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Preparation and characterization of non-linear poly(ethylene glycol) analogs from oligo(ethylene glycol) functionalized polyisocyanopeptides

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ABSTRACT

This contribution describes the synthesis and full characterization of oligo(ethylene glycol) functionalized polyisocyanopeptides. The thermal behavior of the resulting semi-flexible polymers was investigated in diluted aqueous conditions and features a tunable Lower Critical Solution Temperature (LCST). In line with previously described oligo(ethylene glycol) decorated polymers, the LCST of the materials shows a very small hysteresis effect and directly correlates with the oligo(ethylene glycol) side-chains length; short oligo(ethylene glycol) substituents are associated with lower LCST. In contrast with poly[oligo(ethylene glycol) methacrylate], a significant effect of the degree of polymerization (*DP*) of the poly(isocyanopeptide) core on the LSCT of the materials was observed. Most remarkably, poly(isocyanopeptide)-*graft*-oligo(ethylene glycol) chains of high *DP* lead to the reversible formation of strong hydrogels above the transition temperature, even at very low polymer concentration (0.1 wt.%). AFM studies point towards the formation of a highly organized fibrillar network in the gel-state, reminiscent of structures observed for low molecular weight gelators, polysaccharides, and protein-based (hydro)gels. It is proposed that the stiff and well-defined helical poly(isocyanopeptide) backbone avoids the collapse of the chains into globules at the transition temperature as usually observed for more flexible systems. Thus, above a critical *DP* the semi-flexible non-linear PEGs chains are getting kinetically trapped in an extended fibrillar network, when the oligo(ethylene glycol) corona hydrophilicity is lowered at higher temperature. As a result these polymers exhibit a strong ability to gel water at extremely low polymer concentrations.

1. Introduction

Non-linear poly(ethylene glycol) (PEG) have recently attracted a great deal of attention for the development of innovative water soluble materials [1]. Such PEG analogs are classically prepared from the (co)polymerization of macro-monomers bearing oligo(ethylene glycol) substituents. The solution properties of the resulting *comb*-like polymers arise from the fine balance between the hydrophilic/hydrophobic characters of the grafted side-chains and of the polymeric core. The hydrophilicity of oligo(ethylene glycol) groups is temperature dependent and their incorporation as side arms grafted from a polymer backbone offers a simple and elegant way to trigger the overall hydrophilic/hydrophobic balance of these materials, and therefore provides a straightforward approach to the development of a variety of thermo-responsive systems [1,2]. So far, most non-linear PEG analogs have been derived from vinyl [3,4], styrene [5,6] and (meth)acrylate [5,7–11], monomers and led to the formation of rather flexible

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macromolecules, featuring a classical coil to globule transition above their transition temperature in dilute aqueous conditions [12]. Methacrylate derived PEG analogs have been studied extensively and can be considered as the reference for this class of materials. They feature easily tunable Lower Critical Solution Temperature (LCST), sharp phase transition with a very small hysteresis effect, little dependence of the polymer's degree of polymerization (*DP*) and experimental conditions on the LCST, and high biocompatibilty [13]. These characteristics were related to the rather inert nature of oligo(ethylene glycol) arms that do not develop much specific inter- or intramolecular interactions such as hydrogen bonding or other non-covalent interactions [14]. The rather flexible nature of these materials combined with very low specific interactions between the chains explains the inability of these polymers to form gel phases even at high polymer concentration [15]. When associated with purely hydrophilic segments within linear or star-like architectures, poly[oligo(ethylene glycol) methacrylate] based macro-gelators with well-defined and easily tunable transition temperatures could, however be prepared. The latter *co-polymers* led to the formation of strong hydrogels at high (polymer) concentrations ([polymer] > 15-wt.%) [16,17]. Recently we showed that the use of a more rigid polymer backbone which possess a well-defined helical secondary structure, i.e., polyisocyanopeptides, permits the generation of thermo-responsive *homopolymers* that exhibit exceptionally efficient hydro-gelation capabilities ([polymer] = 0.1-wt.%) [18]. The resulting hydrogels were found to fully mimic the mechanical properties of the cytoskeleton's intermediate filament network which was unprecedented thus far for man-made materials [19]. Hence, this class of non-linear PEG analogs opens an avenue towards the development of biomimetic networks that can be easily tuned and functionalized.

