Facile Synthesis of Polymethionine Oxides through Polycondensation of Activated Urethane Derivative of α-Amino Acid and their Application to Antifouling Polymer against Proteins and Cells

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We have developed a facile synthesis of poly(methionine) and poly(methionine oxide), including poly(methionine sulfoxide), and poly(methionine sulfone) through polycondensation of the corresponding *N*-phenoxycarbonyl derivatives of α-amino acids in the presence of amines. These urethane derivatives were readily synthesized through *N*-carbamylation of onium salt of methionine with diphenyl carbonate. Oxidation of sulfide on the urethane derivative with a hydrogen peroxide provided selectively the corresponding sulfoxide and sulfone in the high yield. Heating of their urethane derivative successfully gave the corresponding polypeptide through polycondensation accompanying the elimination of phenol and CO_2 in high yield. The molecular weight of polypeptide was adjusted by varying feed ratio of urethane derivative to amine. MALDI-TOF mass analysis appeared that the added amine was successfully incorporated into the terminal end of polypeptide. Taking advantage of our facile synthetic route to polypeptide, we have synthesized a polystyrene bearing oligo(L-methionine sulfoxide) in the side chain, and investigated their application for surface-coating polymer that leads to antifouling property against proteins and cells. Its polystyrene was readily synthesized through polycondensation of urethane derivative of L-methionine sulfoxide in the presence of 4-vinylbenzylamine, followed by the radical polymerization with water-soluble azo initiator. The inhibition of protein (hRP-IgG) adsorption and F9 cells adhesion was observed on the surface of polymer-coated PS plate, because of a hydrophilic nature of L-methionine sulfoxide segment. In addition, the result of CCK-8 assay appears a low cytotoxicity against F9 cell, indicating the polymer possesses a high biocompatibility.

Introduction

In recent years, there has been considerable interest for the synthesis of polypeptides-based material and their application in the biomaterial including a drug delivery system, biocatalyst, tissue engineering and biosensor.[1] For the construction of polypeptide component, the most frequently used method is a ring-opening polymerization of α-amino acid *N*-carboxyanhydrides (NCAs).[2] The polymerization of NCAs generally proceeded to produce polypeptide by primary amines and provides some advantages such as the precious control of molecular weight by varying feed ratio of NCAs to amines and the well-controlled terminal structure as a result of their living nature of polymerization. However, the use of highly toxic phosgene or its derivatives to synthesis the NCAs has been a major drawback for application of NCAs on a large scale.[3] In addition, susceptible nature of NCAs to moisture and heat has prevented the formation of polypeptide in the living nature.

For these reasons, we have aimed to utilize the safe diphenyl carbonate (DPC) as the alternative of phosgene to synthesize NCAs and polypeptide. In our previous work, we have reported that the synthesis of NCAs was successfully achieved by the selective intramolecular cyclization of activated urethane derivative, *N*-phenoxycarbonyl α-amino acids, that can be easily synthesized by *N*-carbamylation of onium salts of α-amino acid with DPC.[4] Furthermore, these activated urethane derivatives of α-amino acid can be used not only as precursors of NCAs, but also as accessible monomers for a more straightforward synthesis of polypeptides, where heating these urethane derivatives in the presence of amines gives directly the corresponding polypeptides through the *in situ* formation of NCAs and their polycondensation along with the elimination of

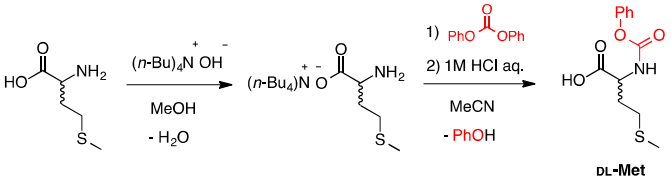
phenol and CO_2.[5] As a result of the investigation on polycondensation behavior, we have found that this approach has the following advantages: (1) the successful incorporation of amine residue in the chain end, (2) the control of molecular weight by varying feed ratio between the urethane derivative and amine, and (3) the easy handling and simple procedure without the use and formation of any toxic compounds. This polycondensation could be applicable for the synthesis of various kinds of polypeptide from the corresponding urethane derivatives.

Among polypeptide-based polymers, polymethionine-containing polymer has received much attention as biomedical materials including contact lenses, artificial skin, etc., due to their high biocompatibility. Also, Oxidation of methionine into the methionine sulfoxide or methionine sulfone could afford the possibility to use in a wide rage of application.[6] For example, Pitha and co-worker reported the utilization of poly(L-methionine sulfoxide) as a solubilizer and carrier of lipophilic compound into water and cells.[7] Deming et al reported an enzyme-triggered cargo release from copolypeptide vesicles that is composed of poly(L-methionine sulfoxide) and poly(L-leucine-*stat*-L-phenylalanine).[8]

Herein, we report the convenient synthesis of polymethionine and polymethionine oxides through polycondensation of the corresponding urethane derivative along with the elimination of phenol and CO_2 in the presence of amines such as *n*-$BuNH_2$ and poly(ethylene glycol) with the terminal of amino group. In addition, the facile synthesis of oligo(L-methionine sulfoxide)-*grafted* polystyrene on the side chain and utilization as new class of surface-coating material that leads to the antifouling property against proteins and cells is also demonstrated.

