Possible bioactive conformations of α-melanotropin

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By comparing the results of energy calculation for α-MSH and its semirigid analogues Ac-[Cys^4, Cys^{10}]-α-MSH$_{4-10}$-NH$_2$, Ac-[Cys^4, Cys^{10}]-α-MSH$_{4-13}$-NH$_2$, and [Cys^4-Cys^{10}]-α-MSH, a detailed description of two possible bioactive conformations for the 'specific' central site of α-MSH$_{6-9}$ is proposed representing variants of chain-reversal structure. A possible explanation of the rise in melanotropic activity of the latter two semirigid analogues is presented.

α-Melanotropin *Energy calculation* *Conformation-function relationship* *Cyclic analog* *Melanotropic activity*

1. INTRODUCTION

An important landmark in investigation of conformation-function relationships for the α-melanotropin molecule (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$_2$) has been the synthesis and biological testing of semirigid α-MSH analogues such as Ac-[Cys^4-Cys^{10}]-α-MSH$_{4-10}$-NH$_2$ (I), Ac-[Cys^4, Cys^{10}]-α-MSH$_{4-13}$-NH$_2$ (II) and [Cys^4, Cys^{10}]-α-MSH (III) described in [1,2]. The aim of this study was to determine the bioactive conformation of a specifically active fragment of the molecule, namely His^6-Phe^7-Arg^8-Trp^9, which allegedly represents a β-turn-like structure. It has been found that the above analogues exhibit melanotropic activity in vitro in the frog (*Rana pipiens*) skin assays equal to 0.07, ~10^4 and ~10^4, respectively, as compared to α-MSH activity. Furthermore, compounds II and III, but not I also showed a substantial prolongation of action [2]. Apparently, the following stage of conformation-function studies with α-MSH should, in our view, involve a comparison of the spatial structures of α-MSH and I–III analogues aimed at identification and detailed description of bioactive conformations of the α-MSH$_{6-9}$ fragment.

2. METHODS AND RESULTS

The sets of low-energy backbone structures of compounds I–III described here were obtained by means of similar energy calculations to the procedure applied to the α-MSH molecule in the study of [3]. An additional modification of the calculation technique was the use of 'closing' potentials for disulphide bond description (cf. [4]). The general outline of calculations implied stepwise elongation of the peptide chain by the following steps Ac-[Cys^4, Cys^{10}]-α-MSH$_{4-10}$-NH$_2$ → Ac-[Cys^4, Cys^{10}]-α-MSH$_{4-13}$-NH$_2$ → [Cys^4, Cys^{10}]-α-MSH accompanied by optimization of side chain spatial arrangement and selection of low-energy ($\Delta U \leqslant 10$–15 kcal/mol) backbone structures at each stage; thereby the intermediate results of calculations reported in [3] were employed extensively.

A detailed description of the calculation procedure and the obtained sets of low-energy structures for compounds I–III will be published elsewhere. Listed below (table 1) are only the sets of low-energy backbone conformations for compounds I ($\Delta U \leqslant 10$ kcal/mol), II and III ($\Delta U \leqslant 12$ kcal/mol) in terms of local minima of potential maps of amino acid residues: B ($\varphi \sim -140°$, $\psi \sim 140°$), R ($\varphi, \psi \sim 60°$), L ($\varphi, \psi \sim -60°$) and H ($\varphi \sim 140°, \psi \sim -80°$) (internal rotation angle values are estimated according to [5]).

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3. DISCUSSION

As bioactive conformations of the α-MSH_{6-9} fragment in this case may be apparently regarded as the backbone structures of this fragment listed in table 1 for all the compounds included in it (for the description of the 'conventional' procedure for bioactive conformation identification see e.g. [6]). Similar structures – conformations of RRBR and RRRB type – are treated in greater detail in table 2 and are depicted in fig.1 for the Ac-[Cys^4, Cys^{10}]-α-MSH_{4-13}-NH_2 molecule. It should be pointed out in this connection that the backbone conformations of the N-terminal tripeptide appear inessential for the general spatial organization of compound III and hence the list of low-energy molecular structures in table 1 comprises only variants of these conformations optimal with regard to the C-terminal decapeptide.

Similarity (or difference) of both types of structures with the bioactive conformation suggested for III in [1] can be easily deduced from fig.1. Thus, hydrogen bonding between NH_{Trp} and CO_{His} postulated in [1] is absent in both cases, although $NH_{Glu} \cdots CO_{Arg}$ (fig.1a) or $NH_{Trp} \cdots CO_{Glu}$ (fig.1b) bonds are conceivable. Interaction of the lipophilic regions on the side chains of Lys and Trp, but not Phe is possible only for the structure in fig.1b, while attraction of the Glu and Lys side chains is feasible only for the structure in fig.1a. It must be pointed out that close spatial arrangement of ionogenic functional groups Glu(−) ··· (+)Arg is observed in almost all the structures listed in table 1, whereas in the cyclic compounds II and III only one type of structure permits the same arrangement of Glu(−) ··· (+)Lys. Hence, in most cases the Lys side chain rather acts as a spacer involved in direct interaction with receptors than as a factor of additional stabilization of the bioactive conformations of the central α-MSH_{6-9} fragment. This restriction on conformational mobility of the Lys residue may be possibly ascribed to additional steric hindrance provided by the disulphide bridge or to the substitution in II and III of the 'conformational hinge' Gly^{10} for a Cys residue. It is also noteworthy too that one of the hypothetical bioactive conformations proposed by us for the central α-MSH fragment (RRRB for α-MSH_{6-9}) within cyclic compounds reproduces more accurately the steric organization of the linear α-MSH (see table 2). Incidentally, in this particular structure the side chain of Lys is located far from the α-MSH_{6-9} region (fig.1b).

