Synthesis of well-defined conjugated copolymers by RAFT polymerization using cysteine and glutathione-based chain transfer agents†

Youliang Zhao and Sébastien Perrier*

Received (in Cambridge, UK) 31st May 2007, Accepted 6th August 2007
First published as an Advance Article on the web 17th August 2007
DOI: 10.1039/b708293b

Novel cysteine and glutathione-based chain transfer agents were synthesized and successfully applied to the straightforward synthesis of well-defined conjugates *via* a RAFT process.

The synthesis and properties of conjugates comprising synthetic polymer and peptide/protein have attracted increasing attention due to their potential applications from nanotechnology to tissue engineering.[1] Depending on their architecture and chemical composition, the conjugates can self-assemble into tapes, nanotubes, fibrils, micelles, vesicles, and some other morphologies.[2] For the synthesis of peptide–polymer conjugates, the polypeptide segment is usually synthesized by ring-opening polymerization of *N*-carboxy anhydrides of amino acids or solid-phase-supported synthesis techniques, followed by conjugating the polymeric segment with the peptide *via* either (1) direct coupling/addition reaction,[3] or (2) living radical polymerization using a polypeptide-based macro initiator or macro chain transfer agent (CTA).[4–6] However, both approaches suffer from serious setbacks. The coupling/addition approach usually leads to low yield of conjugates due to steric hindrance and relatively low reactivity of polymeric chains, so further purification procedures are required to separate unreacted polymer and peptide. The living radical approach, using the peptide sequence as an initiator (nitroxide mediated polymerization, NMP, and atom transfer radical polymerization, ATRP)[4] or the R group of a CTA,[5] provides higher yields, however, the formation of side products is unavoidable since termination and irreversible chain transfer reactions during polymerization lead to the formation of a variety of polymeric structures as side products. These impurities are of great concern for the use of the bioconjugates since they can affect both their interactions with the environment and the type and quality of the self-assemblies achievable.[2] This effect is even further aggravated by the production of block copolymers, which requires chain extension of the primary chain, thus increasing the probability of termination reactions.

A promising approach to addressing this problem is to introduce the peptide sequence into the CTA as a Z group,[6] in which all the resultant living chains obtained *via* a RAFT (reversible addition–fragmentation transfer) process bear a single peptide sequence at their chain end, while the number of dead chains without a peptide unit can be kept very low under optimized reaction conditions. Therefore, this route gives unprecedented control over the structure of homopolymer- and multiblock copolymer-based conjugates. This approach is specific to RAFT, and difficult to achieve by other living radical routes. This adds to the great versatility of the RAFT process, which, by comparison to NMP and ATRP, can control the polymerization of a wider range of vinyl monomers, under milder reaction conditions and without the need for a catalyst.[7]

We report in this communication the exploitation of the thiol functionality of cysteine to produce CTAs for RAFT polymerization. Cysteine residues are frequently targeted for site-specific modification of proteins, using thiol chemistry. If a protein lacks free thiols for conjugation, genetic engineering can incorporate cysteine residues in specific positions. The same approach can be adopted for peptide sequences. We demonstrate the potential of two routes for the synthesis of peptide-based CTAs: (a) the synthesis of cysteine-based CTAs, which can be used as synthetic amino acids in the production of peptide sequences, and (b) the direct synthesis of a peptide-based CTA by modification of the cysteine residue. We then demonstrate the versatility of these CTAs to produce well-controlled homopolymers and block copolymers with a cysteine residue as the chain end functionality, or the straightforward synthesis of peptide–polymer conjugates.

Four novel CTAs (Fig. 1), namely, 2-acetamido-3-(benzylsulfanylthiocarbonylsulfanyl)propionic acid (ABSPA, a), 2-acetamido-3-(methoxycarbonylphenylmethylsulfanylthiocarbonylsulfanyl)-propionic acid (AMSPA, b), *N*-acetyl-*S*-(benzylsulfanylthiocarbonyl)glutathione (ABSG, c), and *N*-acetyl-*S*-(methoxycarbonylphenylmethylsulfanylthiocarbonyl)glutathione (AMSG,