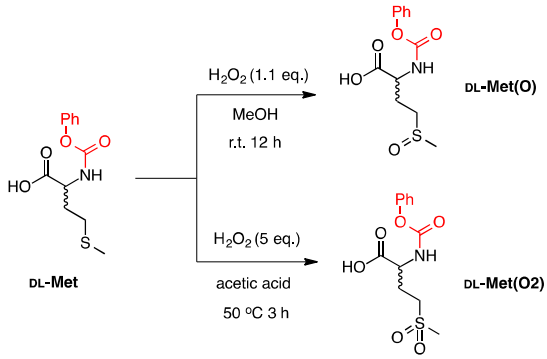
Results and Discussions

According to our previous reports for the synthesis of analogous compound, an activated urethane derivative, *N*-phenoxycarbonyl-DL-methionine (**DL-Met**), was readily synthesized by *N*-carbamylation of tetrabutylammonium salt of racemic methionine with diphenyl carbonate (DPC) in acetonitrile (Scheme 1).[5]



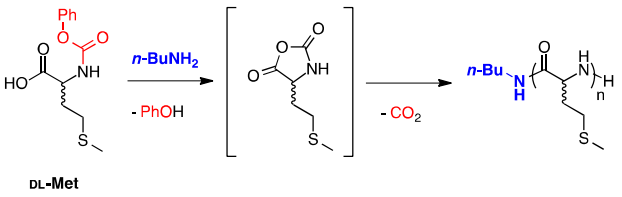
Scheme 1. Synthetic route to urethane derivative of racemic methionine (**DL-Met**).

After the purification with column chromatography, the corresponding urethane derivative was isolated as colorless oil in 69% yield, which crystallizes on standing. Selective oxidation of sulfide moiety of methionine to sulfoxide was conducted with a traditional procedure using 35% aq. hydrogen peroxide (Scheme 2). Treatment of **DL-Met** with a stoichiometric amount of hydrogen peroxide was performed in methanol solution at room temperature. The sulfide moiety of methionine was completely transferred into the sulfoxide moiety for 12 h without any peaks of sulfone moiety as confirmed in ^{1}H NMR spectrum. The corresponding *N*-phenoxycarbonyl-DL-methionine sulfoxide (**DL-Met(O)**) was isolated in good yield (87%) as white powder. The further oxidation to give *N*-phenoxycarbonyl-DL-methionine sulfone (**DL-Met(O2)**) was carried out by heating **DL-Met** in an acetic acid at 50 °C with excess amount of hydrogen peroxide. After complete oxidation, the corresponding **DL-Met(O2)** was isolated in high yield (91%). Chemical structures of all synthesized compounds were confirmed using ^{1}H NMR, ^{13}C NMR, FT-IR, elemental analysis and high-resolution mass spectrometry. These urethane derivatives are storable at room temperature over two months without any side reaction.



Scheme 2. Selective oxidation of **DL-Met** with hydrogen peroxide into **DL-Met(O)** and **DL-Met(O2)**.

Previously, we have reported that urethane derivative of L-methionine was efficiently converted into the corresponding poly(L-methionine) through polycondensation along with the elimination of phenol and CO_2, by heating them in *N*,*N*-dimethylacetamide (DMAc) at 60 °C in the presence of amine.[5] The resulting poly(L-methionine) showed a poor solubility in common organic solvents due to a strong interchain aggregation. We first investigated the polycondensation behavior of **DL-Met** in the presence of *n*-$BuNH_2$ at the different feed ratio ($[M]_0/[amine]_0$ =5-100) (Scheme 3). The progress of polycondensation is tracked by monitoring the amount of phenol eliminated from a urethane derivative. Analyses of ^{1}H NMR spectra from reaction mixture appear that **DL-Met** is completely consumed in all cases to give poly(DL-methionine) without any formation of precipitation. All results were summarized in Table 1. The synthesized polypeptides were collected as ether-insoluble part in excellent yield (83-97 %).



Scheme 3. Synthesis of polypeptide through polycondensation of urethane derivative (**DL-Met**) in the presence of *n*-$BuNH_2$ with the elimination of phenol and CO_2.

As shown in size exclusion chromatography (SEC) of Figure 1, the peak top was gradually shifted into higher molecular weight region when the feed ratio of **DL-Met** to *n*-$BuNH_2$ increased. Their polydispersity index (PDI) remains a relatively narrow value (M_w/M_n) =1.20-1.55. In our previous report for the synthesis of poly(L-methionine) at the same condition, the control of their molecular weight was much difficult due to the precipitation of formed polypeptide from the reaction mixture of DMAc during polycondensation. However, molecular weight of racemic poly(DL-methionine) could be tailored by varying feed ratio between **DL-Met** and *n*-$BuNH_2$, because the disordered chain conformation of racemic polypeptide give rise to a higher solubility in DMAc than that of poly(L-methionine).

