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Thiol−Ene Clickable Polypeptides

Jing Sun and Helmut Schlaad*

Max Planck Institute of Colloids and Interfaces, Department of Colloid Chemistry, Research Campus Golm, 14424 Potsdam, Germany

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Polypeptide-based homopolymers and copolymers are important materials for biomedical applications[1] as well as for biomimetic materials research.[2,3] Synthetic polypeptides can nowadays be produced with a great level of sophistication applying ring-opening polymerization of amino acid *N*-carboxyanhydrides (NCA),[4,5] however, with certain limitations in diversity of the side-chain functionalities. Especially few are synthetic examples of sugar−peptide conjugates or glycopolypeptides,[6,7] which are potential candidates for advanced life science applications and glycomics.[8] Up to the present, the synthesis of carbohydrate functionalized NCAs[9] and controlled polymerization to well-defined glycosylated polypeptides remains a challenging task.[7]

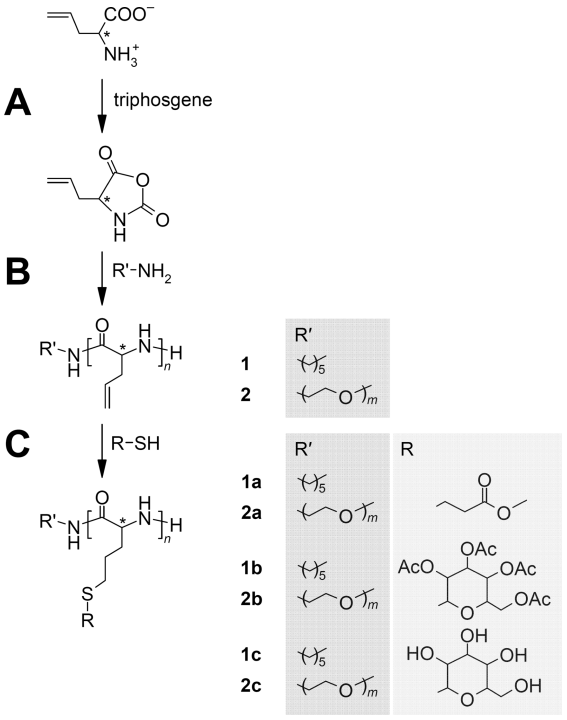
An alternate route to functional polypeptides is the chemical modification of well-defined polypeptide precursors, for instance aminolysis[10−12] or transesterification[13] of poly(γ-benzyl L-glutamate). Recently, poly(ethylene glycol) and also sugars were efficiently grafted onto poly(γ-propargyl L-glutamate) using copper-catalyzed azide−alkyne click chemistry (CuAAC).[14,15]

Thiol−ene chemistry has emerged as a versatile and very efficient tool for the functionalization of polymers,[16−18] including biomacromolecules,[19] as well as of colloids[20,21] and surfaces.[22−24] The natural amino acid being designed for this chemistry is cysteine. In fact, Heise et al.[25] showed that ∼20 mol % cysteine-containing synthetic polypeptides can be coupled with acrylic ester derivatives. Coupling was, however, carried out under basic reaction conditions, promoting nucleophilic Michael addition, in the presence of a radical source (azobisisobutylonitrile, AIBN) at elevated temperature. Disadvantages connected with polycysteines are the inevitable use of protecting group chemistry during synthesis and the slow oxidative cross-linking (disulfide bridging) under atmospheric conditions.

Another possibility is to use an amino acid with an α-olefinic side chain, for instance the commercially available DL-allylglycine (2-aminopent-4-enoic acid, CAS number 7685-44-1). Protecting group chemistry would not be needed, and the corresponding poly(DL-allylglycine) should be rather stable against cross-linking. Poly(DL-allylglycine) exhibits a similar structure as the thiol−ene clickable pseudopeptide poly[2-(3-butenyl)-2-oxazoline],[26] but its solubility may be limited to organic acid media.[27,28] Notably, polydepsipeptides composed of allylglycine and glycolic acid have been synthesized and modified through thermal thiol−ene addition.[29] Conversion of the allyl groups was in the order of 15−100%, depending on the structure of the ω-functional thiol.

