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Design of cyclic and other templates for potent and selective peptide α-MSH analogues

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Abstract

For over three decades, the design of linear peptide ligands often has incorporated cyclic constraints to improve potency, receptor selectivity, proteolytic stability and biodistribution. Its importance has been so well established that modern day schemes for ligand-based drug design often start with cyclization of linear peptides to rigidify peptide structure, to limit its conformational possibilities, and to find key pharmacophore elements in three-dimensional space. In the past several years, cyclic constraints have been used to develop ligands with improved efficacy, binding affinity, biostability and receptor selectivity for α-melanocyte-stimulating hormone (α-MSH). Furthermore, potent cyclic α-MSH analogues, such as MT-II and SHU-9119, have made structure–activity relationship studies and molecular modeling more useful for creating new three-dimensional, topographical pharmacophore templates.

Introduction: constrained cyclic peptides

The design and study of constrained cyclic peptides has had a revolutionary impact in the field of peptide chemistry, receptor–ligand interactions, and drug design in the past three decades [1–6]. Cyclic analogues of linear, biologically active peptides of, for example, somatostatins, melanotropins and enkephalins, have demonstrated that cyclic peptides possess potential to: (1) increase agonist and antagonist potency; (2) withstand proteolytic degradation; (3) increase receptor selectivity; (4) enhance bioavailability; and (5) provide conformational insight for receptor binding (see, for example [1,7,8]). Constrained cyclic peptides have been used to stabilize secondary structures and have been modelled to mimic β-turns [1–3,6–8] and other peptide structures. Results from these studies have been exploited to expand the possibilities of developing peptidomimetics with increased efficacy and binding affinity to protein receptors [1,4,7,9].

If the structure of a receptor's protein-binding site is known, one can design potential ligands by theoretically synthesizing a ligand that can complement the binding pocket by an induced fit [4,9]. In principle, this makes ligand design easier because the shape of the ligand simply has to fit the shape of the binding site. The ideal ligand in return, should either increase bioactivity or inhibit receptor response, making it an efficacious agonist and antagonist respectively. The known structures of many bioactive peptide hormones and neurotransmitters, the first of which was oxytoxin, and their proposed receptor-binding sites can now be found in searchable databases such as the RCBS Protein Data Bank (PDB) [10]. Although recent advances in crystallography and NMR have greatly increased the number of three-dimensional (3-D) structures found in these databases, the percentage of solved structures compared with the number of known proteins is less than ideal [11,12]. Hence, drug design derived from 3-D structural conformation is not feasible for proteins such as G-protein-coupled receptors (GPCRs) whose structures have not been solved. Here, constrained cyclic peptides have proven

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useful in two main ways. First, cyclic peptides possess less structural freedom than linear peptides and, hence, the ligand can be 'locked' into a limited number of possible conformations allowing one to derive testable hypothesis in the design of better receptor pharmacophores [3,4,6,11]. Secondly, the restraint placed on a cyclic peptide can create a 'fit' for ligand–receptor recognition that increases binding affinity, efficacy and receptor selectivity.

Factors affecting 3-D conformation

The spatial conformation of a peptide begins with its backbone. The atoms that map the backbone are the α carbons (C_α), the carbonyl carbons (C′), and the amide nitrogens (N). The pharmacophore elements within a potent ligand are then dictated by the topographical structure of the side chains and where they exist in 3-D space [13].

