Journal of Colloid and Interface Science 387 (2012) 180–186

Contents lists available at SciVerse ScienceDirect

Journal of Colloid and Interface Science

www.elsevier.com/locate/jcis

Investigation of the reversibility of the unimer-to-aggregate transition in block copolymers by surface tension-measurements

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ARTICLE INFO

Article history:
Received 7 May 2012
Accepted 5 July 2012
Available online 25 July 2012

Keywords:
Block copolymer
Polymer nanoparticle
Self-assembly
Reversibility
DNA
Oligonucleotide

ABSTRACT

Two DNA-block copolymers, poly(caprolactone)-DNA and poly(methyl metacrylate)-DNA, were synthesized by conjugation of a short single strand of DNA (12 or 22 mer) to a single reactive group at one end of the synthetic polymer. These polymers self-assemble in water, without the need of any cosolvent, forming micelle-like aggregates that were imaged by TEM. The solution behavior of the bioconjugated polymers was investigated by surface tension measurements. In the direction of dilution, the surface tension was measured using a down-scaled Wilhelmy plate method. To proceed in the reverse direction (concentration), we measured the surface tension of a sessile drop during its evaporation. This latter method was firstly validated using ionic and non-ionic surfactants, including polymeric surfactants. It was then applied to investigate the unimer to micelles transition of the DNA-block copolymers. In all cases, a reversible transition was observed demonstrating the existence of a critical micellar concentration, close to 0.01 mmol L^{-1} for all the conjugates. The CMC was only slightly influenced by the length of the hydrophilic DNA block.

1. Introduction

Block copolymers self-assemble when placed in selective solvents that dissolve only one of the blocks [1–3]. Different structures can be formed, including spherical and rod-like micelles, sheets, vesicles, and other more exotic morphologies. Amphiphilic block copolymer micelles have proven to be very efficient for the solubilization [4,5] of substances in incompatible solvents and have been used as transfer agent [5,6], chemical microreactor [7–9], and vehicles for drug delivery [10–12]. Recently, bioconjugated polymers formed by association of organic polymers with biopolymers such as polypeptides, oligonucleotides, or oligosaccharides blocks were described [13–15]. Interest in such hybrids has been continuously growing and is motivated by their unique structural properties as well as by remarkable advanced functionalities [13,15,16]. Indeed, the biopolymers exhibit secondary structures that influence or even direct the self-assembly of the hybrid copolymers [16,17–19]. The biopolymer block can also impart biological functions such as the recognition of biological targets and undergo enzymatic transformation [20]. In this context, oligonucleotides are particularly interesting since they are able of extremely specific molecular recognition toward other oligonucleotide sequences and also –in the case of aptamers– toward other types of compounds including ions, molecular drugs, and proteins [15,16,21]. Since the early 1980s, automated chemicals methods have been available for the synthesis of arbitrary DNA strands. Later, the specific interest of DNA for building nanoscale structures with a programmable architecture such as origamis and nanoparticles superlattices (mostly from gold nanoparticles) became more and more obvious, leading to a burst of researches in this field [22–28]. More recently, single stranded (ss) oligonucleotides were conjugated to hydrophobic polymers to form block-copolymers with an amphiphilic structure able to self-assemble into vesicles or nanoparticles consisting of a polymer hydrophobic core surrounded by a DNA shell [29–36]. These latter nanoparticles were coined "DNA-micelles", and owing to the recognition abilities of DNA they can easily be equipped with any desired functionalities and are extremely attractive for applications in various fields, including catalysis and sensors [31,36]. Particularly, our group recently demonstrated that DNA micelles made from the poly(ethylene oxide)-poly(caprolactone)-DNA triblock copolymers could graft and ungraft on surfaces via DNA controlled hybridization [36].

