MACROMOLECULAR CHIMERA VIA CONTROLLED RADICAL POLYMERIZATION TECHNIQUES

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Introduction

A "*Macromolecular chimera*" is defined as a macromolecule, which combines both bioorganic and synthetic polymer segments in a single architecture.[1, 2] In particular, tailor-made macromolecular chimera composed of synthetic polymers and sequence defined peptides are a very important class of bioactive polymeric-materials that possess the ability to interact with biological systems.[3] To access macromolecular chimera in a defined manner, known controlled polymerization techniques have to be adapted. Synthetic difficulties are often due to the multiple functional groups present in bioorganic polymers e.g. polypeptides exhibit diverse functionalities in combination with a polyamide backbone. Recently, it has been demonstrated that sequence defined polypeptides can be applied as macroinitiators for nitroxide mediated radical polymerization (NMP[4]) as well as for atom transfer radical polymerization (ATRP[5]).[2,6,7] Here we present an overview, summarizing our efforts in the use of controlled radical polymerization (CRP) techniques to access defined macromolecular chimera. In order to understand the interactions of oligopeptides with the applied CRP systems, model AB-block copolymers were prepared via block extension of a sequence defined pentapeptide (Figure 1).

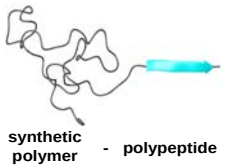


Figure 1. Idealized structure of an AB-block copolymer type of macromolecular chimera.

Experimental

Materials and methods. Materials used for the preparation of the oligopeptide ATRP macroinitiator and the ATRP polymerization have been described previously.[2] 4-cyano-4-((thiobenzoyl)sulfanyl)pentanoic acid was synthesized according to literature procedures.[8]

Size exclusion chromatography, SEC. Molecular weights and molecular weight distributions were determined by SEC using THF as eluent. For calibration, universal calibration methods were used. *^{1}H NMR.* ^{1}H NMR Spectra were recorded in $CDCl_3$ on a Bruker DPX-400 operating at 400.1 MHz. *Electrospray time-of-flight mass spectrometry, ESI-TOF-MS* was performed on a Shimadzu MS-QP8000α instrument.

Preparation of the ATRP peptide macroinitiator (*III*). The oligopeptide was synthesized on an Applied Biosystems ABI 433a peptide synthesizer in N-methyl-2-pyrrolidon (NMP) as solvent following standard FastMoc protocols as described previously.[2] The ATRP initiator group was introduced at the terminal amine of the supported oligopeptide by amidation with of 2-bromopropionic (20 eq. excess) facilitated by DCC. The liberation of the macroinitiator from the support was accomplished by 30 min. treatment with a cleavage mixture (2 v.% of trifluoroacetic acid (TFA) in dichloromethane), followed by isolation via diethyl ether precipitation and lyophilization. Products were identified by ESI-MS-TOF and ^{1}H NMR.

General ATRP procedure. The oligopeptide macroinitiator (0.1 mmol, 0.08 g) was dissolved in DMSO/*n*BA (2.19 mL/1.38 mL (9.7 mmol)) and the solution was degassed. The reaction mixture was preheated to 60°C and 0.5 mL of a catalyst stock solution (CuBr: 1 mmol; $CuBr_2$: 0.05 mmol; PMDETA: 1.05 mmol in 5 mL DMSO/*n*BA) was added. 0.1 mL samples were taken for kinetic analysis (GPC, NMR). After 2 h the polymerization mixture was cooled to room temperature and exposed to air.

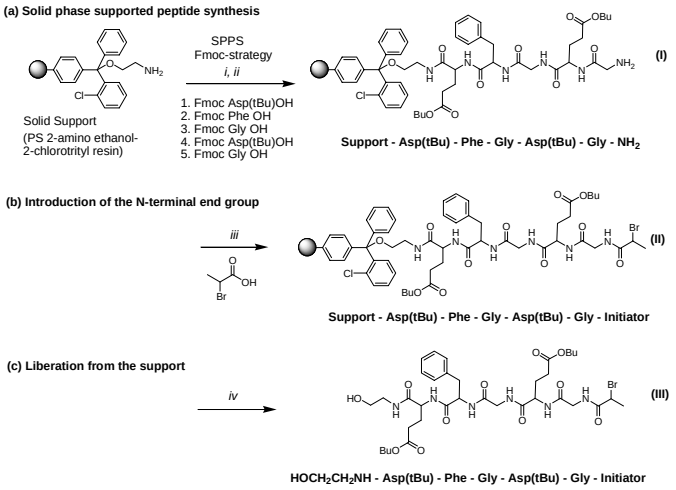
Preparation of the oligopeptide RAFT agents (*IV* and *V*). Route 1: *IV* was synthesized by the reaction of ***I*** (0.1 mmol) and 4-cyano-4-((thiobenzoyl)sulfanyl)pentane carboxylic acid (167 mg, 0.6 mmol) facilitated by 0.3 mmol of DCC in 5 mL NMP. **Route 2: *V*** was synthesized using the Grignard strategy. Therefore, a solution of phenylmagnesium bromide, prepared from bromobenzene (4.47 mmol) and magnesium turnings (4.11 mmol) in 3.5 mL THF was added to 6.7 mmol of carbon disulfide in 2.0 mL of THF. The reaction mixture was stirred for 2 h, then transferred to ***II*** (0.1 mmol) and stirred at 60˚C for 15 h. The liberation from the support and isolation of the final oligopeptide RAFT agents (***IV*** and ***V***) was accomplished as described above.

