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Molecular Modelling of β Turns in a Cyclic Melanotropin

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Abstract

α-Melanocyte stimulating hormone (α-MSH) is a tridecapeptide which interacts with a family of G protein-coupled receptors, the melanocortin receptors, to cause its biological effects.

We have modelled the low energy conformations of the α-MSH derivatives as part of a project to probe the receptor binding conformation of melanocortins, and also to design ligands for targeting cytotoxic drugs to MC1 receptors expressed by melanoma cells. Here we report a molecular dynamics study of β turns in a cyclic lactam analogue [Nle^4, Asp^5, D-Phe^7, Lys^{10}]α-MSH.

The data show that it is possible for a β turn to exist in the ring portion of this molecule which contains the melanocortin conserved sequence -His-Phe-Arg-Trp-, even though the lowest energy conformers lack a β turn.

Melanocortins are known for their role in control of melanocyte pigmentation, and stimulation of glucocorticoid production in the adrenal cortex (by adrenocorticotropic hormone, ACTH). In addition these hormones have been reported to participate in a variety of other physiological functions, including analgesia, thermoregulation, control of the cardiovascular system, higher cortical functions such as attention, learning, and memory, immuno-modulation, and foetal development and parturition (Clark et al 1978; Cannon et al 1986; Walker et al 1990).

α-Melanocyte stimulating hormone (α-MSH) is a tridecapeptide that is widely distributed within the CNS (O'Donohue & Dorsa 1982) and other regions of the body (Eberle 1988). Receptors which bind this peptide are also widely distributed (Tatro & Reichlin 1987). Recent molecular cloning experiments have revealed that there are at least five related melanocortin receptors, three of which are found in the central nervous system (Mountjoy et al 1992; Chhajlani & Wikberg 1992; Mountjoy et al 1992; Roselli-Rehfuss et al 1993; Chhajlani et al 1993; Gantz et al 1993a, b; Chhajlani et al 1993; Roselli-Rehfuss et al 1993). The roles and significance of these receptors are yet to be determined. Although the physiological role of α-MSH in the periphery of humans is still poorly understood, the presence of specific MC1 receptors for the hormone on human melanoma cells is of interest in diagnosis and treatment of melanoma.

All melanocortins, including α-MSH, have a conserved sequence -His-Phe-Arg-Trp- which is essential for ligand binding and biological activity, although both the Phe and Trp residues can be replaced by their D-isomers without loss of activity (Sugg et al 1986). The structure-activity relationships within a variety of linear α-MSH analogues have been studied using both bioassays and binding and activity assays (Eberle 1988), but a breakthrough came when cyclic compounds were investigated. Initially these were cyclised by disulphide bonds between two cysteines as in [Cys^4, Cys^{10}]α-MSH (Eberle 1988). However one cannot be confident of the stability of these compounds so a further advance was made when lactam cyclized analogues were shown to be active in frog and lizard skin bioassays (Al-Obeidi et al 1989). These compounds have not been studied using mammalian cell culture systems but are expected to be active at the corresponding mammalian MC1 receptor. The cyclic compounds being more constrained by the cyclised ring are valuable leads for the discovery of new compounds. Molecular modelling studies can also make use of the constraints implied by the ring structure which restricts the conformational space when contrasted with that available to the linear compounds.

Here we report a modelling study of a cyclic lactam analogue of α-MSH. We regard this as a useful activity as a prelude to modelling the docking of melanocortins with models of the melanocortin receptors. In particular we were keen to examine the likelihood that α-MSH analogues adopt a β-turn in the active conformation. A β-like structure has been proposed to exist in the conserved region in solution (Sugg et al 1986, 1988) and a molecular dynamics study by our group on the [Cys^4, Cys^{10}]α-MSH analogue revealed a β turn in a low energy conformation (unpublished results). This has encouraged us to model a side-chain amide cyclized (lactam) analogue of α-MSH, [Nle^4, Asp^5, D-Phe^7, Lys^{10}]α-MSH, first synthesised by Hruby's group (Al-Obeidi et al 1989), to investigate the effect of introducing β turns at different positions in the molecule on its overall energy, making use of molecular dynamics simulations. This lactam derivative includes a 23-membered ring of atoms involving six amino acid residues.