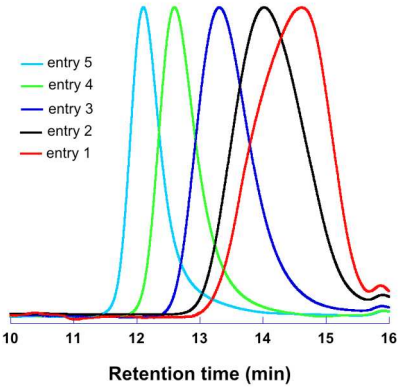


Figure 1. SEC trace of poly(DL-methionine) obtained from polycondensation of **DL-Met** in the presence of *n*-$BuNH_2$. (entries 1-5, in Table 1)

In order to confirm the terminal structure of the obtained poly(DL-methionine) in detail, MALDI-TOF mass spectrometry was performed. As shown in Figure 2, the only one series of signals were observed, which are regularly located with a mean distance corresponding to repeating unit of poly(DL-methionine) (131Da). These mass numbers were in good agreement with the polypeptide structure possessing the *n*-$BuNH_2$-incorporated initiating end and the amino group at the terminal end. Introduction of *n*-$BuNH_2$ residue into terminal end of polypeptide was also confirmed in ^{1}H NMR spectrum of poly(DL-methionine) in DMSO-d_6 (Figure S1 in the Supporting Information). This terminal structure is the same as that of polypeptides produced by a typical ring polymerization of NCAs that was initiated with *n*-$BuNH_2$. These results suggested that *n*-$BuNH_2$ serves as an initiator at the initial stage of the polycondensation system to give the well-defined terminal structure.

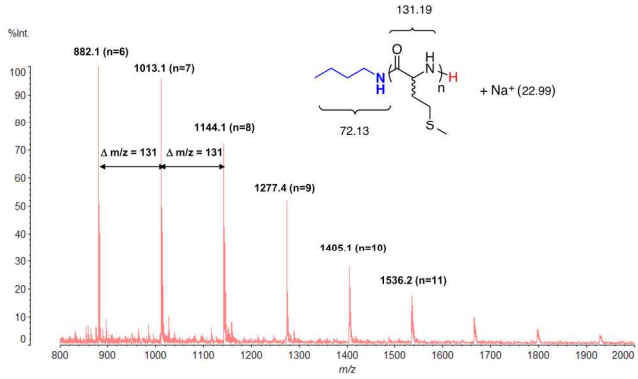


Figure 2. MALDI-TOF mass result of poly(DL-methionine) obtained from polycondensation of **DL-Met** with *n*-$BuNH_2$ (entry 2 in Table 1).

We further investigated polycondensation behavior of **DL-Met(O)** and **DL-Met(O2)** for synthesis of racemic polymethionine oxides in the same manner. In case of polycondensation of **DL-Met(O)**, their polycondensation proceeded smoothly to give the corresponding poly(DL-methionine sulfoxide) in high yield (87-92 %), as shown in Table 2. The resulting polypeptide shows the high solubility in water, DMAc, DMF, DMSO and alcoholic solvents. To determine their molecular weight, an aqueous SEC with phosphate buffered saline as eluent was used. SEC trances are unimodal and their PDIs are relatively narrow (1.17-1.75). The molecular weight was adjusted by varying feed ratio between **DL-Met(O)** and *n*-$BuNH_2$. However, for the synthesis of high molecular weight ($[\textbf{DL-Met(O)}]_0/[n\text{-}BuNH_2]_0$= 50, entry 4, in Table 2), a slight amount of precipitation was formed. Furthermore, we have studied the polycondensation behavior of urethane derivative of L-methionine sulfoxide, **L-Met(O)** as compared with that of racemic one. The polycondensation was carried out in the same manner, however, a white precipitation was formed during polycondensation because of poor solubility for DMAc than that of poly(DL-methionine sulfoxide). The aqueous SEC analyses of resulting poly(L-methionine sulfoxide) appeared multimodal peaks with large PDI > 1.5, indicating uncontrolled chain growth due to precipitation of polypeptide during polycondensation happened (Figure S2 in the Supporting Information).

Polycondensation behavior of **DL-Met(O2)** also conducted in the same manner. Polycondensation of **DL-Met(O2)** was successfully achieved to give the corresponding poly(DL-methionine sulfone). The resulting polypeptide possesses high solubility in high polar solvent such as DMAc, DMF and

DMSO, which allows the control of molecular weight by varying feed ratio (Figure S3 and Table S1 in the Supporting Information). On the other hand, polycondensation of **L-Met(O2)** gave the corresponding polypeptides, which are insoluble in common organic solvents except for TFA, owing to a strong interchain aggregation. As confirmed in MALDI-TOF mass analysis, their polymerization degree was distributed in a wide range from 10 to 35 due to precipitation of polypeptide during polycondensation.