The backbone conformation in a peptide is governed by a series of torsional angles, denoted as φ, ψ, ω, and the side chain conformations by chi (χ) torsional angles. Bond rotation around C′–N–C_α–C′ is defined by the φ angle. The torsional angle for rotation around N–C_α–C′–N is known as the ψ angle. The torsional angle between C'_i and C'_{i+1} is referred to as the ω angle. Torsional angles coding the relationship between side chains and the backbone are designated as χ_1 for N–C_α–C_β–C_γ, χ_2 for C_α–C_β–C_γ–C_δ, etc. As with all other molecules, peptide ligands must obey certain rules for steric and electrostatic interactions. Thus, a peptide chain is restricted to certain allowable or energetically favourable conformations. Ramachandran and co-workers were the first to qualitatively evaluate the contribution of peptide sequences for backbone secondary structures [14,15]. They showed that a plot of the φ angle versus the ψ angle revealed tendencies for most amino acids to fall within specific regions of φ, ψ space. In addition to spatially labeling allowable torsional angles for amino residues, the Ramachandran plot defines regions for secondary structure. For example, the right-handed α helix tends to have a φ angle around − 63° and a ψ angle around −42°.

It should now be noted that although the number of possible conformations for a small, linear peptide sequence can be limited by certain steric hindrances and allowable torsional angles, the overall number often is still large because of the flexibility of the peptide chain. To further restrict the number of possible conformations into a handful of allowed backbone structures, which future ligands can be modelled after, other restrictions such as cyclic constraints must be incorporated [2,3]. Figure 1 outlines a systematic approach to peptidomimetic design [2–4,7]. It must be mentioned that conformationally constrained peptides, whether involving a global constraint using cyclization, or a local constraint with incorporation of specialized amino acids, all play a crucial role in designing functional ligands [4,7,8,13].

Global constraint cyclization

There are four main ways to form constrained cyclic peptides. These cyclizations, as illustrated in Figure 2, involve bridging between (1) two side chains; (2) a side chain to N-terminus; (3) a side chain to C-terminus; or (4) two backbone residues either C-terminal to N-terminal, or backbone internal residue to internal residue (the N and C_α can be used as sites for cyclization). The simplest method used to introduce global constraint into a peptide chain is formation of a covalent bond between two distant segments in the sequence. Such cyclic introduction can be made with lactam bridges, disulfide bridges and other chemical moieties including use of spacer regions in constrained portions of a molecule [2,3,7,8,16]. Furthermore, ring size can be controlled depending on the region for cyclization.

Cyclization of a peptide chain can dramatically change the overall conformation of a ligand favouring certain secondary structures. Table 1 lists some types of structural constraint that lead to specific secondary elements. Greatly reducing the degrees of freedom in a newly formed cyclic structure can stabilize the bioactive conformation of the ligand.

α-Melanocyte-stimulating hormone (historical background)

An excellent example of the increased potency, efficacy and other desirable pharmacological and biological properties due to substitution and cyclization of a linear parent peptide ligand can be seen in the studies of α-melanocyte-stimulating hormone (α-MSH; see Box 1 for a glossary showing peptide sequences). This tridecapeptide was first isolated in the pituitary gland, with primary physiological function in skin pigmentation [17,18]. However, more recently it has been shown that α-MSH also affects feeding behaviour, male and female sexual behaviour, erectile function, immune response, memory and learning, and other biological effects [19–21,22•,23,24•, 25]. This mini-review focuses on work from our laboratory in which cyclic constraints were used to explore a variety of templates and the effects of these constraints on bioactivity.

One of the first α-MSH analogues reported to have increased potency by up to 60 times the activity of native α-MSH tested in frog skin was MT-I. Replacement of Met with Nle at the 4 position and substituting Phe with D-Phe at the 7 position increased the hydrophobicity and created a hypothesized reverse β-turn conformation that improved potency and efficacy [26]. Although MT-I was super potent, it lacked selectivity for melanocortin receptor [27]. This initial study led to a series of cyclic analogues which were designed to obtain a constrained cyclic peptide that would have the necessary conformation held rigidly in space, and which would be a selective agonist (or antagonist) for directed receptor(s) [28].