Methods

Modelling was performed on a Silicon Graphics Iris Indigo R3000 workstation with Biosym's INSIGHT II and DISCOVER molecular modelling packages using the CVFF forcefield (Dauber et al 1981).

Fig. 1 shows the naming and numbering scheme for the amino acid residues of [Nle^4, Asp^5, D-Phe^7, Lys^{10}]α-MSH

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Ac-Ser[1]-Tyr[2]-Ser[3]-Nle[4]-Asp[5]-His[6]-D-Phe[7]-Arg[8]-Trp[9]-Lys[10]-Lys[11]-Pro[12]-Val[13]-NH_2

Ac[1]-Ser[2]-Tyr[3]-Ser[4]-Nle[5]-Csp[6]-His[7]-D-Phe[8]-Arg[9]-Trp[10]-Lac[11]-Lys[12]-Pro[13]-Val[14]-NH_2

FIG. 1. Sequence of [Nle[4], Asp[5], D-Phe[7], Lys[10]]α-MSH. The upper sequence shows the conventional numbering and the lower sequence shows the numbering adopted by the modelling program.

adopted during this modelling study. The modelling program occasionally requires different abbreviations and numbering to those conventionally applied to peptides. This numbering system is used in graphical presentation of structures. Csp and Lac refer to aspartic acid and lysine respectively which form the lactam bridge. The conformational space of [Nle[4], Asp[5], D-Phe[7], Lys[10]]α-MSH was explored by molecular mechanics techniques.

The strategy adopted was to model the ring structure alone during initial experiments to generate low energy structures of the ring. The N- and C- terminals were then added in the second phase of the study.

The ring residues were first assembled using the fragment library within INSIGHT and then the required turns introduced at three separate positions, namely Csp, His and D-Phe. For clarity, these structures are referred to as A, B and C respectively. An intramolecular amide bond between Csp and Lac was formed by adjusting the backbone and the side chain (Csp and Lac) torsion angles. This initial structure was energy minimized by 200 iterations of steepest descents with no cross-term energies and morse potential for bond energies and then conjugate gradients until the maximum derivative was less than 0·005 kcal mol^{-1} $Å^{-1}$ to relieve any steric interactions. Forcing was used to maintain the dihedral angles, (ϕ, φ), of the middle residues to the required turn with 20 kcal rad^{-2}. The minimized structure was then initialized for dynamics using 1000 iterations at 750 K before running a constrained molecular dynamics trajectory at 750 K for 20 ps. Structures were isolated at 1 ps intervals and energy minimized under the conditions mentioned above. The dielectric constant used for the Coulombic interactions is set to 1·000000. Data for the lowest energy structures for each type of turn are presented in Table 1 and the lowest energy structures are highlighted in bold. The N-terminal (Ac-Ser[1]-Tyr[2]-Ser[3]-Nle[4]-) and the C-terminal (-Lys[11]-Pro[12]-Val[13]-NH_2) were then added to each lowest energy structure obtained for A, B and C, and the initial minimisation and dynamics trajectory repeated under the conditions described above.

Based on the results from Table 2, which show that structures A and C had the lowest energies when modelled as full length peptide, we decided to look at a particular starting conformation which included two consecutive β turns. This structure is referred to as D and has a type II β turn starting at Asp and a type I β turn at D-Phe. The results were compared with those obtained with structure E which had no turn introduced. The dynamics trajectory of the whole peptide E ran for 60 ps instead of 20 ps. Finally the minimized structures A to D were further minimized without torsion forcing under the conditions mentioned above to see whether the β turns would survive in the absence of the constraints.

Results

β turns contain four residues with a hydrogen bond between the i and i + 3 amino acids and are classified according to the sign and magnitude of the dihedral angles (ϕ, φ), of the middle residues. The chirality of the residues in the second and third positions of a β turn affects the conformation of the turn. If both are of the L-configuration, a type I turn is preferred, whereas a type II or II′ turn can result from an LD or DL pair respectively (Rose et al 1985).

