Simultaneous and Reversible Functionalization of Copolymers for Biological Applications†

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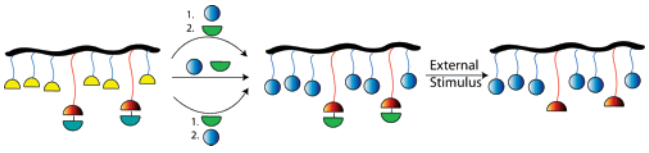
Received June 23, 2006
Revised Manuscript Received July 3, 2006

Simultaneous modification of more than one functionality in a molecular framework with high fidelity is of importance in all areas of synthetic chemistry. This is especially true in the case of polymer synthesis, where the functional group interconversions are carried out in the postpolymerization steps, i.e., on the polymer backbone.[1] Developing such strategies allows for easy access to a variety of copolymers without having to optimize the polymerization conditions for each functional monomer. These methodologies also allow one to carry out a systematic structure−property relationship study on side-chain functionalities without worrying about the complications of differences in PDI or molecular weights influencing the polymer behavior. We disclose here the details of a new methodology for incorporating multiple functionalities on a polymer backbone. Because of the growing interest in using polymers in the biological arena, we stipulated that (i) the reactive groups in the polymer backbone are complementary to common biologically relevant functional moieties and (ii) incorporation of at least one of the functionalities, which is reversible by a biologically relevant stimulus. The latter feature is relevant to applications such as drug delivery[2] and gene delivery.[3] We report here on polymers that are complementary to amines and thiols, two of the most common reactive functionalities found in biological systems.[4] We demonstrate that substituents can be incorporated on to the polymer in a stepwise fashion in either order or in a single pot using a mixture of reagents (Chart 1).

Monomers containing functionalities complementary to thiols and amines are represented by **1** and **2**, respectively (Figure 1a). The *N*-hydroxysuccinimide methacrylate (NHSMA, **2**) has been previously copolymerized[5] using reversible addition−fragmentation termination (RAFT) polymerization[6] and atom transfer radical polymerization (ATRP) methods.[7] To identify whether monomer **1** would be compatible with these methods, we carried out homopolymerization of monomer **1** by RAFT and ATRP. Monomer **1** was synthesized from commercially available Aldrithiol-2 in two simple steps, by taking advantage of the stepwise exchange of a thiol of the disulfide in the latter compound.[8]

When polymerized under RAFT conditions, the resultant polymer was completely insoluble. This was attributed to the possible cross-linking triggered by the initiator containing the dithioester functionality. However, when the polymerization reaction was carried out under ATRP conditions (using ethyl bromoisobutyrate (EBIB) as the initiator in the presence of copper(I) bromide and pentamethyldiethylenetriamine (PMDETA) in anisole at 65 °C), the expected polymer **P1** (Figure 1b) was obtained with a PDI of 1.2 and molecular weight of 5560 (expected M_n = 6760).