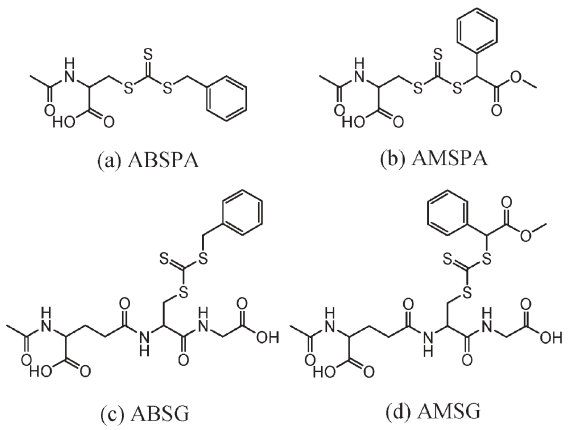

Fig. 1 Chemical structures of various chain transfer agents.

Department of Colour and Polymer Chemistry, University of Leeds, Woodhouse Lane, Leeds, UK LS2 9JT. E-mail: s.perrier@leeds.ac.uk; Fax: +44 113 343 2947; Tel: +44 113 343 2932

† Electronic supplementary information (ESI) available: Experimental details, polymerization results, NMR and FT-IR spectra, and GPC traces. See DOI: 10.1039/b708293b

d) were designed and synthesized using *N*-acetyl cysteine and *N*-acetyl glutathione as starting materials. When the reaction was conducted in aqueous solution, higher temperature, such as 60 °C or more, was necessary to perform the alkylation of $ZC(=S)S^{-}M^{+}$ (where Z = *N*-acetyl cysteine or *N*-acetyl glutathione, M = Na or K) with benzyl bromide or methyl α-bromophenylacetate, and the yield of CTAs (b–d) was quite low (usually less than 20%) except for ABSPA (a, 51% of yield), due to significant thermal degradation and heterophase reaction conditions. To address the synthetic difficulty, a more compatible solvent, methanol, was chosen as the reaction medium in which the alkylation reaction could be quantitatively conducted in 5–8 h even at ambient temperature, as monitored by ^{1}H NMR spectroscopy and TLC. After purification, the target CTAs were obtained with isolated yield larger than 98%, and their chemical structures were confirmed by NMR, ESI-MS, FT-IR and elemental analysis.

These CTAs were used to mediate RAFT polymerization of various vinyl monomers such as methyl acrylate (MA), *n*-butyl acrylate (BA), *N*-isopropylacrylamide (NIPAM), *N*,*N*-dimethylacrylamide (DMA), styrene (St) and methyl methacrylate (MMA) in dioxane at 60 °C. It was found that all these CTAs were good RAFT agents for polymerization of monomers except MMA, for which the polymerization led to well-defined polymers with controllable molecular weight and low polydispersity (PDI < 1.25). For polymerization of MMA mediated by ABSPA and ABSG, the molecular weight values were much higher than those expected, and the polydispersity indices were very high. In the cases of AMSPA and AMSG, however, the control on molecular weight of PMMA was much better and the polydispersities were relatively low (PDI = 1.3–1.4).

When ABSG and AMSG were used to mediate the polymerization of acrylate and acrylamido monomers, the polymerization kinetics was investigated in detail. Fig. 2 depicts the kinetic plots of RAFT polymerization conducted in dioxane at 60 °C. The pseudo first-order kinetics was kept until high conversion (up to 80–90%), after which a deviation from first-order kinetics was observed, and the rates of polymerization decreased in the order of DMA > NIPAM > MA > BA. In all cases, no significant induction period was observed for polymerization mediated by ABSG, in which benzyl was the R group, however, a significant induction period (about 3–4 h) was usually observed from the kinetics curves for AMSG-mediated polymerization, where methoxycarbonylphenylmethyl was the R group, indicating that RAFT polymerization was significantly affected by the chemical structures of the chain transfer agents. Fig. 3 shows the evolution of degree of polymerization (DP) and polydispersity of the conjugates with monomer conversion. The DP values of glutathione–polymer conjugates (GSH–PA) increased linearly with increasing conversion, and the polydispersity indices were usually less than 1.2, indicating that well-defined conjugated polymers could be achieved by the control over monomer conversion.

