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# Synthesis of water soluble and multi-responsive selenopolypeptides *via* ring-opening polymerization of *N*-carboxyanhydrides†

Guangqi Wu,[a] Chenglong Ge,[b] Xingyi Liu,[a] Shuo Wang,[a] Letian Wang,[a] Lichen Yin *[b] and Hua Lu *[a]

**We report here the synthesis of water soluble selenopolypeptides *via* the ring-opening polymerization of *N*-carboxyanhydrides. The oligoethylene glycol-bearing selenopolypeptides are thermally responsive in aqueous solutions with tunable lower critical solution temperatures. The polymers can also undergo rapid and reversible helix–coil transitions upon responding to the added redox cycle.**

Selenium has recently drawn tremendous attention in broad areas of biology and chemistry.[1,2] Compared with its chalcogen sibling sulfur, selenium has a larger atomic radius and is prone to polarization, making it more reactive in many chemical transformations such as nucleophilic substitutions, redox reactions, and radical-mediated exchange reactions.[1–4] In the human body, selenium is a necessary trace element that relates to many diseases such as cancer, diabetes and infection.[5] Selenocysteine (Sec), widely known as the 21st proteinogenic amino acid, has been discovered in proteins (so-called selenoproteins) such as glutathione peroxidases (GPx), iodothyronine deiodinases, and thioredoxin reductases which are involved in important biochemical processes including antioxidation and immunoregulation.[6] Often, these proteins utilize the special reactivity of selenium to execute specific functions.[1] For example, GPx has been found to reduce oxidative stress by taking advantage of the reversible redox reaction of Sec.

Because of these interesting properties, there is growing interest in introducing selenium to polymers for applications such as drug delivery systems, enzyme mimics, self-adaptive and shape-memorizing materials, and catalysts.[7–9] Many chemical forms of selenium in polymers have been reported such as selenoether (R–Se–R), diselenide (R–Se–Se–R),[8] and more recently selenourea (RNHC(=Se)NHR′).[10] Notably, the introduction of selenium often causes dramatic physiochemical changes of materials in response to various stimuli such as oxidation, reduction, light, and γ-radiation.[8]

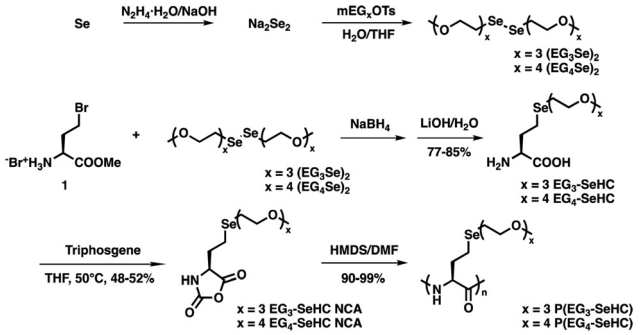
Synthetic polypeptides prepared by the ring-opening polymerization (ROP) of *N*-carboxyanhydrides (NCAs) are promising protein mimics owing to their peptidic backbones.[11] They have been widely used as excellent carriers for drug and nucleic acid delivery, hydrogels for tissue engineering, and stealth materials for protein modification.[12–25] Compared to most synthetic polymers, polypeptides are unique in their hierarchical structures. To date, the introduction of selenium in polypeptides has been achieved mainly through genetic engineering, Sec-based native chemical ligation (NCL), and chemical modification.[26] However, there have been very few reports, if any, on the preparation of selenium containing polypeptides through the ROP of NCAs. Herein, we report the design and synthesis of water-soluble and multi-responsive selenopolypeptides through the controlled ROP of NCAs derived from selenohomocysteine (SeHC). The polypeptides are thermally responsive in aqueous solution and can undergo rapid and reversible helix–coil transitions upon the treatment of an oxidant and/or a reductant.

