JPP Journal of Pharmacy and Pharmacology

JPP 2001, 53: 949–953

Received December 19, 2000
Accepted April 1, 2001
ISSN 0022-3573

Conformational analysis of tripeptide Ac-Lys-Pro-Val-NH_2, COOH-terminal sequence of alpha-MSH

Philippe Chavatte, Saïd Yous, Daniel Lesieur and Jean-Pierre Hénichart

Abstract

Alpha-melanocyte stimulating hormone (alpha-MSH) is an endogenous linear tridecapeptide which interacts with the melanocortin receptors (MC1-R to MC5-R) to mediate its biological effects. Antipyretic and anti-inflammatory activities of alpha-MSH are due to the COOH-terminal peptide sequence, Lys-Pro-Val (alpha-MSH[11–13]). This tripeptide might be useful as a therapeutic agent in the control of fever and inflammatory reactions. With this aim, a theoretical conformational study of the tripeptide has been carried out using molecular dynamics. The obtained conformational space has been classified into families according to the letter-code convention to partition the ϕ–ψ map. The lowest energy conformations of each family were used as templates to design six models of conformationally constrained non-peptide analogues.

Introduction

Alpha-melanocyte stimulating hormone (alpha-MSH), or alpha-melanotropin, is an endogenous linear tridecapeptide (Ac-Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val-NH_2) that is responsible for skin pigmentation in most vertebrates and has been reported to enhance the metastatic behaviour of melanoma cells in man (Cone et al 1996; Abdel-Malek et al 1999). Alpha-MSH is found in the brain where it acts to suppress appetite (Vergoni & Bertolini 2000). It also modulates fever (Richard & Lipton 1984; Huang et al 1998) and all forms of inflammation by acting on peripheral inflammatory cells, glial inflammatory cells, and on central receptors that activate descending anti-inflammatory neural pathways (Hiltz & Lipton 1990; Macaluso et al 1994). The biological effects are mediated by binding of the hormone to the melanocortin receptors (MC1-R to MC5-R) that have been identified and cloned (Wikberg et al 2000). Much research has been dedicated to obtaining either agonists or antagonists of the different subtypes (Sahm et al 1994a; Hadley et al 1996; Hruby et al 1998; Nikiforovich et al 1998). The COOH-terminal sequence, the Lys-Pro-Val (alpha-MSH[11–13]) tripeptide, is more important for binding and biological activities than the NH_2-terminal sequence (Sahm et al 1994b; Schiöth et al 1997) and is responsible for the antipyretic and anti-inflammatory activities of alpha-MSH (Richard & Lipton 1984; Hiltz & Lipton 1990). This tripeptide might be useful as a therapeutic agent in the control of fever and inflammatory reactions. However, peptides are limited in their use as drugs because they show metabolic instability, poor bioavailability and relative selectivity for receptors. From the conformational knowledge of the bioactive peptides when

Institut de Chimie Pharmaceutique Albert Lespagnol, 3 rue du Professeur Laguesse, BP 83, 59006 Lille Cédex, France

Philippe Chavatte, Saïd Yous, Daniel Lesieur, Jean-Pierre Hénichart

Correspondence: P. Chavatte, Institut de Chimie Pharmaceutique Albert Lespagnol, 3 rue du Professeur Laguesse, BP 83, 59006 Lille Cédex, France.

Acknowledgment: We thank Prof. Gérard Vergoten for his critical reading of the manuscript and many valuable suggestions.

bound to the receptor, a design of analogue non-peptidic structures that can mimic the functions of bioactive peptides may be considered (Hruby & Balse 2000). Biologically active conformation can be attempted in two complementary ways: using theoretical methods such as molecular modelling and experimental methods such as vibrational spectroscopies (infrared and Raman), NMR or X-ray crystallography. Using molecular modelling techniques, the possible conformations adopted by a flexible molecule may be obtained, particularly those of lowest energy and the most different from a structural point of view. Molecular dynamics, which simulates the motions expected in a molecule, provides a useful means of exploring the conformational space of flexible peptides such as alpha-MSH (Chan et al 1996; Al-Obeidi et al 1998; Pascutti et al 1999; Prabhu et al 1999a, b). In this paper, we report the theoretical conformational study for the Ac-Lys-Pro-Val-NH_2 tripeptide, COOH-terminal sequence of alpha-MSH, using molecular dynamics. Theoretically predicted structures of this tripeptide constituted the starting point for the design of non-peptidic compounds. Peptide side chains were constrained to fulfil the three dimensional arrangement given by the peptidic template.

