Incorporating amino acid sequences into the backbone chain of polymers through thiol-ene chemistry

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ABSTRACT

Peptide sequences containing at least two cysteines have been copolymerized by means of thiol-ene click chemistry. Well-defined and degradable polymers containing the amino acid sequences within the backbone chain were obtained when the reaction proceeded according to the Michael addition pathway.

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Here, we report a novel synthetic route for incorporating peptides into polymers based on the use of click chemistry. According to Sharpless, who coined the term, a click reaction is one that affords the product in very high yield and in which the by-products can be removed without chromatography, is wide in scope and simple to perform, and can be conducted in easily removable or benign solvents [1]. Thiol-ene reactions [2] are one type of click chemistry that are becoming increasingly important in the synthesis of materials [3]. Thiol-ene click reactions may proceed via a radical or a Michael addition-type pathway. Endo and co-workers used the former to copolymerize diolefins containing a single amino acid with dithiols in the presence of azobisisobutyronitrile (AIBN) as a radical source [4]. To our knowledge, this is the only example of peptide incorporation into a polymer's backbone chain via click chemistry. Polymers with number average molecular weights ($\overline{M}_n$) up to ca. 60,000 and polydispersity indices (PDI) lower than 2 were obtained in high yields. The resulting poly(ester-amide-sulfide)s could be degraded by the lipase *Rhizopus arrhizus*. Inspired by these findings, we envisioned that a peptide sequence containing at least two cysteines might act as conventional dithiols

and thus be incorporated into the backbone chain of a synthetic polymer via thiol-ene click chemistry.

In order to find suitable experimental conditions, a first series of thiol-ene polymerizations were carried out with ethylene glycol dimethylacrylate (EGDMA) and 2,2′-(ethylenedioxy)diethanethiol (EDDET) as the monomers (Scheme S1). The polymerization via the radical pathway in the presence of AIBN yielded, in all the experimental condition tested, insoluble materials likely due to chain cross-linking. On the other hand, as reported for similar systems [5], soluble polymers were obtained via the Michael addition pathway in the presence of hexylamine as the catalyst [6]. To optimize the polymerization conditions in terms of yield and molecular weights, different solvents, polymerization time, polymerization temperature and molar ratios of the monomers were employed (Table S1). As to solubility of the resulting copolymer, DMSO afforded the best results. The yield increased, whereas the molecular weight decreased, with increasing polymerization time and temperature. An acceptable trade-off between these two opposite effects was obtained by carrying out the polymerization at 20 °C for 12 h. The monomers' molar ratio also had an effect on the $\overline{M}_n$, the highest molecular weights being obtained when a slight excess (4 mol%) of EDDET was employed. This might be due to a non-perfect alternation of the two monomers resulting from the formation of disulfide bridges between EDDET units or the formation of an EDDET homopolymer. On the one hand, both the ^{1}H and ^{13}C NMR spectra (Fig. S1) support the existence of S—S bridges since

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they contain signals that are compatible with the presence of ethylene units next to a disulfide bond. On the other hand, the unimodality of the size exclusion chromatography (SEC) traces suggests that the formation of a non-perfectly alternating copolymer is the most likely hypothesis. The experimental conditions were subsequently employed to copolymerize EGDMA and EDDET with the model peptide L-cysteine-L-phenylalanine-L-cysteine (CFC) or L-cysteine-L-leucine-L-cysteine (CLC) as depicted in Scheme 1. In either case, 46 mol% of EGDMA, 44 mol% of EDDET and 10 mol% of peptide were used (Table 1). The resulting poly(β-thioester)s were purified by dialysis in methanol so as to remove, among other possible impurities, possible traces of unreacted peptide. In the case of the CFC-containing polymer (**P1**), a mono-modal mass distribution with $\overline{M}_n = 7300$ and PDI = 2.72 was observed by size exclusion chromatography (Table 1). The copolymer was isolated with an overall 71% yield. The ^{1}H NMR (Fig. 1A) is consistent with a terpolymer containing EDDET, EGDMA and CFC units. By comparing the area underneath the peaks around 7.29 ppm relative to the five protons of the phenyl group in the CFC moiety with the area underneath the doublet around 1.22 ppm relative to the six protons of methyl groups in the EGDMA moiety, it was found that CFC makes up ca. 5 mol% of the whole copolymer. When the ^{1}H NMR experiment was carried out in the diffusion-edited mode, that is, recorded applying a flow-compensated double-stimulated-echo with a gradient strength up 40% [7], the peaks relative to CFC did not fade away (Fig. 1B). With such a gradient strength, the signals relative to low molecular weight species are normally suppressed [8], which indicates that the peptide is incorporated into the polymer rather than forming a physical blend with it. The thermogravimetric analysis showed two distinct mass losses, one at 184 °C corresponding to a 6 w% loss, and the other at 342 °C corresponding to the remaining 94 w% loss (Table 1). The former is attributable to the thermal degradation of CFC whereas the latter corresponds to the thermal degradation of the EDDET and EGDMA moieties. This can be deduced from the direct comparison with the TGA traces of unreacted CFC and poly(EDDET-*alt*-EGDMA), respectively (Fig. 2A). The DSC thermogram displayed a single glass transition occurring at −46 °C (Fig. S2). This is sensibly higher than the one measured for poly(EDDET-*alt*-EGDMA) (−50 °C) even when the different molecular weights of the two copolymers are taken into account. The presence of the CFC moiety is likely to lower the chain flexibility, and thus to increase the T_g, due to a reduction of the number of ether links in the backbone chain and the introduction of large as well as polar side groups [9].