The [Nle[4], Asp[5], D-Phe[7], Lys[10]]α-MSH structure was modelled in stages, using the strategy described above. Table 1 shows the lowest energy structures for each type of turn. There are no energy values for type IVa and IVb turns in structure A as the distance between the side chains of Csp and Lac is too great to form an intramolecular amide bond. Table 2 shows the lowest energy of both the ring residues and the corresponding full length peptides and the types of turn associated with structures A to E.

Structure A with an LD pair of amino acids included a type II turn in the lowest energy structure for the ring. Structure C with an LL pair included a type I turn. For structure B with a DL pair of amino acids, the lowest energy structure had a type III turn but the results indicated that the energy of a type II′ turn was only 0·5 kcal mol^{-1} higher and type I only 2·7 kcal mol^{-1} higher in energy.

Table 3 shows the ϕ, φ torsion angles of the ring residues

Table 1. Energy of [Asp[5], D-Phe[7], Lys[10]]α-$MSH_{(5-10)}$ with different types of turns.

	Residues in turn	Energy (kcal mol^{-1})									
		I	I′	II	II′	III	III′	IVa	IVb	γ	Inverse γ
A	Asp[5], His[6], D-Phe[7], Arg[8]	74·6	74·1	65·6	75·0	78·1	73·3	–	–	72·8	78·7
B	His[6], D-Phe[7], Arg[8], Trp[9]	67·8	73·4	72·4	65·6	65·1	76·0	71·3	72·3	69·6	72·3
C	D-Phe[7], Arg[8], Trp[9], Lys[10]	62·3	73·7	72·2	66·9	68·1	78·5	70·7	75·6	68·6	67·9

Table 2. Lowest energy of the ring residues and the whole peptide of [Nle^4, Asp^5, D-Phe^7, Lys^{10}]α-MSH with constrained β turns.

	Residues in turn	Type of turn	Energy (kcal mol^{-1})	Energy of whole peptide (kcal mol^{-1})
A	Asp^5, His^6, D-Phe^7, Arg^8	II	65·6	203·8
B	His^6, D-Phe^7, Arg^8, Trp^9	III	65·1	209·2
C	D-Phe^7, Arg^8, Trp^9, Lys^{10}	I	62·3	203·6
D	Asp^5, His^6, D-Phe^7, Arg^8	II, I*	58·2	205·0
E	[Nle^4, Asp^5, D-Phe^7, Lys^{10}]α-MSH	–	64·2	206·9

Table 3. Torsion angles φ and ψ (degrees) for Asp, His, D-Phe, Arg, Trp, Lys residues of the minimized conformations.

Residues in turn	Torsion angle											
	Asp^5		His^6		D-Phe^7		Arg^8		Trp^9		Lys^{10}	
	φ	ψ	φ	ψ	φ	ψ	φ	ψ	φ	ψ	φ	ψ
A Asp^5, His^6, D-Phe^7, Arg^8	−83·6	35·6	−64·7	121·9	83·4	−3·8	−153·6	74·8	−83·3	74·7	−87·0	−42·6
B His^6, D-Phe^7, Arg^8, Trp^9	−134·2	83·7	−112·0	149·7	−54·1	−28·7	−62·1	−29·4	−126·6	−39·5	−142·2	124·9
C D-Phe^7, Arg^8, Trp^9, Lys^{10}	67·6	−75·7	−73·8	82·1	151·3	−106·0	−63·1	−31·4	−86·9	−11·7	−98·7	−53·0
D Asp^5, His^6, D-Phe^7, Arg^8	78·3	−92·7	−57·0	116·5	77·5	−7·3	−74·7	−56·1	−108·4	− 7·8	−83·7	−55·3
E [Nle^4, Asp^5, D-Phe^7, Lys^{10}]α-MSH	−96·3	110·3	−83·0	83·1	87·2	−114·1	72·6	−88·3	−153·9	76·4	−108·8	−63·2

Table 4. Torsion angles φ and ψ (degrees) for Asp, His, D-Phe, Arg, Trp, Lys residues of the minimized conformations without torsion forcing.