It was found very early on that the tetrapeptide, His-Phe-Arg-Trp formed the essential active core for α-MSH [29]. Hence, new cyclic analogues were fashioned to incorporate this tetrapeptide into various rings. The first cyclic analogues to be designed involved the replacement of Met^4 and Gly^{10} with cysteine residues to form a disulfide bond. The resulting c[Cys^4, Cys^{10}]-α-MSH analogue was also found to be superpotent but lacked prolonged biological potency [28]. It wasn't until the late 1980s when the first successful shortened sequence and cyclic constrained analogue of α-MSH was developed. The new compound, MT-II, was found to be a superpotent agonist in the lizard, frog and tyrosinase assays, with prolonged biological potency in frog skin assays as well [30,31]. Recent studies of MT-II in the sciatic nerve crush lesion and cisplatin-induced neuropathy in rats found MT-II also to be an effective agent in nerve regeneration and neuroprotection [32•].

All the first-generation cyclic analogues possessed great potency, but lacked selectivity to specific melanocortin receptor sites. Replacement of D-Phe in MT-II with D-Nal-(2′) led to SHU-9119, the first selective antagonist for MC3R and MC4R (the human melanocortin 3 and 4 receptors), although potency at the MC1R remained [33,34]. Recently it has been reported that through steady-state and time-resolved fluorescence spectroscopy studies, both the D-Nal and Trp residues show signs of deep lipid bilayer penetration, an important role for selective hMC4R binding [35]. Furthermore, appropriately labeled derivatives of MT-II and SHU-9119 could be used to evaluate the biological transduction mechanism of these ligands by two-photon fluorescence spectroscopy [36•].

α-MSH cyclic analogues

The discovery of MT-II and SHU-9119 began an era of ligand design directed toward obtaining peptide and peptidomimetic ligands (agonists and antagonists) for MC1R, MC3R, MC4R and MC5R (also, non-peptide ligands were sought with considerable success but are not reviewed). Several analogues based on the MT-II template have been reported that are more selective and highly potent (e.g. [37,38•,39–43]. For example, penta-c[Asp-5-ClAtc-D-Phe-Arg-Trp-Lys]-NH_2 and penta-c[Asp-5-ClAtc-D-Phe-Cit-Trp-Lys]-NH_2, closely related analogues of MT-II were generated by replacing His^6 with 5-ClAtc and substitution of Arg^8 with Cit to the parent

MT-II. Both these analogues were found to be potent hMC4R agonists with weak partial agonist activities in hMC1R, hMC3R and hMC5R *in vitro* agonist assays [38•].

Hruby *et al*. found that although PG-931 was considerably less potent than MT-II, its selectivity for hMC4R versus hMC3R was at least 100-fold higher, making the cyclic peptide a potentially useful ligand for distinguishing binding site activities between the hMC4 and hMC3 receptors [42].

In a separate study, a series of β-substituted prolines replaced His^6 and $_D$-Phe^7 in the MT-II scaffold to test the relationship between conformationally and topographically constrained ligands and selective receptor binding [44]. Overall, binding affinities for the new analogues were weak compared with the parent ligand, suggesting that the newly introduced constraint resulted in an unfavourable 3-D arrangement in chi space. Interestingly, increasing the bulky substituents on proline caused selectivity for hMC5R to increase. These studies demonstrate that hMC3 and hMC4 receptors are more easily affected by conformational changes to the ligands with reduced binding affinity, whereas hMC5R seems to be capable of accommodating bulkier residues [44].

Recent SAR studies

Extensive NMR studies in conjunction with structure–activity relationships (SAR) of α-MSH analogues and molecular modeling have been used to obtain novel peptidomimetic ligand designs (e.g. [44]). For example, Sun *et al*. [45] reported the synthesis of one of the most constrained and hMC4R-selective peptide analogues to date, [penta-cyclo(D-K)-Asp-Apc-(D) Phe-Arg-(2*S*,3*S*)-β-methylTrp-Lys-NH_2]. The backbone is reported to feature a β-turn that incorporates the pharmacophore region needed for activity. The rigidity of the conformation causes the Asp residue to form a protrusion that apparently is neither compatible with nor adaptable to the hMC1R binding site [45]. Further examination of conformational and topographical space should prove fruitful in the design of more selective ligands for the melanocortin receptors since it appears that each receptor has its own special requirements.

