Macromolecules **2006,** *39,* 1311−1312

Thermoresponsive Semitelechelic Polymers Containing Single Terminal Cysteine Derivatives Including a Cys-Gly-OH Dipeptide

Justine Fenech and Fabian Fischer*

Life Technologies Institute, University of Applied Sciences
Sion, Route du Rawyl 47, 1950 Sion, Switzerland

Received December 6, 2005
Revised Manuscript Received January 9, 2006

Macromolecules with a single end group attached to the polymer chain are described as semitelechelic polymers. The terminal functions are chemically different from the rest of the polymer and thus have a relatively important influence in short polymer chains. Semitelechelic polymers have a unique feature that allows a further functionalization based on their particular chemical properties, enabling the development of applications in various fields. The approach with peptide fragments in presented research is promising for applications such as downstream processing in biotechnology or the delivery of therapeutic drug molecules.

Chain transfer polymerization with cysteine derivatives including a cysteine containing dipeptide yielded 24−74% polymer−peptide−conjugates linked by a sulfur atom bridge. The terminal group contains in all examples a cysteine as a template structure, being part of a very short peptide type moiety. The conjugates were formed in particular with cysteine, L-cysteine−methyl ester, guanidyl−cysteine, and cysteine−glycine. The results are compared with the well-known chain transfer reagent 3-mercaptopropionc acid, whose structure is found as backbone in all four chain transfer reagents examined. MALDI-TOF mass spectroscopy is applied to determine the exact size of the polymer including the terminal group and to calculate average molecular mass and the number of chains, the basis for a polydispersity determination.[1] In addition to the chemical specificity, a physical property was recorded in form of a variable lower critical solution temperature (LCST) of aqueous polymer mixtures. The standard LCST value for poly(*N*-isopropylacrylamide) in pure water is 32 °C. The investigations reveal an increase of these temperatures up to ∼35 °C depending on the terminal group. The slightly elevated phase transition temperatures (Δ ∼ 1−3 °C) depend in a first approximation on the mass ratio of the end group with the overall average mass of the polymer.

The use of cysteine as chain transfer reagent is described for *N*-acetyl-L-cysteine[2] in the polymerization of methyl methacrylate and styrene and for cysteine[3] of acrylamide. The combination of cysteine derivatives with thermoresponsive or smart polymers into poly(*N*-isopropylacrylamide) is not described. In addition, the use of a peptide as chain transfer reagent like Cys-Gly-OH offers an interesting method for protein immobilization in general.

The tests started with unprotected cysteine, subsequently employing derivatives such as the protected L-cysteine−methyl ester, guanidyl−cysteine, and the dipetide cysteine−glycine−OH (Scheme 1). The polymerization is possible as cysteine contains a thiol function as side chain, which can be transformed into a thio radical by thermolysis of 2,2′-azobis(isobutyronitrile) (AIBN) in superheated (80−120 °C) methanol or other solvents. Preliminary experiments showed that cysteine in combination with *N*-isoproylacrylamide does not react rapidly by the radical mechanism that is already known from other thiols like 3-mercaptopropionic acid.[4] The standard reaction conditions for this kind of polymerizations are reflux/1 atm, producing the desired polymers in about 5 h. A faster radical reaction is possible in superheated methanol,[5] providing high yields. In a typical procedure 2 mmol of chain transfer reagents was reacted with 50 mmol of monomer in 15 mL of methanol (HPLC grade) employing small amounts of AIBN as initiator. The reaction mixtures were rapidly heated in an autoclave to 100 °C for 1 h. The isolated yields with underivatized cysteine are slightly lower than with mercaptopropionic acid, but with 74% in a productive range (Table 1). With increasing molecular weight of the chain transfer reagent the polymer yields decrease, but average chain lengths remain in a comparable range.