*Corresponding author: Fax +49(0)331.567.9502, e-mail schlaad@mpikg.mpg.de.

Scheme 1. Synthetic Pathway of (A) Synthesis and (B) Polymerization of Allylglycine NCA and (C) Subsequent Radical Thiol Addition[a]



[a] **Reaction conditions are described in the text.**

In the present contribution, we wish to report on the improved synthesis and controlled polymerization of DL-allylglycine NCA[27,28] and exemplary modifications of poly(DL-allylglycine) and poly(ethylene oxide)-*block*-poly(DL-allylglycine) with either (I) methyl 3-mercaptopropionate and (II) 1-thio-β-D-glucopyranose (and the *O*-acetyl-protected derivative) (Scheme 1). Radical thiol−ene additions were performed applying thermal as well as photochemical conditions. Examples are chosen to demonstrate the "clickability" of poly(DL-allylglycine) and general applicability for the synthesis of complex glycopolypeptides.

Synthesis of DL-Allylglycine NCA (*Scheme 1A*). DL-Allylglycine and bis(trichloromethyl)carbonate (triphosgene) were reacted in a mixed solvent of dry tetrahydrofuran (THF) and α-pinene under a constant stream of dry argon.[30] The recrystallized product exhibited the expected chemical structure of DL-allylglycine NCA, as confirmed by ^{1}H NMR spectroscopy (Supporting Information S1). It is especially worth being mentioned that the hydrochlorination of the allyl side chain could be completely avoided by the presence of α-pinene (sacrificial olefin) and argon stream.

Synthesis of Poly(DL-allylglycine) (*Scheme 1B*). DL-Allylglycine NCA was polymerized in dry *N*,*N*-dimethylformamide (DMF) solution at room temperature using *n*-hexylamine as the initiator (molar ratio [NCA]/[NH_2] = 30). The isolated poly(DL-allylglycine) (**1**) exhibited the expected chemical structure (^{1}H NMR, Figure 1A) and a narrow molecular weight distribution with a polydispersity index $PDI^{apparent} \sim 1.1$ (SEC, Figure 1B). The absolute number-average molecular weight was M_n = 1840 g/mol, as calculated

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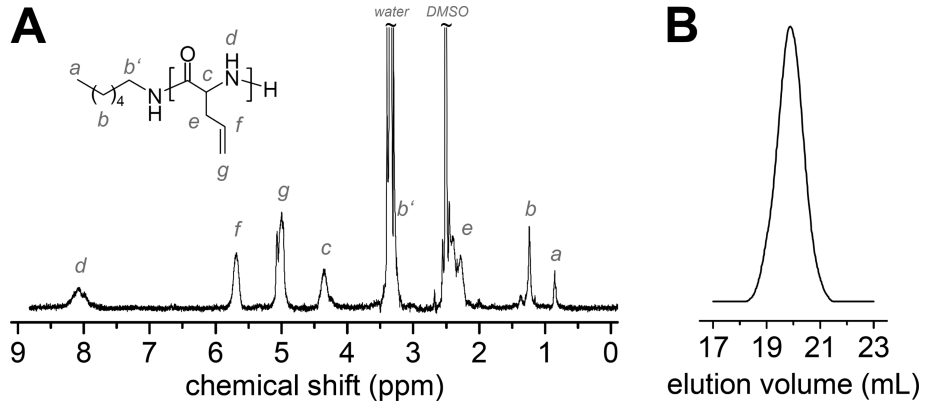

Figure 1. (A) ^{1}H NMR spectrum (400.1 MHz, DMSO-d_6) and (B) size exclusion chromatogram (NMP, 70 °C, RI) of poly(DL-allylglycine) sample **1**.