Development of chimeric analogues

Another approach we have taken is to place the key structural moieties of α-MSH on novel templates. The idea is to use the key pharmacophores of well-established receptor recognition moieties, and link them together on alternate templates to examine whether selectivity or potency can be increased. We have previously called these ligands chimeric analogues. Recent examples in this context are the novel chimeric melanotropin–deltor-phin analogues by Han *et al*. [46]. The chimeric ligand was designed based on the main pharmacophore elements of melanotropin and deltorphin, and had the initial peptide sequence His-Phe-Arg-Trp-Glu-Val-Val-Gly-NH_2. This structure was then cyclized to reduce the number of possible conformation by incorporating a lactam bridge between the acidic functional group of a glutamic acid side-chain and the free amide group at the N-terminus. Figure 3 depicts the design for this chimeric analogue. Two of the 19 chimeric analogues made [46] are listed in Table 2. GXH-32B and GXH-38B were found to have the most potent agonist and antagonist activities, respectively, at each of the MC receptors. GXH-32B was found to be a potent agonist with 30-fold higher binding affinity than GXH-15, which was a weak antagonist for MC1R with an IC_{50} of 8.2 μM. Subsequent substitution of the dipeptide, β-Ala-His, at the N-terminus with Gly-Cpg (cyclopentylglycine) gave GXH-38B, which was found to be the most potent antagonist. Although GXH-38B carried antagonist activity for all the melanocortin receptors, it was particularly selective for MC1R. Table 3 illustrates the potency of GXH-32B and GXH-38B at three human MC receptor sites.

Other chimeric analogues involving α-MSH and somatostatin derivatives have been examined [47]. Using CTAP and CTOP, which are μ opioid receptor antagonists derived from somatostatin, a series of 25 chimeric ligand were synthesized using the design template H-D-Phe-c[XXX-YYY-ZZZ-Arg-Trp-AAA]-Thr-NH_2 [47], and several were shown to be potent agonists.

Conclusion

Incorporation of cyclic constraints into peptidic scaffolds, when properly designed, can provide novel ligands with novel biological profiles, especially with previous insight into key pharmacophore structural moieties. Constrained cyclic peptides with appropriate backbone or template rigidity and relatively fixed side-chains in chi space often have enhanced efficacy, selectivity, binding affinity and bioavailability, as outlined and demonstrated in many other studies (e.g. [1,7]). In addition, highly potent and selective ligands have helped elucidate proposed bioactive conformations at the receptor-binding sites. This, in return, further facilitates identification of the 3-D relationships of key pharmacophores, and contributes greatly to a better understanding for design and synthesis of peptides and peptide mimetics [7]. A major issue with these peptides is the appropriate methods for drug delivery of these ligands. Because most of the biological actions of these compounds appear to be hormonal and/or neurotransmitter-like in their biological actions, a bolus administration, whether oral or otherwise, may be counter indicated, and slow and/or pulsatile administration may be needed for a potent response.

Box 1. Peptide structures covered in this article

α-MSH

Ac-Ser^1-Tyr^2-Ser^3-Met^4-Glu^5-His^6-Phe^7-Arg^8-Trp^9-Gly^{10}-Lys^{11}-Pro^{12}-Val^{13}-NH_2

CTAP/CTOP

H-D-Phe-c[Cys-Tyr-D-Trp-XXX-Thr-Pen]-Thr-NH_2, where XXX = Arg, Orn, respectively

MT-I

Ac-Ser^1-Tyr^2-Ser^3-Nle^4-Glu^5-His^6-D-Phe^7-Arg^8-Trp^9-Gly^{10}-Lys^{11}-Pro^{12}-Val^{13}-NH_2