Materials and Methods

Molecular modelling studies were performed using the SYBYL software version 6.2 running on Silicon Graphics Indigo 2 workstation. The starting conformation for dynamics simulation was built from the standard fragments library and the geometry was subsequently optimized using the all-atom Kollmann force field (Weiner et al 1984, 1986) including the electrostatic term. The partial charges were calculated by the semi-empirical MOPAC package version 6.0 using the AM1 hamiltonian. The Powell algorithm of the Maximin2 procedure was used for energy minimization until gradient value smaller than 0.005 kcal mol^{-1} Å. The molecular dynamics simulation was performed at constant temperature (300 K) in-vacuo for a total 600 ps period. The target temperature was achieved by slowly heating the system in stages of 50 K for 4 ps. The atomic velocities were initialized in a Maxwell distribution consistent with the chosen interval temperature. The Verlet algorithm was used with a step of 0.001 ps and the bonds lengths were constrained to their equilibrium values using the SHAKE algorithm. The results (coordinates, energies and velocities) were collected every 0.025 ps during simulation. The last 500 ps (corresponding to 20000 conformers) were subjected to analysis. These numerous conformations have been classified using the COMPARE program implemented in DGEOM95 (Blaney et al 1995). COMPARE clusters a set of conformations (molecular dynamics trajectory) into families. It calculates all pairwise RMS least-squares fit errors between the conformers heavy atoms and generates a distance matrix from the RMS matrix. A set of nearest neighbours is extracted from the distance matrix and this list is clustered using the Jarvis-Patrick algorithm to group the conformations into families. An RMS value of 1.35 Å was taken as the upper limit value. The families' centres were retained as to represent the molecular dynamics trajectory and subjected to energy minimization. They were then ordered by increasing energy and the similarity between the minimized structures was evaluated by comparing the RMS measured between all heavy atoms. Equivalent conformations (with RMS < 0.40 Å) are discriminated; the remaining low-energy conformations are used for further investigations. All energies are expressed as $\Delta E = E–E°$, where E° is the energy corresponding to the most stable conformer.

Results and Discussion

The outcome produced by the COMPARE program for clustering the molecular dynamics trajectory consists of 191 conformations. After energy minimization, 155 duplicates are removed on the basis of their similarity with the lower energy conformation. The 36 remaining conformations spread over an energy range of 9.8 kcal mol^{-1} above the most stable one. These 36 conformations are grouped into 12 families according to the

Table 1 Conformers families of tripeptide Ac-Lys-Pro-Val-NH_2.

Family	Number of conformers	Code	E° (kcal mol^{-1})	ΔE (kcal mol^{-1})
1	5	DCC	−5.1	0.0
2	3	ECC	−2.8	2.3
3	5	DCA	−1.2	3.9
4	5	DCG	−0.9	4.2
5	2	EAC	−0.9	4.2
6	3	ECG	0.0	5.1
7	4	DAA	0.3	5.4
8	4	DAC	0.7	5.8
9	1	DAC	1.1	6.2
10	1	DAD	1.9	7.0
11	1	EAA	1.9	7.0
12	2	EAH	3.3	8.4

Table 2 Conformational groups for each amino-acid residue.

Lysine	Proline	Valine
25 D	15 A	10 A
11 E	21 C	15 C
		8 G
		1 D
		2 H

letter-code convention to partition the ϕ–ψ map (Zimmerman et al 1977) as shown in Table 1.

Table 2 shows the conformational groups found for each amino-acid residue according to their respective Ramachandran maps (Figure 1). It appears that lysine conformations are found exclusively in regions D (25/36) and E (11/36). The proline conformations are divided into group A (15/36) and group C (21/36) while the most representative groups for valine are A (10/36), C (15/36) and G (8/36).

Despite the lack of data concerning the structural requirement which is important for binding to the receptor, the backbones of the family heads were constrained to maintain the peptide side chains in their initial spatial orientations. The heads of family are stabilized by one (C_7 or C_{10}) or two (C_7) hydrogen-bonded rings which can be mimicked in several ways as shown in Table 3 and Figure 2.