Scheme 1. General Reaction for the Chain Transfer Polymerization in Superheated Methanol Employing Cysteine Derivatives as Chain Transfer Reagent

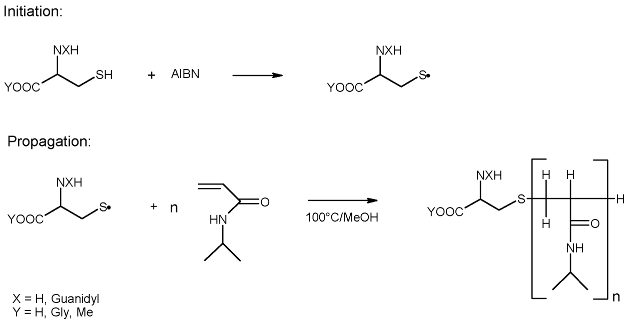

Table 1. M_n and M_w of Polymerized *N*-Isopropylacrylamide with Derivatives of Cysteine Used as Chain Transfer Reagents

chain transfer reagent	M_n [g/mol]	M_w [g/mol]	M_w/M_n[a] PD	yield [%]	T [°C]
cysteine	1762	1849	1.05	74	110
L-Cys-methyl ester	1664	1790	1.08	50	100
guanidyl-Cys	1580	1676	1.06	38	100
Cys-Gly-OH	1679	1897	1.13	24	100

[a] M_w/M_n describes a low polydispersity (PD).

The best information on the analysis of semitelechelic polymers is obtained by high-accuracy mass spectroscopy. MALDI-TOF MS was therefore used for the molecular mass determination, and the M_n and M_w values were derived from the mass spectra[1] to calculate the polydispersity (Table 1). The polydispersity of isolated conjugates of 1.01−1.013 is as low as expected for chain transfer polymers with mercaptans.[6] The precision of the measurements may also depend on the chain length, which increases if the size is relatively low, as lower masses are more easily extracted from the matrix by the laser beam. We found that deamidation is sometimes possible and explained it either that it happens during the polymerization or with the fact that the laser beam adjustment to an optimal threshold is not always possible, as it depends on the size of the analyte and the kind of matrix. The phase transition temperature (LCST) largely depends on the monomer structure and the interaction with solvent molecules (H_2O). The phase transfer phenomenon has been described for compounds such as poly(alkyl ether)phosphazenes,[7] poly(*N*-vinylcaprolactam), and poly(*N*-vinylpyrrolidone)[8] as well as a number of other

---

* Corresponding author: Ph +41(0)27 606 86 58; Fax +41(0)27 606 86 15; e-mail Fabian.Fischer@hevs.ch.

10.1021/ma052609w CCC: $33.50

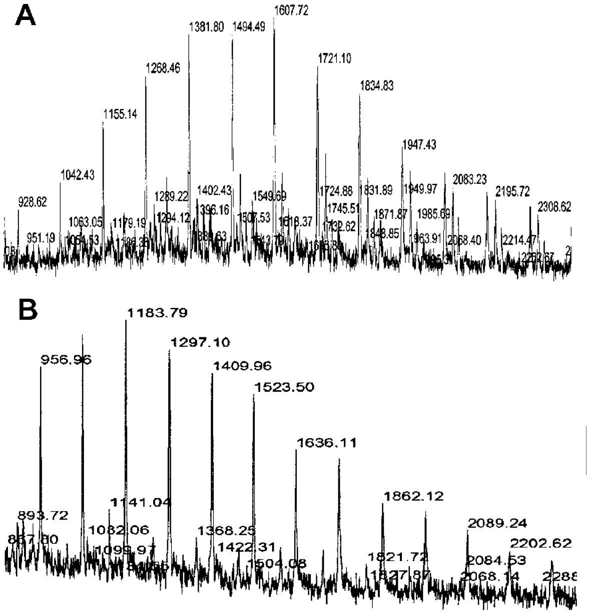

Figure 1. MALDI-TOF MS of semitelechelic poly(*N*-isopropylacrylamide)s: (A) L-cystein−methyl ester and (B) guanidyl−cysteine as terminal functions.

