Easy Access to Bioactive Peptide−Polymer Conjugates via RAFT

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Received January 14, 2008
Revised Manuscript Received January 17, 2008

The combination of sequence-defined peptides with "common" synthetic polymers is an appealing modern strategy for designing advanced polymeric materials.[1−3] Indeed, such peptide−polymer conjugates contain monodisperse peptide segments, enabling one to program microstructure formation in polymers, to generate selective interactions with DNA for delivery applications, or to encode interactions for specific surfaces, e.g., inorganic or organic crystals.[1−3] However, probably most important is the possibility to achieve polymeric materials that exhibit biological activity.[4,5]

The synthesis of peptide−polymer conjugates can be performed using either coupling or polymerization approaches.[6] While the coupling strategy is probably more suitable for the synthesis of conjugates with low molecular weight synthetic blocks (M_n < 1K−5K),[7−9] the polymerization approach covers a much broader range of molecular weight (M_n = 1.5K−38K).[10,11] The latter exploits "grafting from" routes, usually combined with controlled radical polymerization techniques.[6,9,12−14] For instance, chain-transfer agents (CTAs) were linked to peptide segments, allowing the effective mediation of reversible addition−fragmentation chain transfer radical polymerization (RAFT).[15−18] The tolerance against many functional groups, the absence of metal catalysts, and the close relation of the RAFT process to conventional free-radical polymerizations are clear advantages for the synthesis of bioconjugates.[19,20] Yet, the potential of the RAFT process for polymer bioconjugation has not been fully explored. This is probably due intrinsic limitations such as (i) the sensitivity of common CTAs against nucleophiles (e.g., amines are abundant in peptides and proteins) and (ii) the use of multistep synthesis procedures, which frequently require chromatographic purification.

So far, in most of the reported examples of RAFT bioconjugation, CTAs have been linked to biosegments via the Z group (i.e., the activating moiety of the CTA).[16,18] An interesting alternative is indeed conjugation via the R group (i.e., the initiating moiety of the CTA) as this approach leads to α,ω-functional bioconjugates. The latter are highly relevant for biomedical applications as they can be easily derived into α-peptide-ω-thiol-functionalized polymers. In this context, we recently studied the direct coupling of the commonly used carboxylate-functionalized CTA (4-cyano-4-((thiobenzoyl)sulfanyl)pentanoic acid) to the amine terminus of a supported peptide. As expected, such a direct approach led to a significant amount of thioamide as byproduct.[17] However, this obstacle could be detoured by a two step procedure: (i) capping the nucleophilic amine terminus with α-bromo propionate and (ii) substituting the α-bromo group with dithiobenzoate. This reaction proceeds quantitatively and leads—even with complex peptides—to peptide−CTAs in high purity, making chromatographic purification obsolete. However, although efficient, this protocol is nonautomated and therefore remains relatively time-consuming.

Here we present a convenient and fully automated one-step approach to peptide−CTAs, strongly reducing the synthetic efforts and the costs. This approach does not rely on standard dithioester-based CTAs but on trithiocarbonates. The latter have been recently evidenced to be very efficient CTAs for controlling the polymerization of various monomers and moreover exhibit a higher tolerance against nucleophiles than dithiobenzoates.[21,22] Additionally, while the synthesis of RAFT CTAs often require multistep reactions and chromatographic purifications, the S-1-dodecyl-S′-(R,R′-dimethyl-R″-acetic acid) trithiocarbonate (**II**) is readily accessible in a one-pot reaction. The ease of synthesis, the absence of further purification steps, and the cost-effective large-scale accessibility (100 g scales) makes **II** an appealing candidate for the design of peptide-based CTAs.

The coupling of **II** to the N-terminal amine group of the supported peptide GGRGDS (**I**) was investigated (Scheme 1). This particular peptide sequence corresponds to the minimal adhesive domain of fibronectin and therefore allows control over cell adhesion via specific integrin binding.[23] **I** was synthesized by a sequential assembly of Fmoc amino acid derivatives, applying fully automated solid-phase supported peptide synthesis (SPPS). The successful synthesis was verified by electrospray ionization mass spectrometry (ESI-MS) of a small amount of peptide, liberated from the support for analysis purpose (cf. Supporting Information). As outlined in Scheme 1, **II** was coupled to **I** using automated SPPS protocols. The coupling was facilitated by DIC, and quantitative conversion of the terminal amine group was confirmed by a negative Kaiser test. Subsequently, the fully protected peptide−CTA (**III**) was cleaved from the support and lyophilized. The chemical structure of **III** was verified via ^{1}H NMR spectroscopy by comparing the integral intensities of resonances characteristic for the CTA moiety with that of the peptide segment (cf. Supporting Information). Moreover, ESI-MS suggested a highly selective transformation of **I** to **III** and did not show detectable evidence for side products. The chemoselectivity of the coupling reaction was further demonstrated by a control experiment in the absence of the DIC activator. HPLC-MS analysis shows that after 2 h of reaction only 10% dithiocarbamate was formed as a substitution product, despite the 20 equiv excess of **II** with respect to **I**. HPLC-MS analysis of the product **III** indicated that the substitution side product was only present in traces (∼0.3%), which confirms the tolerance of the trithiocarbonate moiety against nucleophilic attacks.