The present study provided six different non-peptide models that are able to mimic the three-dimensional arrangement of the tripeptide representative conformations backbone. The position of the substituents allows the keeping in position of the spatial orientation of side chains that hold putative pharmacophoric features.

The stereochemistry of our non-peptide models must

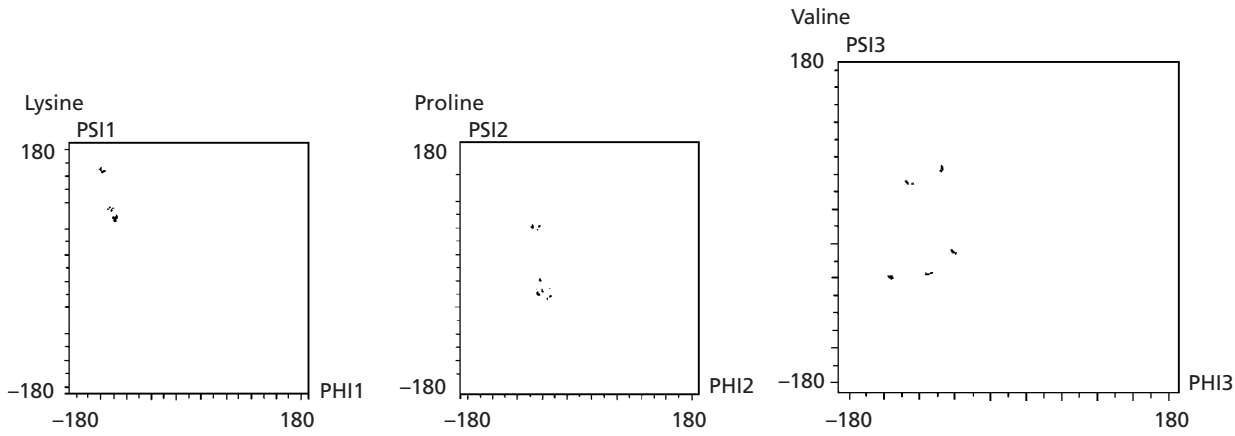
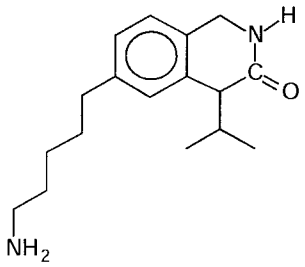


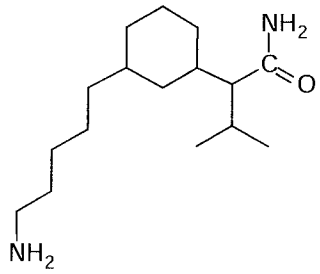
Figure 1 Ramachandran maps for each amino-acid residue.

Table 3 Hydrogen-bonded pseudo-cycle characteristics and mimetic rings for conformer families of tripeptide Ac-Lys-Pro-Val-NH_2.

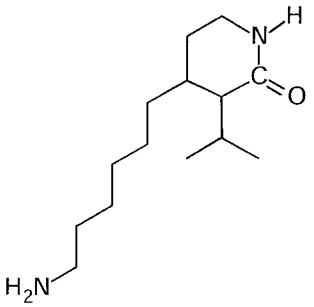
Family	Number of H-bonds	H-bond type	Pseudo-cycle	Mimetic ring
1	2	[Lys]C═O … HN[Val]	C_7	1,2,3,4-tetrahydroisoquinolein-3-one
2		[Pro]C═O … HN[NH_2]	C_7	
3	1	[Lys]C═O … HN[Val]	C_7	cyclohexyl
4				
5	1	[Pro]C═O … HN[NH_2]	C_7	piperidin-2-one
6	1	[Lys]C═O … HN[Val]	C_7	*N*-substituted piperidin-2-one
7	1	[Lys]C═O … HN[NH_2]	C_{10}	1,2,3,4-tetrahydroisoquinolein-3-one
10				
11				
12				
8	1	[Pro]C═O … HN[NH_2]	C_7	piperidin-2-one
9				