Despite numerous advances in the design of new block copolymers (terpolymers, bioconjugated polymers...), few publications address the issue of the thermodynamic state of their assemblies. However, the knowledge of the behavior of polymer nanoparticles including, onset of aggregation, polymer exchange dynamics and

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0021-9797/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jcis.2012.07.009

disassembly on dilution or in presence of serum constituents is a critical issue for many applications such as drug delivery [37,38]. The existence of micelles is generally assessed, in given conditions, by electron microscopy, dynamic light scattering, or fluorescence spectroscopy, using polarity probes such as pyrene. A few authors have investigated the exchange dynamics between polymer aggregates and unimers by mixing two populations of micelles, labeled, and unlabeled. The exchange dynamics between these aggregates is followed either by fluorescence or by neutron scattering using fluorescently labeled polymers or deuterated polymers, respectively [39]. More recently, fluorogenic probes that emit fluorescence on disintegration of the micelles were used to investigate the stability of polymer micelles in presence of serum constituents [38].

Pursuing our efforts to investigate the potential interest of DNA-micelles in DNA technology, we synthesized new conjugates based on PMMA and PCl. Our main objectives were to investigate (1) the conditions for the formation of DNA micelles from these conjugates and (2) their solution behavior, particularly the onset of self-aggregation and its reversibility. Micelle forming DNA block-copolymers were obtained by coupling short oligonucleotides (12 or 22 mer) either to a poly(methyl methacrylate) (PMMA, $M_n \approx 6300$ g mol^{-1}) or to a poly(caprolactone) (PCl, $M_n \approx 3700$ g mol^{-1}) polymer. The formation of DNA-micelles in aqueous solution was probed by TEM and by surface tension measurements. The reversibility of the aggregation was probed by measurements of the surface tension firstly in the direction of dilution using a scaled-down Wilhelmy plate device, then in the direction of concentration using an evaporating sessile drop.

2. Experimental section

2.1. Materials

All single stranded oligonucleotides were purchased from Eurogentec (Belgium). Amino terminated oligonucleotides (DNA–C_6H_{12}–NH_2) were obtained in solution after reverse phase HPLC and hydroxy terminated oligonucleotides (DNA–C_6H_{12}–OH) were obtained on beads. Hydroxy terminated PMMA (M_n = 6300; Polydispersity Index = 1.06, probed by GPC) was purchased from Polymer Source. Methanesulfonic acid was kindly furnished by Arkema and was used as received. All other reactants were bought from Aldrich and used as received.

The concentrations of the oligonucleotide stock solutions were calculated from measurements of the optical density at 260 nm, using the molar extinction coefficient of the corresponding oligonucleotide. NMR spectra were recorded in $CDCl_3$ or CH_2Cl_2 using a Bruker Avance 300 MHz spectrometer at room temperature. ^{1}H chemical shifts were reported in ppm relative to Me_4Si as an external standard.

2.2. Synthesis of the poly(caprolactone) block [33]

ε-caprolactone (5 mL, 45 mmol) was dissolved in 20 mL of toluene together with H_2O (18 μL, 1 mmol). Methanesulfonic acid (75 μL, 1.15 mmol) was then added to the mixture. After 1 h 30 under stirring at 50 °C, the reaction was quenched by the addition of 50 μL of triethylamine. The volatiles were removed under reduced pressure. Dichloromethane (30 mL) was then added to the resultant solid, and the organic solution was poured in 300 mL cold methanol to precipitate the polymer. The precipitate was filtered and washed with cold methanol. After drying, the polymer was analyzed by ^{1}H NMR and SEC.

^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 3.99 (t, J = 6.7 Hz, 2H × 32, $n$$OCH_2$), 3.57 (t, J = 6.7 Hz, 2H, $HOCH_2$), 2.23 (t, J = 7.5 Hz, 2H × 32, $n$$COCH_2$), 1.58 (m, 4H × 32, $n$$CH_2$), 1.32 (m, 2H × 32, $n$$CH_2$).

SEC (THF, reference: polystyrene): $M_n \sim 3700$ g mol^{-1}, $M_w/M_n \sim 1.2$.