Residues in turn	Torsion angle											
	Asp^5		His^6		D-Phe^7		Arg^8		Trp^9		Lys^{10}	
	φ	ψ	φ	ψ	φ	ψ	φ	ψ	φ	ψ	φ	ψ
A Asp^5, His^6, D-Phe^7, Arg^8	−90·1	34·6	−74·9	120·7	97·6	−73·2	−79·4	79·2	−86·2	76·6	−86·8	−45·7
B His^6, D-Phe^7, Arg^8, Trp^9	−137·1	82·8	−112·9	146·9	−57·2	−14·0	−69·1	−30·9	−124·9	−40·7	−142·4	125·6
C D-Phe^7, Arg^8, Trp^9, Lys^{10}	66·4	−77·4	−87·8	84·4	154·3	−71·7	−88·6	−27·3	−83·2	−48·0	−65·2	−42·7
D Asp^5, His^6, D-Phe^7, Arg^8	77·1	−85·4	−53·3	114·4	74·4	16·6	−84·2	−75·6	−88·4	−22·2	−73·3	−54·9

of the minimized conformations and Fig. 2 shows the stereo views of each minimized full length peptide. Although we used constrained molecular dynamics to maintain a required β turn, distorted turns (i.e. with torsion angles greater than 20° from the normal) were still found. The torsion angles of Arg and Trp in structure D with two consecutive turns deviated from the standard angles by approximately 30°. Analysis of the torsion angles of structure E suggests no evidence of a β turn, although the energies of the structures containing β turns are within 5 kcal mol^{-1} of this structure. Table 4 shows the φ, ψ torsion angles of the ring residues of the minimized conformations without torsion forcing. In Table 5, these data are compared with forced experiments. Table 5 shows the energy of the full length peptide after minimization without torsion forcing, and the difference in energy between this and the forced structure. These data show that the β turns disappeared after the torsion forcing was removed, except in the case of structure B. However the difference in energy was never more than 6 kcal mol^{-1} after the torsion forcing had been removed.

Table 5. Lowest energy of the whole peptide of [Nle^4, Asp^5, D-Phe^7, Lys^{10}]α-MSH without torsion forcing and the difference in energy when compared with the one with torsion forcing.

	Residues in turn	Energy of whole peptide (kcal mol^{-1})	Difference in energy (kcal mol^{-1})
A	Asp^5, His^6, D-Phe^7, Arg^8	198·0	5·8
B	His^6, D-Phe^7, Arg^8, Trp^9	208·4	0·8
C	D-Phe^7, Arg^8, Trp^9, Lys^{10}	197·8	5·8
D	Asp^5, His^6, D-Phe^7, Arg^8	201·1	3·9

Discussion

Certain pairs of amino acid configurations are classically associated with particular β turns; however, in other small cyclic peptides, aberrant turns have been found in crystal structures (Karle et al 1988). The usual conventions may not apply due to steric constraints imposed by the ring. For this reason we have compared all the classic β turns at several positions in the ring moiety of the lactam peptide to search for low energy structures.

Although the dynamics ran only for 20 ps, the results show that it is possible that a β turn is present in low energy structures of this lactam peptide; a type II turn is predicted at Asp and a type I turn at D-Phe as expected across LD and LL pairs of amino acid respectively. The expected turn across a DL pair (type II′) was only slightly higher in energy than the lowest energy type III turn found for a β turn at His. A double turn gave an even lower energy structure when only the ring was modelled.

