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Phosgene-free synthesis of non-ionic hydrophilic polyserine†

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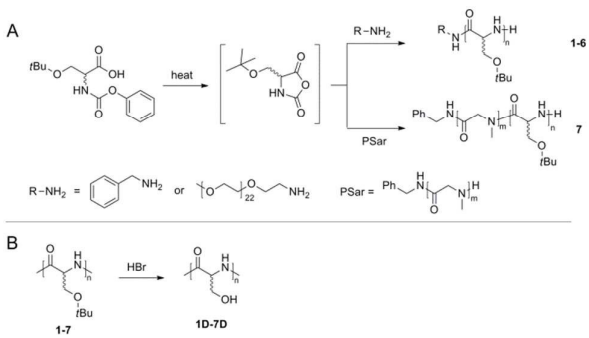
Non-ionic water-soluble poly-DL-serine (PSer) was synthesized by activated urethane-type derivative of serine. SEC, NMR and MALDI-ToF analyses of the polypeptides revealed a controllable polymerization initiated by primary or secondary amine. PSer exhibited excellent solubility in water when the ratios of L-serine/D-serine units ranged from 5/5 to 8/2.

Polypeptide-based materials have been drawing increasing interest in drug and gene delivery,[1-4] tissue engineering,[5] biomineralization,[6] and nanoscaled self-assembly systems.[1, 7-9] The excellent characteristics of polypeptides from their protein-like structures include biocompatibility with blood and tissue, biodegradability properties and biorecognition-like properties.[10-12] Among these materials, polypeptides possessing water-soluble property, such as poly-L-lysine and poly-L-glutamic acid (PLG), have been illustrated by numerous studies to be indispensible and potential as hydrophilic parts when applied *in vivo*.[13, 14] However, due to the amino or carboxyl side groups, they are not water-soluble when the side groups are not ionized, *i.e.* their water-solubility is pH-dependent. To avoid it, some modifications have been reported to introduce oligo(ethylene glycol) side chains onto PLL and PLG to improve their solubility.[15-18] Beyond polypeptides and their derivatives, polysarcosine (PSar) is a pH-independent water-soluble polypeptoid, whose structure is similar to polypeptides, bearing repeating units of sarcosine, an intermediate compound in the metabolism of α-amino acids.[19-25] To the best of our knowledge, there are very few reports about the synthesis of non-ionic hydrophilic polypeptide which comes from natural α-amino acids. Bearing hydroxyl group in side chains, poly-L-serine is the most likely candidate. However, previous study has revealed that it is sparingly soluble when its degree of polymerization (DP) is larger than 7.5 due to its β-sheet folding.[26]

The most popular chemical method to synthesize polypeptides is ring-opening polymerization (ROP) of α-amino acid *N*-carboxyanhydrides (NCAs)[27, 28] for their high reactivity, high productivity, and precision control on molecular weights (MWs). However, the synthesis of NCAs requires highly toxic phosgene or its derivatives. The sensitive nature of NCAs to moisture and heat limits the production and utilization of NCAs on a large scale. In 2008, Endo and coworkers reported a new method to synthesize polypeptides from activated urethane derivatives of α-amino acids (AA-UDs) which produce the corresponding NCAs *in situ* under heating during polymerization.[29-32] Four years later they proposed a refined process to synthesize AA-UDs to avoid toxic reagents.[33]

Herein we present a phosgene-free strategy to synthesize poly-DL-serine with well-defined structure by urethane derivatives of *O-tert*-butyl-DL-serine (Ser(*t*Bu)-UD) to achieve non-ionic hydrophilic polypeptide. Introducing mesomeric repeating units prevents chain folding, which improves the water-solubility of poly-L-serine.

