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Semisynthesis of Peptoid–Protein Hybrids by Chemical Ligation at Serine

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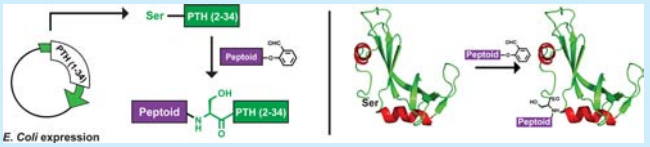
Supporting Information

ABSTRACT: Chemical ligation protocols were explored for generating semisynthetic peptoid–protein hybrid architectures containing a native serine residue at the ligation site. Peptoid oligomers bearing C-terminal salicylaldehyde esters were synthesized and ligated to the N-terminus of the RNase S protein or the therapeutic hormone PTH(1–34) polypeptide. This technique will expand the repertoire of strategies to enable design of hybrid macromolecules with novel structures and functions not accessible to fully biosynthesized proteins.



The incorporation of discrete abiotic chemical moieties within protein architectures provides a valuable tool for the study or modification of protein folding and function. A variety of chemical synthetic and bioconjugation protocols have been developed for the total chemical synthesis of proteins or to introduce desired chemical modifications within biosynthesized protein substrates.[1] Native chemical ligation (NCL) is the most widely utilized strategy for total chemical synthesis of large protein architectures. NCL features a chemoselective reaction between a peptide bearing a C-terminal thioester and a peptide containing an N-terminal cysteine residue, affording a native amide peptide bond at the ligation site.[2] While significant attention has been devoted to total chemical synthesis of proteins using NCL, alternative approaches have been developed to streamline the assembly of large protein constructs.[3] Biosynthesis of one protein fragment via recombinant expression, followed by ligation to a smaller synthetic fragment, can efficiently yield large proteins with minimal peptide synthesis, a technique known as expressed protein ligation (EPL).

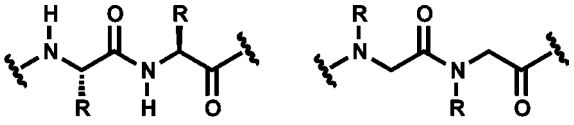


Figure 1. Comparison of peptide and peptoid chemical structures.

EPL can be conducted via two routes: (1) a protein can be expressed as a fusion to an intein and subsequently converted to a C-terminal thioester for ligation with a synthetic peptide containing an N-terminal cysteine residue or (2) a protein can be expressed and processed to liberate an N-terminal cysteine residue for ligation to a synthetic peptide with a C-terminal thioester handle.[4] Unfortunately, this method is limited to generating protein assemblies containing cysteine at the ligation site, although postligation desulfurization can also yield an alanine residue at the site of ligation. More recently, a method was introduced to generate methionine at the ligation site using postligation methylation.[5] Ideally, new EPL protocols would enable ligations at additional (non-cysteine) residues without the need for subsequent modifications. In addition, chemical ligation strategies can be explored to craft hybrid products that comprise both a polypeptide and novel abiotic oligomer constituents, thus providing access to new hybrid macromolecules with attributes not readily attained by biosynthesized proteins.[6]

N-Substituted glycine peptoids are a promising class of biomimetic oligomers composed of tertiary amide linkages that can be designed for enantioselective catalysis, molecular recognition, and intracellular delivery (Figure 1).[7−10] Relative to peptides, peptoids exhibit enhanced proteolyic stability and cell permeability.[11,12] Peptoids are notable for their monodispersity, side-chain diversity, and ability to fold into distinct secondary structures.[13] Extensive efforts are currently devoted to foldamer fragment condensation and to the development of well-ordered tertiary structures for peptoids and other foldamer species.[14] Recently, two peptoid macrocycles were conjoined through triazole linkages to generate a peptoid architecture that begins to resemble folded miniproteins, such as cyclotides.[14e] Although NCL has been utilized to generate protein architectures that incorporate a single peptoid monomer unit at the site of ligation,[15] other attempts to ligate peptoid

