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Thermoresponsive glycopolypeptides with temperature controlled selective lectin binding properties

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ABSTRACT

Linear polypeptide poly(γ-propargyl-L-glutamate) (PPLG) was synthesized by the ring opening polymerization (ROP) of the corresponding N-carboxy anhydride (NCA). Azide-functional galactose and 1-azido-2-(2-methoxyethoxy)ethane (mEO_2) were co-clicked on the polypeptide at different ratios to introduce selective binding and thermoresponsive properties. Selective binding was demonstrated by turbidity assays with Ricinus Communis Agglutinin (RCA_{120}) lectin. Cloud point measurements confirmed the cloud point temperature (T_{cp}) of the polymers increasing with the ratio of mEO_2 to galactose. Moreover, lectin binding experiments at temperatures above and below T_{cp} suggest that binding is suppressed above T_{cp}. This opens opportunities to design functional materials with temperature controlled biological response.

1. Introduction

Stimuli-responsive polymers have been intensively investigated including materials responding to temperature, light, pH, and biological cues such as enzymes [1–4]. A promising application for thermoresponsive polymers is in drug delivery due to the fact that the temperature of infected tissue (e.g. tumors) is slightly elevated [5–7]. One challenge of this strategy is to fine tune the material's response temperature so that the phase transition triggered release of the payload occurs in a very narrow temperature window precisely at the desired temperature. Another challenge arises from the non-specificity of most thermoresponsive materials, which demands the systemic application of higher dosages. A strategy to overcome this is the incorporation of targeting molecules in the thermoresponsive polymer. Carbohydrates (glycans) are promising targeting candidates since the discovery that they regulate numerous biological processes such as cell communication and selective binding of other biological species through highly specific glycan–lectin binding [8–10]. We are interested in exploring ways to manipulate this specific binding event with the long-term goal to design smart materials in which lectin binding can be triggered by an external stimulus.

To the best of our knowledge only two instances of the combination of thermo- and lectin-responsive polymers have been reported. In a first example Schubert described the synthesis of thermoresponsive glycopolymers by atom transfer radical copolymerization (ATRP) of N-allylacrylamide (NAllAm) and N-isopropylacrylamide (NiPAm) [11]. The pendant allyl groups were subsequently utilized for conjugation with thiol-functional glucose and galactose via thiol-ene chemistry. Turbidity measurements confirmed that the copolymers bearing protected sugar moieties had a lower cloud point temperature (T_{cp}) than those bearing deprotected sugars. Biorecognition of the polymers was studied by turbidity assays using Concanavalin (ConA) and Ricinus Communis Agglutinin lectin (RCA_{120}), albeit the influence of the temperature on the lectin binding was not investigated.

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In a second example Zhu described the preparation of the hydrophilic block glycopolymer poly(N-isopropylacrylamide-*co*-6-O-vinyladipoyl-D-glucose)-*b*-poly(N-isopropylacrylamide) using reversible addition-fragmentation chain transfer (RAFT) copolymerization of NIPAm and 6-O-vinyladipoyl-D-glucose (OVAG) [12]. This amphiphilic block copolymer forms micelles in water, which upon heating above the T_{cp} significantly increased in size due to a coil-to-globule transition of the glycopolymer block. Interestingly, Dynamic Light Scattering (DLS) measurements suggested an influence of the temperature on the lectin binding of the micelles, which was better below the T_{cp}. This was explained by the enhanced accessibility of the glycans in the random coil structure below T_{cp} as compared to their accessibility when the glycopolymer block adopts a globular structure in the micelle above the T_{cp}.

Both of the above polymers are polyacrylic in nature and lack biodegradability. An interesting alternative class of polymers is polypeptides obtained from the ring-opening polymerization of amino acid N-carboxyanhydrides (NCA). Progress in NCA polymerization has opened opportunities to synthesize a wealth of well-defined polypeptide structures [13–15]. As a result, polypeptides promise potential in biomedical fields such as drug delivery [16–21], tissue engineering [22] or imaging [23]. The synthesis of glycosylated polypeptides has been achieved using direct polymerization of glycosylated NCA or post-modification by conventional chemistry as well as click chemistry [24–31]. On the other hand, a range of thermoresponsive polypeptides have been described in the literature [21,32–35]. Notably, Hammond described the ring opening polymerization of poly(γ-propargyl-L-glutamate) (PPLG) to obtain a polypeptide platform on which they attached different oligo(ethylene glycols) through copper catalyzed click reaction in order to induce thermoresponsiveness [36]. In an extension of this work, the same authors grafted both 1-azido-2-(2-methoxyethoxy) ethane (mEO_2) and N-(2-azidoethyl)-N-isopropylpropan-2-amine (diisopropylamine) on the PPLG backbone to achieve both thermal and pH responsiveness [37].