L-Ser(*t*Bu)-UD and D-Ser(*t*Bu)-UD were prepared according to the literature with yields of 61% and 59%, respectively (Scheme S1 and Experimental section in Supporting Information)[29] with the structural confirmation by ^{1}H NMR and ^{13}C NMR (Figure S1 in Supporting Information). All the procedures were carried out in open air, which was convenient



Scheme 1. (A) Polymerization of Ser(tBu)-UD initiated by different amines. (B) Deprotection of PSer(tBu)

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Table 1 Polymerization of Ser(*t*Bu)-UD initiated by different initiators

Sample[a]	Initiator	[M]/[I]	DP[b]	Yield (%)	Mn_{SEC}[c] (kDa)	Đ[c]
1	benzyl amine	25	27	78	9.7	1.09
2	benzyl amine	50	49	72	15.6	1.17
3	benzyl amine	100	99	79	20.2	1.27
4	$mPEG_{22}$-NH_2	25	34	68	16.9	1.14
5	$mPEG_{22}$-NH_2	50	54	76	21.2	1.18
6	$mPEG_{22}$-NH_2	100	95	70	22.2	1.29
7	$PSar_{17}$	75	70	93	17.6	1.29

[a] Polymerization conditions: $[M]_0$ = 1.0 mol/L, 48 h. [b] Calculated by ^{1}H NMR analyses. [c] Determined by SEC.

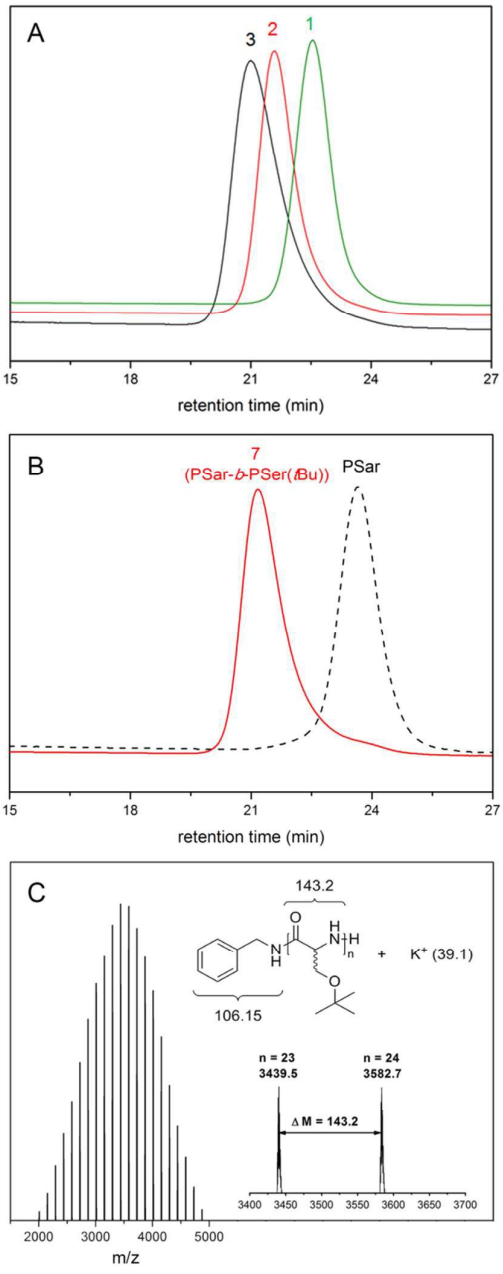

Figure 1. (A) SEC traces of samples **1**, **2** and **3** in Table 1. (B) SEC traces of PSar and **7**. (C) MALDI-ToF MS of sample **1**.

and easy to handle. The monomers were white powder capable to be stored under ambient environment for months without the protection of inert atmosphere. The Ser(*t*Bu)-UD was captured as a 50/50 mixture of L-Ser(*t*Bu)-UD and D-Ser(*t*Bu)-UD and was confirmed to be racemic by HPLC with chiral column (Figure S2).