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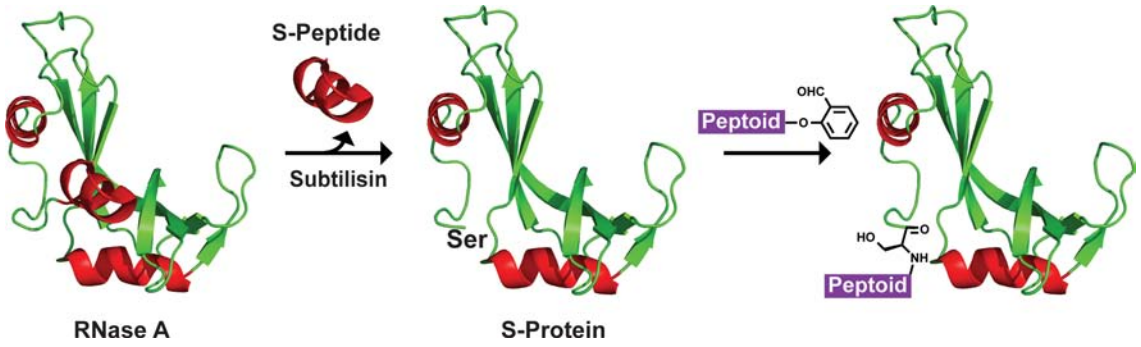
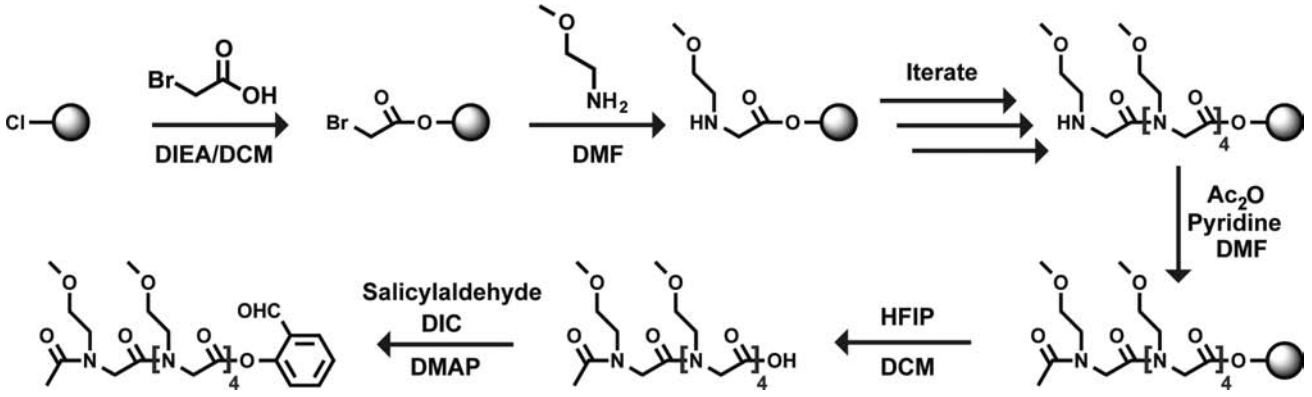


Figure 2. Schematic diagram of serine ligation between a peptoid bearing a C-terminal salicylaldehyde ester and the S-protein containing an N-terminal serine residue. Subtilisin cleavage was utilized to prepare the S-protein bearing the N-terminal serine residue. Ligation conditions: pyridine/acetic acid (1:1) at a final protein concentration of 10 mM (16 h, rt).

Scheme 1. Synthesis of C-Terminal Peptoid Salicylaldehyde Ester Used in RNase Ligation Study[a]



[a]The peptoid oligomer bearing a C-terminal salicylaldehyde ester was synthesized from 2-chlorotrityl resin.

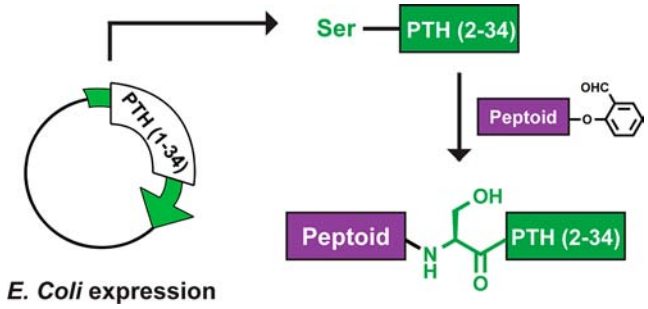


Figure 3. Schematic diagram of Ser/Thr ligation. Functionalization of PTH (1−34) was conducted by ligation with a peptoid containing a C-terminal salicylaldehyde ester.

