Peptides 31 (2010) 1894–1905

Contents lists available at ScienceDirect

Peptides

journal homepage: www.elsevier.com/locate/peptides

Solid-phase peptide head-to-side chain cyclodimerization: Discovery of C_2-symmetric cyclic lactam hybrid α-melanocyte-stimulating hormone (MSH)/agouti-signaling protein (ASIP) analogues with potent activities at the human melanocortin receptors☆

Alexander V. Mayorov[1], Minying Cai, Erin S. Palmer, Zhihua Liu, James P. Cain, Josef Vagner, Dev Trivedi, Victor J. Hruby*

Department of Chemistry and Biochemistry, University of Arizona, 1306 E. University Blvd., Tucson, AZ 85721, USA

ARTICLE INFO

Article history:
Received 21 April 2010
Received in revised form 23 June 2010
Accepted 23 June 2010
Available online 3 August 2010

Keywords:
Agouti-signaling protein
Melanocortin receptors
Cyclodimerization
C_2-symmetry
Macrocyclic peptide

ABSTRACT

A novel hybrid melanocortin pharmacophore was designed based on the pharmacophores of the agouti-signaling protein (ASIP), an endogenous melanocortin antagonist, and α-melanocyte-stimulating hormone (α-MSH), an endogenous melanocortin agonist. The designed hybrid ASIP/MSH pharmacophore was explored in monomeric cyclic, and cyclodimeric templates. The monomeric cyclic disulfide series yielded peptides with hMC3R-selective non-competitive binding affinities. The direct on-resin peptide lactam cyclodimerization yielded nanomolar range (25–120 nM) hMC1R-selective full and partial agonists in the cyclodimeric lactam series which demonstrates an improvement over the previous attempts at hybridization of MSH and agouti protein sequences. The secondary structure-oriented pharmacophore hybridization strategy will prove useful in development of unique allosteric and orthosteric melanocortin receptor modulators. This report also illustrates the utility of peptide cyclodimerization for the development of novel GPCR peptide ligands.

1. Introduction

Peptide cyclization is a well-established approach to improving peptide biological activity [38,53,59], which stems from reduced conformational freedom of the parent peptide and thus a better defined secondary structure required for efficient receptor–ligand interaction, *i.e.*, "bioactive conformation" [39,54]. It has been previously noted that the success of a peptide cyclization depends strongly on the probability of juxtaposition of the reactive groups of the linear peptide precursor, and is usually encumbered by side reactions, most notably, oligomerizations and cyclooligomerizations [20,57]. These side reactions become prevalent in syntheses of small strained cyclic peptides, such as *N*-unsubstituted tri- and tetrapeptides, typically incurring formation of cyclic dimers and trimers (Scheme 1) [57].

