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Ring-Opening Polymerization of γ-(4-Vinylbenzyl)-L-glutamate *N*-Carboxyanhydride for the Synthesis of Functional Polypeptides

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Supporting Information

Polypeptides bearing functional side chains have been broadly used in various biological and biomedical applications.[1] Preparation of such materials in a highly controlled manner has been a great challenge to polymer chemists. Conventional approaches typically involve the ring-opening polymerizations (ROP) of side chain protected, multifunctional α-amino acids *N*-carboxyanhydrides (NCAs),[2] followed by removal of the side chain protecting groups and conjugation to desired functional groups/moieties[3] or direct aminolysis/transesterification.[4] These approaches require the use of harsh deprotection chemistry (e.g., 33% HBr) and may have low grafting efficiency,[3] especially for polypeptides with high molecular weights (MWs). To avoid the deprotection step, there has been growing interest in developing new NCA monomers containing conjugation-amenable functional groups that can stay intact during polymerization and be used for the subsequent grafting of desired moieties after polymerization.[5] In this study we report the controlled polymerization of γ-(4-vinylbenzyl)-L-glutamate *N*-carboxyanhydride (VB-Glu-NCA) and utilization of the resulting poly(γ-(4-vinylbenzyl)-L-glutamate) (PVBLG) to easily access a variety of functional polypeptides (PVBLG-a−g) through controlled vinyl chemistries (Scheme 1).

VB-Glu-NCA was readily prepared and purified in multigram scale in crystalline form (Figure S1).[6] The purified VB-Glu-NCA is very stable in moisture-free conditions and can be stored in the freezer of a glovebox at −30 °C for more than 6 months without noticeable change of properties. The PVBLGs, the resulting polypeptides, are very soluble in common organic solvents (e.g., THF, $CHCl_3$, and DMF), making study of the polymerization and characterization of their products straightforward by standard techniques (e.g., gel permeation chromatography (GPC)).

We previously reported that hexamethyldisilazane (HMDS) is an excellent initiator for the ROP of γ-benzyl-L-glutamate NCA (Glu-NCA).[2i,j] Along this direction, we first attempted to use HMDS to polymerize VB-Glu-NCA. However, at a monomer/initiator (M/I) ratio of 50, the resulting PVBLG had a M_n value of 1.87×10^4 g/mol, which is significantly higher than the expected M_n (1.22×10^4 g/mol, entry 1, Table 1). The molecular weight distribution (MWD = M_w/M_n) was also fairly broad (2.03). The GPC analysis of the obtained PVBLG gave a bimodal curve with the higher MW peak showing a strong light-scattering signal (red, Figure 1a) but a very weak refractive index signal (data not shown). These GPC results suggest the existence of polymers with very high MWs, presumably due to the interchain cross-linking of the vinyl groups of the PVBLG. To eliminate this side reaction, nitrobenzene (NB), a radical retarder, was added to the HMDS-mediated VB-Glu-NCA polymerization solution. As expected, the cross-linking side reaction was completely inhibited, evidenced by the monomodal GPC light-scattering curve of the resulting PVBLG (blue, Figure 1a), which had a MW much closer to the expected value and a narrower MWD (entry 2, Table 1). The amount of NB had very limited effect on polymerization rates and the MWs of the resulting PVBLGs, suggesting that NB functions as a radical inhibitor and does not participate in chain propagation (Figure S2).

Although HMDS-mediated VB-Glu-NCA polymerization in the presence of NB gave controlled polymerization, VB-Glu-NCA has fairly low reactivity as compared to the parent Glu-NCA (Figure S4), making HMDS/NB-mediated polymerization undesirable for the synthesis of high-MW PVBLG (Figure S5). Polymerization at high M/I ratio generally gave low monomer conversion even with extended reaction time (entry 3, Table 1). Our previous study indicated that HMDS-mediated Glu-NCA polymerization proceeds via a trimethylsilyl carbamate (TMS-CBM) terminal group. Polypeptide chains were propagated through the transfer of the TMS group from the terminal TMS-CBM to the incoming monomer to form a new TMS-CBM terminal propagating group.[2i,j] In the case of VB-Glu-NCA, the TMS-transfer process could have been retarded due to the low reactivity of VB-Glu-NCA.

