On-Resin Disulfide Bridge Formation

Application Note 1

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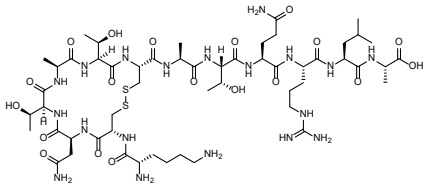
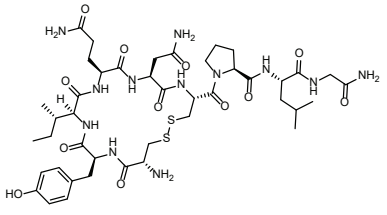
Introduction

Many naturally occurring peptides contain intradisulfide bridges, which play an important role in biological activities. There are many ways to form a disulfide bridge in the solution phase and solid phase. Formation of a disulfide bridge in the solution phase is well known and widely used in the peptide community. However, a method for on-resin disulfide bridge formation has also been developed and would be a great tool for library and screening purposes[1,2]. In this application note, on-resin disulfide bridge formation is demonstrated. The commercially available peptides human amylin (1-13) and oxytocin were synthesized by oxidative cyclization with Thallium(III)$_3$ or Iodine[4] prior to cleavage.

Amylin (1-13), Human: H-KCNTATCATQRLA-OH,
Disulfide Bridge, Cys^2-Cys^7



Oxytocin: *H-CYIQNCPLG-NH$_2$,*



Disulfide Bridge, Cys^1-Cys^6

For on-resin disulfide bridge formation, the Acm protecting group was used to protect the cysteine side chain during the synthesis. The Acm protecting group is stable to TFA, but is removed oxidatively with Tl(III) or I_2 during disulfide formation. Linear and cyclic amylin and oxytocin were prepared and analyzed by reverse phase HPLC and mass spectrometry.

Method

Acm-protected Linear Peptide Synthesis: linear human amylin (1-13), H-KC(Acm)NTATC(Acm)ATQRLA-OH[5] and oxytocin H-C(Acm)YIQNC(Acm)PLG-NH$_2$[5] were synthesized on a Prelude® peptide synthesizer under the following conditions:

Scale: 40 µmol; **Resin:** Fmoc-Rink-MBHA (0.47 mmol/g); **Deprotection:** 20% piperidine in DMF, 3 min then 20 min; **Coupling:** 1:1:2 AA/HCTU/ NMM in DMF, 2 x 45 min. **Cleavage:** 92.5:2.5:2.5:2.5 TFA/EDT/H_2O/ TIS, 2 hours.

Cyclic Peptide Synthesis: Peptides were synthesized the same as the linear peptides, except the disulfide bridge was formed prior to cleavage.

Disulfide Bridge Formation with Iodine:

Treat resin with I_2 (10 eq.) in DMF/H_2O (4:1) for 40 min. Wash resin with DMF x 2, 2% Ascorbic acid in DMF x 2, DMF x 5 and DCM x 2.

Disulfide Bridge Formation with $Tl(CF_3CO_2)_3$:

Treat resin with $Tl(CF_3CO_2)_3$ (1.2 eq.) in DMF for 40 min x 2. Wash resin with DMF x 6 and DCM x 6.

Analysis: Peptides were analyzed on a Varian Microsorb C-18 column (4.5 x 250 mm) on a Varian Pro-Star HPLC using an aqueous acetonitrile, 0.1% TFA buffer system with an increasing gradient of 5-60% acetonitrile over 55 minutes. Detection was at 214 nm. Mass analysis was performed using a Perseptive Biosystems MALDI-TOF mass spectrometer.

Results/Discussion

Mass spectrometry confirmed the successful synthesis of both linear and cyclic peptides. Acm-protected linear human amylin (1-13) and oxytocin results are shown in Table 1, while cyclic peptide results are shown in Table 2.

HPLC results are shown in Figure 1. From these results, it is clear that cyclization with thallium produced a purer product than iodine for both peptides.

Conclusion

This application note demonstrates that on-resin disulfide bridge formation using Thallium or Iodine would be good tool for synthesizing a disulfide bridge-containing library.

[1] S. Gazal, G. Gellerman, E. Glukhov, C. Gilon. J. Peptide Res., 58, 527 (2001).
[2] M. C. Munson and G. Barany. J. Am. Chem. Soc., 115, 10203 (1993).
[3] N. Fujii, A. Otaka, S. Funakoshi, K. Bessho, T. Watanabe, K. Akaji, and H. Yajima. Chem. Pharm. Bull., 35, 2339 (1987).
[4] B. Kamber, A. Hartmann, K. Eisler, B. Riniker, H. Rink, P. Sieber, and W. Rittel. Helv. Chim. Acta, 63, 899 (1980).

