Peptide stabilization by side-chain to side-chain cyclization

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Opportunities and Weaknesses in Developing Peptide Drugs

• Opportunities
  – Readily available leads
    • Synthesis and SAR are straightforward and rapid
  – A large number of diverse unnatural amino acids available to increase stability
  – Can access larger binding surface area than small molecules
    • Well suited for extracellular protein-protein interactions as GPCR agonist and antagonists
  – Possible to achieve high potency (sub-nanomolar) and efficacy
  – High selectivity and low toxicity

• Weaknesses
  – Peptide Therapeutics: It is all in the Delivery
  – High clearance: requires extensive optimization, fusion/conjugation and/or formulation

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Strategies to Increase Peptide Half-Life

- **Lipidation**
  - Liraglutide (phase 3/registration) (Novo Nordisk)

- **Pegylation**
  - Hematide (phase 3, renal failure) (Affymax)

- **Albumin Conjugation/Complexation**
  - Albumin binding peptides (Genentech)
  - Domain Anti-albumin fusions (Domantis, GSK)
  - Albumin fusion proteins (Human Genome Sciences, GSK)
  - Covalent attachment (CJC-1411, Conjuchem)

- **Antibody Conjugation/Complexation**
  - Fc-fusions (Mimetibody, Centocor)
  - Ab-covalent attachment
  - Anti-digoxigen antibodies (Roche)

W. Danho, PIPS 2014, University of Cergy-Pontoise
Cyclization: Learnings from Nature

- orally bioavailable marketed cyclic peptide (11 aa)
- intramolecular H-bonds reduce desolvation penalty when leaving water

Cyclosporin A: %F = 29

- Many intramolecular side-chain to side-chain cycles

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Side-chain to side-chain cyclization

- Rigidification reduces susceptibility to proteolytic enzymes thus increasing the metabolic stability in vitro and more significantly in vivo
- Restriction to active conformation in cyclic peptides can give superpotent analogues in matched cases
- Basis for receptor selectivity: often different receptors bind the same flexible substrate in different conformations
Cyclization types

- Carbon-carbon bridge
- Disulfide bridge
- Lactam bridge
- Triazolyl bridge
carbon-carbon bridge
by Ring Closing Metathesis

Starting material:
unnatural amino acids:
allyglycine

Cyclization:

Presence of metal (Grubbs catalysts)
Dicarba-analogs of octreotide

• octreotide: octapeptide analog of the disulfide-bridged somatostatine hormone

• cell growth inhibitor in a few cancer types and carrier of radionuclides

Dicarba group instead of disulfide bridge

Keeping pharmacophore region and type II’ β-turn conformation

Xaa = Thr, Tyr(Bzl), Phe

Increased stability (more than 30h in human serum)
Possible labelling with $^{99m}$Tc and $^{188}$Re (no disulfide cleavage in reducing medium)

Another type of carbon-carbon bridge: 1,3-butadiyne

- Glaser oxidative coupling = a click reaction never explored to constrain peptide backbone
- Catalyzed by copper(I) salt in the presence of oxygen (micro-wave assisted reaction)
- The diyne tether combines high rigidity and limited occupied space

β-turn stabilization

- including the minimal epitope RNGH for antibody detection in Multiple Sclerosis
- disulfide bridged hexapeptide / diyne bridged hexa or octapeptides
- NMR conformational analysis in water:

\[
\text{Ac-Cys-Arg-Asn-Gly-His-Cys-NH}_2 : \ \text{β-turn centered on Asn-Gly}
\]

\[
\text{Ac-N-CH}_2\text{-CO-Arg-Asn-Gly-His-N-CH}_2\text{-CONH}_2 : \ \text{β-turn centered on Arg-Asn}
\]

\[
\text{Ac-NH-CH-CO-Arg-Asn-Gly-His-NH-CH-CONH}_2 : \ \text{no turn stabilization for the hexapeptide but...}
\]

β-turn stabilization

...octapeptide

\[ \text{Ac-NH-CH-CO-Arg-Arg-Asn-Gly-His-Thr-NH-CH-CNH}_2 \]