We next screened various substrates that have been used as nucleophilic organic catalysts in various acyl-transfer or acyl-activation reactions.[7] We found that 1,5,7-triazabicyclo-[4.4.0]dec-5-ene (TBD) assisted faster polymerizations with excellent control over MWs when it was used in conjunction with HMDS for VB-Glu NCA polymerization. At a VB-Glu-NCA/HMDS molar ratio of 200:1 along with a catalytic amount of TBD (0.1 equiv), the polymerization was noticeably faster and completed within 20 h with quantitative conversion of VB-Glu-NCA (entry 4 vs 3, Table 1). The GPC analysis of the polymerization solution *in situ* revealed that the resulting PVBLG had a narrow MWD ($M_w/M_n = 1.08$) and an M_n value of 4.7×10^4 g/mol,

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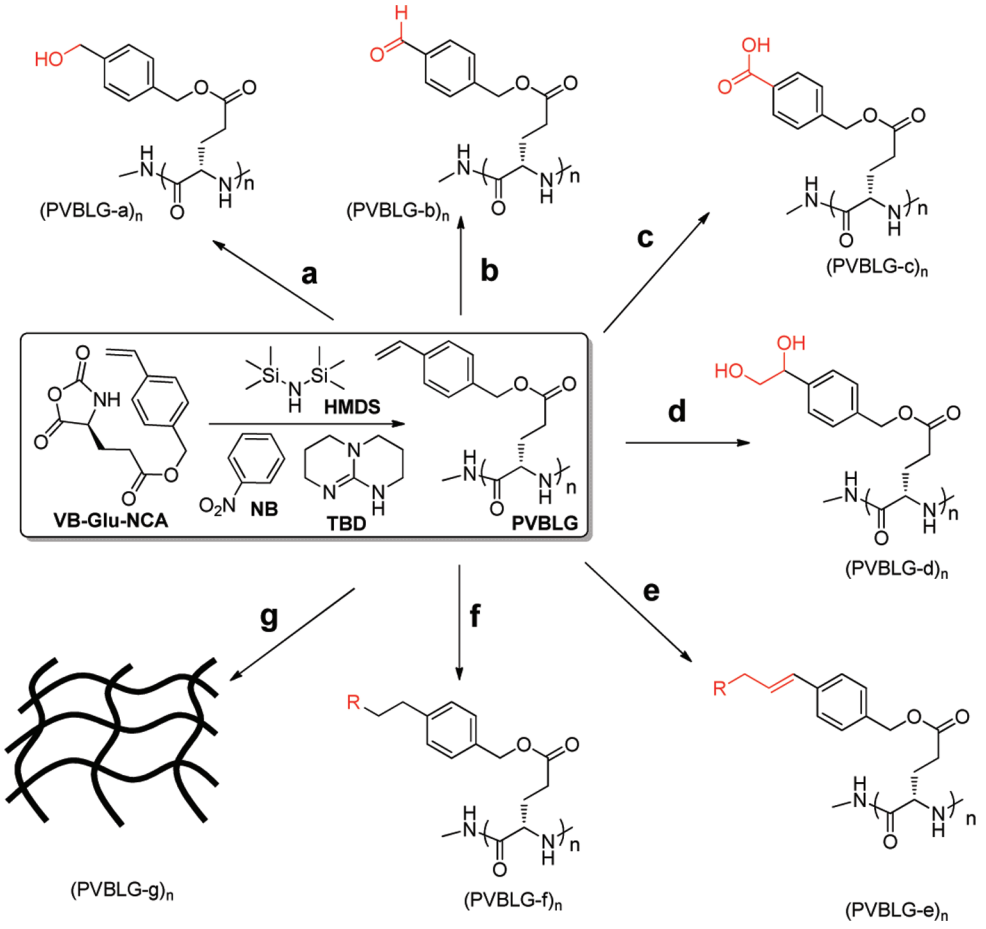
Scheme 1. Synthesis and Functionalization of PVBLG[a]



[a] Reagents and conditions: (a) i. O_3, −78 °C, 1−5 min; ii. $NaBH_4$, rt, 16 h; (b) i. O_3, −78 °C, 1−5 min; ii. PPh_3, rt, 2−3 h; (c) OsO4, oxone, rt, 48 h; (d) OsO_4, NMO, rt, 20 h; (e) second-generation Grubbs catalysts, *cis*-RCH═CHR, rt, 24 h; (f) i. 9-BBN, rt, 16 h; ii. Ar−Br, $Pd(PPh_3)_4$, $NaHCO_3$(aq), N_2, 70 °C, 20 h; (g) UV.

