N- and C-Terminal Biotinylation on the *Prelude*®

INTRODUCTION

Biotinylation is the process of covalently binding biotin to a protein or other molecule. Due to the specificity and high binding affinity of biotin to the proteins avidin and streptavidin, biotinylation is commonly used in immunoanalytical techniques such as ELISA, ELISPOT and western blots. Biotin has been recognized for its poor solubility in peptide synthesis solvent (i.e. DMF), and as a result can be introduced using a pre-formed active ester, such as N-(biotinyloxy) succinimide (biotin-OSu) or biotin p-nitrophenyl ester (biotin-ONp) [1]. However, these alternative forms of biotin are significantly more expensive than pure biotin, which can be used directly by simply dissolving it in DMSO.

In this application note, we show how biotin can be incorporated on the N- or C-terminus of two peptides in a completely automated fashion using the Prelude® parallel peptide synthesizer. The Prelude®'s Single-Shot™ delivery feature and extra amino acid bottle positions make it perfect for adding expensive special monomers to a peptide sequence or performing post-translational modifications such as biotinylation. The Prelude®'s Single-Shot™ delivery feature can deliver the entire contents of the special 10 mL Single-Shot™ amino acid vial to the reaction vessel of your choice without priming or wasting a drop. In the first example, pure biotin is coupled directly to the N-terminus of the G-LHRH peptide (Biotin-GHWSYGLRPG-NH_2). In the second example, biotin is added to the C-terminus of a modified histone peptide (Ac-SGRGK(Ac)GGKGL GK(Ac)GGAKRHRK VLR-PEG-Biotin) [2] along with a PEG-linker using Biotin-PEG-NovaTag resin.

METHOD

Synthesis and N-Terminal Biotinylation of G-LHRH Peptide: Biotinylated G-LHRH was synthesized on a Prelude® parallel peptide synthesizer (Protein Technologies, Inc.) at the 20 µmol scale using Rink Amide MBHA resin (0.33 mmol/g). Deprotection was performed for 2 x 30 sec with 20% piperidine in DMF. Coupling was performed for all amino acids for 2 x 1 min, and 2 x 30 min for biotin, using 1:1:4 0.05 M AA (or biotin)/ 0.05 M HCTU/0.2 M NMM in DMF at a 5x excess. 2 x 30 sec DMF top washes were performed after the deprotection and second coupling step for each cycle except for biotin which had 6 x 30 sec DMF top washes. Also, a 30 sec DMF top wash was performed between couplings. All reagents were dissolved in DMF except biotin which was dissolved in DMSO right before the coupling step, with the aid of the pause and Email Notification features present on the Prelude®.

Synthesis of a C-Terminally Biotinylated Modified Histone Peptide: A modified histone peptide was synthesized at the 20 µmol scale on a Prelude® parallel peptide synthesizer using Biotin-PEG NovaTag resin (0.47 mmol/g). Deprotection was carried out for 2 x 7 min using 20% piperidine in DMF. Washes with DMF were performed for 6 x 30 sec. Coupling was performed using 1:1:4 0.05 M AA/0.05 M HCTU/0.2 M NMM in DMF at a 5x excess for 1 x 50 min for all amino acids except Lys(Ac)-OH, which was performed for 2 x 50 min. N-terminal acetylation was performed by treating the resin with 1:1:3 acetic anhydride/NMM/DMF for 30 minutes after the final deprotection.

Analysis: The resins were cleaved on the instrument for 2 hours using 95/2.5/2.5 TFA/TIS/water. Crude peptides were analyzed on a Varian HPLC, equipped with a C18, 300 Å, 5 µm, 250 x 4.6 mm column (Varian Microsorb-MV), run for 60 minutes with a flow rate of 1 mL/min and using a gradient of 5-95% B, where Buffer A was 0.1% TFA in water and Buffer B was 0.1% TFA in acetonitrile. Detection was at 214 nm. Mass analysis was performed on a Shimadzu LCMS-2020 single quad mass spectrometer in the positive ion mode. Separation was performed on a C18, 100 Å, 2.6 µm, 50 x 2.1 mm column (Phenomenex Kinetex), run for 9 minutes with a flow rate of 1 mL/min and using a gradient of 5-95% B, where Buffer A was 0.1% formic acid in water and Buffer B was 0.1% formic acid in acetonitrile.