Table. 1 Synthesis of poly(DL-methionine) by polycondensation of **DL-Met** in the presence of *n*-$BuNH_2$

entry	feed ratio [**DL-Met**]$_0$/[amine]$_0$	reaction time (h)	conversion (%)[a]	yield (%) [b]	M_n [c]	M_w/M_n [c]
1	5	5	>99	83	1,500	1.55
2	10	12	>99	97	2,000	1.38
3	25	20	>99	91	4,400	1.31
4	50	24	>99	95	12800	1.23
5	100	48	>99	96	23000	1.20

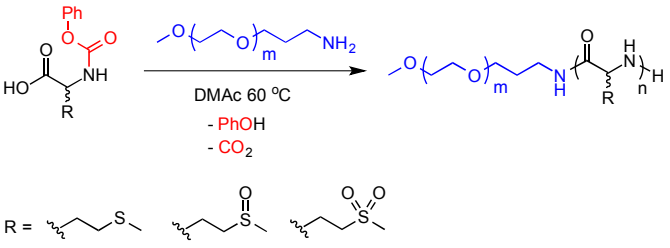
[a] Calculated by ^{1}H NMR spectra
[b] Ether-insoluble parts
[c] Estimated by SEC (eluent: DMF solution of LiBr (10 mM), calibrated by Polystyrene standards)

Table. 2 Synthesis of poly(DL-methionine sulfoxide) and poly(L-methionine sulfoxide) by polycondensation of **DL-Met(O)** and **L-Met(O)**, respectively, in the presence of *n*-$BuNH_2$

entry	urethane derivative	feed ratio $[M]_0/[amine]_0$	reaction time (h)	conversion (%) [a]	yield (%) [b]	M_n [c]	M_w/M_n [c]
1	**DL-Met(O)**	5	5	>99	90	510	1.75
2	**DL-Met(O)**	10	12	>99	91	1,210	1.22
3	**DL-Met(O)**	25	20	>99	97	2,000	1.17
4	**DL-Met(O)**	50	24	>99	91	3,000	1.23
5	**L-Met(O)**	5	5	>99	95	530	2.30
6	**L-Met(O)**	10	12	>99	92	1,300	1.51
7	**L-Met(O)**	25	20	>99	97	1,900	1.50
8	**L-Met(O)**	50	24	>99	96	3,200	1.89

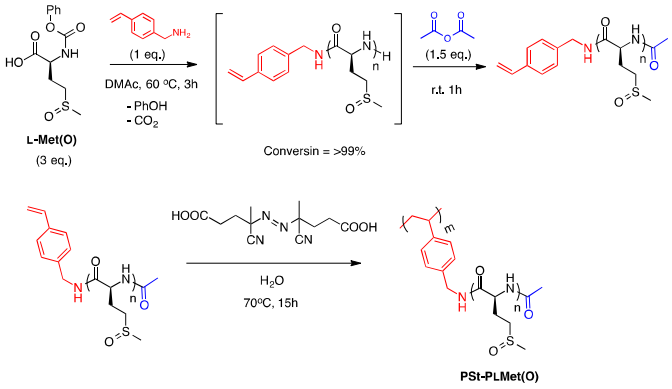
[a] Calculated by ^{1}H NMR spectra
[b] Ether-insoluble parts
[c] Estimated by SEC (eluent: PBS, calibrated by Poly(ethylene glycol) standards)

Based on the successful synthesis of polypeptides with well-defined terminal structure, the employment of an amine-terminated poly(ethylene glycol) (PEG-NH_2) in place of *n*-butylamine was also conducted to synthesis of diblock copolymers composed of polyether and polypeptide segments (Scheme 4). According to the procedure described above, **DL-Met, DL-Met(O)**, and **DL-Met(O2)** were dissolved in DMAc/PEG-NH_2 (M_n = 5,300 and PDI =1.04) solution with various feed ratios and each mixture was then stirred at 60 °C. All results of polycondensation were summarized in Table S3 of Supporting Information. The obtained diblock copolymer shows unimodal and narrow peaks (PDI =1.12-1.32) in the SEC analysis (Figure S5-S7 in the Supporting Information). These peaks are clearly shifted to higher molecular weigh region from that of the original PEG-NH_2, indicating the successful conjugation of polyether segment and polypeptide segment and polypeptide chain length was adjusted by varying feed ratio. Degree of polymerization for polypeptide was estimated between the proton of $-CH_2-CH_2-O-$ and the methine proton of polypeptide around δ= 4.50 ppm. These values are close to the predicted one based on feed ratio between urethane derivative and PEG-NH_2.



Scheme 4. Utilization of PEG-NH_2 for synthesis of diblock copolymer through polycondensation of urethane derivative.