Compound **III** was subsequently investigated as CTA for the RAFT polymerization of various monomers. First, *n*-butyl acrylate (*n*BA) was studied as a model monomer. The polymerization was performed under highly dilute conditions in DMF at 65 °C with AIBN as a source of primary radicals. The reaction kinetics was followed by GPC and ^{1}H NMR. As shown in Figure 1, the polydispersity index M_w/M_n of the formed polymer **IV**

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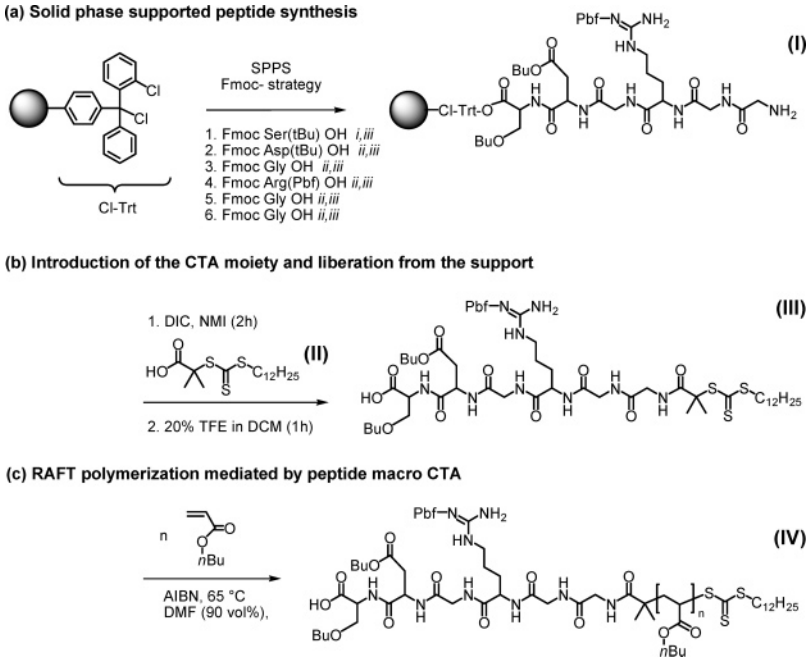
10.1021/ma8000934 CCC: $40.75
Published on Web 02/01/2008

Scheme 1. Synthesis of the Oligopeptide−P*n*BA Conjugate (IV)[a]



[a] Reagents and conditions: 2-polystyrene-(2-chlorotrityl chloride) resin; i. Fmoc-Ser OH, DIPEA/DCM, 90 min; ii. Fmoc-Aa OH, HBTU/DIPEA/NMP, 20 min; iii. 20 vol % piperidine/NMP.

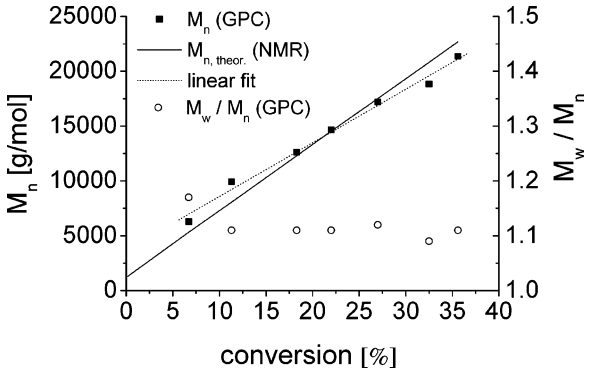


Figure 1. Dependence of M_n and M_w/M_n on monomer conversion for the RAFT polymerization of *n*BA, mediated by **III** in DMF solution at 65 °C ([*n*BA]$_0$/[**III**]$_0$/[AIBN]$_0$ = 470/1/0.1, DMF = 90 vol %).

was low (∼1.1) and the molecular weight M_n increased linearly with monomer conversion throughout the polymerization. Moreover, the semilogarithmic plot of conversion vs time was linear (cf. Supporting Information). These observations indicate that the RAFT polymerization was efficiently controlled by **III**.