Prior to this work, only a few studies have been reported on the synthesis of oligo(ethylene glycol)-functionalized polyisocyanides. These were prepared either by post-modification of a pre-formed polyisocyanide backbone by peptidic coupling [20] and the copper catalyzed Huisgen 1,3-dipolar cycloaddition [21], or through the direct polymerization of crown-ether appended isocyanides [22]. The thermo-responsive properties of the resulting materials were, however, not explored in detail [23]. In addition the direct polymerization of oligo(ethylene glycol)-functionalized isocyanides led to materials with only limited degree of polymerization [22,23].

Polyisocyanides form a well-known class of static helical polymers [24,25]. They consist of poly(imine) chains in which every carbon atom of the backbone bears a substituent, resulting in an extremely dense *comb*-like architecture; helical folding of the backbone permits the minimization of steric repulsion between the pendant side groups [26–30]. The introduction of peptide-containing side chains further leads to materials with unprecedented stiffness [31,32]. This effect has been attributed to the development of an intramolecular hydrogen-bonding network between the peptides pendants, which adopt a twisted β-sheet arrangement along the helical poly(imine) core [31–33]. Such a well-defined structure has naturally designated these materials as versatile synthetic platforms to order various photo- and electro-active species for optoelectronics applications [34–37]. With the aim of developing non-ionic water soluble biocompatible analogs, we investigated the synthesis of oligo(ethylene glycol)-coated polyisocyanopeptides. In this contribution, we describe an optimized protocol for the preparation of polyisocyanopeptides-*graft*-oligo(ethylene glycol) and discuss the basic properties of this intriguing class of non-linear PEG analogs.

2. Experimental

2.1. General methods and materials

Dichloromethane and chloroform were distilled over $CaCl_2$. Tetrahydrofuran, diethyl ether and toluene were distilled on sodium, in the presence of benzophenone. Water was purified with a Milipore MiliQ system, (mQ water 18.2 MΩ). All the other chemicals were used as received from the suppliers. Column chromatography was performed using silica gel (40–60 μm) purchased from Merck or silica gel (0.060–0.200 mm) provided by Baker. TLC analyses were carried out on silica 60 F_{254} coated glass obtained from Merck and the compounds were visualized using Ninhydrine or basic aqueous $KMnO_4$ solutions. ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker AC-300 MHz instrument operating at 200 or 300 MHz and 75 MHz, respectively. FT-infrared spectra of the pure compounds were recorded on a ThermoMattson IR300 spectrometer equipped with a Harrick ATR unit. Solution IR spectroscopy was carried out in sealed KBr cuvette (1 mm) on a Bruker Tensor 27 spectrometer operated with Opus software. Solutions of ***poly*-1a–c** and the respective isocyanides **1a–c** were prepared in chloroform, tetrahydrofuran, or toluene at a concentration of 30 mM. Melting points were measured on a Buchi B-545 and are reported uncorrected. Mass spectrometry measurements were performed on a JEOL AccuTOF instrument (ESI). Optical rotations were measured on a Perkin Elmer 241 Polarimeter at room temperature and are reported in 10^{-1} deg cm^2 g^{-1}. CD spectra were recorded on a Jasco 810 instrument equipped with a Peltier temperature control unit. The cell was thermostated at 20 °C or heated/cooled within the desired temperature range at a temperature gradient of 1 °C/min. Gel permeation chromatography was conducted on a system equipped with a Waters 1515 Isocratic HPLC pump, a Waters 2414 refractive index detector, a Waters 2707 autosampler, a PSS PFG guard column followed by a ReproGel column (300 × 8 mm, 5 μm, linear, Dr. Maisch Gmbh, Ammerbuch-Entrigen, Germany) run at 55 °C with THF containing 0.25% (v/w) tetrabutyl ammonium bromide (TBAB). Calibration: poly (ethylene glycol) standards. AFM experiments were performed using a dimension 3100 or multimode microscope operated with nanoscope III or nanoscope IV control units (Digital Instruments). Solutions of ***poly*-1a–c** ($\sim 10^{-6}$ M in $CHCl_3$) were spin-coated (1600 rpm) onto freshly cleaved Muscovite Mica to determine the contour lenght (L_n) of isolated polymers chains. ***Poly*-1b** hydrogels were deposited by direct contact with freshly cleaved HOPG or Muscovite Mica. All images were recorded with the AFM operating in Tapping Mode™