Facile synthesis of antifouling polymers against biological matters to construct biosensor application and so on are recently required.[9] Hydrophilic and water-soluble poly(methionine sulfoxide) is expected to be a promising candidate. Therefore, we have attempted to apply our synthetic strategy of poly(methionine sulfoxide) to produce an antifouling surface-coating material against proteins and cells. We have designed a novel polystyrene bearing oligo(L-methioinie sulfoxide) in the side chain (**PSt-PLMet(O)**), as shown in Scheme 5, where the hydrophobic main chain of polystyrene permits the adsorption into the hydrophobic surface such as polystyrene (PS) plate, and the oligo(L-methioinie sulfoxide) of the side chain can act as a hydrophilic parts that prevents protein adsorption and cell adhesive. The synthesis of polymer was conducted in the two step reaction: (1) polycondensation of **L-Met(O)** in the presence of 4-vinylbenzylamine as an initiator to incorporate a polymerizable group at the terminal end (feed ratio: [**L-Met(O)**]$_0$/[amine]$_0$=3) and (2) subsequent radical polymerization with azo initiator in aqueous solution. In the first step, acetic anhydride (three equivalents, relative to 4-vinylbenzylamine) was added into the reaction mixture at room temperature for the acetylation of the terminal amine that gives rise to the reduction of the undesired interaction to proteins. The isolated oligo(L-methionine sulfoxide) exhibited the characteristic three signals from 5.2 to 6.8 ppm, assignable to styrene C=C double bonds at the terminal end (Figure S8 in the Supporting Information). From ^{1}H NMR peak integrals of methine group and styrene moiety, polymerization degree of oligo(L-methionine sulfoxide) was estimated to be 3. Its M_n and PDI as estimated by SEC were found to be 630 and 1.56, respectively.



Scheme 5. Synthetic route to polystyrene bearing oligo(L-methionine sulfoxide).

Subsequently, radical polymerization of this oligopeptide with a polymerizable styrene group was carried out in aqueous solution (10 wt.%) using 4,4-azobis(4-cyanopentanoic acid) (V-501) to give a **PSt-PLMet(O)** (M_n = 84,000 and PDI = 1.30). The resultant **PSt-PLMet(O)** was covered on the surface of PS plate by using aqueous solution (0.01-1.0 w%). The water contact angle of PS plate after polymer coating was reduced to around 50° from 89° of the original contact angle, suggesting successful adsorption of the **PSt-PLMet(O)** on the surface of PS plate.

First, protein adsorption behavior on the polymer-coated surface was investigated using hRP-IgG. The amount of adsorbed hRP-IgG was calculated. As shown in Figure 3, adsorption of hRP-IgG on the coated PS plate was obviously suppressed as compared with the original PS plate even when a low concentration aqueous solution (0.01 wt%) was used. From these results, we confirmed that the hydrophilic nature of oligo(L-methionine sulfoxide) brings about the decreasing of adsorbed amount of hRP-IgG. Next, this polymer was used for

studying a cell adhesion property. F9 cells were cultured on the PS plate modified with 0.01 wt.% polymer aqueous solution. In the case of the original PS plate, F9 cells were found to be adhered and proliferated on the surface through nonspecific interaction (Figure 4(a) and (b)). Conversely, F9 cells adhesion was completely inhibited on polymer-coated PS plate even after incubation for 24 hour as shown in Figure 4(c) and (d). These results indicated that the hydrophilic oligo(L-methionine sulfoxide) segment plays an important role for suppression of both hRP-IgG adsorption and F9 cell adhesion. In addition, cell cytotoxicity was studied with F9 cell to evaluate the biocompatibility of the resulting polymer using CCK-8 assay. Figure S10 in the Supporting Information shows that the cell viability in the presence of the resulting polymer (1mg/mL) is over 97%, indicating almost no cytotoxicity against F9 cells. From these results, these novel polymers are expected to be used as surface-coating materials, leading to antifouling property against biological matters.

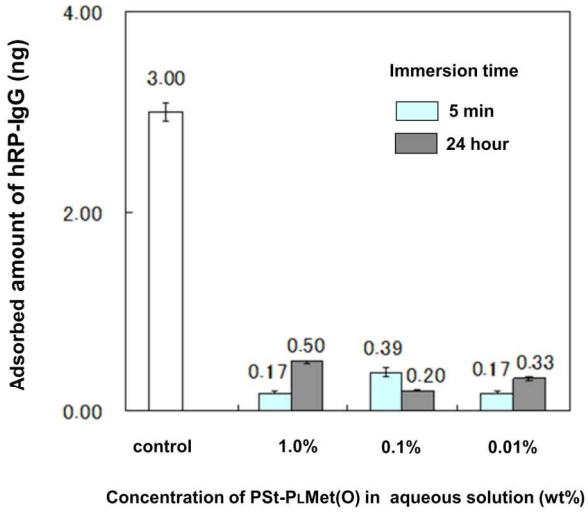


Figure 3. Adsorbed amount of hRP-IgG on the surface of PS plate coated with various concentrations of **PSt-PLMet(O)** aqueous solution.

