Melanin Concentrating Hormone; Molecular Modelling and Experimental Analysis of Conformation

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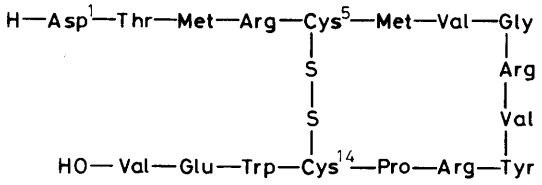
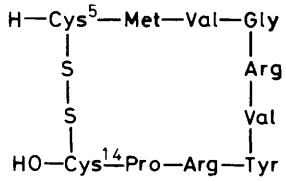
Molecular dynamics analyses and associated energy minimizations for the peptide hormone MCH and the related cyclic decapeptide MCH(5—14) suggest that it may access two major families of conformations; experimental support comes from n.m.r. studies of MCH(5—14).

Melanin concentrating hormone (MCH) (**1**) is a neuropeptide produced in the hypothalamus. In teleosts it serves to concentrate melanin within the pigment cells of the skin.[1—3] It also induces melanosome dispersion within tetrapod melanophores.[4] MCH also acts as a potent pituitary hormone, inhibiting the release of ACTH in mammals,[5] and stimulating growth hormone release in rats.[6]

As the first phase in elucidating the shape and charge characteristics of MCH receptors we have performed theoretical analyses of the conformational features of the cyclic decapeptide, MCH(5—14) (**2**), and of the intact peptide (**1**). Additionally, we have synthesized these two peptides,[7] and a range of fragments, for biological assay and for n.m.r. analysis of conformation. In this communication we report the results of the theoretical predictions from molecular dynamics analyses, together with structural information obtained from n.m.r. spectroscopy.



(**1**)



(**2**)

Molecular dynamics methods have been employed recently to reveal accessible conformations and to characterize the dynamic conformational transitions of proteins and peptide hormones.[8] We therefore applied molecular dynamics simulations and energy minimization procedures using a valence force field software package for MCH and for the cyclic decapeptide MCH(5—14). In the valence force field,[9] the potential energy of a molecular system is represented as a sum of the internal or valence degrees of freedom and the interatomic distances. The analytical expression of the potential energy[10] includes (a) strain energies arising from deformations of internal co-ordinates (like bond length, bond angle, and torsion angle) and 'cross terms' caused by coupling between deformations of two or more internals and (b)

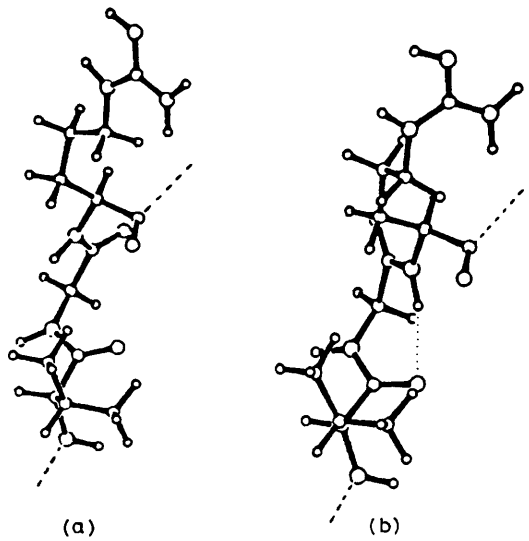


Figure 1. Region of the peptide around Val[7]-Gly[8]-Arg[8] when Gly is in conformations which are (a) an α-helical and (b) a γ turn.

Table 1. Chemical shift assignments for MCH(5—14) in DMSO solution at 293 K.

	NH	α	β	γ	δ	ε
5 Cys		4.54	~3.0			
6 Met	—[b]	4.52	1.90, 2.09	2.96, 2.56		
7 Val	7.73	4.12	1.90	0.83		
8 Gly	8.41	3.90, 3.65				
9 Arg[a]	7.83	4.55	1.78, 1.55	2.40	~3.1	9.23 or 8.70
10 Val	8.10	4.10	1.92	0.70		
11 Tyr	8.16	4.52	2.90, 2.78			
12 Arg[a]	8.12	4.17	1.77, 1.52	2.43	~3.2	9.23 or 8.70
13 Pro		4.33	2.02, 1.97	2.40	~3.5	
14 Cys	8.08	3.98	2.92, 3.24			

[a] Assignment uncertain. [b] Cross-peak absent.