MT-II

Ac-Nle-c[Asp-His-D-Phe-Arg-Trp-Lys]-NH_2

penta-c[Asp-5-ClAtc-DPhe-Arg-Trp-Lys]-NH_2

$CH_3CH_2CH_2CH_2C(=O)$-c[Asp-5-ClAtc-DPhe-Arg-Trp-Lys]-NH_2

PG-931

Ac-Nle-c[Asp-Pro-D-Phe-Arg-Trp-Lys]-Pro-Val-NH_2

SHU-9119

Ac-Nle-c[Asp-His-D-Nal(2′)-Arg-Trp-Lys]-NH_2

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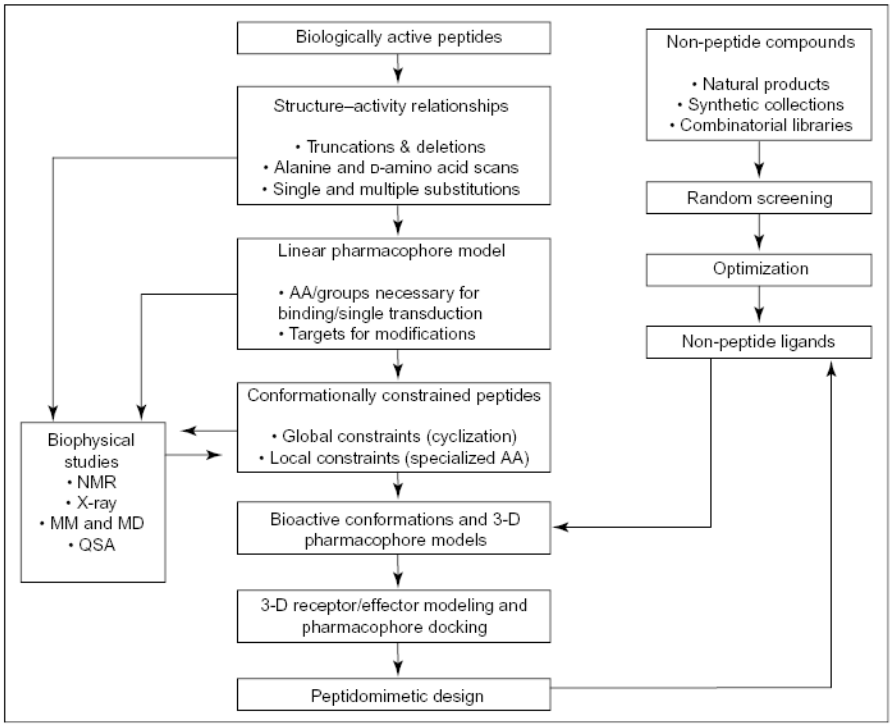

Figure 1.
Systematic approach to peptidomimetic design. Adapted from [7] with permission. © 2002 *Nature: Reviews Drug Discovery*. www.nature.com/reviews.