Theoretically, the ω-terminal of the living macro CTA chains in glutathione–polymer conjugates should quantitatively carry the thiocarbonyl thio functionality originating from the CTA. In this study, the end group analysis and chain extension polymerization were used to confirm the existence of glutathione in the conjugates. In ^{1}H NMR spectra, the signal of methylene protons (2 H, benzyl group) was shifted from 4.67 ppm to 2.2–2.5 ppm after

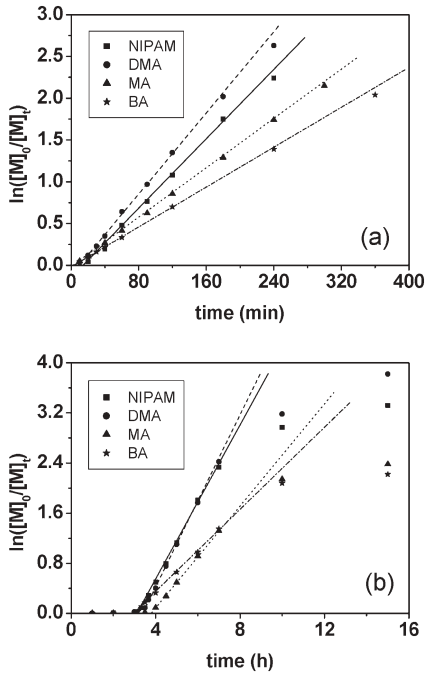

Fig. 2 Pseudo first-order kinetics for RAFT polymerization mediated by ABSG (a) and AMSG (b). Polymerization conditions: $[M]_0 : [CTA]_0 : [AIBN]_0 = 300 : 1 : 0.2$, $[M]_0 = 1.60$ mol L^{-1}, in dioxane at 60 °C.

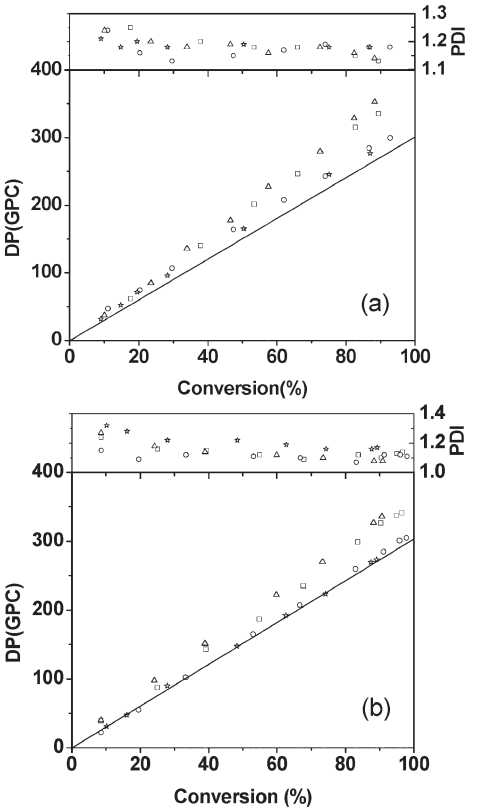

Fig. 3 DP and PDI evolution with conversion for RAFT polymerization of NIPAM (□), DMA (○), MA (△) and BA (☆) mediated by ABSG (a) and AMSG (b) in dioxane at 60 °C. The line indicates the theoretical DP value.

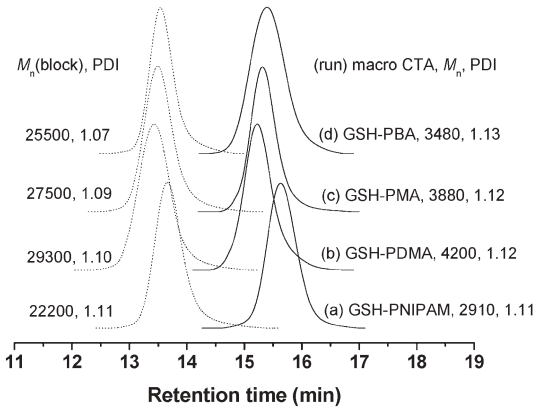

Fig. 4 GPC traces of macro CTAs (—) and block copolymers (···) obtained by chain extension polymerization of DMA (a) and NIPAM (b–d). See ESI for polymerization conditions.†

polymerization, the characteristic signals of NH, CH and CH_2 protons in glutathione unit were quantitatively noted, and the signals of methine proton connected to the trithiocarbonate (SC(=S)SC*H*, 1 H) in GSH–PNIPAM, GSH–PDMA, GSH–PMA and GSH–PBA conjugates were observed at 4.45, 5.04, 4.74 and 4.68 ppm, respectively (see ESI†). Moreover, the molecular weight estimated by ^{1}H NMR spectroscopy using the functional glutathione end group was very close to the theoretical value, suggesting glutathione was almost quantitatively present in the macro CTA chains.