I’ β-turn stabilization centered on Asn-Gly

\[ \text{Ac-N-CH}_2\text{-CO-Arg-Arg-Asn-Gly-His-Thr-N-CH}_2\text{-CONH}_2 \]

β-turn structure around Asn-Gly

- Diyne bridged cyclic peptides allow stabilization of various β-turn structures in water
- Optimization of on-resin Glaser oxidative coupling: libraries of stable constrained butadiyne peptides can be generated

α-helix stabilization

- PTH = 84-aa hormone increasing Ca\(^{2+}\) concentration in blood
- PTHrP = 139-173 aa hormone causing humoral hypercalcemia of malignancy

- N-terminal portion essential for interaction with PTHR1 receptor

- Chorev et al. demonstrated that an α-helical motif is essential for the bioactive conformation:

\[ \text{[Lys}^{13}(\&^1),\text{Asp}^{17}(\&^2),\text{Tyr}^{34}]\text{hPTHrP(7-34)NH}_2 \]

a potent PTHR1 antagonist containing an extended and stabilized α-helical conformation increasing efficiently peptide interactions

Schievano E.; Rosenblatt M.; Chorev M.; Peggion E. J. Peptide Sci. 1999, 5, 330-337
Bisello A.; Nakamoto C.; Roseblatt M.; Chorev M.; Am. Chem. Soc. 1997, 36, 3293-3299
Mierke D.F.; Bisello A.; Mammi S.; Peggion E.; Chorev M.; Am. Chem. Soc. 1997, 36, 10372-10383
Maretto S.; Rosenblatt M.; Chorev M.; Mierke D.F.; Am. Chem. Soc. 1997, 36, 3300-3307
α-helix stabilization

- NMR studies of Ac-hPTHrP(11-19)NH₂ derived cyclopeptides in water:HFA

- α-helical structures in the cyclic part of the molecules

- slight difference of the backbone arrangement but common spatial orientation of side-chains

Unnatural amino acids for various azido-alkynyl intramolecular peptide cyclization

\[
\begin{align*}
N^\alpha\text{-Fmoc-Apr} & (\beta-N_3)\text{-OH} & N^\alpha\text{-Fmoc-Abu} & (\gamma-N_3)\text{-OH} & N^\alpha\text{-Fmoc-Ava} & (\delta-N_3)\text{-OH} & N^\alpha\text{-Fmoc-Nle} & (\varepsilon-N_3)\text{-OH} \\
N^\alpha\text{-Fmoc-Pra} & \text{-OH} & N^\alpha\text{-Fmoc-Abu} & (\gamma-\text{yl})\text{-OH} & N^\alpha\text{-Fmoc-Ava} & (\delta-\text{yl})\text{-OH} & N^\alpha\text{-Fmoc-Nle} & (\varepsilon-\text{yl})\text{-OH}
\end{align*}
\]

**α-helix stabilization**

- Variation in the size of the triazol-containing bridge, the location and orientation of the triazol in the bridge:

  ![Chemical structures](image)

- **NMR** studies of Ac-hPTHrP(11-19)NH₂ derived cyclopeptides in water:HFA

Peptide stabilization by side-chain to side-chain cyclization

- β-turn stabilization
- disulfide bridge
- dicarba-analog
- diyne tether
- lactam bridge
- triazolyl bridge
- α-helix stabilization
PeptLab@UCP platform

• Created thanks to ANR chaire d’excellence Pepkit 2009-2014

• in Neuville-Université (RER A), Cergy-Pontoise

• Missions:
  ✓ research, development or expertise services for industries or academics
  ✓ Scientific equipment provision
  ✓ Training courses in peptide synthesis
Equipment

Peptide synthesis

- Biotage SyroWave™
- Biotage Syro II
- Liberty Blue CEM

Purification/Characterisation

- Autopurifier HPLC Waters 2767
- UPLC-MS Waters Acquity
Equipment

Peptide-protein interaction analysis

- TECAN (ELISA)
- Surface acoustic wave SAW instruments SamX
- Microcalorimetry GE Healthcare ITC200
PEPTLAB Plateforme

Design, Synthesis, Purification and Characterization of peptides and proteins

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