To prepare water soluble selenopolypeptides, we decided to replace the methyl group of selenomethionine (SeMet) with methoxy-capped oligoethylene glycol ($EG_x$, $x$ = 3 or 4) (Scheme 1). Previously, Li and Deming have separately pioneered a series of $EG_x$-containing polypeptides starting from thiol-containing amino acids such as cysteine[27,28] and homocysteine[29] derivatives. Given the expense of selenohomocysteine, here, we prepared methoxy oligoethylene glycol substituted diselenide $(EG_xSe)_2$ as a scalable and practical selenium source (Scheme 1).[30] Briefly, $(EG_xSe)_2$ was synthesized from selenium powder and methyl *p*-toluenesulfonate of oligoethylene glycol monomethyl ether ($mEG_xOTs$). Next, $(EG_xSe)_2$ was reduced with sodium borohydride *in situ* and reacted with 2-amino-4-bromobutyric acid methyl ester **1**

[a] *Beijing National Laboratory for Molecular Sciences, Center for Soft Matter Science and Engineering, Key Laboratory of Polymer Chemistry and Physics of Ministry of Education, College of Chemistry and Molecular Engineering, Peking University, Beijing 100871, People's Republic of China. E-mail: chemhualu@pku.edu.cn*

[b] *Jiangsu Key Laboratory for Carbon-Based Functional Materials & Devices, Institute of Functional Nano & Soft Materials (FUNSOM), Collaborative Innovation Center of Suzhou Nano Science & Technology, Soochow University, Suzhou 215123, P. R. China. E-mail: lcyin@suda.edu.cn*

† Electronic supplementary information (ESI) available: Additional data, synthesis of monomers and their characterization data (Fig. S6–S19), polymerization procedures, and additional experimental procedures. See DOI: 10.1039/c9cc03767e




Scheme 1 Synthesis of P(EG$_x$-SeHC).

*via* one pot through an $S_N2$ reaction. Two selenium-containing amino acids EG$_x$-SeHC ($x$ = 3, 4) were then successfully obtained on a gram scale after the hydrolysis of the methyl ester with LiOH. The overall yield of the three steps was reasonable, typically ~40% (calculated based on the selenium fed). The amino acids were then converted to NCAs, denoted as EG$_x$-SeHC NCA ($x$ = 3 or 4, Scheme 1), using the standard triphosgene protocol and purified with silica gel chromatography. In most cases, EG$_x$-SeHC NCA was obtained as colorless oil in 56–60% yield after column purification. The intermediates and NCA monomers were carefully characterized by mass spectrometry and $^1$H NMR and $^{13}$C NMR spectroscopy (see the ESI†).

Next, we performed the ROP of EG$_x$SeHC-NCA in *N*,*N*-dimethylformamide (DMF) using hexamethyldisiloxane (HMDS) as the initiator.[31] Smooth polymerizations with complete (>95%) monomer conversion proceeded readily at room temperature to afford the corresponding selenopolypeptides, denoted as P(EG$_x$-SeHC)$_n$ where $n$ is the degree of polymerization (DP). The molar mass ($M_n$) of the polypeptides coincided well with the feeding monomer-to-initiator ratios (M/I) as shown in Table 1. The highest $M_n$ of the selenopolypeptides we obtained was 33.4 kg mol$^{-1}$. The dispersity ($Đ$) values of the polymers were generally 1.10–1.16, indicating well-controlled ROPs under the practical conditions (Table 1 and Fig. S5, ESI†).