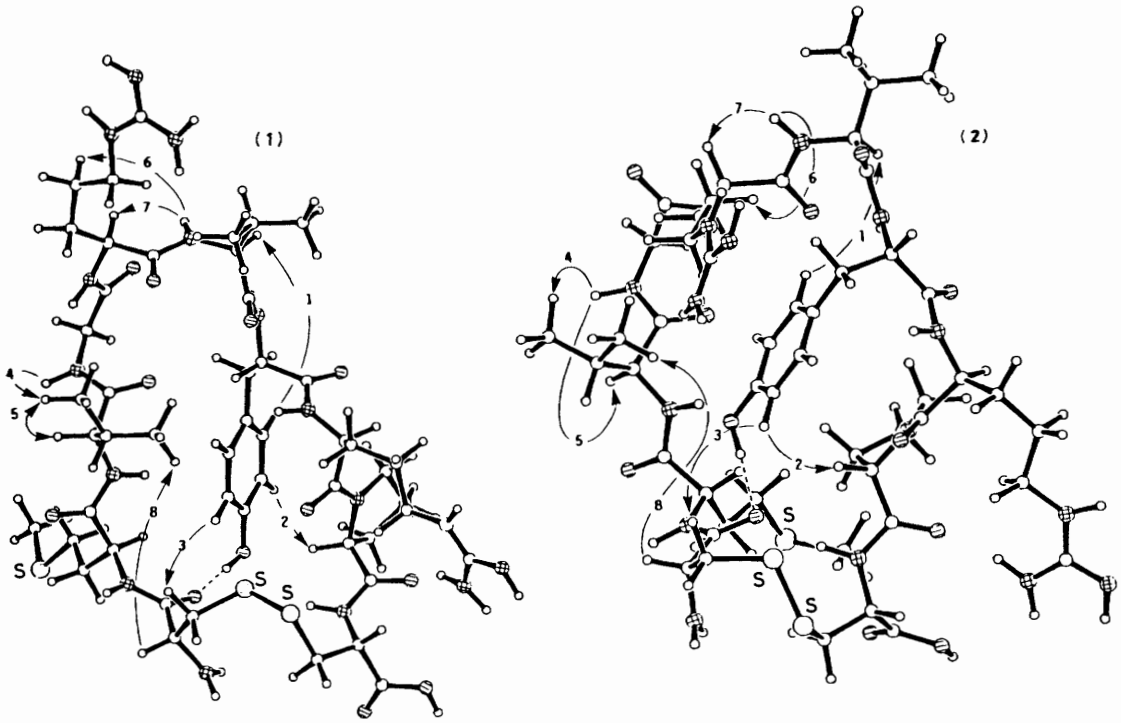


Figure 2. Typical conformations of MCH(5—14) showing n.O.e.s.

interaction energies which are a result of the exchange repulsion, dispersion, and coulomb interactions between the non-bonded atoms. The hydrogen bond energy term is represented as a general non-bonded interaction but with specific parameters. A fixed dielectric with a dielectric constant of 1 was used in the calculations. The parameters included in the various energy terms have been determined by fitting experimental crystal structure data, sublimation energies, molecular dipole moments, vibrational spectra, and strain energies of small organic compounds. *Ab initio* molecular orbital calculations have also been used to provide information on charge distributions, energy barriers, and coupling terms.[11] The effect of solvent has not been modelled. Thus, given the potential energy of a molecular system, the force exerted on each atom by all the other atoms in the system is defined and consequently the equations of motion can be solved. This yields a detailed description of the dynamical behaviour of the system. The conformations accessed at different instances in the dynamics trajectory can then be minimised and studied. Here main chain torsion angles of energy minimized conformations were analysed at picosecond intervals along a dynamics trajectory of 50 picoseconds. Each conformation was minimized to a maximum first derivative of less than 0.05 kcal/Å (cal = 4.184 J) and (ϕ/ψ) angles analysed for all amino acids.

The initial conformation of MCH was built on an E&S PS330 picture system, using the INSIGHT package. The residues in the two tails (*i.e.* residues 1—4 and 15—17) were then removed to generate the initial structure of cyclic MCH(5—14). This structure was partially minimised (to a maximum first derivative of 0.5 kcal mol^{-1} $Å^{-1}$) to relieve excessive strain and eliminate unrealistic motion. Initial random velocities consistent with a Maxwell–Boltzmann distribution for an average temperature of 300 K were assigned to each of the atoms. Molecular dynamics simulations with a time step of 10^{-15} s were performed for a total time period of 50 picoseconds (50×10^{-12} s). The results of the simulations showed some interesting structural features. Firstly, the maximum conformational change occurred in the Gly^8–Arg^9 region of the peptide. Transitions between two conformational states persisted throughout the simulations. The Gly^8 residue existed in either a γ turn (−80,50) or an α-helical (−70,−40) type of conformation (Figure 1). The Arg^9 residue also existed in two conformations, with (ϕ,ψ) values of (−155,100) and (−80,100). In other words the φ of Gly^8 and the ψ of Arg^9 do not undergo much change, whereas the ψ of Gly^8 and the φ of Arg^9 undergo concerted transitions. Whenever the near type I Gly^8–Arg^9 β-turn occurs the Arg^9–Val^{10} forms a near type II β-turn. The other mobile residue in this region is Val^7. Met^6 and Val^7 exist mainly in a left-handed helix or in a β-sheet structure.

