Improved OPA Cyclization for Bioactive Peptide Analogues

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Abstract

The protocol of a recently developed chemoselective peptide cyclization method using *ortho*-phthalaldehyde (OPA) and peptides with Lys and Cys residues developed by Prof. Xuechen Li's group was improved. Three bioactive analogues and one model peptide were synthesized through the improved protocol. The compatibility between Fmoc and N_3 protecting groups (PG) and OPA cyclization conditions was also demonstrated.

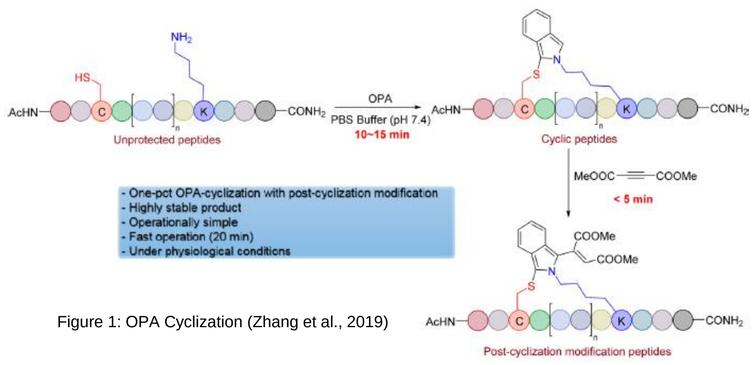

Figure 1: OPA Cyclization (Zhang et al., 2019)

Introduction

Cyclic peptides have gained popularity in drug development due to their unique conformation, and hence, possibly higher binding affinity to target cells. Chemoselective peptide cyclization using OPA and peptides with Lys and Cys residues were reported.

Despite its convenience, its limitations include product instability, sequence-dependent low solubility in reaction buffer, and lack of orthogonal PGs developed for this method. Hence, this project aims at improving OPA cyclization protocol and explore the use of orthogonal PGs in OPA cyclization.

Methodology

1. Solid Phase Peptide Synthesis (SPPS)

Linear peptides were synthesized using standard Fmoc-SPPS protocol.

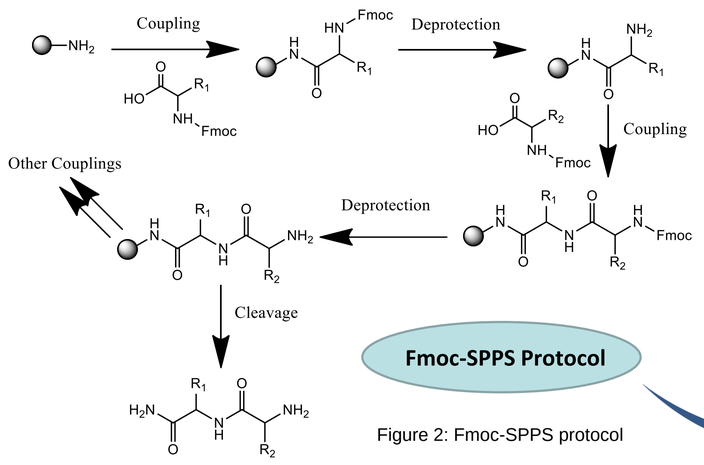

Figure 2: Fmoc-SPPS protocol

2. OPA Cyclization

The linear peptides were cyclized with excess OPA in PBS (pH 7.4) / Phosphate (pH 8.0) buffer. Guanidine could be added to improve solubility of peptide, and hence, the overall yield. If no PG removal was required, HPLC would be conducted to purify the product.

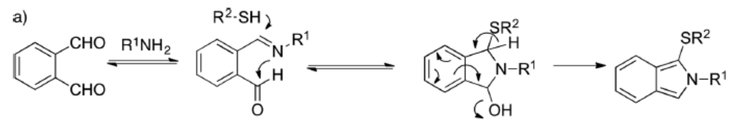

Figure 3: OPA Cyclization Mechanism

3. One-pot PG removal

For oxytocin and the model peptides, the PG was removed after OPA cyclization.

Fmoc could be removed with 20% ACN and 10% DEA (1 hour), while N_3 could be reduced with 50 mM TCEP (pH 10, 40 minutes).

HPLC was then conducted to purify the product.

Results and Discussion

1. Guanidine enhances product yield, but lowers reaction rate

Table 1 shows that product yields for OPA cyclization were higher with guanidine for all three bioactive peptides. This could be attributed to improved peptide solubility in the cyclization buffer. Yet, the presence of guanidine slowed the rate of cyclization, as demonstrated in Table 2.