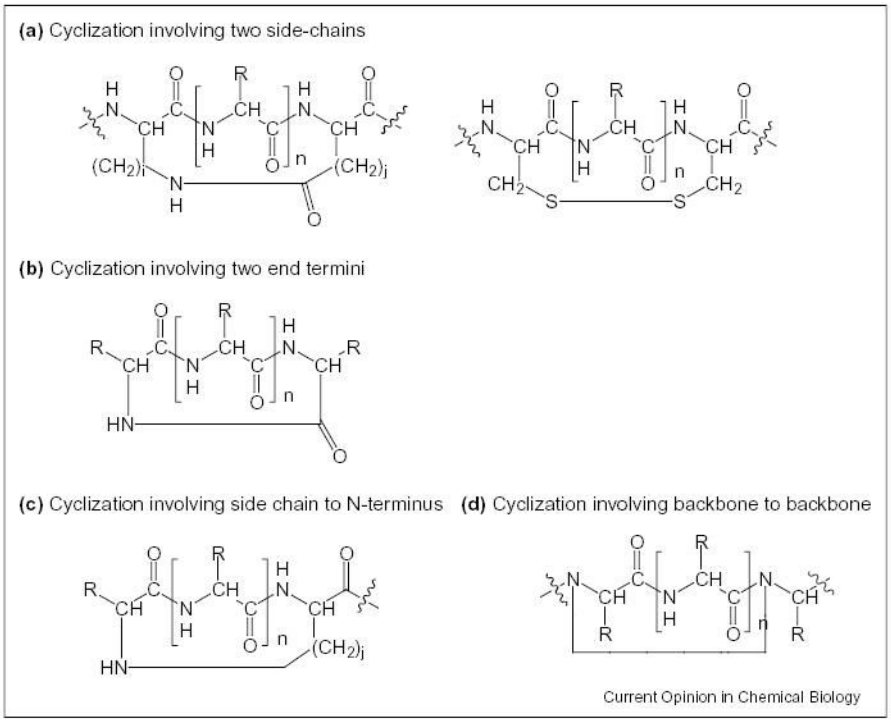

Figure 2.

Types of covalent cyclization. **(a)** Cyclization involving two side chains. **(b)** Cyclization involving two end termini. **(c)** Cyclization involving side chain to N-terminus (or C-terminus). **(d)** Cyclization involving side chain to backbone to backbone (or side chain to backbone).

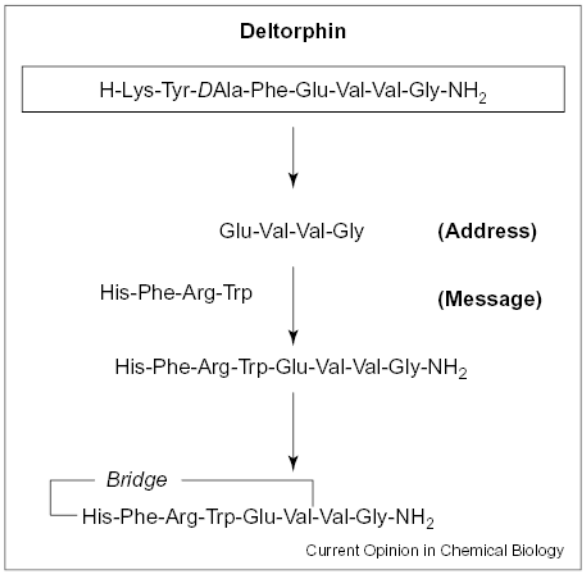

Figure 3.
Design of novel chimeric melanotropin analogues.

Table 1

Incorporation of certain structural constraints can introduce or stabilize specific secondary conformations.

Constraints	Secondary structures
Disulfide bridging	β-turns, γ-turns, α-helices, β-sheets
Lactam bridging (side-chain to side-chain or side-chain to N- or C-terminus)	α-helices, β-turns
Backbone to backbone cyclization	β-turns, γ-turns, β-sheets

Table 2

High-throughput screening of chimeric α-MSH analogues at *Xenopus* frog MC1R.

Peptide	Sequence	EC_{50} (nM)	IC_{50} nM[a] (0.5 nm α-MSH)
GXH-32B	c[β-Ala-His-D-Nal(2′)-Arg-Trp-Glu]-Val-Val-Gly-NH_2	2 (SE 0.3)	None
GXH-38B	c[Gly-Cpg-D-Nal(2 μ)-Arg-Trp-Glu]-Val-Val-Gly-NH_2	> 1000	43 (SE 5)

[a] IC_{50} is the competitive binding in the presence of 500 pM of α-MSH. From [46].

Table 3

Bioassay results of selected analogues at human MC receptors.

Peptide	Sequence	EC_{50} or IC_{50}[a] (nM)		
		MC1	MC3	MC4
GXH-32B	c[β-Ala-His-D-Nal(2′)-Arg-Trp-Glu]-Val-Val-Gly-NH_2	75	3.8	77
GXH-38B	c[Gly-Cpg-D-Nal(2′)-Arg-Trp-Glu]-Val-Val-Gly-NH_2	12	44	1300

[a] EC_{50} for GXH-32B; IC_{50} for GXH-38B. From [46].