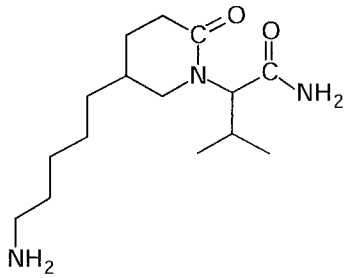
1 1,2,3,4-tetrahydroisoquinolein-3-one



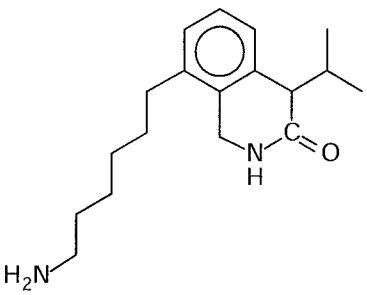
2 cyclohexyl



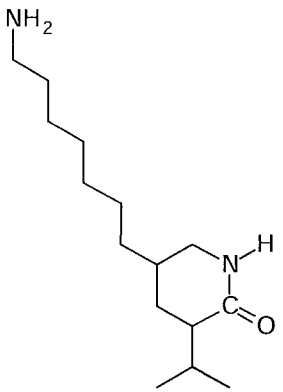
3 piperidin-2-one



4 *N*-substituted piperidin-2-one model



5 1,2,3,4-tetrahydroisoquinolein-3-one model



6 piperidin-2-one model

Figure 2 Non-peptide suggested models.

be considered since it determines the best superposition of side chains on the peptide templates. All models contain chiral centres. The two tetrahydroisoquinolein-3-one models (**1** and **5**) contain one chiral centre, respectively *S* and *R*, according to the preferred orientation of the isopropyl group that corresponds with the valine side-chain residue. The three piperidin-2-one models, (**3**, **4** and **6**), contain two chiral centres and we have retained the corresponding diastereoisomers (3*S*, 4*R*), (2*R*, 5′*S*) and (3*S*, 5*S*). The cyclohexyl model (**2**) presents three chiral centres (2*S*, 1′*S*, 3′*R*).

In conclusion, the conformational study using molecular dynamics allowed us to find several possible conformations for the Ac-Lys-Pro-Val-NH_2 tripeptide, COOH-terminal sequence of alpha-MSH. The synthesis of molecules derived from these theoretical

models, followed by their preliminary pharmacological evaluation, would be helpful to investigate the specific three-dimensional requirement necessary to obtain a biological response. The best compound might constitute a starting point for the design of novel antipyretic and anti-inflammatory drugs.

References

Abdel-Malek, Z., Suzuki, I., Tada, A., Im, S., Akcali, C. (1999) The melanocortin-1 receptor and human pigmentation. *Ann. NY Acad. Sci.* **885**: 117–133

Al-Obeidi, F., O'Connor, S. D., Job, C., Hruby, V. J., Pettitt, B. M. (1998) NMR and quenched molecular dynamics studies of superpotent linear and cyclic alpha-melanotropins. *J. Pept. Res.* **51**: 420–431

Blaney, J. M., Crippen, G. M., Dearing, A., Dixon, J. S., Spellmeyer, D. C. (1995) DGEOM95 - Distance geometry, QCPE program 590, Bloomington

Chan, N. C., Branch, S. K., Moss, S. H., Pouton, C. W. (1996) Molecular modelling of beta turns in a cyclic melanotropin. *J. Pharm. Pharmacol.* **48**: 218–222

Cone, R. D., Lu, D., Koppula, S., Vage, D. I., Klungland, H., Boston, B., Chen, W., Orth, D. N., Pouton, C., Kesterson, R. A. (1996) The melanocortin receptors: agonists, antagonists, and the hormonal control of pigmentation. *Recent Prog. Horm. Res.* **51**: 287–317

Hadley, M. E., Hruby, V. J., Jiang, J., Sharma, S. D., Fink, J. L., Haskell-Luevano, C., Bentley, D. L., Al-Obeidi, F., Sawyer, T. K. (1996) Melanocortin receptors: identification and characterization by melanotropic peptide agonists and antagonists. *Pigment Cell Res.* **9**: 213–234

Hiltz, M. E., Lipton, J. M. (1990) Alpha-MSH peptides inhibit acute inflammation and contact sensitivity. *Peptides* **11**: 979–982