oligomers to the N-terminus of the ribonuclease S protein using NCL were unsuccessful due to incompatibilities with standard peptoid synthesis protocols. Instead, the authors developed a catalytically active semisynthetic hybrid protein by introducing a non-native thiazolidine linkage in the backbone.[16]

Recently, a robust Ser/Thr ligation strategy, based on an original aldehyde capture technique, has been introduced by Li et al. for total protein chemical synthesis.[17,18] We have previously adopted this strategy to show that peptoid oligomers bearing C-terminal salicylaldehyde esters can be ligated to peptides containing N-terminal serine or threonine residues, establishing native amide linkages at the ligation site.[19] The chemoselective reaction proceeds rapidly, even with peptoid fragments that incorporate β-branched side chains at their C-terminus. The *N*,*O*-benzylidene acetal intermediates are quantitatively converted into their native amide products. Importantly, this ligation strategy is compatible with 19 of the 20 proteinogenic amino acids and is orthogonal to cysteine-based NCL.[18] We now seek to evaluate whether peptides or proteins generated via recombinant expression systems can be ligated to synthetic oligomer fragments utilizing Ser/Thr ligation.

First, we evaluated if peptoid oligomers can be ligated through a native amide linkage to biosynthesized proteins, a current limitation in peptoid synthesis.[16] As a model, we selected bovine pancreatic ribonuclease A (RNase A). The N-terminal portion (residues 1−20), dubbed the S-peptide, can be readily removed by enzymatic cleavage with subtilisin, providing the S-protein which contains an N-terminal serine residue (Figure 2).[20] Following high-performance liquid chromatography (HPLC) purification and characterization by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF), the corresponding S-protein was obtained (calc. *m*/*z*: 11,534.3; obs. *m*/*z*: 11,533.2, Supporting Information).

We then synthesized an *N*-acetylated peptoid pentamer incorporating methoxyethyl side chain groups (Scheme 1). The pentamer was converted to the salicylaldehyde ester and coupled to the S-protein, forming the corresponding *N*,*O*-benzylidene acetal intermediate. This reaction was monitored by analytical HPLC and MALDI-TOF, which established that coupling was complete after 16 h (see the Supporting Information). Conversion of the acetal group to the amide

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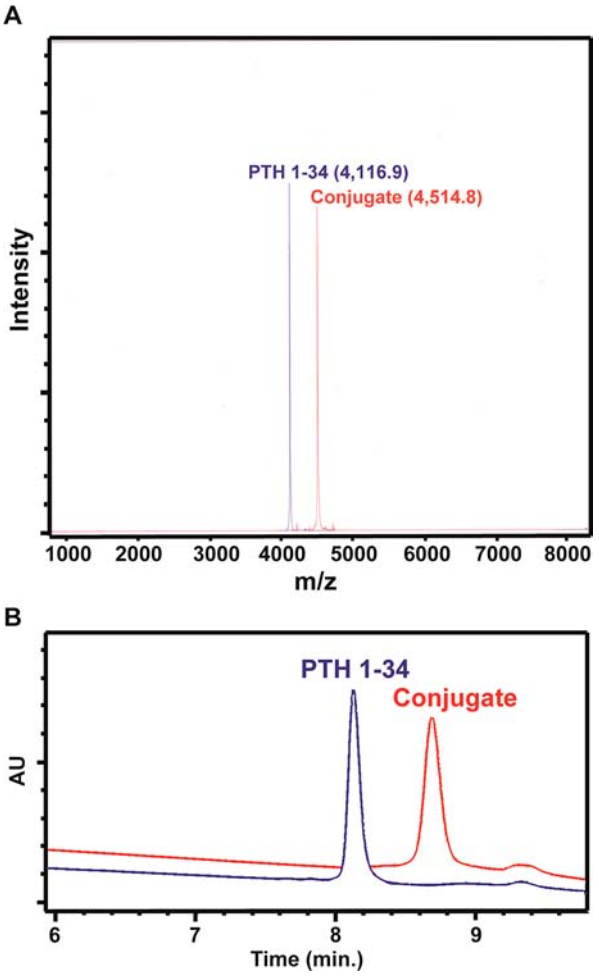

Figure 4. Characterization of ligation between PTH (1−34) and a peptoid containing a C-terminal salicylaldehyde ester. (A) MALDI-TOF analysis of purified PTH (1−34) (Forteo) before (blue) and after ligation (red). (B) Analytical HPLC analysis of purified PTH (1−34) (blue) and purified PTH conjugate (red). Upper trace of PTH conjugate offset in *y*-direction for clarity. AU indicates absorbance units.