DISCUSSION/RESULTS

N-Terminal Biotinylation: Biotin was dissolved in DMSO and loaded on the instrument right before the biotin addition step, using the Email

Notification and Single-Shot™ delivery features present on the Prelude® peptide synthesizer. An Email Notification step was programmed right before a pause to alert the user it was time to prepare the biotin. Biotin can only couple for about an hour before an insoluble precipitate forms. It is therefore important not to couple pure biotin for more than an hour. The HPLC result (Figure 1) shows one major product peak with excellent purity, and no significant impurity peaks. The product mass (MW = 1354 g/mol) was confirmed via LC/MS analysis as $[M + H]^{+}$ = 1355 for the singly charged ion and $[M + 2H]^{2+}$ = 678 for the doubly charged ion (data not shown).

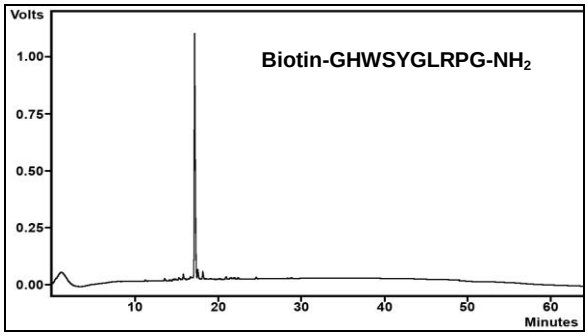

Figure 1: HPLC results for crude biotinylated G-LHRH synthesized on the Prelude®.

C-Terminal Biotinylation: The modified histone peptide was successfully synthesized on the Prelude® using biotinylated resin and the Single-Shot™ delivery feature to deliver the special amino acid K(Ac). The HPLC result for the crude peptide is shown in Figure 2.

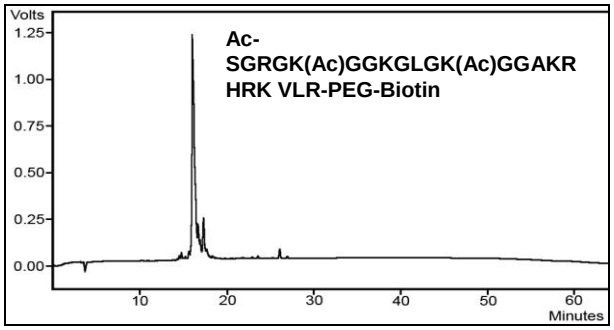

Figure 2: HPLC result for crude histone peptide synthesized on the Prelude®.

CONCLUSION

Biotin was successfully added to the N- & C-termini of two peptides in a fully automated fashion on the Prelude® peptide synthesizer. The Prelude®'s Single-Shot™ Delivery Feature was used to deliver biotin and special expensive amino acids, while the Email Notification feature and pause were used to alert the user when it was time for the biotinylation reaction.

REFERENCE

[1] Baumeister B, Beythien J, Ryf J, Schneeberger P, and White PD. Evalution of Biotin-OSu and Biotin-ONp in the Solid Phase Biotinylation of Peptides. *Int. J. Pept. Res. Ther.,* 2005; **11**: 139-141.

[2] Modified version of the H4 (*Homo sapiens*) [1-23] histone peptide (sequence 70) originally synthesized as part of a library by Dr. Brian Strahl at the University of North Carolina School of Medicine.
http://www.med.unc.edu/~bstrahl/Arrays/Pep_t.htm