The polymerization of Ser(*t*Bu)-UD was successfully carried out at 80 °C in *N*,*N*-dimethylacetamide (DMAc) by means of Schlenk techniques. Amines were active initiators including benzyl amine, PEG-NH_2, and PSar with secondary amine chain end. Both primary and secondary amines were efficient to initiate polymerizations and the results are summarized in Table 1. DPs of PSer(*t*Bu) can be controlled by various feed ratios of Ser(*t*Bu)-UD and amine.

PSer(*t*Bu) samples were characterized by size-exclusion chromatography (SEC) and matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-ToF MS) as shown in Figure 1B and 1C. The SEC traces of all the samples are unimodal with narrow distributions between 1.09 and 1.29, indicating the polymerizations of Ser(*t*Bu)-UD are in good control. When amine-ended PSar initiates polymerization, the SEC curve of the resulted diblock polypept(o)ide[34, 35] PSar-*b*-PSer(*t*Bu) obviously moves to high MW region compared to that of PSar (Figure 2A), keeping narrow distribution. It proves that both primary and secondary amines are efficient initiators for polymerization of Ser(*t*Bu)-UD.

MALDI-ToF MS measurement confirms the structure of PSer(*t*Bu). The spectrum of sample **1** shown in Figure 1C exhibits one prominent population with the interval mass of 143, the MW of one Ser(*t*Bu) repeating unit. The chain end is a residue of benzylamine initiator suggesting a merit of introducing functional groups. The other end of PSer chain is a primary amine which leaves a possible site for post-polymerization modification or chain extension. The structure is further confirmed by ^{1}H NMR analysis (Figure 2A(I)). The signals at 0.8-1.4 (H^d), 3.3-3.8 (H^c), 4.4-4.6 (H^b) and 7.2-8.2 ppm (H^{a+e}) with correct intensities are assigned to protons of Ser(*t*Bu) repeating units. All above suggest controlled polymerizations of Ser(*t*Bu)-UD via a normal amine mechanism[36, 37] experiencing NCA intermediate.[32]

The *tert*-butyl protecting groups of PSer(*t*Bu) are removed after treatment by HBr in $CHCl_3$ (c.a. 3%) (Scheme 1B). The corresponding deprotected samples of **1**-**7** are named as **1D**-**7D**. Figure 2A compares the ^{1}H NMR spectra of **1** and **1D**. The disappearance of the *tert*-butyl proton signal at 0.8-1.4 confirms the complete deprotection. Meanwhile, the structure of PSer can be further confirmed by MALDI-ToF MS analysis. The spectrum of **1D** (Figure 2B) shows two prominent populations, either of which is regularly located with an interval mass of 87, the MW of a repeating unit of serine. Two populations are contributed to PSer chains end-capped by amine and acetamide groups. The acetylization of the amine chain end occurs during the deprotection in acetic acid.

The obtained PSer is highly water-soluble in all pH ranges between 0 and 14 with the solubility of 600 mgPSer/mLH_2O, *i.e.* 37.5 wt%.