In this work we present the synthesis of a polypeptide with combined lectin recognition and thermoresponsive properties. The polymers are obtained by ring-opening polymerization of γ-propargyl L-glutamate NCA and conjugated with mEO_2 and galactose through copper catalyzed click chemistry. Varying the ratios of the two units on the polymer backbone, the hydrophilicity as well as the T_{cp} could be modified. Moreover, we demonstrate that the lectin binding can be thermally controlled.

2. Experimental section

2.1. Materials

All chemicals were purchased from Sigma–Aldrich and used as received unless otherwise noted. Diethyl ether was purchased from VWR. DMSO, ethyl acetate and ethanol were used directly from the bottle under an inert and dry atmosphere. 1-β-Azido-2,3,4,6-tetraacetyl-D-galactose was synthesized following a literature procedure [16]. 1-Azido-2-(2-methoxyethoxy)ethane (mEO_2) was synthesized using a similar procedure as the one reported by Hammond [37]. All chemicals were used without any purification unless otherwise noted.

2.2. Methods

^{1}H NMR spectra were recorded at room temperature with a Bruker Avance 400 (400 MHz), DMSO-d_6, $CDCl_3$ and D_2O were used as solvents and signals were referred to the signal of residual protonated solvent signals (D_2O). TMS was used as an internal standard for DMSO-d_6 and $CDCl_3$. ATR-FTIR spectra were collected on a Perkin–Elmer Spectrum 100 in the spectral region of 650–4000 cm^{-1} and were obtained from 4 scans with a resolution of 2 cm^{-1}. A background measurement was taken before the sample was loaded onto the ATR unit for measurements. CD-spectroscopy was performed on a Jasco J-815 spectrometer with 0.0050 mM solution of the polypeptide in demineralized water. Mean residue ellipticities were calculated from the CD spectra following a literature procedure [38] using the equation $[\Theta]_{MRW} = (\Theta \times M_{MRW})/(10 \times c \times l)$, Θ: experimental ellipticity in mdeg, M_{MRW}: molecular weight in g/mol, c: concentration in mg/ml, l: path length 0.5 cm. M_{MRW} used for calculations: 19,925 g/mol (mEO_2), 20,217 g/mol (10% galactose), 20,305 g/mol (13% galactose), 20,627 g/mol (24% galactose), 21,096 g/mol (40% galactose). Helicities were calculated at $\lambda = 222$ nm using $f\alpha = (-[\Theta 222]_{MRW} + 3000)/39{,}000$. All turbidity assays were carried out on an Perkin Elmer Lambda900 UV–VIS instrument using UV quartz cuvettes. Assays with lectin were monitored at 450 nm in PBS buffer solution, while assays without lectin were monitored at 500 nm in deionised (DI) water.

2.3. Synthesis of γ–propargyl L-glutamate hydrochloride

The preparation of γ-PLG-HCl was carried out following a modified procedure reported by Hammond and coworkers [36]. To a solution of L-glutamic acid (10 g, 68 mmol) suspended in propargyl alcohol (300 mL, 5.2 mol), 17 mL of chlorotrimethylsilane was added drop-wise under nitrogen. The solution was stirred at 40 °C overnight and then the crude product was precipitated into diethyl ether. To avoid the presence of free propargyl alcohol, the product was extensively washed with ether, filtered, recrystallized from ethanol and dried under vacuum to obtain a white powder (dark grey powder when propargyl alcohol has not been removed). Yield: 13.3 g (60 mmol, 88%). ^{1}H NMR (D_2O, δ ppm): 2.06 (2H, $CHCH_2CH_2$), 2.53 (2H, $CHCH_2CH_2$), 2.84 (1H, C≡CH), 3.80 (1H, $CHCH_2CH_2$), 4.59 (2H, $OCH_2C{\equiv}CH$).

2.4. Synthesis of N-carboxyanhydride of γ-propargyl L-glutamate (PLG-NCA)

γ-propargyl L-glutamate hydrochloride (3.6 g, 16 mmol) was suspended in dry ethyl acetate (120 mL) and the solution was heated to reflux under nitrogen. Triphosgene (2.2 g, 7 mmol) was added and the reaction was left to