Table 1. HMDS-Mediated VB-Glu-NCA Polymerization

entry	monomer	M/HMDS/catal	catal	NB[a] (μL)	time (h)	conv (%)	M_n(M_n*) ($\times10^{-4}$)[b]	MWD
1	VB-Glu-NCA	50/1/0	NA	0	30	>98	1.87 (1.22)	2.03
2	VB-Glu-NCA	50/1/0	NA	30	30	>98	1.43 (1.22)	1.10
3	VB-Glu-NCA	200/1/0	NA	30	40	67	3.30 (4.9)	1.08
4	VB-Glu-NCA	200/1/0.1	TBD[c]	30	24	>98	4.68 (4.9)	1.08
5	Lys-NCA/VB-Glu-NCA	(20/1 + 50/1)/0.02[d]	TBD	30	8 + 12[e]	>98	0.61/2.10 (0.52/1.74)[f]	1.05/1.18[g]

[a] NB = nitrobenzene. [b] Obtained MW (expected MW*). [c] TBD = 1,5,7-triazabicyclo[4.4.0]dec-5-ene. [d] Feed ratio of (Lys-NCA/HMDS + VB-Glu-NCA/HMDS)/catal. [e] Lys-NCA polymerization time + VB-Glu-NCA polymerization time. [f] Obtained MW of PZLL/PZLL-*b*-PVBLG (expected MW of PZLL/PZLL-*b*-PVBLG). [g] MWD of PZLL/PZLL-*b*-PVBLG.

which was very close to the expected M_n of 4.9 × 10^4 g/mol (entry 4, Table 1). As shown in Figure 1b, the obtained M_n's, which were the average of the M_n's of the PVBLGs prepared in three separate polymerization experiments at the corresponding M/I ratios, agreed almost perfectly with the expected M_n's. Furthermore, the very small error bars of M_n's indicate that the polymerizations were highly reproducible. The resulting PVBLGs all had very narrow MWDs (1.08−1.27). The MWs of PVBLG also showed linear correlation with the conversions of VB-Glu-NCA and agreed well with the expected MWs (Figure 1c), demonstrating that PVBLG chains were propagated through living chain ends. Block copolypeptides, such as poly(ε-cbz-L-lysine)-*block*-PVBLG (PZLL-*b*-PVBLG), can be readily prepared with predictable MWs and narrow MWDs (entry 5, Table 1). These experiments demonstrated that the HMDS/TBD mediated well-controlled, living polymerizations of VB-Glu-NCA. It is unclear if HMDS/TBD can be applied to other NCAs for accelerated polymerization and the mechanism of action of TBD. These studies are underway and will be reported later.

With the successful establishment of ROP of VB-Glu-NCA for the controlled synthesis of PVBLG, we next performed the

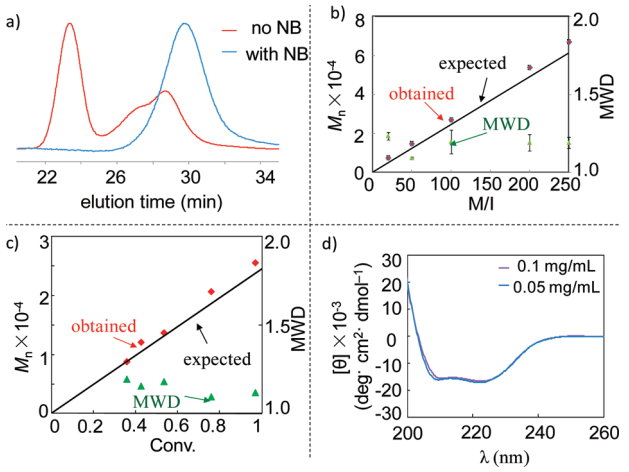


Figure 1. (a) GPC (multiangle laser light scattering (MALLS) detector) curves overlay of HMDS-mediated VB-Glu-NCA polymerizations at M/I ratio of 50/1 in the presence (blue) and absence (red) of NB. (b) Plot of MW and MWD versus M/I in the HMDS/TBD initiated VB-Glu-NCA polymerization; the experiment was repeated three times at each M/I ratio, and the error bars were presented as the standard deviation. (c) Plot of MW and MWD versus conversion in the HMDS/TBD initiated VB-Glu-NCA polymerization. (d) CD curves of $(PVBLG\text{-}d)_{70}$ at the concentrations of 0.05 mg/mL (blue) and 0.1 mg/mL in water (purple).