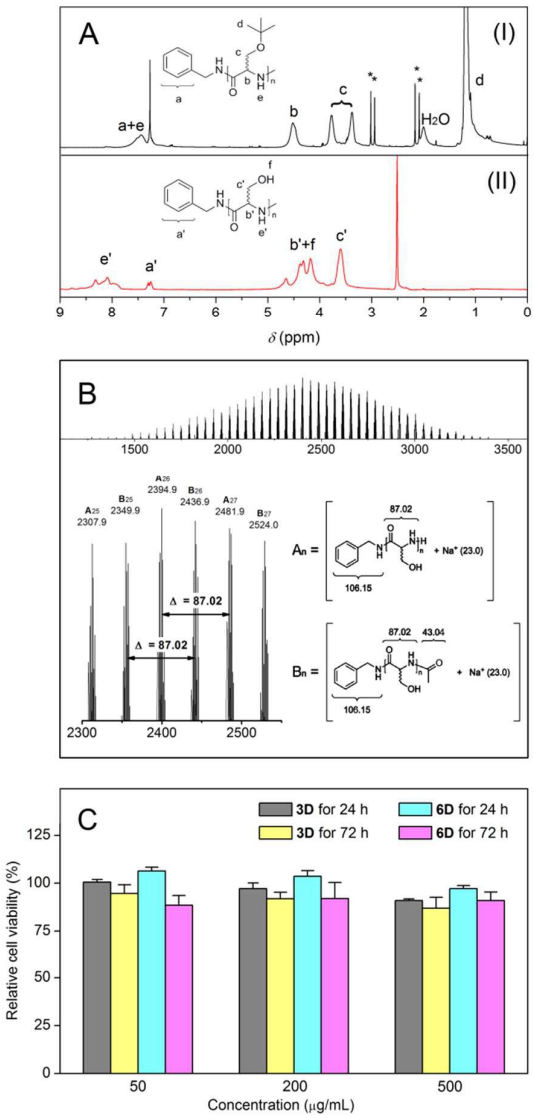

Figure 2. (A) ^{1}H NMR spectra of sample **1** (I) and **1D** (II). (B) MOLDI-ToF MS of sample **1D**. (C) MTT assays of sample **3D** and **6D**.

More significantly, when the feed ratio of D-Ser(*t*Bu)-UD/L-Ser(*t*Bu)-UD decreases to 2/8, the obtained PSer is still water soluble. In other words, PSer with L-repeating units between 50-80% has good solubility in water. On the contrary, we find that homopolymer of poly-L-serine with DP over 7.5 is water insoluble. It is worthy of mentioning that PEG-*b*-PSer (**4D**-**6D**) and PSar-*b*-PSer (**7D**) are two new types of non-ionic double-hydrophilic diblock copolymers.[19] Their properties are under investigation. Especially the self-assembly behaviors of these polymers are worth to study, for the reason that they are water permeable systems that might be suitable for use as cellular mimics.[38]

MTT assay was carried out to evaluate the cytotoxicity of PSer. The results of PSer (**3D**) and PEG-*b*-PSer (**6D**) reveal their very low cytotoxicity at a concentration up to 500 μg/mL (Figure 2C).

PSer containing 50-80% L-serine is a novel non-ionic and hydrophilic polypeptide with repeating units of natural α-amino acid. PSer with narrow MW distributions and predetermined DPs can be synthesized via a phosgene-free and easily handling polymerization of Ser(*t*Bu)-UD followed by deprotection. Two double-hydrophilic diblock copolymers, *i.e.* PEG-*b*-PSer and PSar-*b*-PSer are also prepared. Highly hydrophilic and low toxic properties make PSer a candidate material for biomedical applications.

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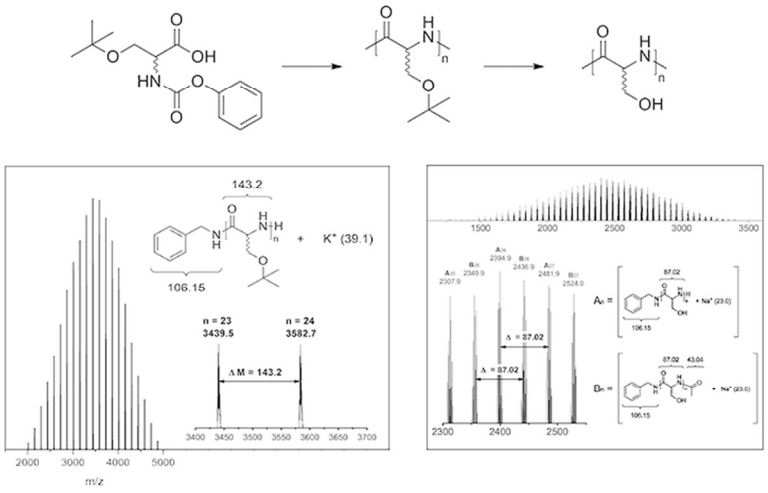

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