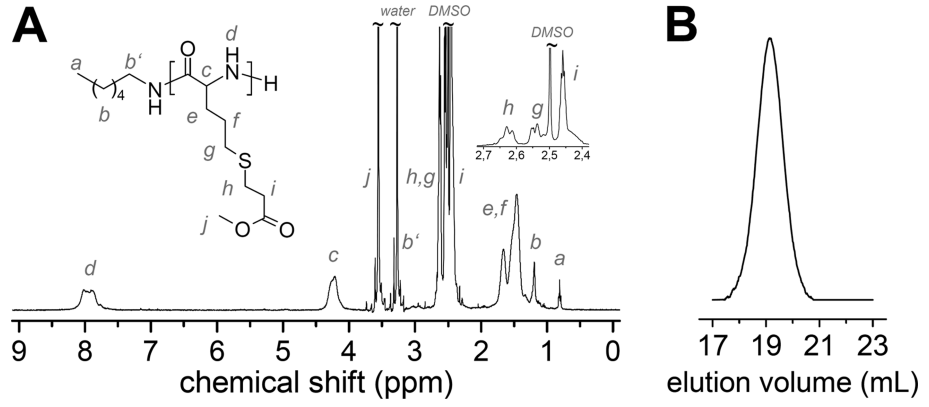

Figure 2. (A) ^{1}H NMR spectrum (400.1 MHz, DMSO-d_6) and (B) size exclusion chromatogram (NMP, 70 °C, RI) of the methyl 3-mercaptopropionate-modified poly(DL-allylglycine) sample **1a**.

from ^{1}H NMR peak integrals of the CH_3 end group and =CH monomer unit (peaks *a* and *f* in Figure 1A). Solvents for poly(DL-allylglycine) include DMF, *N*-methyl-2-pyrrolidone (NMP), dimethyl sulfoxide (DMSO), and trifluoroacetic acid (TFA); nonsolvents are heptane, chloroform, diethyl ether, THF, ethanol, and water.

Poly(ethylene oxide)-*block*-poly(DL-allylglycine) (**2**) was synthesized by the same protocol using amino-terminated poly(ethylene oxide) (PEO-NH_2; M_n = 3600 g/mol) as the initiator ([NCA]/[NH_2] = 15). The chemical structure of the isolated block copolymer (M_n = 4860 g/mol) was confirmed by ^{1}H NMR spectroscopy (Supporting Information S2). SEC indicated a narrow molecular weight distribution with $PDI^{apparent}$ = 1.09 (Supporting Information S2).

All these results seem to suggest that the polymerization of DL-allylglycine NCA proceeds in a controlled manner to yield well-defined poly(DL-allylglycine) (co)polymers.

Radical Thiol−Ene Addition (*Scheme 1C*). (I) The first attempt involved functionalization of poly(DL-allylglycine) (**1**) with methyl 3-mercaptopropionate (**a**) using AIBN as radical source at elevated temperature. A ~1 wt % solution of **1** in DMF was mixed with methyl 3-mercaptopropionate and AIBN (molar ratio [C=C]:[SH]:[AIBN] = 1:2:1), put under an argon atmosphere, and stirred for 1 day at 70 °C. The ^{1}H NMR spectrum of the isolated product **1a** (Figure 2A) showed the characteristic signals of the newly formed CH_2SCH_2 linkage at $\delta \sim 2.6$ ppm. As further indicated by the complete lack of double bond signals (δ = 5.0−5.7 ppm), the allylglycine units were modified in a virtually quantitative yield thus clicked. The narrow molecular weight distribution of the poly(DL-allylglycine) precursor has also been preserved during the thiol−ene reaction (SEC, Figure 2B).

The direct addition of methyl 3-mercaptopropionate onto **1** using a mercury medium pressure UV lamp at room temperature appeared to be, at first, far less effective. The degree of modification was just 21% (NMR, not shown) but could be improved to 92% when working with higher concentrations of the reactants (~2 wt % **1** in DMF, [C=C]:[SH] = 1:2). The presence of residual double bonds indicates that the less than quantitative addition may be a matter of reaction kinetics or sterics, not of side reactions. Higher reactant concentrations (however, > 2 wt % polymer solutions are not applicable) and the presence of a photoinitiator (vide infra) should help to accelerate the thiol−ene photoaddition. It is worth being mentioned that the degree of modification of **1** reached 81% after 1 day of irradiation with the Osram Ultra-Vitalux lamp (reaction temperature ~40 °C); this light bulb is on the market as a substitute for sunlight and is considerably less dangerous and expensive as the mercury lamp.[31]

Similar results were obtained for the modification of the poly(DL-allyglycine) block copolymer **2** with methyl 3-mercaptopropionate (→ **2a**). The degrees of modification obtained were either quantitative (thermal route; Supporting Information S3) or about 80% (photochemical route: mercury lamp ~75%, UV hand lamp ~85%; data not shown).