Chart 1. Orthogonal and Reversible Functionalities in Copolymers



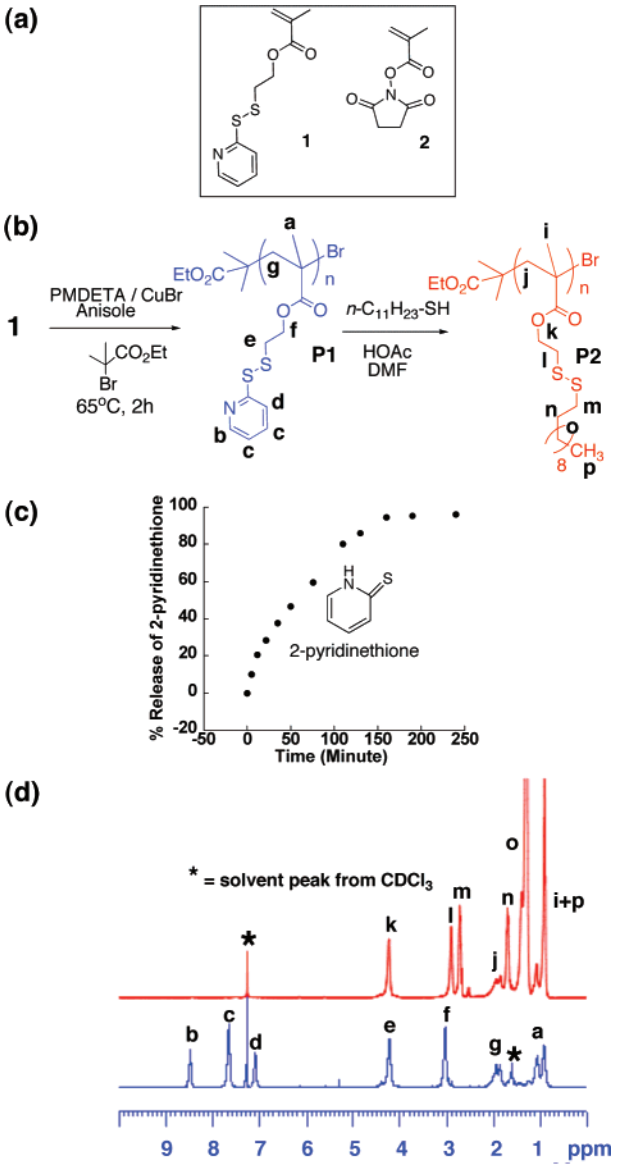


Figure 1. (a) Structure of the monomers **1** and **2**. (b) Polymerization of monomer and exchange of pyridyl disulfide with alkanethiol in the polymer. (c) Progress of the thiol-exchange reaction with time, by UV−vis spectroscopy. (d) NMR plot for the conversion of **P1** (bottom) to **P2** (top).

The premise on which this methodology was conceived is based on the fact that the pyridyl disulfide unit in **1** and polymer **P1** can be exchanged in high yields by reaction with a thiol compound of interest. To test this possibility, we treated polymer **P1** with 1-undecanethiol in DMF in the presence of catalytic amount of acetic acid. The progress of the reaction was monitored by absorption spectroscopy, since the spectroscopic signature of the released 2-pyridinethione (λ_{max} = 375 nm) is

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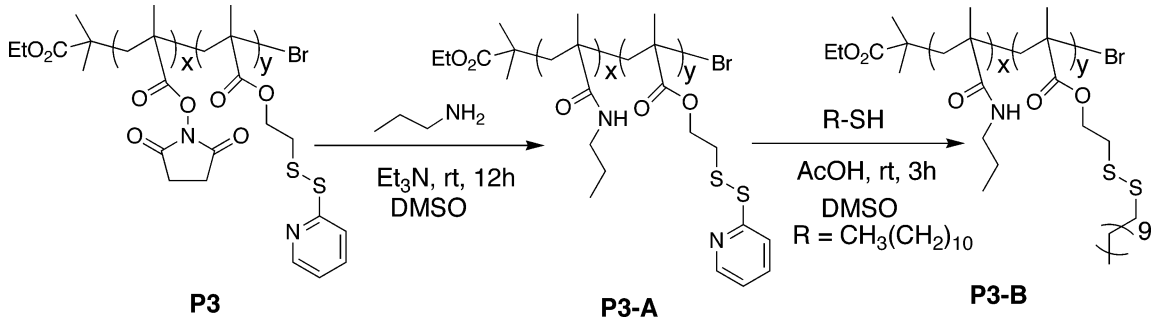
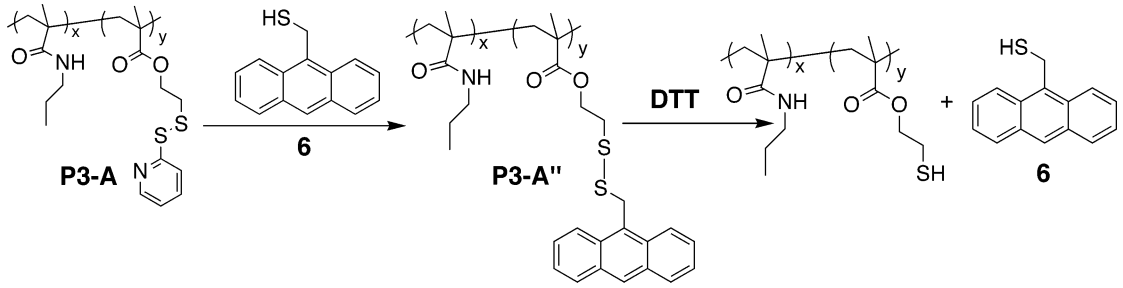
† Dedicated with great regard and respect to Professor Peter Beak on the occasion of his 70th birthday.

10.1021/ma061420x CCC: $33.50
Published on Web 07/26/2006

Scheme 1. Representation of the Functionalization of Random Copolymer P3



Scheme 2. Incorporation and Cleavage of Fluorophore



distinctly different from the spectrum of the pyridyl disulfide functionality ($\lambda_{max} = 280$ nm) in the polymer **P1**. The exchange reaction seemed to be complete in less than 3 h. In addition to the fact that no change in the absorbance of the peak at 375 nm was observed after 3 h, the maximum absorbance observed is also consistent with the expected amount of 2-pyridinethione released from this reaction (Figure 1c). The high conversion efficiency was also confirmed by ^{1}H NMR of the precipitated polymer. No peaks in the aromatic region are seen, consistent with the removal of the thiopyridine units (Figure 1d). Similarly, the peaks in the aliphatic region are consistent with the incorporation of the undecyl moiety onto the polymer backbone.[8]