General RAFT polymerization procedure. The oligopeptide RAFT agent (10 mg, 10.5 µmol) was dissolved in DMF (2.12 mL). After the addition of *n*BA (11.1 mmol) and AIBN (0.091 mg, 0.55 µmol) the reaction mixture was carefully degassed and heated to 60° C. Samples of 0.2 mL were taken for kinetic analysis (GPC, NMR).

Results and Discussion

Atom Transfer Radical Polymerization (ATRP): The oligopeptide ATRP macroinitiator was prepared via solid phase supported peptide synthesis, followed by a N-terminal modification reaction (Scheme 1, ***I*** and ***II***). The CRP reaction of acrylic monomers was subsequently performed homogeneously in solution after the polypeptide was liberated from the support (Scheme 1, ***III***).[2]

Scheme 1. Solid phase supported synthesis of oligopeptide ATRP macroinitiator (C*onditions: i* Fmoc-Aa-OH, HBTU, DIPEA (N,N-diisopropyl-ethylamine), NMP 7.5 min.; *ii* 20% piperidine in NMP, 2-3×1.5 min. (UV-monitored at 301 nm). *iii* DCC, NMP, 12 h.; *iv* 2% trifluoroacetic acid (TFA) in DCM, 30 min.)



The kinetics of the solution ATRP of *n*-butyl acrylate initiated by ***III*** were investigated (Figure 2).[2] These studies revealed a controlled polymerization process leading to poly(*n*-butyl acrylate-*block*-polypeptide) samples with controlled molecular weights and relatively low polydispersities ($M_w/M_n \approx 1.20$). Although well-defined products could be obtained, interactions between the copper catalyst and the peptide were evident.[2] This was indicated by a relative slow overall rate of polymerization and a curvature of the first order kinetic plot. To analyze these further a series of additional ATRP reactions were performed in the presence of various amounts of oligopeptides (data not shown).[2] In this series the added oligopeptide had a comparable sequence to ***III*** but did not possess any initiator moiety. Instead the polymerization was initiated by methyl 2-bromopropionate. These experiments reveal a decrease of the overall rate of polymerization with increasing oligopeptide concentration. Most likely this is due to the inherent property of the polyamide backbone of the peptides representing a multi-dentate ligand for metal ions. This probably causes a ligand exchange reaction

with the ATRP metal complex leading to a partial inhibition of the catalyst. A comparable behavior has already been reported in ATRP processes of (meth) acrylamides.[9]

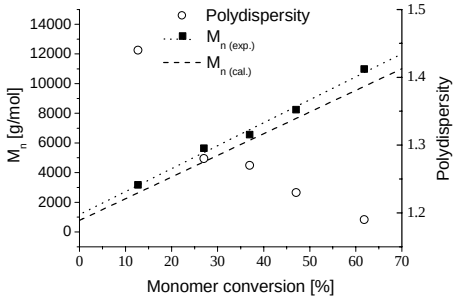
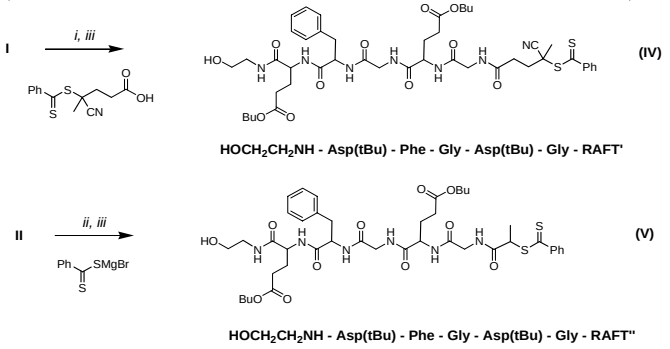


Figure 2. ATRP of *n*BA initiated by ***III*** at 60 °C (Conditions: $[n\text{BA}]_0/[\text{In}]_0/[\text{CuBr}]_0/[\text{CuBr}_2]_0/[\text{PMDETA}]_0 = 114/1/1/0.05/1.05$ DMSO = 60 vol.%).