The GSH–PA conjugates were used as macro CTAs to mediate chain extension polymerization of a second monomer such as NIPAM and DMA to synthesize block copolymers. The molecular weights of the resultant block copolymers determined by GPC are in good agreement with those expected, and the polydispersity indices were less than 1.12 (see ESI†). The GPC traces (Fig. 4) of the block copolymers obtained were completely shifted to the higher molecular weight side, and no obvious tailings or shoulders were observed, suggesting the target block copolymers were successfully achieved. The highly efficient chain extension polymerization also demonstrated that the glutathione group was quantitatively present in the original macro CTAs as the Z group.

In summary, we have demonstrated a versatile synthetic route to produce synthetic amino acid and peptide sequences that allows for the direct polymerization of vinyl monomers. Chain transfer agents based on cysteine and glutathione were firstly synthesized and utilized to mediate RAFT polymerization of various monomers. The use of the RAFT process allows for a unique control over the architecture of the polymer–peptide conjugates, providing polymeric chains with a high degree of end group functionality. A series of well-defined block copolymers and glutathione–(co)polymer conjugates with controlled molecular weight and low polydispersity were also successfully prepared.

We gratefully acknowledge the financial support of this work by the University of Leeds and EPSRC.

Notes and references

1 (*a*) Z. L. Ding, R. B. Fong, C. J. Long, P. S. Stayton and A. S. Hoffman, *Nature*, 2001, **411**, 59; (*b*) H. Schlaad and M. Antonietti, *Eur. Phys. J. E*, 2003, **10**, 17; (*c*) J. M. Smeenk, D. Lowik and J. C. M. van Hest, *Curr. Org. Chem.*, 2005, **9**, 1115; (*d*) D. Bontempo, K. L. Heredia, B. A. Fish and H. D. Maynard, *J. Am. Chem. Soc.*, 2004, **126**, 15372; (*e*) D. Bontempo and H. D. Maynard, *J. Am. Chem. Soc.*, 2005, **127**, 6508; (*f*) K. L. Heredia, D. Bontempo, T. Ly, J. T. Byers, S. Halstenberg and H. D. Maynard, *J. Am. Chem. Soc.*, 2005, **127**, 16955; (*g*) B. S. Lele, H. Murata, K. Matyjaszewski and A. J. Russell, *Biomacromolecules*, 2005, **6**, 3380; (*h*) S. Kulkarni, C. Schilli, B. Grin, A. H. E. Müller, A. S. Hoffman and P. S. Stayton, *Biomacromolecules*, 2006, **7**, 2736; (*i*) L. Tao, G. Mantovani, F. Lecolley and D. M. Haddleton, *J. Am. Chem. Soc.*, 2004, **126**, 13220; (*j*) G. Mantovani, F. Lecolley, L. Tao, D. M. Haddleton, J. Clerx, J. J. Cornelissen and K. Velonia, *J. Am. Chem. Soc.*, 2005, **127**, 2966; (*k*) J. Nicolas, G. Mantovani and D. M. Haddleton, *Macromol. Rapid Commun.*, 2007, **28**, 1083 and references therein.

2 (*a*) R. Langer and D. A. Tirrell, *Nature*, 2004, **428**, 487; (*b*) K. T. Kim, C. Park, G. W. M. Vandermeulen, D. A. Rider, C. Kim, M. A. Winnik and I. Manners, *Angew. Chem., Int. Ed.*, 2005, **44**, 7964; (*c*) F. Chécot, S. Lecommandoux, Y. Gnanou and H.-A. Klok, *Angew. Chem., Int. Ed.*, 2002, **41**, 1339; (*d*) J. Rodríguez-Hernández and S. Lecommandoux, *J. Am. Chem. Soc.*, 2005, **127**, 2026; (*e*) M. G. J. ten Cate, N. Severin and H. G. Börner, *Macromolecules*, 2006, **39**, 7831; (*f*) J. Hentschel and H. G. Börner, *J. Am. Chem. Soc.*, 2006, **128**, 14142; (*g*) J. Hentschel, E. Krause and H. G. Börner, *J. Am. Chem. Soc.*, 2006, **128**, 7722; (*h*) Y. Geng, D. E. Discher, J. Justynska and H. Schlaad, *Angew. Chem., Int. Ed.*, 2006, **45**, 7578.

