Controlled synthesis of phosphorylcholine derivatives of poly(serine) and poly(homoserine)

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Supporting Information Placeholder

ABSTRACT: We report methods for the synthesis of polypeptides that are fully functionalized with desirable phosphorylcholine, PC, groups. Due to inherent challenges in the direct incorporation of the PC group into α-amino acid N-carboxyanhydride (NCA) monomers, we developed a synthetic approach that combined functional NCA polymerization with efficient post-polymerization modification. While poly(L-phosphorylcholine serine) was found to be unstable upon synthesis, we successfully prepared poly(L-phosphorylcholine homoserine) with controlled chain lengths, and found these to be water soluble with disordered chain conformations.

Phospholipids are ubiquitous in biology as major components of cell membranes, and many contain phosphatidylcholine. Due to the biomimetic and biocompatible properties of the phosphorylcholine, PC, functional group, it has been incorporated into a wide variety of polymeric materials for use in applications including drug delivery,1-3 imaging,4-6 and for preparing surfaces that resist protein adsorption and cell adhesion.7-9 Although they possess promising properties, most PC polymers are non-degradable, which limits the scope of their use especially for applications in vivo.10 Recently, there has been much interest in the preparation of functional polypeptide materials that may be used as degradable and more biologically relevant alternatives to non-degradable functionalized polymers.11 Hence, we sought methods to incorporate PC functionality into polypeptides to prepare zwitterionic, potentially degradable polymers bearing biocompatible groups that may resist protein and cell adhesion. We describe our results on the synthesis of PC derivatives of poly(serine), PC-S, and poly(homoserine), PC-SH, via a combination of functional NCA monomer polymerization and post-polymerization amination (Figure 1).

Most studies on PC polymers have focused on poly(2-methacryloyloxyethyl phosphorylcholine), PMPC, which has excellent non-fouling properties, yet also a non-degradable polymer backbone.12,13 Degradable PC polysters have been prepared, but required the post-polymerization addition of the PC groups using long tethers.14 Polypeptides with PC functionality have only been prepared as derivatives of polylysine and polyglutamate), where PC groups were covalently attached to side-chain functional groups post-polymerization.15 In these materials, long spacers were also needed between the PC groups and the polypeptide backbone, and the highest degree of functionalization attained was 80%. Otherwise, phosphate16 and phosphonate17,18 containing polypeptides, mainly based on poly(phosphoserine) or its analogs, are the only other phosphorous containing polypeptides that have been prepared. These polypeptides were prepared from the corresponding functionalized α-amino acid-N-carboxyanhydride (NCA) monomers and could be obtained with controlled lengths and low polydispersities in some cases.

Figure 1. Structures of phosphorylcholine containing lipids and polypeptides. PC = phosphorylcholine; R = lipid tails; S = serine; SH = homoserine.

To enable an efficient synthesis of fully functionalized PC polypeptides, especially those with structures similar to the natural phosphoserine, we undertook an approach that combined the preparation and polymerization of a functionalized NCA monomer with an efficient post-polymerization modification.19 The main challenge associated with incorporating PC groups into polypeptides is that this functionality is not easily protected, and there is no precedent for polymerization of NCA monomers containing charged quaternary ammonium groups. Hence, our strategy necessitated preparation of a suitably protected phosphoserine NCA, which after polymeriza-
tion could then be directly converted to a PC serine polypeptide by reaction of bromoethyl groups with trimethylamine (eq. 1). Success of the approach required identification of an appropriate phosphate protecting group that could be removed without loss of the bromoethyl functionality.

Our initial studies revealed that alkyl protecting groups for phosphoserine NCAs, such as methyl, ethyl, and isopropyl, gave polymers with poor solubility in common solvents. Attempts to use other groups such as 2-chlorophenyl\(^{20}\) and 2-trimethylsilylethyl\(^{25}\) gave NCA monomers that were unstable and challenging to purify. Following these initial setbacks, we developed a more modular approach toward quickly testing suitable protecting groups. Building on methods developed for DNA and nucleotide synthesis\(^{22,23}\) we prepared chlorophosphoramidite reagent 1, which could be stored for long periods at -20 °C and also functionalized with a variety of different protecting groups (eq. 2). We found that the benzyl protecting group was well suited for subsequent steps, and so phosphoramidite reagent 2 was synthesized for use in NCA synthesis (eq. 2).

