

On-Resin Disulfide Bridge Formation

INTRODUCTION

Many naturally occurring peptides contain intra-disulfide 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 Iodine⁴ prior to cleavage.

Amylin (1-13), Human: H-KCNTATCATQRLA-OH, Disulfide Bridge, Cys²-Cys⁷

Oxytocin: H-CYIQNCPLG-NH₂, Disulfide Bridge, Cys¹-Cys⁶

For on-resin disulfide bridge formation, the Ac_m protecting group was used to protect the cysteine side chain during the synthesis. The Ac_m protecting group is stable to TFA, but is removed oxidatively with TI(III) or I₂ during disulfide formation. Linear and cyclic amylin and oxytocin were prepared and analyzed by reverse phase HPLC and mass spectrometry.

METHOD

Ac_m-protected Linear Peptide Synthesis: linear human amylin (1-13), (H-KC*NTATC*ATQRLA-OH)⁵ and oxytocin (H-C*YIQNC*PLG-NH₂)⁵ were synthesized on a *Prelude*TM peptide synthesizer under the following conditions:

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).

⁵ C* represent Ac_m protected Cys.

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/H₂O/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 I₂ (10 eq.) in DMF/H₂O (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 TI(CF₃CO₂)₃:** Treat resin with TI(CF₃CO₂)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. Ac_m-protected linear human amylin (1-13) and oxytocin results are shown in Table 1, while cyclic peptide results are shown in Table 2.

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

Peptide	Expected m/z	Observed m/z
Amylin	1522	1547 [Na ⁺]
Oxytocin	1154	1176 [Na ⁺]

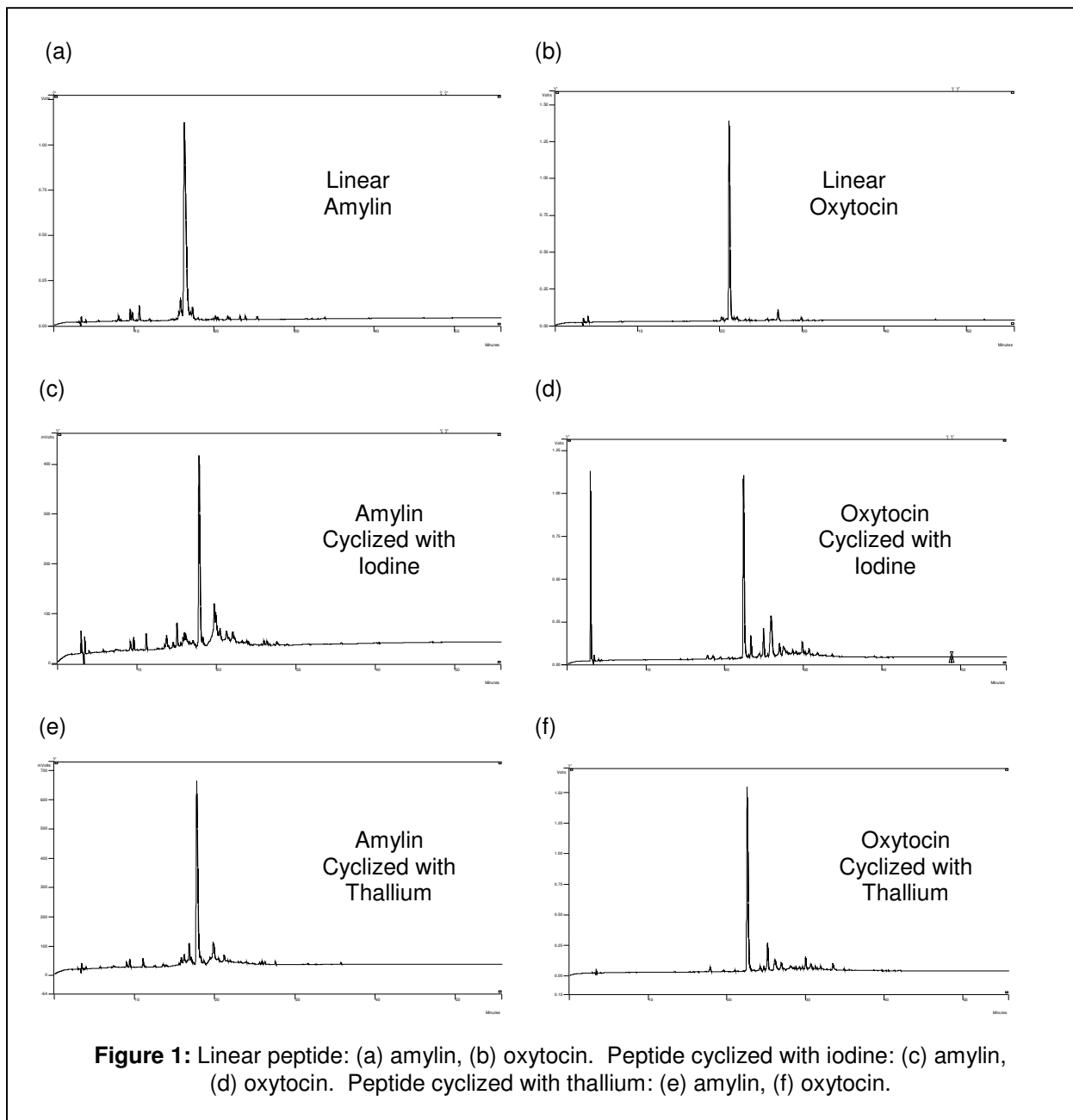


Figure 1: Linear peptide: (a) amylin, (b) oxytocin. Peptide cyclized with iodine: (c) amylin, (d) oxytocin. Peptide cyclized with thallium: (e) amylin, (f) oxytocin.

Table 2: Mass spectrometry analysis of cyclic peptides.

Peptide	Expected m/z	Observed m/z
Amylin	1378	1378.9
Oxytocin	1007	1029.6 [Na ⁺]

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.