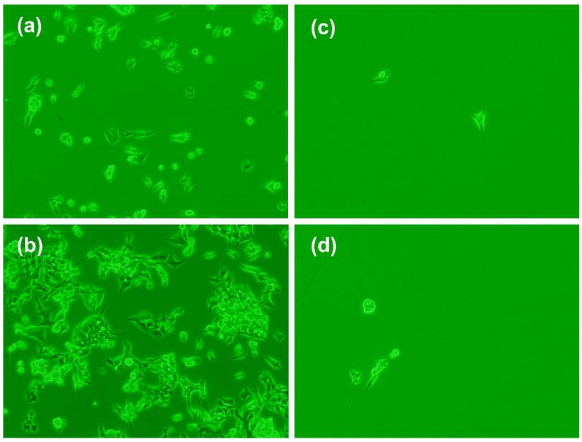


Figure 4. Morphology of F9 cells cultured on PS plate as a control for 4 h (a) and 24 h (b), and on PS plate coated with **PSt-PLMet(O)** for 4 h (c) and 24 h (d).

Conclusions

We have developed a facile synthesis of poly(methionine), poly(methionine sulfoxide), and poly(methionine sulfone) using polycondensation of the corresponding urethane derivatives in the presence of amines such as butylamine, polyethylene glycol with amino group at the terminal. Heating of their urethane derivatives gave polypeptides through polycondensation with the elimination of phenol and CO_2 in high yield. The molecular weight of polypeptide was adjusted by varying feed ratio of urethane derivative to amine. These amines were successfully incorporated into terminal end of polypeptide. Based on our synthetic method, polystyrenes bearing oligo(L-methionine sulfoxide) structure in the side chain were also synthesized utilizing 4-vinylbenzylamine and subsequent radical polymerization. It was appeared that the resultant polymer showed low cytotoxicity as the result of CCK-8 assay, and the excellent antifouling property against proteins (hRP-IgG) and F9 cells was achieved, because of hydrophilic nature of methionine sulfoxide segment.

Experimental Section

Materials and Instruments.

Polyethylene glycol containing amino group at terminal was purchased from NOF Corporation. Diphenyl carbonate was kindly provided from Asahi Kasei Corporation. All other chemicals and solvents were purchased from Tokyo Chemical Industry and Watanabe Chemical Industry, and used without further purification unless otherwise denoted below. A *n*-butylamine (*n*-$BuNH_2$) and *N*,*N*-dimethylacetamide (DMAc) were purified by the distillation over calcium hydride (CaH_2), prior to use. Analytical TLC was performed on commercial Merck plates coated with silica gel (TLC Silica gel 60 F_{254}). Column chromatography was carried out by using Kanto Silica Gel 60N (spherical, neutral, 63-210 μm). ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded with a JEOL ECS-400 spectrometer, and chemical shifts were recorded in ppm units using tetramethylsilane (TMS) as an internal standard. Fourier transform infrared spectroscopy (FT-IR) analysis was recorded on Thermo Scientific Nicolet iS10 in a range from 600 to 4000 cm^{-1}. Size exclusion chromatography (SEC) was carried out on TOSOH HLC-8220 system equipped with three consecutive polystyrene gel columns [TSK-gels (bead size, exclusion limited molecular weight); super-AW4000 (6 μm, > 4 × 10^5), super-AW3000 (4 μm, > 6 × 10^4) and super-AW2500 (4 μm, >

2×10^3)] and refractive index and ultraviolet detectors at 40 °C. This system was operated using DMF containing 10 mM LiBr as eluent at a flow rate of 0.5 mL/min. Polystyrene standards were employed for calibration. For the poly(methionine sulfoxide), aqueous SEC was carried out on CTO-20A column oven (Shimadzu Cooperation) equipped with TSKgel SuperMultiporePW-M. This system was operated using phosphate buffered saline (PBS) as eluent at a flow rate of 0.4 mL/min at 25°C. Poly(ethylene glycol) standards were used for calibration. Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry was performed at room temperature on a Shimadzu Biotech AXIMA Confidence using *α*-cyano-4-hydroxycinnamic acid (CHCA) and sodium trifluoroacetate, as a matrix material and a cationization agent, respectively. In order to prepare the sample for MALDI-TOF mass analysis, the obtained polypeptide was dissolved in TFA (2 mg/mL), and then the solution was mixed with CHCA/THF solution (5 mg/mL) and sodium trifluoroacetate/THF solution (1 mg/mL) at a ratio of 1:2:1. The final mixture was deposited onto sample plates and allowed to air dry. The resulting sample was irradiated with 337 nm of nitrogen laser and detected on positive mode at 20 kV. Melting point was measured on Bibby Stuart scientific melting point apparatus SMP3. Elemental analysis was performed on LECO CHNS-932 analyzer. High-resolution mass spectrometry was conducted using a JEOL JMS-700 mass spectrometer.