Thus, the peptide−CTA **III** was further studied to mediate the RAFT polymerization of other monomers such as *N*-isopropylacrylamide (NIPAM) and oligo(ethylene glycol) acrylate (OEGA). The resulting peptide−polymer conjugates are certainly of interest for bioapplications as they exhibit biorelevant features such as biocompatibility or stimuli responsivity. For instance, PNIPAM displays a lower critical solution temperature (LCST) at ∼32 °C in water. The polymerizations of NIPAM and OEGA were monitored by ^{1}H NMR spectroscopy and stopped at ∼50% conversion in order to reach molecular weights, suitable for further bioapplications (e.g., 8K−9K is an interesting range to prepare temperature-responsive surfaces). Both polymerizations proceed in a controlled fashion as indicated by GPC data (the resulting conjugates exhibit $M_w/M_n \sim 1.2$). Moreover for both conjugates, after deprotection of the peptide segment, the ratio peptide/polymer measured by ^{1}H NMR matches with theoretical values (cf. Supporting Information).

To demonstrate the biorelevance of the obtained bioconjugates, PNIPAM−GGRGDS was used for controlling cell adhesion on gold surfaces. For that, the ω-trithiocarbonate moiety was first hydrolyzed under reductive means. The resulting thio-functionalized conjugate (HS−PNIPAM−GGRGDS) was grafted onto planar gold surfaces.[24] Figure 2a shows effective adhesion of L929 mouse fibroblasts, cultivated

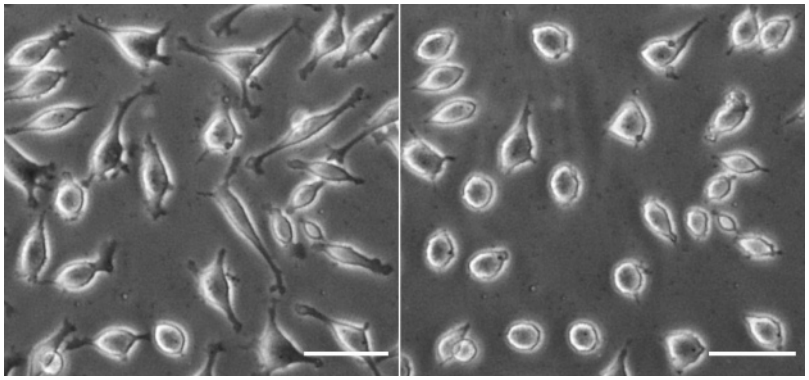


Figure 2. Representative micrographs of L929 mouse fibroblasts on PNIPAM−GGRGDS grafted Au surface: after 18 h cultivation at 37 °C (left) and after 30 min of cooling at 25 °C (right). Scale bar: 100 μm.

at 37 °C on the resulting HS−PNIPAM−GGRGDS grafted surfaces. The cells adhered fast and spread well after 18 h of cultivation. The observed adhesion rate is clearly faster than what is found for standard PNIPAM surfaces,[24,25] indicating a beneficial effect of the GGRGDS. Upon temperature decrease to 25 °C, rapid cell rounding was evident within ∼30 min (Figure 2b). No cell rounding of spread fibroblasts occurs on plain gold surfaces upon a temperature decrease from 37 to 25 °C. Thus, the polymer-modified Au surface exhibits a switchability from a dehydrated/cell attractant to a hydrated/cell repellent surface. Standard competition assays suggested peptide mediated cell−surface interactions, because cell adhesion to HS−PNIPAM−GGRGDS modified surfaces was blocked by the addition of free GGRGDS into the culture medium.

In summary, it was shown that well-defined oligopeptide−polymer conjugates can be accessed via a convenient and cost-effective RAFT polymerization route, utilizing a novel type of R-group-anchored peptide−CTA. This strategy opens up a wide range of possibilities for the design of biorelevant materials.

Acknowledgment. This work was supported by the German Research Foundation (DFG, Emmy Noether Program BO 1762/2-3), the Max Planck Society, and Max Planck Society−Fraunhofer Society (project bioactive surfaces).

Supporting Information Available: Full experimental part and characterization data for **I**, **III**, and the formed polymer bioconjugates. This material is available free of charge via the Internet at http://pubs.acs.org.

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MA8000934