NCA monomer synthesis began with commercially available Boc-L-serine 3, where the α-carboxylate was protected in situ using tert-butyldimethylsilyl chloride, TBSCI, followed by phosphorylation with reagent 2. The resulting phosphate 3a was then oxidized to the phosphate with tert-butyl hydroperoxide, tBuOOH, and the TBS protecting group was removed in aqueous workup, yielding NCA precursor 4 in nearly quantitative yield over this 3 step, one-pot reaction. NCA synthesis was achieved in reasonable yield using phosgene and the product was purified using anhydrous silica chromatography in a glove box (Scheme 1).\(^{24}\) Polymerizations of NCA 5 using Co(PMe\(_3\))\(_4\) in THF proceeded readily at ambient temperature to give corresponding soluble homopolypeptides 6 with complete monomer conversion and no reactions at the side-chain phosphate or alkyl bromide groups (Scheme 1).\(^{25}\) It is worth noting that the polymerization of 5 may not be feasible using amine-initiated NCA polymerization methods due to possible S\(_2\)2 side reactions at the primary alkyl bromide groups.

Scheme 1. Attempted synthesis of poly(L-phosphorylcholine serine), PC-S\(_n\). (a) i) TBSCI, THF, N-methylmorpholine; ii) 2, tetrazole and MeCN; iii) tBuOOH (95 % yield). (b) COCl\(_2\), N-methylmorpholine, THF, 40 °C (63 % yield). (c) Co(PMe\(_3\))\(_4\), THF, see SI for yields. (d) multiple attempts, see text.

We next attempted the transformation of 6 to the desired product poly(L-phosphorylcholine serine), PC-S\(_n\). We reasoned that due to the known short half-lives of phosphotriesters in aqueous solutions,\(^{26}\) we would have been able to remove the labile benzyl group and aminate the 2-bromoethyl group in one step. Thus 6 was subjected to 20% aqueous trimethylamine, which caused the water-insoluble polymer 6 to form a clear solution within 10 minutes. However, no polymer was isolated following dialysis. A stepwise debenzylization of 6 followed by attempted amination with trimethylamine yielded similar results. It was apparent that chain cleavage reactions were occurring under these basic conditions. A possible mechanism for this is via β-elimination of the phosphate group, which is known to occur in phosphoserine derivatives.\(^{27,28}\) We have also previously observed similar behavior in phosphonate-containing serine analogs.\(^{10}\) In polymer 6, β-elimination of phosphates would yield dehydroalanine residues. These groups would likely rehydrate to give DL-serine repeats, which are known to decompose under both basic and acidic conditions (see eq. S1).\(^{30}\) To eliminate this problem, we recognized that β-elimination and subsequent polymer degradation can be avoided by the homologation of serine to homoserine. β-elimination on serine derivatives yields dehydroalanine, which contains a stabilized α,β-unsaturated olefin, while a homoserine derivative would not yield such a stabilized product.

Consequently, a homoserine polypeptide analog of 6 was synthesized using a similar procedure, starting with the commercially available Boc-L-homoserine 7, which was elaborated to 8 using the aforementioned 3 step one-pot reaction. NCA 9 was synthesized using Ghosez’s reagent\(^{19}\) and was purified using anhydrous silica chromatography (Scheme 2).\(^{24}\) Polymerizations of NCA 9 using Co(PMe\(_3\))\(_4\) in THF proceeded readily at ambient temperature to give the corresponding soluble homopolypeptides 10 with complete monomer conversions with no detectable side reactions.\(^{25}\) Residual cobalt salts were readily removed by washing the polypeptides with water. In order to gauge if chain lengths could be controlled, 9 was polymerized to completion at different monomer to initiator, M:I, ratios, and active chains were then end capped with isocyanate terminated polyethylene glycol monomethyl ether (PEG-NCO, \(M_n = 1000\) Da).\(^{32}\) The polymers were purified and analyzed by \(^1\)H NMR to obtain the number average molecular weights, which increased linearly with M:I stoichiometry (Table 1). Chain length distributions of samples of 10 were obtained by GPC/RI analysis and polydispersity indices were