Synthesis of Urethane Derivative: *N*-(phenoxycarbonyl)-DL-methionine (DL-Met)

The urethane derivative was synthesized using procedure previously reported for analogous compound:[5] To a stirred suspension of DL-methionine (1.9 g, 12.5 mmol) in methanol 15 mL, tetrabutylammonium hydroxide (37% in methanol) (8.8 g, 12.5 mmol) was slowly added at room temperature. After stirring for thirty minutes, the reaction mixture was concentrated under reduced pressure. The resulting residue was dissolved into acetonitrile (15 mL), and then the resulting solution added dropwise to a stirred solution of diphenyl carbonate (DPC) (2.7 g, 12.5 mmol) in acetonitrile (15 mL) at room temperature. After stirring the solution for an hour, the distilled water (100 mL) was added into the resulting mixture. The resulting mixture was acidified to pH 2-3 with 1M HCl aqueous solution, and then extracted with ethyl acetate (3 × 20 mL). The combined organic layer was dried over Na_2SO_4, filtrated, and concentrated under reduced pressure. The crude products were purified by column chromatography (eluting with a gradient from 30-70% ethyl acetate in *n*-hexane), and then concentrated to give 2.3 g (8.6 mmol, 69%) of urethane derivative, **DL-Met**, as a colorless oil which crystallized on standing, mp: 97.5-98.5. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 2.00-2.17 (m, 4H), 2.25 (m, 1H), 2.62 (m, 2H), 4.59 (m, 1H), 5.83 (d, 1H, J = 8.0 Hz), 7.07-7.16 (m, 2H), 7.17-7.24 (m, 1H), 7.31-7.40 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 15.54, 30.06, 31.48, 53.30, 121.62, 125.76, 129.47, 150.77, 154.61, 176.70. IR (neat, cm^{-1}): 3355, 2918, 1725, 1651, 1531, 1489, 1405, 1199, 1177, 1046, 857, 782, 717, 688. Anal. calcd for $C_{12}H_{15}NO_4S$: C, 53.52; H, 5.61; N, 5.20. Found: C, 53.26; H, 5.50; N, 5.17.

Synthesis of Urethane Derivative: *N*-(phenoxycarbonyl)-DL-methionine sulfoxide (DL-Met(O))

Urethane derivative of DL-methionine, **DL-Met** (2.7 g, 10 mmo) was dissolved in methanol (25 mL). After the solution was cooled to 0°C using ice bath, 35% hydrogen peroxide (1.1 g, 11 mmol) in water was dropwise added to the solution under stirring. The reaction mixture was warmed to room temperature and stirred for 12 h. The distilled water (100 mL) was added into the resulting mixture. The aqueous layer was saturated with NaCl and extracted with EtOAc (3 × 50 mL). The combined organic layer was dried over Na_2SO_4 and concentrated under reduced pressure to give 2.5 g, (8.7 mmol, 87%) of target urethane derivative, **DL-Met(O)**, as a white amorphous solid that tends to adsorb a moisture. ^{1}H NMR (400 MHz, $CDCl_3$, mixture of diastereomers, δ, ppm): 2.23-2.54 (m, 2H), 2.67/2.69 (total 3H, ratio 48: 52, s), 2.84-3.15 (m, 2H), 4.42-4.65 (m, 1H), 6.34/6.44 (total 1H, ratio 48: 52, d, J = 7.4/7.4 Hz), 7.04-7.14 (m, 2H), 7.15-7.24 (m, 1H), 7.28-7.39 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$, mixture of diastereomers, δ, ppm): 25.86, 26.10, 37.36, 37.59, 49.21, 49.29, 52.94, 53.17, 121.68, 121.71, 125.71, 129.47, 150.83, 154.66, 154.73, 172.79, 172.86. IR (neat, cm^{-1}): 2919, 1712, 1531, 1484, 1200, 983, 688. HRMS (FAB) *m/z* calcd for $C_{12}H_{16}NO_5S$, 286.0749 $(M + H)^+$; found, 286.0748.

In the same manner described above, *N*-(phenoxycarbonyl)-L-methionine sulfoxide (**L-Met(O)**) was synthesized in high yield (91%) as a white amorphous solid that tends to adsorb a moisture.

Synthesis of Urethane Derivative: *N*-(phenoxycarbonyl)-DL-methionine sulfone (DL-Met(O2))

To the solution of DL-Met (2.7 g, 10 mmol) in acetic acid (25 mL), 35% hydrogen peroxide in water (4.9 g, 50 mmol) was added, and the mixture was heated at 50 °C for three hours. After cooling to room temperature, 1M sodium thiosulfate aqueous solution (100 mL) was mixed into resulting solution and stirred for half an hour to eliminate the excess hydrogen peroxide. The resulting mixture was extracted with EtOAc (3 × 50 mL). The combined organic layer was washed with water (2× 50 mL) and brine (50 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The obtained residue was recrystallized from hot water to give 2.7 g, (9.1 mmol, 91%) of target urethane derivative, **DL-Met(O2)**, as a white powder. mp: 149.5-150.8 ºC. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.99-2.14 (m, 1H), 2.15-2.31 (m, 1H), 3.01 (s, 3H), 3.12-3.34 (m, 2H), 4.18 (m, 1H), 7.11 (d, 2H, J = 7.5 Hz), 7.20 (t, 1H, J = 7.4 Hz), 7.38 (t, 2H, J = 7.8 Hz), 8.20 (d, 1H, J =8.2 Hz). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 23.83, 40.28, 50.62, 52.59, 121.64, 125.12, 129.32, 150.88, 154.39, 172.48. IR (neat, cm^{-1}): 3335, 1698, 1520, 1482, 1312, 1239, 1123, 966, 909, 758, 690. Anal. calcd for $C_{12}H_{15}NO_6S$: C, 47.83; H, 5.02; N, 4.65. Found: C, 47.78; H, 4.87; N, 4.68.