2.3. Activation of the poly(caprolactone) block

Poly(ε-caprolactone) (1 g, 0.27 mmol) was dissolved with dicyclohexylcarbodiimide (123.6 mg, 0.6 mmol) and *N*-hydroxysuccinimide (69 mg, 0.6 mmol) in 5 mL DMSO. The solution was stirred overnight at 50 °C. After cooling to room temperature, the solution was poured in 50 mL cold methanol. The resulting white precipitate was immediately filtered, washed with cold methanol, and dried under vacuum. The activated polymer was characterized by ^{1}H NMR. It was checked that no resonance corresponding to the formation of the methyl ester appeared in the 3.5 ppm region of the ^{1}H NMR spectra.

^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 3.99 (t, J = 6.7 Hz, 2H × 32, $n$$OCH_2$), 3.57 (t, J = 6.7 Hz, 2H, $HOCH_2$), 2.77 (broad, 4H, CH_2(NHS)), 2.23 (t, J = 7.5 Hz, 2H × 32, $n$$COCH_2$), 1.58 (m, 4H × 32, $n$$CH_2$), 1.32 (m, 2H × 32, $n$$CH_2$).

2.4. Synthesis of the DNA block copolymer PCl-DNA$_{22}$

The activated polymer (30 mg, 8.6 μmol) was dissolved in hot (50 °C) DMSO (1.2 mL). The amino-terminated oligonucleotide (60 mmol) was dissolved in 1.5 ml of carbonate buffer (1 M, pH = 10) and added to the hot polymer solution, which resulted in the formation of a precipitate. The mixture was then heated at 70 °C, and more DMSO (about 500 μL) was added if necessary to yield a homogeneous solution. The mixture was heated at 70 °C overnight. The solvents were then removed under vacuum and the conjugate was mixed with 1.5 ml of water. After 2 days of incubation, the system was filtered (Anopore filter, cutoff = 200 nm) to remove unsoluble unreacted polymer. The conjugate solution was purified by polyacrylamide gel electrophoresis (PAGE) and then desalted on a Sephadex G25 column. Unfortunately, it was not possible to find a suitable matrix for the Maldi-TOF analysis of the PCl-DNA compounds.

2.5. Synthesis of the DNA block copolymers PMMA-DNA$_{12}$ and PMMA-DNA$_{22}$

Hydroxyl terminated PMMA (1 g, 0.15 mmol) was dissolved in dry dichloromethane (6 ml) under an argon atmosphere. *N*,*N*-diisopropylethylamine (131 μl, 0.75 mmol) was added to the solution. The solution was stirred vigorously while 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (67 μl, 0.3 mmol) was added dropwise. The solution was stirred vigorously for 4 h until all of the phosphoramidite was reacted (checked by ^{31}P NMR). The reaction mixture of the activated polymer was washed with a saturated aqueous $NaHCO_3$ solution (10 ml) and brine (10 ml). After drying over $MgSO_4$, the organic layer was filtered and solvents were evaporated under reduced pressure, yielding the activated polymer as a white solid. ^{31}P NMR (81.0 MHz, CH_2Cl_2): 148 ppm.

Oligonucleotides immobilized on beads (200 mg, 6.6 μmol) were placed into a dry Schlenk flask and a solution of the activated polymer (0.198 mmol). Then, dry dichloromethane (6 ml), tetrazole (5.0 ml, 1.2 mmol), and dry acetonitrile (5 ml) were added under an argon atmosphere. The suspension was left to react for 2.5 h at room temperature. The beads were filtered and washed three times with dichloromethane (5 ml) and acetonitrile (5 ml). Then, 2 ml of an I_2 solution in H_2O/THF (1:2 v/v) was added to the beads. The suspension stood for 30 min and the beads were filtered. The beads were then washed with acetonitrile (30 ml) until the washing mixture remained colorless. Then, the beads were