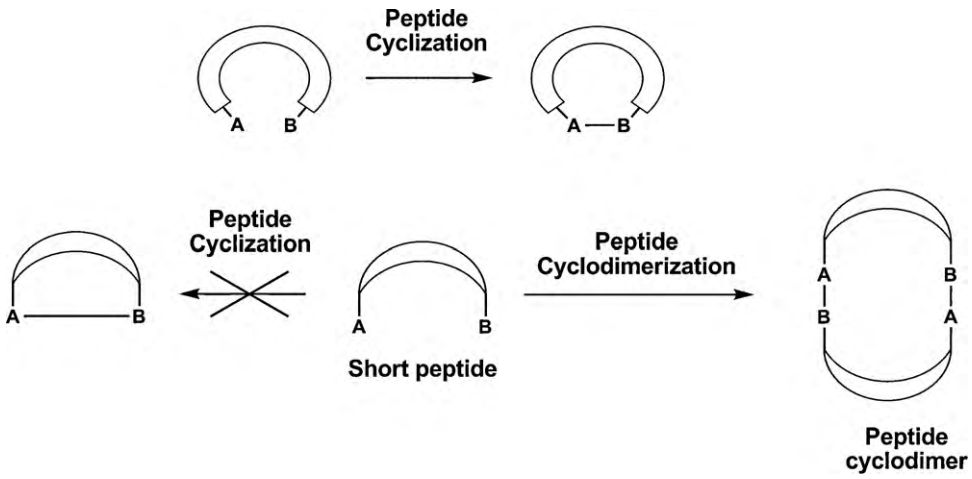
Although several peptide natural products, such as gramicidin S [27] and marine cyclodepsipeptide IB-01212 [19], are known to possess C_2-symmetry, there are few examples of peptide cyclodimerizations pertinent to development of peptides with enhanced biological activities, which include syntheses of cyclic biphalin [70] and morphiceptin [96] analogues. Thus, practical aspects of this area of peptide chemistry and its applications to development of novel GPCR ligands remain relatively unexplored. Recent reports on preparative peptide cyclodimerizations including syntheses of dimeric disulfides [76,80,91], depsipeptides [19], lactams [98], and peptide cyclodimerizations involving azide-alkyne cycloaddition "click chemistry" [14,62,77] prompted us to disclose our results on preparation of structurally unique C_2-

Abbreviations: All, allyl; AgRP, Agouti-related protein; ASIP, agouti-signaling protein; Boc, *tert*-butyloxycarbonyl; Fmoc, fluorenylmethoxycarbonyl; CH_3CN, acetonitrile; Cl-HOBt, 1-hydroxy-6-chlorobenzotriazole; DCM, dichloromethane; DIPEA, diisopropylethylamine; DMF, *N*,*N*-dimethylformamide; DIC, diisopropyl carbodiimide; HOBt, *N*-hydroxybenzotriazole; *h*MCR, human melanocortin receptor; *m*MCR, mouse melanocortin receptor; MSH, melanocyte-stimulating hormone; Nal(2′), 2′-naphthylalanine; Pbf, 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl; TFA, trifluoroacetic acid; SPPS, solid-phase peptide synthesis; RP-HPLC, reverse-phase high performance liquid chromatography; hMC3R, human melanocortin-3 receptor; α-MSH, α-melanocyte-stimulating hormone Ac-Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val-NH_2; NDP-α-MSH, Ac-Ser-Tyr-Ser-Nle-Glu-His-*D*-Phe-Arg-Trp-Gly-Lys-Pro-Val-NH_2; *h*ASIP (116–123), human agouti-signaling protein c [Cys-Arg-Phe-Phe-Arg-Ser-Ala-Cys]; MT-II, Ac-Nle-c [Asp-His-*D*-Phe-Arg-Trp-Lys]-NH_2.

☆ Abbreviations used for amino acids and designation of peptides follow the rules of the IUPAC-IUB Commission of Biochemical Nomenclature in J. Biol. Chem. 1972, 247, 977–983.

* Corresponding author. Tel.: +1 520 621 6332; fax: +1 520 621 8407.
E-mail address: hruby@u.arizona.edu (V.J. Hruby).
[1] Current address: Department of Chemistry, The Scripps Research Institute, La Jolla, CA 92037, USA.

0196-9781/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.peptides.2010.06.026



Scheme 1. Peptide cyclization and cyclodimerization.

symmetrical hybrid analogues of α-MSH/agouti-signaling protein *via* solid-phase head-to-side chain lactam cyclodimerization, and their biological evaluation at the human melanocortin receptors.