(II) Glucosylation of poly(DL-allylglycine) **1** (~1 wt % in DMF) with 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranose (**b**) was first attempted using AIBN at 70 °C ([C=C]:[SH]:[AIBN] = 1:2:1); reaction time was 1 day. The isolated protected glucopolypeptide **1b** appeared to be just partially glucosylated with a degree of modification of ~35%.

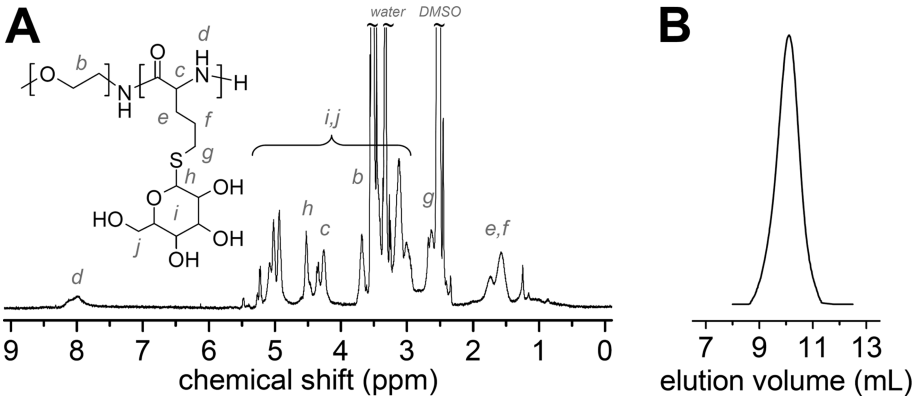

Figure 3. (A) ^{1}H NMR spectrum (400.1 MHz, DMSO-d_6) and (B) size exclusion chromatogram (DMSO, 70 °C, RI) of the 1-thio-β-D-glucopyranose-modified poly(ethylene oxide)-*block*-poly(DL-allylglycine) **2c**.

Applying the same procedure to the block copolymer **2** (→ **2b**), however, yielded an even lower degree of modification of just ~27% (data not shown).

Rather as expected, the photoaddition of 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranose onto **2** (~2 wt % in DMF) was less effective as compared to thermal addition. Irradiation with either the mercury medium pressure lamp or the UV hand-lamp for 1 day at room temperature afforded very poor degrees of modification of about 10%. The residual allylglycine units remained intact, pointing again to slow reaction kinetics rather than problems with the occurrence of side reactions. The presence of phenyl bis(2,4,6-trimethylbenzoyl)phosphine oxide (Irgacure 819, 1 equiv with respect to double bonds) helped to improve the glucosylation of **2** with mercury medium pressure UV light, reaching a degree of modification of ~50% after 1 day. However, this product carried detectable amounts of aromatic fractions of the initiator (^{1}H NMR, Supporting Information S4).

The low degrees of glucosylation could possibly be explained by poor solvent quality. Apparently good solvents for poly(DL-allylglycine) are, apart from amide solvents, DMSO and TFA. DMSO is known as radical scavenger and thiol oxidation agent,[32] leaving TFA as the solvent of choice. TFA would also offer the possibility to directly use the sodium salt of 1-thio-β-D-glucopyranose (**c**) (being protonated in situ) instead of the *O*-protected derivative. Poly(DL-allylglycine) and also the sugar appear to be stable in TFA solution at room temperature (data not shown). The photoaddition of 1-thio-β-D-glucopyranose onto the poly(DL-allylglycine) in TFA solution (2 wt %; [C=C]/[SH] = 1:2) yielded the glucopolypeptide **1c** (after evaporation of TFA in vacuum, dialysis against water, and freeze-drying) with a degree of modification of ~50% (Supporting Information S5). Glucosylation of poly(ethylene oxide)-*block*-poly(DL-allylglycine), on the other hand, reached ~90% yield after 1 day and was virtually quantitative after 2 days (→ **2c**), as indicated by ^{1}H NMR spectroscopy (absence of =CH signals at δ = 5.7 ppm, Figure 3A). SEC showed a monomodal molecular weight distribution for **2c** with a polydispersity index $PDI^{apparent}$ = 1.3 ($M_n^{apparent}$ = 15 000 g/mol) (Figure 3B).