Reversible Addition Fragmentation Transfer Radical polymerization (RAFT): We explored the potentials of the RAFT[10] polymerization process as a synthesis tool to access well-defined macromolecular chimera. Recently, Perrier *et al.* have reported the application of amide based transfer agents for RAFT polymerization of diverse monomers.[11] However, the reported strategy can not be easily extended for the synthesis of oligopeptide based RAFT agents since chromatographic purification procedures are necessary. Nevertheless, we describe herein a straightforward solid-phase supported synthesis approach to oligopeptide based RAFT agents, avoiding the chromatographic purification step and therefore offering potentially a versatile route to fragile and/or complex RAFT agents.

Scheme 2. Solid phase supported synthesis of the oligopeptide transfer agents (C*onditions: i* DCC, NMP, 4 h, rt.; *ii* THF 15 h, 60° C*; iii* 2% TFA/DCM).



Two different synthetic strategies based on solid phase supported chemistry have been evaluated: *i.* The coupling of a preformed carboxyl functionalized RAFT agent to the N-terminus of a peptide (Scheme 2, ***IV***) and *ii.* the transformation of an ATRP initiator group present in an oligopeptide macroinitiator into a RAFT functionality (Scheme 2, ***V***).

The first route (Scheme 2, ***IV***) lead to byproduct formation that could not be completely suppressed. This result from a nucleophilic attack of the peptide amine terminus on the dithioester, yielding the substitution product as it was confirmed by LC-MS analysis.

Since it is not expected that the substitution byproduct interfere with the RAFT process a polymerization of *n*BA was performed. Figure 3 shows the kinetics indicating a controlled polymerization that yields products with M_w/M_n of around 1.2. The molecular weight increases linearly with reaction time following the theoretical values. However, the fist order kinetic plot (data not shown) exhibited an obvious inhibition phase of roughly 4 hours. Afterwards, the semilogarithmic expression of monomer conversion increased linearly with time reaching 50% conversion after 32 h.

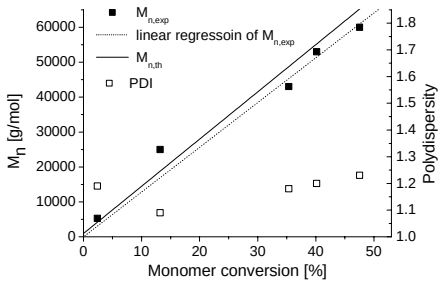


Figure 3. RAFT polymerization of *n*BA controlled by ***IV*** at 60°C (Conditions: $[n\text{BA}]_0/[\boldsymbol{V}]_0/[\text{AIBN}]_0 = 1050/1/0.05$ DMF = 60 vol.%).

The second synthesis route (Scheme 2, ***V***) comprises the functionality switch of the oligopeptide ATRP macroinitiator into an oligopeptide transfer agent (***V***). Since the presence of a nucleophilic amine is avoided within this route, substitution side reactions can be neglected. The analysis of the product by ESI-TOF-MS allows the verification of the chemical structure of the targeted oligopeptide macro transfer agent (***V***) and quantitative consumption of the precursor (***II***) was proved by HPLC analysis. Preliminary polymerization experiments with ***V*** as oligopeptide transfer agent show a linear consumption of monomer with reaction time. However, further studies are in progress.

These results demonstrate a novel, straightforward synthesis route towards multifunctional RAFT agents exhibiting high potentials to be broadened to diverse systems including polypeptides, PNA's and polysaccharides.

Conclusions

It was shown that well-defined macromolecular chimera comprising sequence defined oligopeptides and synthetic polymers can be accessed via either ATRP or RAFT polymerization techniques. Therefore, defined oligopeptide macroinitiators, for the ATRP process as well as macro transfer agents for the RAFT polymerization were synthesized. The latter could be accessed via novel solid phase supported synthesis approaches, allowing the straightforward preparation of RAFT agents without any chromatographic purification step. Kinetic investigations were presented, revealing an efficient control of the polymerization processes, but also inherent difficulties.

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