Scheme 2. Synthesis of poly(L-phosphorylcholine homoserine), PC-S\(_n\)\(_\beta\). (a) i) TBSCI, THF, N-methylmorpholine; ii) 2, PPTS and MeCN; iii) tBuOOH (85 % yield), (b) Ghosez Reagent, THF (80% yield). (c) Co(PMe\(_3\))\(_4\), THF, see Table 1 for yields. (d) 1:1 TFA/DCM (95% yield). (e) 20 % aq. NMe\(_3\) (95% yield). (f) 20% aq. NMe\(_3\) (90% yield).
found to range from 1.14 to 1.24, indicating that well-defined polypeptides were formed (Table 1, Figure 2).

Modification of polymer 10 to give poly(L-phosphorylcholine homoserine), PC-S, was carried out by the removal of the benzyl protecting group to yield 11, followed by amination using aqueous trimethylamine. The modification can also be accomplished directly by exposing 10 to aqueous trimethylamine. Both methods work well and provide fully functionalized PC-S in excellent yield and high purity (Scheme 2). Samples of PC-S were found to be stable in aqueous media for prolonged periods (ca. 1 month) with no signs of degradation.

Table 1. Synthesis of polymer 10 at different monomer to initiator (M:I) ratios.

<table>
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<tr>
<th>M:I</th>
<th>M&lt;sub&gt;n&lt;/sub&gt;</th>
<th>M&lt;sub&gt;n&lt;/sub&gt;/M&lt;sub&gt;w&lt;/sub&gt;</th>
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<td>49 100</td>
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*Equivalent of monomer 9 per Co(PMe<sub>3</sub>)<sub>3</sub>. Molecular weight determined for PEG end-capped samples using 'H NMR. Polydispersity index determined by GPC/RI analysis. DP = degree of polymerization. Total isolated yield of purified polypeptide.

Figure 2. Chain length control and polydispersity index of polymer 10. (A) Molecular weight (M<sub>n</sub>, ■) and polydispersity index (M<sub>n</sub>/M<sub>w</sub>, ▲) of 10 as a function of monomer to initiator ([M]/[I]) ratio using Co(PMe<sub>3</sub>)<sub>3</sub> in THF at ambient temperature. (B) GPC chromatogram (RI intensity in arbitrary units (a.u.) versus elution time) of polymer 10 samples from Table 1 (20:1, solid red and 40:1, dotted blue).

The chain conformation of PC-S was examined in aqueous media using circular dichroism, CD, spectroscopy. PC-S gave CD spectra consistent with a disordered chain conformation over a broad pH range of 3 to 11 (Figure 3). This behavior is expected due to the permanent charges of the PC groups, which disrupt the formation of ordered chain conformations. Similar to other PC functionalized polymers, PC-S was also found to display minimal toxicity to cells in vitro (see Figure S1).

Figure 3. Circular dichroism spectra of PC-S in different aqueous media (DP = 34, 0.1 mg/ml) at 20 °C.

The PC-S polymers reported here are notable in that they are the first polypeptides containing PC functionality at every residue. The strategy to prepare these polymers also allows for control over PC-S chain length and incorporation of these residues into block copolymers, such as with PEG as shown here. Upon initial examination, these polypeptides appear to have properties characteristic of other PC polymers, and may prove to be useful materials for biomedical research due to the potential biodegradability of their polypeptide backbone.

ASSOCIATED CONTENT
Supporting Information Experimental procedures and data for all new compounds, as well as polymerization data, M<sub>n</sub>, calculation data, and cell viability data. This material is available free of charge via the Internet at http://pubs.acs.org.

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