In summary, we described the synthesis of well-defined poly(DL-allylglycine) homopolymer and block copolymer (PDI ~ 1.1) using the NCA method and subsequent modification through radical thiol−ene chemistry. Eventually, for properly chosen reaction conditions, the thiol addition onto poly(DL-allylglycine) can have the characteristics of a click reaction. Importantly, this approach allows the direct synthesis of glucopolypeptides without need for protecting group chemistry or transition metal catalysis. Current investigations deal with the optimization of reaction conditions for direct "click" glycosylation and synthesis of more complex glycopolypeptides.

Experimental Section. *Materials*. DL-Allylglycine (99%), α-pinene (98%), methyl 3-mercaptopropionate (98%), 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranose (97%), 1-thio-β-D-glucopyranose sodium salt hydrate, Irgacure 819 (97%) (Sigma-Aldrich), triphosgene (96%, Merck Schuchardt OHG), TFA (99.9%, Fisher Scientific), and diethyl ether (Carl Roth GmbH & Co. KG) were used as received. AIBN (98%, Fluka) was recrystallized from methanol. *n*-Hexylamine (99.9%, Sigma-Aldrich), DMF (peptide grade; IRIS Biotech), ethyl acetate (p.a., Th. Geyer GmbH & Co. KG), and heptane (isomers, 99%, Carl Roth GmbH & Co. KG) were distilled from CaH_2. THF (p.a., VWR International) was distilled in the presence of sodium prior to use. Amino-terminated poly(ethylene oxide) (PEO-NH_2) was received from RAPP Polymere GmbH (Tübingen, Germany); M_n = 3600 g/mol, PDI = 1.3 (as determined by SEC in THF, calibration with PEO standards).

NCA Synthesis. DL-Allylglycine (5.0 g) and triphosgene (7.0 g) were stirred in a mixed solvent of dry THF (50 mL) and α-pinene (15 mL) for 1.5 h at 50 °C; the mixture was constantly flushed with a stream of dry argon. The crude product was precipitated from heptane, recrystallized three times from ethyl acetate/heptane 1:1 (v/v), and dried in vacuum; gravimetric yield: 3.5 g (58%). Melting point: 88−90 °C (lit.[27] 92−94 °C). ^{1}H NMR (400.1 MHz, $CDCl_3$): δ/ppm = 2.51, 2.74 (m, 2H, CH_2), 4.38 (m, 1H, CH), 5.29 (m, 2H, $H_2C=$), 5.74 (m, 1H, =CH), 6.35 (b, 1H, NH).

NCA Polymerization. Poly(DL-allylglycine): To a solution of DL-allylglycine NCA (1.0 g) in dry DMF (100 mL) was added *n*-hexylamine (24 mg) via syringe, and the mixture was stirred for 2 days at room temperature under an argon atmosphere. The polymerization was quenched by precipitation into diethyl ether under vigorous stirring. The polypeptide was collected by filtration as a white powder and dried in vacuum at room temperature; gravimetric yield: 0.66 g (92%). ^{1}H NMR (400.1 MHz, DMSO-d_6): δ/ppm = 0.83 (t, CH_3 hexyl), 1.21 (b, CH_2 hexyl), 2.26, 2.39 (m, CH_2), 4.36 (m, CH), 5.02 (m, $H_2C=$), 5.67 (m, =CH), 8.04 (b, NH); M_n = 1840 g/mol. SEC (NMP): $M_n^{apparent}$ = 1800 g/mol, $PDI^{apparent}$ = 1.12.