Entry	Sequence (K-C / (K-Pen / Native)	OPA: Guanidine	Yield
Oxytocin	Native (S-S)	/	2.3 mg (21.7%)
	K-C (one-pot deFmoc)	0 M	Cannot be purified
		6 M	1.4 mg (33.0%)
	K-Pen (one-pot deFmoc)	0 M	Cannot be purified
		3 M	1.6 mg (26.8%)
ER-α36	Native	/	74.5 mg (53.5%)
	K-C	0 M	0.6 mg (12.0%)
		6 M	1.5 mg (27%)
	K-Pen	0 M	1.5 mg (39.5%)
		1 M	2.2 mg (58%)
PSD-95	K-Pen	0 M	1.6 mg (28%)
		1 M	2.4 mg (36%)

Table 1: Summary of synthesized bioactive peptide analogues

Entry	Peptide	Buffer pH	Guanidine	Reaction Time	Remarks
1	Model Peptide (N_3)	7.4 (PBS)	0M	35 m	90< % cyclization
2		7.4 (PBS)	3M	40 m	60% cyclization
3				80 m	70% cyclization
4				150 m	90% cyclization

Table 2: Cyclization condition screening for model peptide (N_3)

2. Pen increases product stability

Figure 4 demonstrates superior stability of the K-Pen cyclized product comparing with K-C cyclized products that degraded. This can be attributed to the bulkier dimethyl group at the C3 position for Pen.

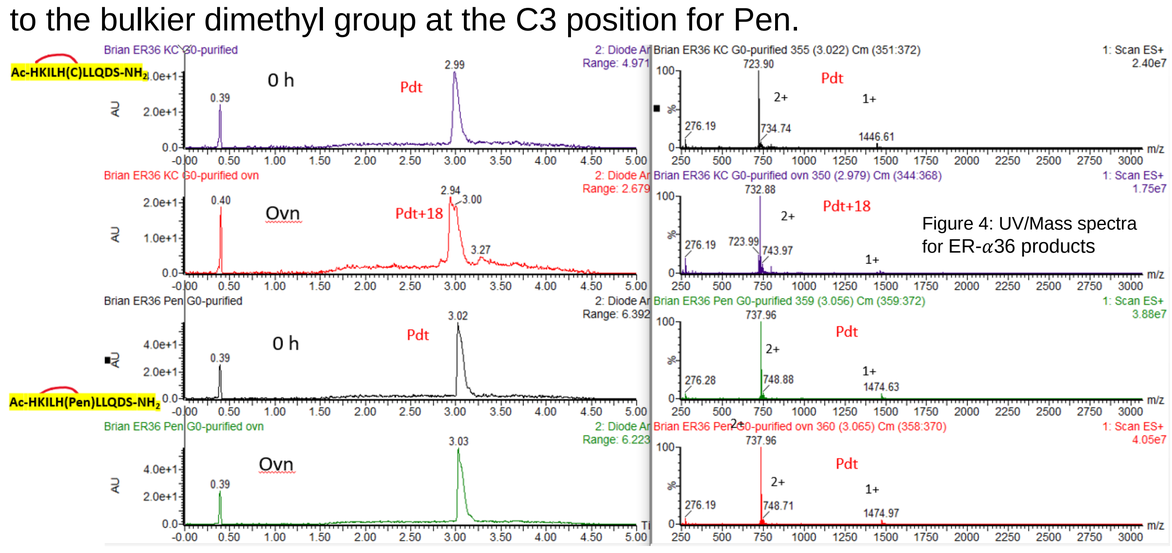

Figure 4: UV/Mass spectra for ER-α36 products

3. Fmoc and N_3 are compatible PGs in OPA cyclization

Figure 5 showed one-pot Fmoc removal after OPA cyclization. Successful removal of Fmoc and N_3 implied that OPA cyclized products could withstand basic conditions. For quenching of OPA in both cases, Gly was found to be the best quenching reagent.

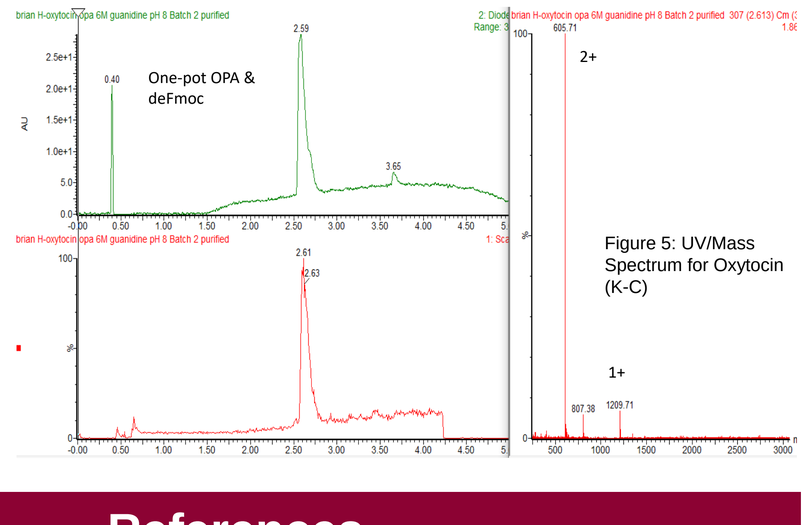

Figure 5: UV/Mass Spectrum for Oxytocin (K-C)

Summary

Through synthesizing various bioactive peptide analogues, the author demonstrated that the use of Pen instead of Cys increases product stability, which allows OPA cyclization suitable for bioactive cyclic peptide synthesis. The use of Gn improves yield, but also reduces reaction rates. Also, Fmoc and N_3 are compatible orthogonal PGs for OPA cyclization, and they can be easily removed in one-pot. Further work would focus on exploring more orthogonal PGs for OPA cyclization, testing the binding affinity of cyclized peptides and to carry out OPA cyclization on more complex sequences.

References

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