EG$_x$-moieties are known to impart thermoresponsive behaviors to polypeptides.[27,29,32–40] As expected, all of the P(EG$_x$SeHC)$_n$ polypeptides possessed reversible phase transition behaviors in aqueous solutions. Upon heating the aqueous solution of P(EG$_x$SeHC), a sharp transition from clear to opaque was observed at various onset temperatures (Fig. 1a inset). The same solutions underwent a rapid and reversed phase transition from opaque to clear upon cooling to lower temperatures. All of the samples showed slight hysteresis in the transition temperatures. This is probably because the redispersion of the EG unit requires more energy to overcome the energy barriers.[32,41] The LCST can be influenced by various factors. For example, P(EG$_3$-SeHC)$_{40}$ showed an LCST in water of 22 °C, considerably lower than the 40 °C of P(EG$_4$-SeHC)$_{40}$ (Fig. 1a). Moreover, the LCST was slightly lower in phosphate buffered saline (PBS) than that in pure water, likely a consequence of the salt-out effect (Fig. S2, ESI†).[29] Similar to many other polymers, the LCST of P(EG$_4$-SeHC)$_n$ was also found to be DP-dependent as shown in Fig. 1b, with an earlier transition following a greater DP. Overall, the LCST can be easily manipulated in a relatively broad range by simply adjusting the length of the EG, the DP, and/or the buffer species. In one example, the LCST of P(EG$_4$-SeHC)$_{120}$ in PBS was found to be ~37 °C, which is close to body temperature (Fig. S1, ESI†). Circular dichroism (CD) spectroscopy of P(EG$_4$-SeHC)$_{40}$ in water indicated that it maintained the α-helical conformation above the LCST but with a decreased signal-to-noise ratio due to the phase transition (aggregation). This result suggested no conformational switches during the thermal induced phase transition (Fig. S4, ESI†).[27,29,32] Owing to the excellent water solubility of P(EG$_4$-SeHC) at room temperature, we mainly focused on this polymer in the following research.

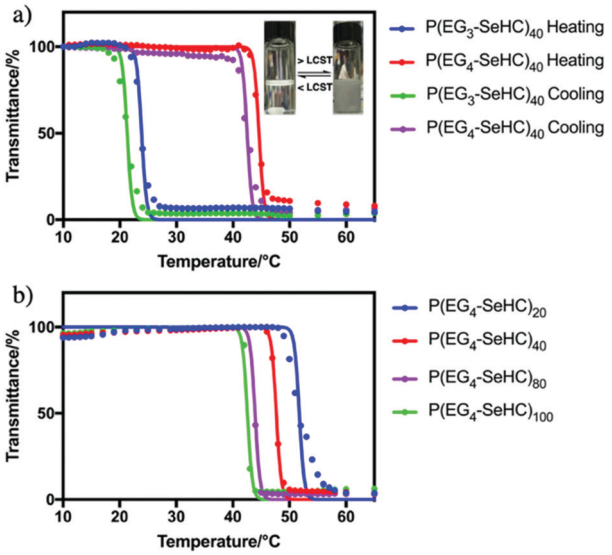



Fig. 1 Thermal-responsiveness of the selenopolypeptides (1.0 mg mL$^{-1}$ in water). (a) Temperature-dependent transmittance of P(EG$_3$-SeHC)$_{40}$ and P(EG$_4$-SeHC)$_{40}$, inset: photographs of P(EG$_3$-SeHC) showing phase transition below and above its LCST. (b) The DP-dependent thermally responsive behavior of P(EG$_4$-SeHC).

Table 1 HMDS-mediated ROP of EG$_x$-SeHC NCA

| Entry | Monomer | DP$_{exp.}$ [a] | DP$_{obt.}$ [b] | $M_n$ [c] (g mol$^{-1}$) | $Đ$ [d] |
|---|---|---|---|---|---|
| 1 | EG$_3$-SeHC | 40 | 35 | 10 900 | 1.16 |
| 2 | EG$_3$-SeHC | 80 | 72 | 22 300 | 1.14 |
| 3 | EG$_3$-SeHC | 100 | 88 | 27 200 | 1.15 |
| 4 | EG$_4$-SeHC | 40 | 37 | 15 200 | 1.11 |
| 5 | EG$_4$-SeHC | 80 | 79 | 28 100 | 1.12 |
| 6 | EG$_4$-SeHC | 100 | 94 | 33 400 | 1.10 |

[a] Expected degree of polymerization. [b] Obtained degree of polymerization. [c] Absolute molar mass based on the d$n$/d$c$ values determined using SEC equipped with a multi-angle light scattering detector; 0.0770 mL g$^{-1}$ for P(EG$_3$-SeHC) and 0.0711 mL g$^{-1}$ for P(EG$_4$-SeHC). [d] Dispersity, determined by SEC.

