solutions of PB_{85}-*b*-PG_{55} in more detail by small-angle neutron scattering (SANS) (see Figure 4). The form factors found at all examined concentrations (1−4 wt %) are in perfect agreement with the form factor of vesicles (see solid lines in Figure 4). Data analysis yields an outer vesicle radius $R_0 = 62$ nm and an inner radius $R_i = 34$ nm with particle size distributions of 33% for any sample. This yields an average vesicle diameter of 124 nm and a bilayer thickness of 28 nm, which goes well with the molecular dimensions of those polymers in a brushlike morphology. The peptosomes of PB_{85}-*b*-PG_{55} could be visualized in electron micrographs which were taken from freeze-fractured specimens of the aqueous polymer solution (see Figure 5). The micrographs show spherical aggregates that are 110−190 nm in diameter and are thus in accord with the results obtained from scattering experiments.

Both scattering and microscopy therefore strongly support the presence of vesicles with moderate polydispersity in the screened concentration range of 0.1−4.0 wt %. It must be pointed out that those structures are generated by simple dissolution; i.e., there is a driving minimum of free energy and an exchange of single block copolymer units which establishes the present equilibrium situation, independent of the history of the sample. The strong tendency of water-soluble amphiphilic block copolymers to form vesicular aggregates was already

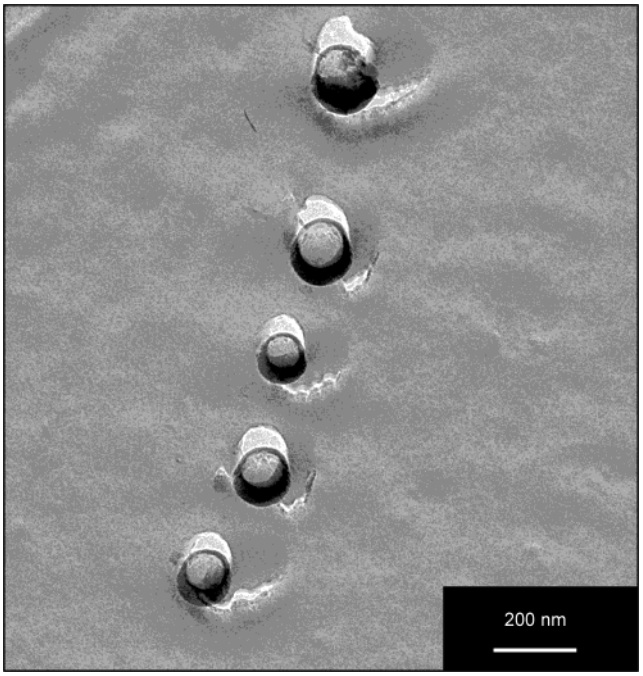

Figure 5. Freeze-fracture electron micrograph of a vesicular solution of PB_{85}-*b*-PG_{55} (sample **2**) in pure water (pH ∼6).

documented for other systems by a number of working groups.[33−38] The novelty in the present case is that the solvating chains are polypeptides, including their ability to interface with biological systems. In addition, the systems can perform a helix−coil transition, dependent on pH, without serious change of the vesicle morphology, as indicated by the pH-dependent DLS measurements. In the notation of Eisenberg, who named his structures "crew-cut vesicles", we name these structures "dreadlock vesicles".

Conclusions and Outlook

We described the multistep synthesis of novel amphiphilic polybutadiene-*block*-poly(L-glutamate) copolymers via anionic polymerization of butadiene and ring-opening polymerization of *N*-carboxyanhydrides. The molecular structure of the samples was determined by NMR, FT-IR, and SEC, and the pH-dependent secondary structure of the polypeptide segment (coil ↔ α-helix) was characterized by circular dichroism. In dilute aqueous solutions, the investigated samples form either spherical micelles ($R_h = 16$ nm) or large vesicular aggregates ($R_h = 70$−90 nm, DLS), depending on the chemical composition of the sample. The "peptosomes" were clearly identified by SANS and freeze-fracture TEM measurements and are considered to be equilibrium structures. In addition, these systems can perform a coil−helix transition, dependent on pH, without serious change of the morphology.

Future work shall cover a more advanced molecular characterization of the block copolymer samples, in particular the determination of molecular weight distributions. The copolymer aggregates shall be analyzed by static light scattering in order

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to gain information on aggregation numbers and radii of gyration. The evaluation of the stability of the vesicular regime in the phase diagram, the fixation of vesicular structures by cross-linking of the polybutadiene domain, and the examination of "giant peptosomes" shall also be investigated.

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