Hruby, V. J., Balse, P. M. (2000) Conformational and topographical considerations in designing agonist peptidomimetics from peptide leads. *Curr. Med. Chem.* **7**: 945–970

Hruby, V. J., Han, G., Hadley, M. E. (1998) Design and bioactivities of melanotropic peptides agonists and antagonists: design based on a conformationally constrained somatostatin template. *Lett. Pept. Sci.* **5**: 117–120

Huang, Q. H., Hruby, V. J., Tatro, J. B. (1998) Systemic alpha-MSH suppresses LPS fever via central melanocortin receptors independently of its suppression of corticosterone and IL-6 release. *Am. J. Physiol.* **275**: R524–R530

Macaluso, A., McCoy, D., Ceriani, G., Watanabe, T., Biltz, J., Catania, A., Lipton, J. M. (1994) Antiinflammatory influences of alpha-MSH molecules: central neurogenic and peripheral actions. *J. Neurosci.* **14**: 2377–2382

Nikiforovich, G. V., Sharma, S. D., Hadley, M. E., Hruby, V. J. (1998) Studies of conformational isomerism in alpha-melanocyte stimulating hormone by design of cyclic analogues. *Biopolymers* **46**: 155–167

Pascutti, P. G., El-Jaik, L. J., Bisch, P. M., Mundim, K. C., Ito, A. S. (1999) Molecular dynamics simulation of alpha-melanocyte stimulating hormone in a water-membrane model interface. *Eur. Biophys. J.* **28**: 499–509

Prabhu, N. V., Perkyns, J. S., Pettitt, B. M. (1999a) Modeling of alpha-MSH conformations with implicit solvent. *J. Pept. Res.* **54**: 394–407

Prabhu, N. V., Perkyns, J. S., Pettitt, B. M., Hruby, V. J. (1999b) Structure and dynamics of alpha-MSH using DRISM integral equation theory and stochastic dynamics. *Biopolymers* **50**: 255–272

Richard, D. B., Lipton, J. M. (1984) Effect of alpha-MSH 11–13 (lysine-proline-valine) on fever in the rabbit. *Peptides* **5**: 815–817

Sahm, U. G., Qarawi, M. A., Olivier, G. W., Ahmed, A. R., Branch, S. K., Moss, S. H., Pouton, C. W. (1994a) Synthesis and biological evaluation of alpha-MSH analogues substituted with alanine. *Peptides* **15**: 1297–1302

Sahm, U. G., Olivier, G. W., Branch, S. K., Moss, S. H., Pouton, C. W. (1994b) Influence of alpha-MSH terminal amino acids on binding affinity and biological activity in melanoma cells. *Peptides* **15**: 441–446

Schiöth, H. B., Muceniece, R., Mutulis, F., Prusis, P., Lindeberg, G., Sharma, S. D., Hruby, V. J., Wikberg, J. E. (1997) Selectivity of cyclic [D-Nal^7] and [D-Phe^7] substituted MSH analogues for the melanocortin receptor subtypes. *Peptides* **18**: 1009–1013

SYBYL 6.2 – Molecular modeling software (1995), Tripos Inc., St Louis

Vergoni, A. V., Bertolini, A. (2000) Role of melanocortins in the central control of feeding. *Eur. J. Pharmacol.* **405**: 25–32

Weiner, S. J., Kollman, P. A., Case, D. A., Singh, U. C., Ghio, C., Alagona, G., Profeta, S., Weiner, P. (1984) A new force field for molecular mechanical simulation of nucleic acids and proteins. *J. Am. Chem. Soc.* **106**: 765–784

Weiner, S. J., Kollman, P. A., Nguyen, D. T., Case, D. A. (1986) An all atom force field for simulations of proteins and nucleic acids. *J. Comput. Chem.* **7**: 230–252

Wikberg, J. E., Muceniece, R., Mandrika, I., Prusis, P., Lindblom, J., Post, C., Skottner, A. (2000) New aspects on the melanocortins and their receptors. *Pharmacol. Res.* **42**: 393–420

Zimmerman, S. S., Pottle, M. S., Nemethy, G., Scheraga, H. A. (1977) Conformational analysis of the 20 naturally occurring amino acid residues using ECEPP. *Macromolecules* **10**: 1–9