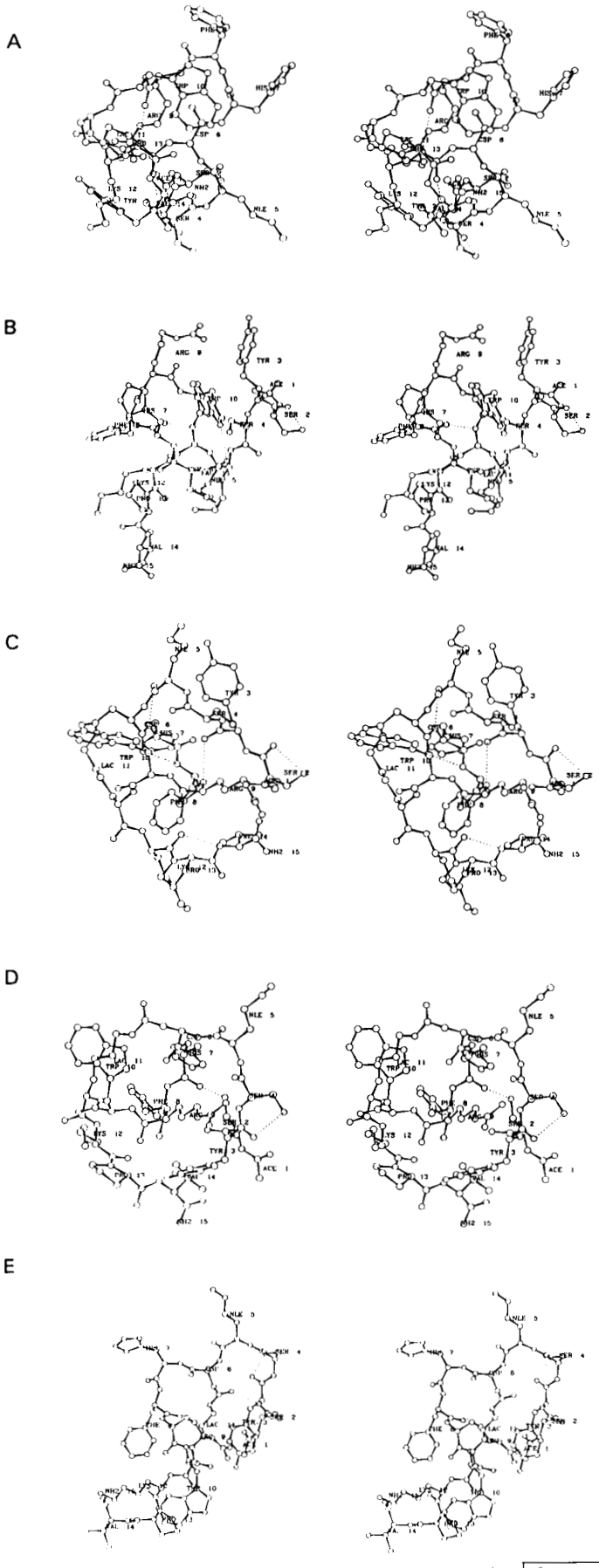

FIG. 2. Stereoviews of energy-minimized conformations for structures A to E of [Nle^4, Asp^5, D-Phe^7, Lys^{10}]α-MSH after the molecular dynamics simulations (only heavy atoms are shown for clarity). The figure is in cross-eyed stereo. Hydrogen bonds are indicated by dotted lines.

From this study, we have found that structures A and C had the lowest energy, followed by those with double turns (structure D) or without any turn (structure E). When the minimum energy structures were subjected to a second energy minimization in the absence of torsion constraints, another energy minimum was reached in which β-turns were absent. It is generally recognised that differences of up to 5 kcal mol^{-1} can be accommodated in ligand-receptor binding interactions. After removal of torsion constraints, structure B adopted a conformation only 0·8 kcal mol^{-1} lower in energy. The relevant energy differences for structures A and C were both larger at 5·8 kcal mol^{-1}, and it is noticeable that these were the overall lowest energy structures. Our interpretation of these data is that the energy differences are too small to rule out the possibility that a β-turn could be present in the active conformation of cyclic lactam melanocortins, although it is clear that the lowest energy structures found lacked a β-like structure in the conserved ring region. Further studies will be necessary to draw a firm conclusion on the involvement of β-turns in ligand binding.

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