3 (*a*) A. Rösler, H.-A. Klok, I. W. Hamley, V. Castelletto and O. O. Mykhaylyk, *Biomacromolecules*, 2003, **4**, 859; (*b*) G. W. M. Vandermeulen, C. Tziatzios and H.-A. Klok, *Macromolecules*, 2003, **36**, 4107; (*c*) J. H. Collier and P. B. Messersmith, *Adv. Mater.*, 2004, **16**, 907; (*d*) J. M. Smeenk, M. B. J. Otten, J. Thies, D. A. Tirrell, H. G. Stunnenberg and J. C. M. van Hest, *Angew. Chem., Int. Ed.*, 2005, **44**, 1968; (*e*) D. Eckhardt, M. Groenewolt, E. Krause and H. G. Börner, *Chem. Commun.*, 2005, 2814.

4 (*a*) M. L. Becker, J. Liu and K. L. Wooley, *Biomacromolecules*, 2005, **6**, 220; (*b*) M. L. Becker, J. Q. Liu and K. L. Wooley, *Chem. Commun.*, 2003, 180; (*c*) K. Qi, Q. Ma, E. E. Remsen, C. G. Clark, Jr. and K. L. Wooley, *J. Am. Chem. Soc.*, 2004, **126**, 6599; (*d*) S. Venkataraman and K. L. Wooley, *Macromolecules*, 2006, **39**, 9661; (*e*) H. Rettig, E. Krause and H. G. Börner, *Macromol. Rapid Commun.*, 2004, **25**, 1251; (*f*) Y. Mei, K. L. Beers, H. C. M. Byrd, D. L. VanderHart and N. R. Washburn, *J. Am. Chem. Soc.*, 2004, **126**, 3472; (*g*) Y. Wang, B. A. Armitage and G. C. Berry, *Macromolecules*, 2005, **38**, 5846; (*h*) S. Steig, F. Cornelius, P. Witte, B. B. P. Staal, C. E. Koning, A. Heise and H. Menzel, *Chem. Commun.*, 2005, 5420.

5 (*a*) M. G. J. ten Cate, H. Rettig, K. Bernhardt and H. G. Börner, *Macromolecules*, 2005, **38**, 10643; (*b*) M. Bathfield, F. D'Agosto, R. Spitz, M.-T. Charreyre and T. Delair, *J. Am. Chem. Soc.*, 2006, **128**, 2546; (*c*) C. Y. Hong and C. Y. Pan, *Macromolecules*, 2006, **39**, 3517.

6 (*a*) J. Liu, V. Bulmus, C. Barner-Kowollik, M. H. Stenzel and T. P. Davis, *Angew. Chem., Int. Ed.*, 2007, **46**, 3099; (*b*) C. Boyer, V. Bulmus, J. Liu, T. P. Davis, M. H. Stenzel and C. Barner-Kowollik, *J. Am. Chem. Soc.*, 2007, **129**, 7145.

7 (*a*) J. Chiefari, Y. K. Chong, F. Ercole, J. Krstina, J. Jeffery, T. P. T. Le, R. T. A. Mavadunne, G. F. Meijs, C. L. Moad, G. Moad, E. Rizzardo and S. H. Thang, *Macromolecules*, 1998, **31**, 5559; (*b*) Y. K. Chong, T. P. T. Le, G. Moad, E. Rizzardo and S. H. Thang, *Macromolecules*, 1999, **32**, 2071; (*c*) C. Barner-Kowollik, T. P. Davis, J. P. A. Heuts, M. H. Stenzel, P. Vana and M. Whittaker, *J. Polym. Sci., Part A: Polym. Chem.*, 2003, **41**, 365; (*d*) G. Moad, E. Rizzardo and S. H. Thang, *Aust. J. Chem.*, 2005, **58**, 379; (*e*) S. Perrier and P. Takolpuckdee, *J. Polym. Sci., Part A: Polym. Chem.*, 2005, **43**, 5347; (*f*) A. Favier and M. T. Charreyre, *Macromol. Rapid Commun.*, 2006, **27**, 653.