Poly(ethylene oxide)-*block*-poly(DL-allylglycine): DL-Allylglycine NCA (0.9 g) was added to a solution of PEO-NH_2 (M_n = 3600 g/mol) (1.6 g) in dry DMF (90 mL), and the mixture was stirred for 2 days at room temperature under an argon atmosphere. The polymerization was quenched by

precipitation into diethyl ether under vigorous stirring. The polypeptide block copolymer was collected by filtration as a white powder and dried in vacuum at room temperature; gravimetric yield: 2.2 g (88%). ^{1}H NMR (400.1 MHz, DMSO-d_6): δ/ppm = 2.30, 2.41 (m, CH_2), 3.51 (b, CH_2CH_2O), 4.38 (m, CH), 5.02 (m, $H_2C=$), 5.69 (m, =CH), 8.03 (b, NH); M_n = 4860 g/mol. SEC (NMP): $M_n^{apparent}$ = 5900 g/mol, $PDI^{apparent}$ = 1.09.

Radical Thiol−Ene Addition (*Exemplary Procedure*). To a ~1 wt % solution of **1** in DMF were added methyl 3-mercaptopropionate and AIBN (molar ratio [C=C]:[SH]:[AIBN] = 1:2:1). The solution was degassed by three freeze−thaw cycles, put under an argon atmosphere, and stirred for 1 day at 70 °C. Then, the mixture was dialyzed against THF (Spectra/Por dialysis membrane, regenerated cellulose, MWCO 1000) for 3 days and precipitated into diethyl ether under vigorous stirring. The solid product (**1a**) was filtered and dried in vacuum at room temperature; gravimetric yield: 0.1 g (71%). ^{1}H NMR (400.1 MHz, DMSO-d_6): δ/ppm = 0.81 (t, CH_3 hexyl), 1.19 (b, CH_2 hexyl), 1.45, 1.66 (b, CH_2CH_2), 2.46 (t, CH_2CO), 2.54, 2.62 (m, SCH_2), 3.55 (s, CH_3), 4.23 (b, CH), 7.99 (b, NH). SEC (NMP): $M_n^{apparent}$ = 2800 g/mol, $PDI^{apparent}$ = 1.10.

UV Light Sources. (i) Heraeus TQ 150, mercury medium pressure, 150 W (UV-Consulting Peschl, Mainz, Germany); (ii) UV hand lamp VL-215.BL, λ = 365 nm, black light 2 × 15 W, 108 μW/cm^2 (LTF Labortechnik GmBH & Co. KG, Wasserburg, Germany); (iii) Ultra-Vitalux, 300 W, 230 V, E27, FS1 (Osram GmbH, Munich, Germany). Lamps were used in combination with borosilicate glass filters (Duran glass reaction vessels).

Analytical Instrumentation. Melting point was measured in an open capillary with a Mel Temp II, Laboratory Devices. ^{1}H NMR measurements were carried out at room temperature using a Bruker DPX-400 spectrometer operating at 400.1 MHz. $CDCl_3$ (Sigma-Aldrich) and DMSO-d_6 (Carl Roth GmbH & Co. KG) were used as solvents; signals were referenced to the signal of residual protonated solvent at δ = 7.26 and 2.50 ppm, respectively. Size exclusion chromatography (SEC) with simultaneous UV and RI detection was performed in (i) THF at 25 °C, flow rate: 1 mL/min, column set: two MZ-SD*plus* columns, 300 × 8 mm (dimensions), 5 μm (particle size), 10^3 and 10^5 Å (porosity), (ii) NMP + 5 g/L LiBr, 70 °C, 0.8 mL/min, two PSS-GRAM columns, 300 × 8 mm, 7 μm, 10^2 and 10^3 Å, or (iii) DMSO + 5 g/L LiBr, 70 °C, 1 mL/min, one PSS-GRAL column, 10 μm, linear. Solutions containing ~0.15 wt % polymer were stirred overnight and filtered through 0.45 μm filters; injected volume was 100 μL. Calibration was done with poly(methyl methacrylate) standards (PSS, Mainz, Germany).

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Supporting Information Available: ^{1}H NMR spectra and size exclusion chromatograms (S1−S5). This material is available free of charge via the Internet at http://pubs.acs.org.

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