The terpolymerization in the presence of CLC afforded qualitatively similar results. The resulting poly(β-thioester) (**P2**) was found to have a monomodal SEC trace with $\overline{M}_n = 6700$ and

Table 1
Monomer equivalents, yield, number average molecular weight, polydispersity index, degradation temperatures, glass transition temperatures and peptide content for **P1** and **P2**.

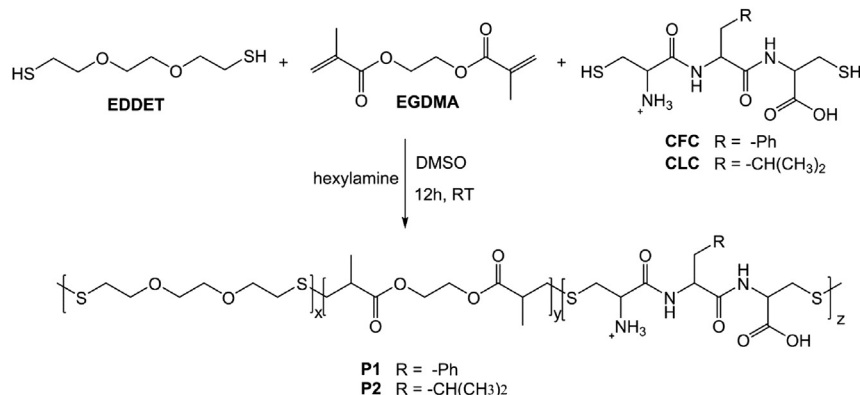| Terpolymer	P1	P2
EGDMA/EDDET/CFC	1.00/0.96/0.22	–
EGDMA/EDDET/CLC	–	1.00/0.96/0.22
Yield[a]	71%	75%
M_n[b]	7300	6700
PDI[b]	2.72	2.41
T_{deg1}	184 °C	165 °C
T_{deg2}	342 °C	344 °C
T_g	−46 °C	−47 °C
Peptide content	5 mol%[c]	5 mol%[d]

[a] After dialysis in MeOH.
[b] Determination by SEC in THF on the basis of polystyrene calibration.
[c] Determination by comparison of the integrated peaks in the ^{1}H NMR spectra of the phenyl group in CFC and the methyl group in EGDMA.
[d] Determination by comparison of the integrated peaks in the ^{1}H NMR spectra of the CH_3- isopropyl unit in CLC and the methyl group in EGDMA.

PDI = 2.41 and was isolated with an overall 75% yield. The DSC thermogram (Fig. S2) was quantitatively similar to the one of the CFC-containing polymer, from which a glass transition temperature of −47 °C was calculated (Table 1). The ^{1}H NMR spectrum (Fig. S3A) appeared to contain the signals relative to CLC, which did not vanish in the diffusion-edited ^{1}H NMR recorded with a gradient strength up to 40% (Fig. S3B). The comparison of the area underneath the multiplet around 0.84 ppm relative to the six protons of the methyls in the isopropyl group of the CLC moiety with the area underneath the doublet around 1.22 ppm relative to the six protons of the methyl groups in the EGDMA moiety yielded a CLC content of ca. 5 mol%. The TGA trace showed, also in this case, two distinct mass losses, one of ca. 10% at 165 °C corresponding to CLC moieties and the other around 344 °C corresponding to EDDET and EGDMA moieties (Fig. 2B). The difference in peptide content between **P1** and **P2** may be due to different decomposition pathways of CFC and CLC during heating, which leave different types and amounts of solid pyrolysis products in the crucibles [10]. A similar trend is indeed observed in the TGA traces of pristine CFC and CLC.

In order to confirm further the incorporation of the peptide, a series of degradation experiments were carried out in the presence of subtilisin, a protease that is able to cleave, selectively, the peptide bond after amino acids with bulky hydrophobic residues such as that in leucine [11]. As expected, when poly(EDDET-alt-EGDMA), which contains no peptide bonds, was incubated with subtilisin, no decrease in the $\overline{M}_n$ of the polymer was observed by SEC (Fig. S4A). In contrast, **P2** degraded almost completely after 48 h of

Scheme 1. Terpolymerization of ethylene glycol dimethacrylate (EGDMA), 2,2′-(ethylenedioxy) diethanethiol (EDDET) and the tripeptides CFC or CLC.