Regions of steric constraint emerged from the simulation. Val^{10} to Cys^{14} showed little change, Tyr^{11} to Cys^{14} being in a stable helix with (ϕ,ψ) around (−80,110) (near the polyproline helix). Interestingly, Pro^{13} which is known to be an initiator of β-turns does not participate in any of these in the simulation. A major conformational feature which persisted throughout the analyses was a transannular hydrogen bond from Tyr^{11} to Cys^5 or Tyr^{11} to Met^6. The preference for this conformationally constraining feature was most significant. Further features leading to relative rigidity in this section of the molecule included the stable +90° disulphide conformation and a stable hydrogen bond between the ε-NH of Arg^9 and the NH of Val^{10}.

Additional molecular dynamics simulations were performed on MCH(5—14) starting from a conformation which was not characterised by the cross-ring Tyr^{11} to Cys^5 hydrogen bond. However, it was found that the conformational features of the minima accessed during the new simulations were very similar to those found previously. In particular, the Tyr^{11} to Cys^5 transannular hydrogen bond was once again found to be present in all the minimum energy conformations. It was further apparent that the overall backbone conformations of MCH(5—14) and MCH were closely similar, and that the

Table 2. Inter-residue 1D nuclear Overhauser enhancements for MCH(5—14) in DMSO solution.

			Enhancement	Interatomic distance/Å[a] Conf. 1	Conf. 2
1	$C^{\delta}_{11}H$	$C^{\alpha}_{10}H$	1	6.6	3.9
2	$C^{\epsilon}_{11}H$	$C^{\alpha}_{13}H$	1	3.1	2.9
3	$C^{\epsilon}_{11}H$	C^{β}_{5} or $C^{\beta}_{14}H$	0.5	4.1[b]	3.2[b]
4	N_8H	$C^{\alpha}_{7}H$	3	3.5	2.1
5	N_8H	$C^{\alpha}_{7}H$	15	2.1	2.4
5[a]	$C^{\alpha}_{7}H$	N_8H	20	2.1	2.4
6	$N_{10}H$	$C^{\alpha}_{9}H$	4	4.0	2.7
7	$N_{10}H$	$C^{\alpha}_{9}H$	6	2.1	2.3
8	$C^{\alpha}_{5}H$	$C^{\alpha}_{7}H$	2	6.0	5.6

[a] The interatomic distances are obtained from conformations observed in molecular dynamics simulations and where more than one hydrogen is designated represents the smallest value. [b] N.m.r. assignments ambiguous. Distances based on $C_{14}H$.

mobile side chains did not induce significant changes. MCH-(5—14) retains significant activity relative to MCH.[1—4]

Independent n.m.r. analyses were performed on MCH-(5—14) at 400 MHz both in water and in dimethyl sulphoxide (DMSO). Chemical shift assignments were made (Table 1) from COSY, long range COSY, and NOESY measurements. The proton signals for MCH(5—14) were highly dispersed in relation to linear MCH(5—14), the α-protons of Gly^8, for example, being strongly differentiated and complete resolution of all NH and Cα-H protons was achieved, consistent with greater conformational restraints. Variable temperature studies of backbone amide and arginine ε-NH protons were performed. In H_2O the shallowest slope was shown by the Arg^9 guanidinium protons, the remaining coefficients being large and therefore not strongly internally hydrogen bonded. In DMSO the guanidinium NH units had the largest temperature coefficients, with uniformly low values for the amide NH units with the exception of Cys^{14} and Gly^8. This may be regarded as indicating more rigid, hydrogen bonded conformations existing in DMSO. Conformationally significant n.O.e. data were obtained only in DMSO. The most interesting of these involved Tyr^{11}, which, by 1D differential n.O.e. showed connectivities from Tyr^{11}-CεH to Pro^{13}-CαH (1.5%), Tyr^{11}-CδH to Val^{10}-CαH (1%), and from Tyr^{11}-CεH to one of the Cys Cβ-H protons (0.5%). These small, but reproducible, effects suggest a conformation in which Tyr^{11} occupies a transannularly hydrogen bonded conformation. (Tyr^{11}OH was not detected.) Other inter-residue n.O.e. effects (Table 2) were also in accord with the two conformations shown in Figure 2 which had previously been predicted by molecular dynamics and are typical of the two different families of accessible, minimized conformations obtained from each of the dynamics trajectories. The great majority of these minimized conformations satisfy the spatial requirements for either six of the eight or seven of the eight observed n.O.e. effects depending upon the family. *All* accessible minimized conformations feature the transannular tyrosine hydrogen bond.

Independent theoretical and experimental studies are thus mutually compatible in these preliminary studies. Constraining features and regions of conformational flexibility have been defined. Further synthetic studies designed to lock one or other conformation, together with n.m.r. analysis of conformation and assessment of biological activity are in progress and will be reported.

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