Deming, Li, Ding and many others have previously explored the redox responsivity of thioether-containing polypeptides.[29,42–44] Specifically, the thioether was oxidized by peroxide to generate sulfoxide which was reducible using thiols[29] or enzymes.[45] However, high concentrations of reagents were usually required for the thioether–sulfoxide cycle with relatively slow kinetics, making them sub-optimal as rapid responsive materials under

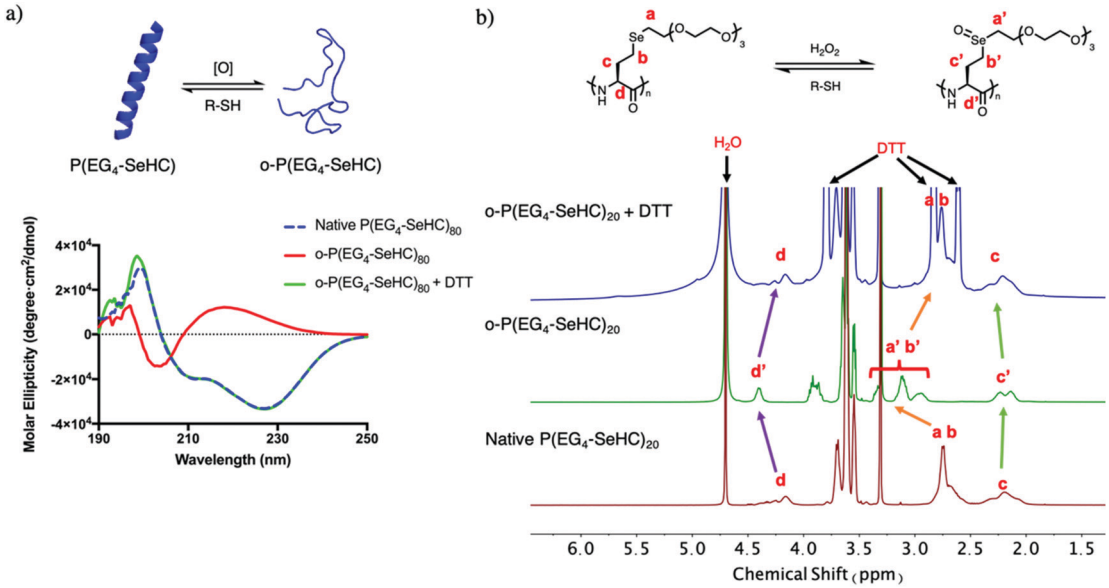



Fig. 2 Oxidation and reduction-induced helix–coil transition of P($EG_4$-SeHC). (a) CD and (b) $^1$H NMR spectroscopy of P($EG_4$-SeHC) after $H_2O_2$ oxidation followed by DTT reduction.

physiological and/or pathological conditions. Here, we expected that P($EG_x$-SeHC) may show more sensitive redox behaviors owing to the higher reactivity of selenium relative to sulfur.[2] To study the oxidative responsiveness of the selenopolypeptides, we first treated P($EG_4$-SeHC)$_{80}$ with 20 mM hydrogen peroxide ($H_2O_2$) in water at room temperature. As revealed by the CD spectra (Fig. 2a and Fig. S2, ESI†), the selenopolypeptide lost its helicity completely 1 h after the $H_2O_2$ treatment. $^1$H NMR spectroscopy confirmed the complete oxidation of selenoether to selenoxide (Fig. 2b). Compared to the oxidation of homocysteine derived polypeptides,[29] which normally required 6 h treatment of 1% acetic acid and 1% $H_2O_2$ (~300 mM) at 38 °C, the oxidation of P($EG_4$-SeHC) is clearly milder and more controllable. No cloudy point up to 65 °C was found for the oxidized selenopolypeptides *o*-P($EG_3$-SeHC) and *o*-P($EG_4$-SeHC) (Fig. S3, ESI†). Treating the oxidized *o*-P($EG_4$-SeHC)$_{80}$ with 30 mM DTT for 5 h completely regained the selenoether-containing polypeptide P($EG_4$-SeHC)$_{80}$, evidenced by both $^1$H NMR and CD spectroscopy (Fig. 2a and b). The NMR peaks of P($EG_4$-SeHC) appeared broader than its oxidized form *o*-P($EG_4$-SeHC), which was possibly a result of P($EG_4$-SeHC) aggregation as indicated by atomic force microscopy (AFM) and light scattering (data not shown).

