NANYANG TECHNOLOGICAL UNIVERSITY SINGAPORE

Butelase 1 from blue pea flower (*Clitoria ternatea*)

Highly Efficient Synthesis of Therapeutic Macrocyles by Ligases

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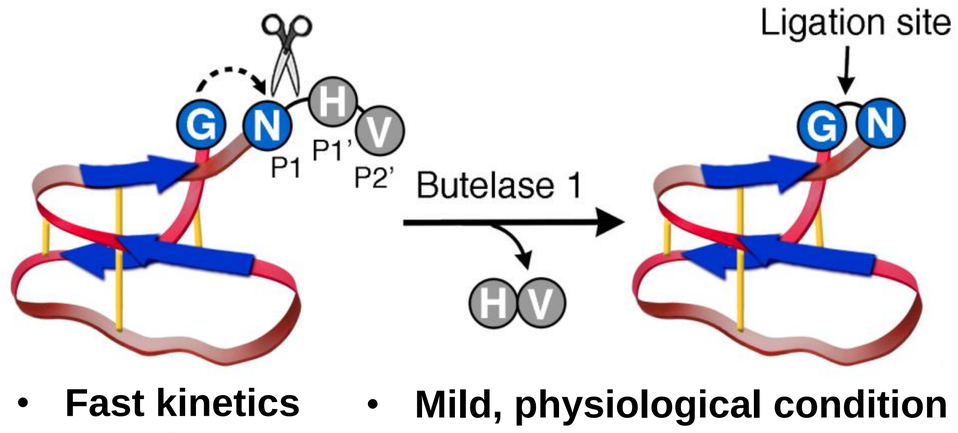
Introduction

Peptide ligases are peptide-bond staplers which enable site-specific bonding of chemicals, polymers, peptides and proteins under physiological conditions. Butelase is a novel Asn/Asp (Asx)-specific ligase discovered from butterfly pea (*Bunga Telang*). Butelase 1 accepts most N-terminal amino acids with D- or L-configuration, exhibits unmatched kinetics with catalytic efficiencies of up to 1,340,000 M^{-1} s^{-1} and is useful for both intra- and intermolecular ligation. Here we report the butelase-mediated cyclization of peptides ranging in size from 8 to >300 amino acids, and in particular, the total synthesis of three largest circular bacteriocins, AS-48, uberolysin, and garvicin ML with the peptide length of 60-70 amino acids.



- **Fast kinetics**
- **Traceless**
- **Mild, physiological condition**
- **Broad substrate tolerance**

Figure 1. Advantages of butelase-mediated synthesis

Results

A. Summary of selected cyclized peptides and proteins using Butelase-mediated ligation (Table 1)

Peptide/ Protein	Length (aa)	Peptide/ Protein	Length (aa)	Peptide/ Protein	Length (aa)
Antimicrobials	8 – 21	Galanin	32	Circular bacteriocins	60 – 70
L/D-SFTI-1	14	Salusin	32	1L-1ra	157
Conotoxin	17	Apelin	40	Somatropin	197
Hormones	28	Histatin	43	GFP	245
Cyclotides	29 – 37	Dermcidin	49	Lipases	395

B. One-pot total synthesis of circular bacteriocins (AS-48, uberolysin, garvicin, carnocyclin)

Table 2. Sequences and ligation sites (in bold) of three selected circular bacteriocins

Name	Sequence	AA	Origin
AS-48	MAKEFGIPAAVAGTVL**NV**VEAGGWVTTIVSILTAVGSGGLSLLAAAGRESIKAYLKKEIKKKGKRAVIAW	70	*E. faecalis S-48*
Uberolysin	LAGYTGIASGTAKKVVDAIDKGAAAFVIISIISTVISAGALGAVSASADFIILTVK**NY**ISRNLKAQAVIW	70	*S. uberis 42*
Garvicin	LVATGMAAGVAKTIV**NA**VSAGMDIATALSLFSGAFTAAGGIMALIKKYAQKKLWKQLIAA	60	*L. garvieae DCC43*