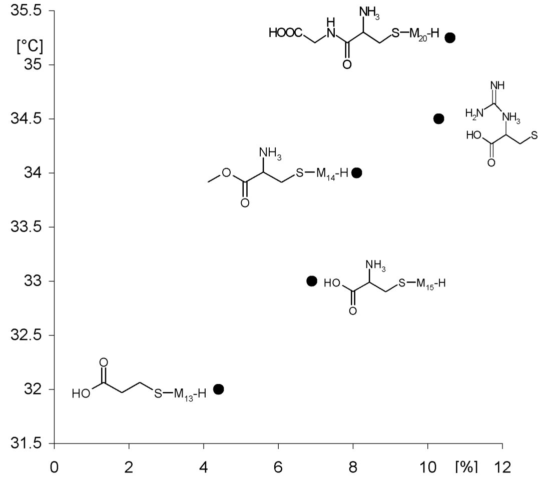

Figure 2. Clouding temperatures of cysteine derivatives. 3-Mercaptopropionic acid is a backbone structure found in all five chain transfer reagents. The *X*-axis describes mass percent of the terminal group/M_n. The *Y*-axis shows the variation of the lower critical solution temperature. M is the monomer (*N*-isopropylacrylamide), and the indices specify how many times on average it is part of the polymer chain.

structures.[9] The LCST with *N*-isopropylacrylamide only works in H_2O, including D_2O, which induces just slightly different physical properties. No other pure solvent is known to cause the same phase transition effect. Aqueous solutions with cosolutes such as inorganic salts, organic compounds such as EtOH, and tensio-active compounds have a pronounced influence on the clouding point, depending on the concentration. In addition, chirality influences the LCST as described for poly-*N*-(*S*)-*sec*-butylacrylamide-*co*-*N*-isoproylacrylamide.[10] Equally the tacticity[11] of the polymer backbone has an influence on the phase transition temperature. The LCST measurements were executed with a Peltier-controlled UV−vis spectrometer and recorded values between 33 and 35 °C. The values are 1−3 °C above the standard of 32 °C for long-chain poly(*N*-isopropylacrylamide). The measurements of the phase transition temperature reveal that the terminal groups cause a detectable difference as if they were different block polymers. For the interpretation of the LCST results several approaches may be considered. For instance, there may be different numbers of ionic interactions or hydrogen bonds, and presumably both play a role in the outcome of the variations of the phase transfer phenomenon. An overall correlation appears to be possible by combining the average mass of the polymer with the mass of the terminal function. We calculated the mass percentage of the end group in relation to the overall mass of the polymer chain. This analysis shows that if the mass percentage of 4.4−10.6% of the terminal group increases, also the LCST temperature is rising (Figure 2).

Acknowledgment. We thank the University of Applied Sciences Sion and the Interreg Foundation for financial support.

Supporting Information Available: A description of a typical MALDI-TOF MS sample preparation, ^1H NMR of PNIPAM, and other information. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Hanton, S. D. *Chem. Rev.* **2001**, *101*, 527.
(2) Glöckner, P.; Metz, N.; Ritter, H. *Macromolecules* **2000**, *33*, 4288−4290.
(3) Okaya, T.; Kikuchi, K.; Morii, Y. *Polym. J.* **1997**, *29*, 545−549.
(4) Fischer, F. *Synlett* **2002**, *8*, 1368−1369.
(5) Fischer, F.; Riad, T.; Freitag, R. *Eur. Polym. J.* **2005**, *41*, 403−408.
(6) Hoyle, Ch. E.; Lee, T. Y.; Roper, T. *J. Polym. Sci., Part A: Polym. Chem.* **2004**, *42*, 5301−5338.
(7) Allcock, H. R.; Dudley, G. K. *Macromolecules* **1996**, *29*, 1313.
(8) Maeda, Y.; Nakamura, T.; Ikeda, I. *Macromolecules* **2002**, *35*, 217.
(9) Galaev, I. Y.; Mattiasson, B. *Enzyme Microb. Technol.* **1993**, *15*, 354.
(10) Aoki, T.; Nishimura, T.; Sanui, K.; Ogata, N. *React. Funct. Polym.* **1998**, *37*, 299−303.
(11) Baltes, T.; Garret-Flaudy, F.; Freitag, R. *J. Polym. Sci., Part A: Polym. Chem.* **1999**, *37*, 2977−2989.

MA052609W