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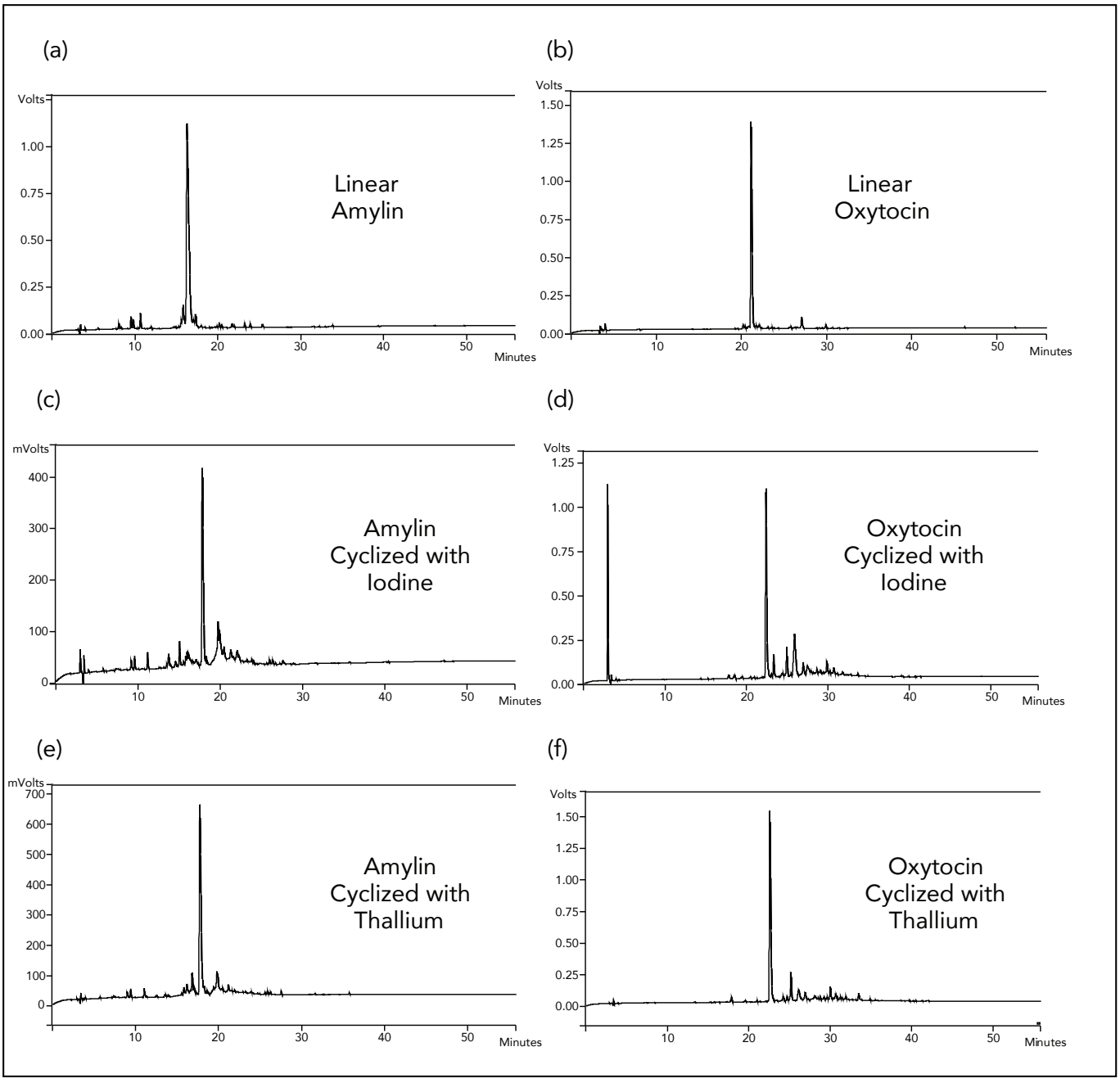

Figure 1: Linear peptides: (a) amylin, (b) oxytocin. Peptides cyclized with iodine: (c) amylin, (d) oxytocin. Peptides cyclized with thallium: (e) amylin, (f) oxytocin.

Peptide	Expected m/z	Observed m/z
Amylin	1522.8	1544.8 [Na+]
Oxytocin	1151.4	1176.5 [Na+]

Table 1: Mass spectrometry analysis of Acm-protected linear peptides.

Peptide	Expected m/z	Observed m/z
Amylin	1378.6	1378.9 [M+]
Oxytocin	1007.2	1029.6 [Na+]

Table 2: Mass spectrometry analysis of cyclic peptides.

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