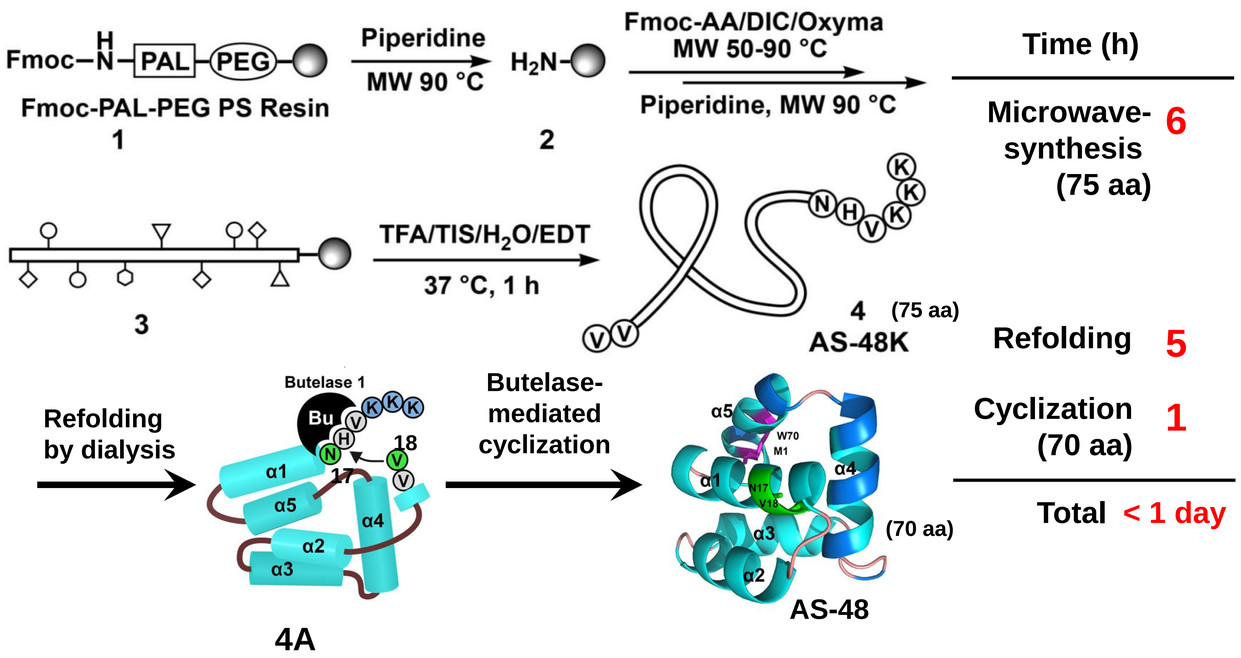


Figure 2. Butelase-mediated synthesis of AS-48

Table 3. Antimicrobial activity and MIC of synthetic AS-48

Bacteria	MIC (μM) AS-48
E. coli	0.42
E. coli DR23975	0.83
S. aureus	0.39
S. aureus DR15686	0.85
E. faecium	1.18
E. faecalis V583	0.49
E. faecalis OG1RF	1.27
L. monocytogenes	0.24