The melanocortin system [16,17,25] remains a challenging target for rational peptide and peptidomimetic design since the 3D-topographical requirements for the specific melanocortin receptor subtype recognition have not been fully elucidated [28,33,100]. At the same time, the numerous multifaceted physiological functions of the five known subtypes of human melanocortin receptors (hMC1-5R), including skin pigmentation [15,32], control of the immune system [15,32], erectile function [25,55,99,100], blood pressure and heart rate [60,74], control of feeding behavior and energy homeostasis [6,16,22,23,94,95,100,102], modulation of aggressive/defensive behavior [71,72], and mediation of pain [50,69,97], continue to provide a strong stimulus for development of potent and selective melanocortin agonists and antagonists. Several general approaches to development of such compounds have been described in literature [9,24,40,41], and include: (a) *D*-amino acid scan/unnatural amino acid substitutions in linear α-, β- and γ-MSH-derived sequences [29,83]; (b) hybridizations of the native MSH sequences with each other and with sequences of other bioactive peptides [4,8,34]; (c) implementation of various global and local conformational constraints *via* peptide cyclizations and employment of constrained amino acids [1,5,7,30,31,65,66,82]; and (d) manipulation of steric factors that influence receptor–ligand interactions [3,64,65]; as well as (e) construction of small molecules based on β-turn peptidomimetics and "privileged structure" scaffolds [10,73,79,85,89]. Until recently, this work had primarily been focusing on the hMC4R due to its direct involvement in the regulation of feeding behavior and energy homeostasis [6,16,22,23,94,95,100,102], as well as sexual behavior [25,55,93,99,100]. The hMC3 receptor has been acknowledged to play a complementary role in weight control [6,21,102], and current reports suggest that hMC3R is an inhibitory autoreceptor on POMC neurons [16] based on the observed stimulation of food intake by peripheral administration of an MC3R-selective agonist [63], and MC3R agonist-induced inhibition of spontaneous action of POMC neurons [18], although the full scope of physiological functions of this receptor is still poorly understood. Development of selective ligands for the hMC1 and hMC5 receptors is also receiving some attention lately due to the roles of these receptors in regulation of skin pigmentation [15,32] and control of the immune system [15,32] (hMC1R), and in regulating exocrine gland function [13], and coordinating central and peripheral signals for aggression (hMC5R) [71,72].

Agouti-signaling protein (ASIP) [61] and agouti-related protein (AgRP) [75] (Fig. 1) were originally described as endogenous antagonists for the melanocortin-1 and melanocortin-3/-4 receptors, respectively, although recent reports ascribe inverse agonist activity to both proteins [11,36]. There is some evidence that AgRP mediates orexigenic signaling of ghrelin [12], which makes it an important regulator of feeding behavior [44]. Initial structure–function relationship studies have attributed the melanocortin receptor antagonist activities to the Arg-Phe-Phe tripeptide pharmacophore, which is a part of the central loop within the inhibitor cystine knot (ICK) motif in both agouti proteins [68], as displacement of these three key amino acids with alanine within the AgRP/ASIP pharmacophore sequence results

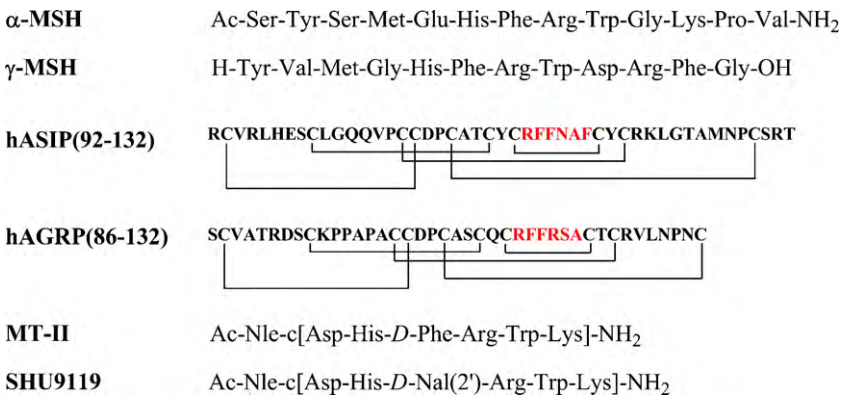


Fig. 1. Sequences of some endogenous and synthetic melanotropin peptides.