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Cyclic Cell-penetrating Peptides: Mechanisms and Synthesis

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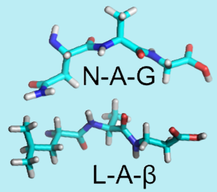

Cyclic Cell-penetrating Peptides: A Review of Mechanisms and Synthesis

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Introduction

Peptides and peptidyl molecules are regarded as the bridge between antibodies and small molecule drugs due to their ability to target regions "undruggable" by the latter two therapeutics (i.e., protein-protein interactions). For peptide therapeutics to be viable, they must be able to cross cellular membranes to reach their targets. Cyclic cell-penetrating peptides have been proven to enter cells more efficiently than their linear counterparts, thus most of the drug development efforts are focused on this type of peptides. Despite this, the mechanisms with which cyclic CPPs enter cells remain elusive, and comprehensive methods to improve their cell delivery rate are yet to be developed.

This review compiled information from published literature on cyclic CPPs to synthesize a comprehensive review on the classification, modes of cellular penetration, and methods to improve cellular entry of cyclic CPPs. The classification focused on *de novo* synthetic cyclic CPPs.

Materials and methods

The outline of this review was synthesized by screening previously published articles on CPPs and cyclic CPPs. Details of each section were compiled from relevant peer reviewed articles identified from Google Scholar and PubMed databases.

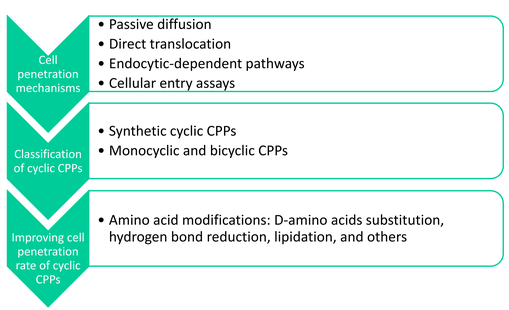

Figure 1. Sections of the review in chronological order.

Results and Discussion

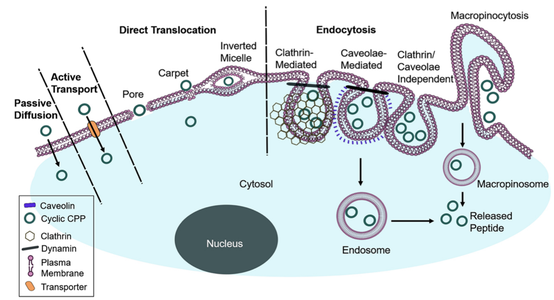

Figure 2. Cellular entry pathways of CPPs. Passive diffusion is when CPPs crosses the plasma membrane without disrupting the lipid bilayer and using ATP. Direct translocation involves disruption of the plasma membrane and temperatures above 4°C. Endocytosis involves CPPs being internalized by the plasma membrane forming endosomes, which the peptides need to lyse to be released into the cytosol. Source: (1)

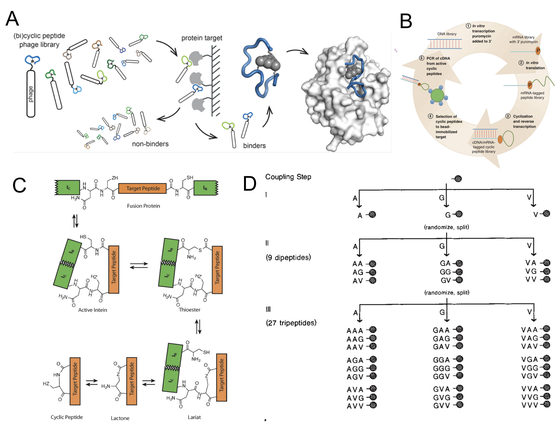

Figure 3. Types of *de novo* peptide synthesis. A) Synthesis from a phage library. B) Synthesis from an mRNA library. C) Synthesis by split-intein circular ligation of peptides and proteins (SICLOPPS). D) Split-and-pool synthesis of one-bead-one-compound (OBOC) libraries. Source: (2),(3),(4),(5)

Table 1. Inhibitors and promoters used to determine cellular entry mechanisms.

Mechanism	Inhibitors/Promoters
Direct translocation	Temperatures below 4°C, ATP depletion, pyrene butyrate (promoter)
Macropinocytosis	Cytochalasin D, amiloride
Lipid raft	Nystatin
Clathrin-mediated	Chlorpromazine, methyl-βcyclodextrin

Table 2. Cyclization strategies of monocyclic and bicyclic peptides.

	Monocyclic	Bicyclic
Naturally derived	Example: cyclosporin A	Example: Romidepsin
Synthetic	• Metal-facilitated head-to-tail cyclization • Difunctional linkers • Copper-catalyzed alkyne-azide cycloaddition ("Click" reaction)	• Trifunctional linkers • Thiol-amine cyclization

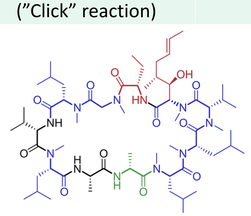

Figure 4. Cyclosporin A, an example of a natural cyclic CPP that can passively diffuse into the cell. The peptide has N-methylated amino acids (in blue), non-canonical and lipophilic side chains (in red), and a D-amino acid (in green). Source: (6)

Conclusions

Advancements in *de novo* peptide synthesis have made the discovery of cyclic CPPs with high specificity and affinity an attainable goal. The issue with peptide therapeutic development is now to improve the peptide's cellular penetration. Depending on the structure and concentration, peptides vary in their cellular entry rate and mechanism. Similarly, there are many strategies to improve the penetration rate of CPPs, including bicyclization, D-amino acid substitution, and non-proteogenic amino acid substitution. Understanding the penetration mechanisms and how to identify which mechanism is taking place will allow for application of the appropriate penetration-enhancement strategies.

Literature cited

1. Dougherty PG, Sahni A, Pei D. Understanding Cell Penetration of Cyclic Peptides. Chem Rev. 2019;119(17):10241-87. Epub 2019/05/16. doi: 10.1021/acs.chemrev.9b00008. PubMed PMID: 31083977; PMCID: PMC6739158.
2. Deyle K, Kong XD, Heinis C. Phage Selection of Cyclic Peptides for Application in Research and Drug Development. Acc Chem Res. 2017;50(8):1866-74. Epub 2017/07/19. doi: 10.1021/acs.accounts.7b00184. PubMed PMID: 28719188.
3. Josephson K, Ricardo A, Szostak JW. mRNA display: from basic principles to macrocycle drug discovery. Drug Discov Today. 2014;19(4):388-99. Epub 2013/10/26. doi: 10.1016/j.drudis.2013.10.011. PubMed PMID: 24157402.
4. Lennard KR, Tavassoli A. Peptides come round: using SICLOPPS libraries for early stage drug discovery. Chemistry. 2014;20(34):10608-14. Epub 2014/07/22. doi: 10.1002/chem.201403117. PubMed PMID: 25043886.
5. Lam KS, Salmon SE, Hersh EM, Hruby VJ, Kazmierski WM, Knapp RJ. A new type of synthetic peptide library for identifying ligand-binding activity. Nature. 1991;354(6348):82-4. Epub 1991/11/07. doi: 10.1038/354082a0. PubMed PMID: 1944576.
6. Vinogradov AA, Yin Y, Suga H. Macrocyclic Peptides as Drug Candidates: Recent Progress and Remaining Challenges. J Am Chem Soc. 2019;141(10):4167-81. Epub 2019/02/16. doi: 10.1021/jacs.8b13178. PubMed PMID: 30768253

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