postpolymerization reactions as illustrated in Scheme 1, aiming to explore the scope and versatility of PVBLG for the synthesis of functional polypeptides. The efficiencies of side chain functionalization illustrated in Scheme 1 were found to be at least 90% as confirmed by the NMR. The separated yields of all the reactions were between 60% and 90% (see Supporting Information). To generate PVBLG-a−g, the N-terminus of $PVBLG_{70}$, a 70-mer of PVBLG, was first protected by a CBZ group immediately after polymerization to prevent undesired side reactions. The $PVBLG_{70}$ was then treated with ozone, and the vinyl group was converted to alcohol in $(PVBLG\text{-}a)_{70}$ in 72% yield (route a, Scheme 1) and aldehyde in $(PVBLG\text{-}b)_{70}$ in 78% yield (route b), when sodium borohydride and triphenylphosphine were used as the reductive reagent, respectively. Notably, the aldehyde functionalized PVBLG-b was very reactive and could be used for further grafting of various functional moieties with amines, hydrazides, and oxyamines through reductive amination.[8] The vinyl group of $PVBLG_{70}$ was also converted to carboxylic acid $(PVBLG\text{-}c)_{70}$ in 81% yield under mild conditions by osmium tetroxide-promoted catalytic, oxidative cleavage of the olefin (route c).[9] 1,2-Bishydroxylation of the vinyl of $PVBLG_{70}$ was performed by following osmium tetroxide-catalyzed oxidation in the presence of *N*-methylmorpholine *N*-oxide (route d), resulting in $(PVBLG\text{-}d)_{70}$ in 79% yield. Remarkably, PVBLG-d is very soluble in water, has very low toxicity (IC_{50} > 1 mM in HeLa cells, see Figure S6), and adopts a helical conformation in aqueous solution (Figure 1d). The molar ellipticity at both 208 and 222 nm remained unchanged when the CD analyses were carried out at two different concentrations of PVBLG-d, indicating that PVBLG-d stays in its monomeric form in water. Thus, we can readily convert PVBLG, a water-insoluble polypeptide, to a water-soluble PVBLG-d via a one-step postmodification reaction, and PVBLG-d can potentially be used as water-soluble, noncharged, rodlike structures in self-assembly or biological applications. Further studies of PVBLG-d are underway in our laboratory. We also performed the metathesis reaction of $PVBLG_{70}$ (route e, Scheme 1). By mixing the polymer solution in dichloromethane with excessive *cis*-1,4-dichlorobutene in the presence of the second-generation Grubbs catalyst, allyl chloride functionalized polypeptide $(PVBLG\text{-}e)_{70}$ was exclusively generated in 78% yield. By treating $PVBLG_{70}$ with 9-borabicyclo[3.3.1]nonane (9-BBN) followed by reaction with 4′-bromoacetophenone and tetrakis(triphenylphosphine)palladium, $(PVBLG\text{-}f)_{70}$ was derived in 60% separated yield via the Suzuki reaction. UV-induced cross-linking reaction of the vinyl group of $PVBLG_{70}$ resulted in formation of an organogel ($(PVBLG\text{-}g)_{70}$).[10] Thus, the functionalization of PVBLG's side chain vinyl group can be a very useful approach to generate a large number of polypeptide materials with a variety of side chain functionalities and moieties through versatile vinyl chemistry.

In conclusion, we report the preparation of polypeptides with poly(L-glutamate) backbone and a variety of different side chains via controlled polymerization of VB-Glu-NCA followed by a variety of highly efficient postfunctionalization reactions. VB-Glu-NCA is readily available in large scale (tens of grams) with satisfactory purity after crystallization. The initiator (HMDS) and the cocatalyst (TBD) of the polymerization are both commercially available and inexpensive and can be used as received to prepare PVBLGs with precisely controlled MWs and narrow MWDs. The side chain postfunctionalization reactions are also straightforward with high efficiency. We believe this streamlined strategy will find widespread utility for the synthesis of a large number of functional polypeptides with tailored side chain structures and desired functions.

■ ASSOCIATED CONTENT

Supporting Information. Supporting figures, tables, and experimental methods. This material is available free of charge via the Internet at http://pubs.acs.org.

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