In the same manner described above, *N*-(phenoxycarbonyl)-L-methionine sulfone (**L-Met(O2)**) was synthesized in high yield (94%) as a white powder. mp: 91.0-93.8 ºC.

Synthesis of polypeptide through polycondensation of urethane derivative

A typical procedure is as follows: Urethane derivative of DL-methionine, **DL-Met** (269 mg, 1 mmol) was dissolved in DMAc (2 mL) and *n*-$BuNH_2$/DMAc solution (20 μL, 1×10^{-3} mmol μL^{-1}) was added. The mixture was placed into flame-dried Schlenk tube and heated at 60 °C for 24 h. The reaction mixture was cooled to room temperature and poured into diethyl ether (100 mL). The resulting white precipitates were collected by filtration and dried at 60 °C under vacuum to give 126 mg of poly(DL-methionine) (95%). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.75-2.09 (br, 5H), 2.34-2.56 (br, 2H), 4.21-4.48 (br, 1H), 7.77-8.11 (br, 1H). IR (neat, cm^{-1}): 3289, 2912, 1651, 1537, 1434, 1279, 957.

Other polypeptides, poly(DL-methionine sulfoxide) and poly(DL-methionine sulfoxide) were obtained by similar procedure as a white powder in high yield (82-95%). The physical data from ^{1}H NMR and IR analysis is listed below. Poly(DL-methionine sulfoxide): ^{1}H NMR (400 MHz, D_2O, δ, ppm): 2.08-2.38 (br, 2H), 2.69-2.80 (br, 3H), 2.87-3.09 (br, 2H), 4.42-4.56 (br, 1H). IR (neat, cm^{-1}): 3256, 1645, 1538, 1444, 1307, 1238, 1000, 942. Poly(DL-methionine sulfoxide): ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.85-2.30 (br, 2H), 2.81-3.23 (br, 5H), 4.22-4.50 (br, 1H), 8.16-8.52 (br, 1H). IR (neat, cm^{-1}): 3306, 1658, 1537, 1272, 1122, 965, 770

Analysis of Protein adsorption on the PS plate coated with polystyrene bearing oligo(L-methionine sulfoxide)

Horseradish peroxidase (hRP)-conjugated goat antimouse IgG was purchased from Merck Millipore. As the substrate, 96-well polystyrene (PS) plate was coated with 200 μL of aqueous solution of polymer (0.01-1.0 wt.%) for 5 minutes at room temperature and rinsed with 350μL of distilled water twice. The 100 μL of hRP-IgG/PBS solution (200 ng/mL) was added into the polymer-coated well of the plate followed by incubation a certain time. After the well of the plate was rinsed three times with 350μL of PBS, each well of the plate is allows to add the 1-Step Ultra TMB-ELISA substrate solution (10 μL/well, Pierce Biotechnology, Inc.) and incubates for 90 seconds at room temperature. The reaction was stopped with 0.5 M sulfonic acid (10 μL/well) and the amount of adsorbed hRP-IgG on the PS substrate was estimated from the measurement of the maximum absorbance at 450 nm.

Cell culture studies

One milliliter of polymer aqueous solution (0.01 wt.%) was added to the PS 6-well plate (Iwaki Cell Biology). The plate was left for overnight. The polymer solution was removed by suction and then the plate was rinsed twice with 2 mL sterilized water. F9 cells were obtained from RIKEN BioResource Center and cultivated in a Dulbecco's Modified Essential Medium (DMEM) at 37 °C in a humidified environment with 5.0% CO_2. The concentration of F9 cells in DMEM was adjusted to be 25×10^4 cell/mL. The F9 cells suspension in DMEM (1.5 mL) were seeded into each wells and then cultured in 5% CO_2 at 37 °C for 4h. After 4 h incubation, the medium was removed to separate non-adherent cells and then 1.5 mL of 10% fetal bovine serum (FBS) containing DMEM was added. The morphology change of cell under incubation was observed with a phase-contrast microscope (Olympus IX71).

Cell viability studies against F9 cell

Cell viability of F9 cells was investigated using a standard cell-counting kit 8 (CCK-8) assay. F9 cells were seeded into each 96-well of the plate using 100 μL of cell suspension (25×10^4 cell/mL) in DMEM and cultured for 24 h at 37°C in a humidified environment with 5.0% CO_2. Culture medium was removed with suction and the solution 100 μL of 10% FBS/DMEM was added. Then aqueous solution with polymer (1 mg/mL) was mixed in the volume ratio 9 to 1 or 10% FBS/DMEM as the control sample. After incubation for 24 h, CCK-8 assay solution (10μL) was added into each well of the plate. The cell was incubated further for 4 h to measure absorbance at 450 nm using a microplate reader. The cell viability was calculated relative to the control sample.

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Notes and references

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† Footnotes should appear here. These might include comments relevant to but not central to the matter under discussion, limited experimental and spectral data, and crystallographic data.

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