Grey: drug-resistant strains/ Yellow: food-borne pathogen

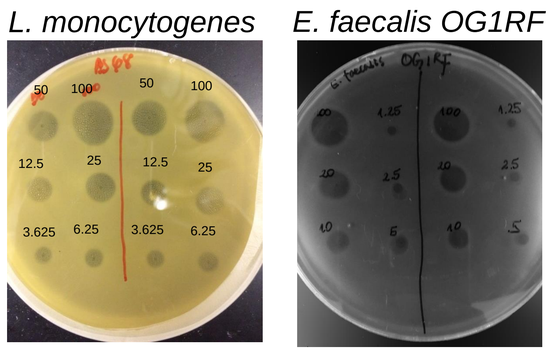


Figure 4. Radical-diffusion assays of synthetic AS-48

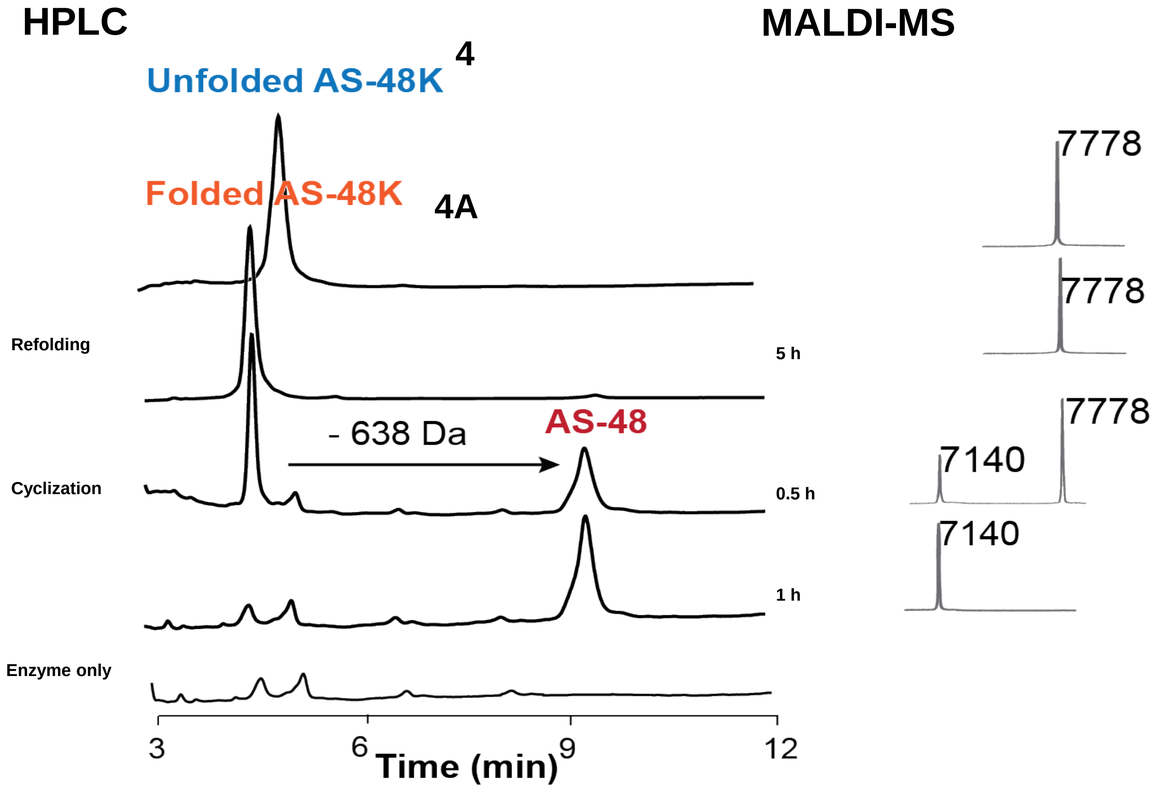


Figure 3. LC/ MS monitoring of AS-48 synthesis

Discussion and Conclusion

An advantage of our butelase-mediated cyclization is the flexibility and convenience of preparing their linear precursors by recombinant methods or microwave stepwise peptide synthesis. Examples include therapeutic peptides, proteins and enzymes. In the synthesis of circular bacteriocins, the overall process including cyclization is completed within a day. Antimicrobial assays showed that the AS-48 linear precursor is inactive at concentrations up to 100 μM whereas the macrocyclic AS-48 is potently active against pathogenic and drug-resistant bacteria including the food-borne pathogens and drug-resistant CREC and MRSA with minimal inhibitory concentrations in a sub-micromolar range. Our butelase-mediated approach of preparing circular bacteriocins could accelerate the development and application of circular bacteriocins as novel biopreservatives.

References

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