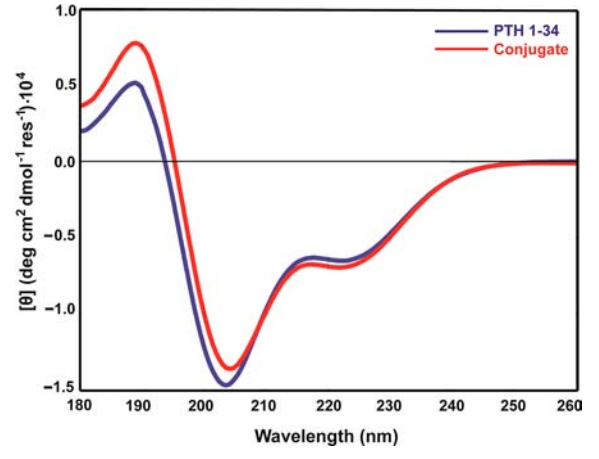

Figure 5. Circular dichroism spectra of PTH (1−34) (blue) and conjugate (red). Scans were performed at 25 °C.

via TFA treatment (see the Supporting Information) afforded the hybrid protein in 13.5% yield, confirming that peptoid oligomers can be ligated to biosynthesized proteins through native amide linkages. These results were reproduced in triplicate.

Next, we utilized Ser/Thr ligation to generate a variant of parathyroid hormone 1−34 or PTH (1−34) (Figure 3). PTH (1−34) agonizes the class B G-protein-coupled receptor PTHr1 and is currently marketed (Forteo) to enhance bone density and formation in patients diagnosed with osteoporosis, although it has a poor half-life in serum (∼5 min).[21] Following HPLC purification of recombinant PTH (1−34) and characterization by MALDI-TOF (calcd *m*/*z* 4,117.7; obsd *m*/*z* 4,116.9), an *N*-(methoxyethyl)glycine (Nme) trimer peptoid oligomer containing a C-terminal salicylaldehyde ester was ligated to recombinant PTH (1−34). We chose to ligate a peptoid oligomer comprising Nme side chains to PTH (1−34) because they share chemical similarities to polyethylene glycol (PEG) units, increase aqueous solubility, and have previously been shown to enhance the bioavailability of therapeutic peptides.[22] Chemoselective fragment condensation was monitored by analytical HPLC and MALDI-TOF, which determined that coupling was complete after 12 h (Figure 4). Conversion of the acetal group under acidic conditions provided the desired semisynthetic conjugate of PTH (1−34) (calcd *m*/*z*: 4,515.9; obsd *m*/*z*: 4,514.8, 27.7% yield), confirming that Ser/Thr ligation can be accomplished with recombinant polypeptide fragments.

To understand the impact on secondary structure of conjugating the peptoid oligomer to the N-terminus of PTH (1−34), we used far-UV circular dichroism (CD) spectroscopy. Wild-type PTH (1−34) and the conjugate were purified to >95% by HPLC and CD spectra were obtained at 25 °C in 10 mM PBS buffer (Figure 5). When compared to the CD spectrum of PTH (1−34), which exhibits typical helical character with minima near 208 and 222 nm, the conjugate displayed similar secondary structural features as PTH (1−34).[23] These results suggest that this modification at the N-terminus of PTH (1−34) has negligible impact on the structure of the protein. This result is desirable as the helical character at the C-terminus of PTH (1−34) may be critical for recognition by PTHr1.[24]

We report the semisynthesis of a hybrid peptoid−polypeptide and peptoid−protein conjugate containing native amide linkages. The products are obtained via a modification of a chemoselective Ser/Thr ligation protocol, conducted between peptoid oligomers bearing C-terminal salicylaldehyde esters and either RNase A or the recombinant polypeptide PTH (1−34). Importantly, no postligation modifications, such as desulfurization or methylation, are required. These results demonstrate that Ser/Thr ligation is amenable to the semisynthesis of proteins via modifications of current expressed protein ligation techniques. In addition, these methods can be applied to create hybrids of polypeptides and abiotic oligomer types that will expand the range of synthetically accessible macromolecular structures and functions.

■ ASSOCIATED CONTENT

Supporting Information

Synthetic protocols and additional characterization data. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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