With the successful homopolymerization of **1**, copolymerizations of the amine-reactive monomer **2** and the thiol-reactive monomer **1** were carried out in different ratios under the ATRP conditions mentioned above, to obtain polymers **P3**−**P7**.[8] The ratio of the monomers incorporated in the polymer and molecular weights closely correlated with the feed ratio of the monomers and the monomer: initiator ratio, respectively, as shown in Table 1. The incorporation ratio of the monomers onto the polymer backbone was estimated by the relative integration of the peaks at 2.7 and 8.4 ppm, corresponding to the succinimide protons and one of the pyridyl ring protons, respectively.[8]

We then tested the possibility of reaction of the NHS ester with the amines and of thiols with pyridyl disulfide moiety in one of these copolymers (**P3**) (Scheme 1). Polymer **P3** was treated with propylamine in DMSO, which resulted in complete conversion of the *N*-hydroxysuccinimidyl ester moiety to the corresponding amide, as ascertained by ^{1}H NMR.[8] Similarly, the exchange of the pyridyl disulfide by 1-undecanethiol was also found to be complete from the disappearance of the aromatic peaks with the concomitant appearance of the undecanethiol peaks in the ^{1}H NMR.[8] These results demonstrate the high fidelity of the reactive functionalities complementary to amines and thiols in these copolymers. We then tested the possibility of substituting the pyridyl disulfide moiety first with 1-undecanethiol. It was observed that this substitution proceeded quantitatively without any reaction with the NHS ester moiety.[8] When we attempted this reaction by simultaneously adding the undecanethiol and propoylamine in the presence of Et_3N and catalytic amount of HOAc in DMSO, this reaction indeed provided the same polymer product, further illustrating the versatility of the methodology. We do realize that we mixed the Et_3N base needed for the amide formation with catalytic amount of acid needed for the disulfide exchange in a single pot. Our hypothesis was that the equilibrium between Et_3N and its conjugated acid $Et_3NH^+OAc^-$ would be sufficient for the acid-catalyzed exchange. We also carried out this reaction by adding the amine and thiol in a single pot, but stepwise. Not surprisingly, this reaction also provided the identical polymer.[8]

Table 1. Details of the Random Copolymers P3−P7

	feed ratio of monomers	ratio determined by proton-NMR	M_n(theory)	M_n(obsd)	PDI
P3	90:10	85:15	11 639	9 700	1.6
P4	80:20	72:28	12 202	13 400	1.3
P5	70:30	67:33	12 419	10 000	1.5
P6	60:40	60:40	12 548	8100	1.4
P7	40:60	42:58	13 501	19 000	1.5

Reversibility of the incorporation of at least one of these incorporated functionalities is the next feature we were interested in because of its relevance in biomedical applications. We were particularly interested in disulfide-based reversibility, since drug delivery based on differential glutathione levels in various cell types has been an attractive approach in targeted drug delivery.[2] For this purpose, we incorporated an anthracene moiety through the disulfide bond and monitored the cleavage of the disulfide bond from the polymer backbone in the presence of dithiothreitol (DTT) as glutathione mimic, using fluorescence anisotropy (Scheme 2). When the fluorescent anthracene is attached to the polymer backbone (**P3-A″**), the anisotropy is understandably an order of magnitude higher than the small molecule, thiomethylanthracene (**6**). Indeed, when the polymer was treated with DTT, the anisotropy from anthracene changed from 80×10^{-3} to 6×10^{-3}. The latter value corresponds to the anisotropy obtained for the small molecule control. This result is consistent with the fact that the fluorescent anthracene was indeed released from the polymer backbone. To further confirm this, we obtained the NMR of the polymer precipitated from this reaction and

found no aromatic peaks that correspond to the anthracene probe moiety.

In summary, we have shown that monomers **1** and **2** can be copolymerized in different ratios by ATRP, where the reactivity of the functional groups are specific to thiols and amines, respectively. These polymers can be quantitatively substituted in one pot or by a sequential addition strategy and is independent of the order of addition. As mentioned before, the motivation for choosing these two functionalities in particular is due to the fact that these are two of the most commonly seen reactive functionalities in biology. One could imagine utilizing such methods in polymer−protein conjugates, where more than one protein molecule can be attached to a single polymer chain reversibly.[9] To further demonstrate a specific example in which such polymers could find use in biological applications, we have shown that the disulfide linkage could be used for the reversible incorporation of a molecule. It is conceivable that a similarly attached drug molecule is then released in a reducing environment, e.g., cancer cells. The versatility of the polymers and the simplicity of the methodology by which these can be prepared are likely to significantly enhance the repertoire of synthetic polymer chemistry in biological applications. Pursuing these polymers for delivery and bioconjugate applications is a part of the ongoing efforts in our laboratory.

Acknowledgment. We thank the U.S. Army Research Office and NSF-MRSEC for the financial support for this work.

Supporting Information Available: Synthetic and other experimental details. This material is available free of charge at http://pubs.acs.org.

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MA061420X