Many selenium-containing materials showed different degrees of GPx-like activity, in which selenium served as the catalytic redox center.[9] The GPx activity of P($EG_4$-SeHC)$_{40}$ was tested by employing a well-established multi-enzymatic biochemical assay involving the oxidation of GSH by peroxide (see the ESI†). The result showed that P($EG_4$-SeHC)$_{40}$ efficiently catalyzed the GSH oxidation using $H_2O_2$ (Fig. 3a). Safety is of vital importance for biomaterials. The cytotoxicity of P($EG_4$-SeHC)$_{40}$ was tested on several cell lines including RAW264.7, HeLa, and HUVEC. The results revealed excellent biocompatibility of P($EG_4$-SeHC)$_{40}$ in all of the cell lines at a concentration up to 250 μg mL$^{-1}$ (Fig. 3b).

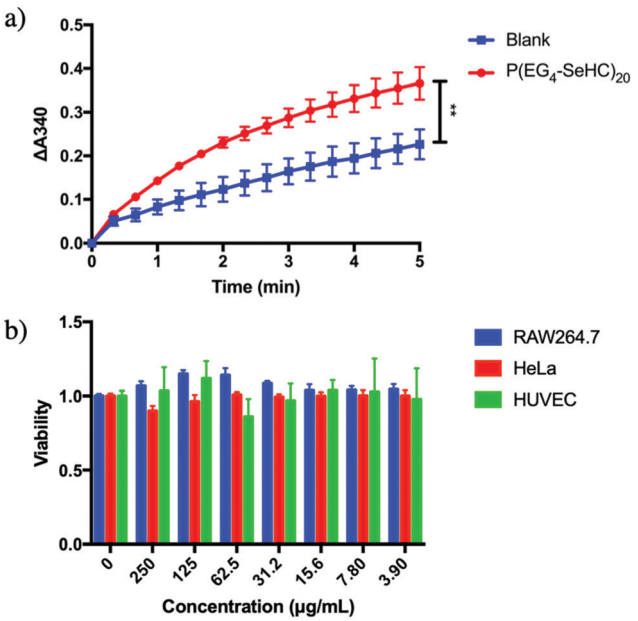



Fig. 3 GPx-like activity and safety of P($EG_4$-SeHC). (a) GPx activity of P($EG_4$-SeHC)$_{20}$ (0.05 mg mL$^{-1}$) measured by a colorimetric assay. $N$ = 3, **: $p < 0.01$; statistical analysis was performed by the $t$ test. (b) Viability of different cell lines after the treatment of P($EG_4$-SeHC)$_{80}$ at indicated concentrations for 24 h. $N$ = 6.

In conclusion, we report here the synthesis of selenopolypeptides P($EG_x$-SeHC)s with well-defined structures and controlled molar masses. The polypeptides can undergo sensitive and reversible helix–coil transition in a biologically relevant redox environment. The $EG_x$-bearing P($EG_x$-SeHC)s exhibit thermally responsive properties in aqueous solutions. The LCST is readily tunable in a broad range including that near body temperature. The polymers are safe in different cell lines and demonstrate moderate

GPx-like catalytic activities. With all these unique and intriguing features, the selenopolypeptides are potentially useful for various biomedical and bioengineering applications including chiral materials, stimuli-responsive carriers, autoxidation and anti-inflammatory agents.

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## Conflicts of interest

There are no conflicts of interest to declare.

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