Table 1

Low-energy backbone structures of α-MSH [3] and semirigid analogues

Compound	Structural group	Structure	Sequential number													ΔU (kcal/mol)	Distance (Å)	
			1	2	3	4	5	6	7	8	9	10	11	12	13		C^{δ}_{Glu}–C^{η}_{Arg}	C^{δ}_{Glu}–N^{ϵ}_{Lys}
α-MSH	I	1	B	L	R	R	B	B	R	R	B	L	B	R	B	0.0	3.3	8.8
		2	B	R	R	B	B	B	R	R	B	H	B	B	B	4.4	3.3	10.9
		3	B	B	B	B	B	B	R	R	B	B	B	R	B	6.7	4.4	3.8
		4	B	B	R	B	B	B	R	R	B	R	B	B	B	7.0	3.4	12.4
	II	5	B	B	R	B	B	R	B	R	B	L	B	R	B	5.0	3.8	3.5
	III	6	B	L	R	R	B	B	R	R	R	R	B	B	B	5.0	3.4	8.5
	IV	7	B	R	R	B	B	R	R	R	B	B	B	R	B	5.4	3.1	4.4
	V	8	B	R	R	R	B	B	L	R	B	L	B	B	B	6.6	5.3	2.9
		9	B	R	R	R	B	B	L	R	B	H	B	B	B	10.9	5.5	3.0
	VI	10	B	R	R	R	B	B	L	R	R	R	B	B	B	8.0	5.5	3.4
	VII	11	B	R	R	B	L	R	B	B	B	L	B	R	B	9.3	4.0	3.5
		12	R	L	R	B	B	R	B	B	B	L	B	R	B	9.8	4.1	3.5
	VIII	13	B	B	R	B	L	R	R	B	R	B	B	R	B	11.2	3.7	3.6

Table 1 (Continued)

Compound	Structural group	Structure	Sequential number													ΔU (kcal/mol)	Distance (Å)	
			1	2	3	4	5	6	7	8	9	10	11	12	13		$C^{\delta}_{Glu}-C^{\eta}_{Arg}$	$C^{\delta}_{Glu}-N^{\epsilon}_{Lys}$
I	I	1				B	B	L	L	R	B	B				0.0	4.5	–
		2				R	L	L	L	R	B	B				4.7	3.9	–
	II	3				B	B	L	L	R	R	B				2.6	4.8	–
		4				R	L	L	L	R	R	B				7.8	3.7	–
	III	5				R	B	R	R	B	B	B				3.7	8.0	–
	IV	6				B	B	B	R	R	R	B				4.1	3.5	–
	V	7				R	B	R	R	R	R	B				4.4	3.2	–
	VI	8				B	B	R	R	R	B	B				4.7	2.7	–
	VII	9				B	B	L	L	B	B	B				7.9	3.8	–
		10				B	L	L	L	B	B	B				8.9	3.3	–
	VIII	11				B	B	R	R	R	L	B				8.0	4.3	–
	IX	12				B	R	B	R	R	B	B				8.3	5.0	–
	X	13				B	R	R	R	L	R	B				9.5	4.8	–
	XI	14				B	R	R	B	R	B	B				9.6	8.3	–
II	I	1				R	L	L	L	R	R	B	B	R	B	0.0	3.6	9.9
		2				B	B	L	L	R	R	B	B	R	B	0.8	4.7	12.5
		3				B	B	L	L	R	R	B	B	B	B	1.0	4.5	12.6
		4				R	L	L	L	R	R	B	B	B	B	1.3	3.6	10.2
		5				B	B	L	L	R	R	B	L	B	B	4.5	4.8	16.0
		6				R	L	L	L	R	R	B	L	B	B	6.6	3.6	15.2
		7				B	B	L	L	R	R	B	L	R	B	8.6	4.6	17.4
	II	8				R	B	R	R	B	R	B	B	R	B	1.9	4.7	3.6
	III	9				B	B	R	R	R	B	B	B	B	B	7.0	4.0	18.1
		10				B	B	R	R	R	B	B	B	R	B	10.8	3.9	16.6
	IV	11				B	B	L	L	R	B	B	B	B	B	9.0	4.6	20.5
		12				B	B	L	L	R	B	B	B	R	B	10.0	4.7	20.4
		13				R	L	L	L	R	B	B	B	R	B	10.6	3.7	12.1
		14				R	L	L	L	R	B	B	B	B	B	11.6	3.8	18.5
	V	15				R	B	R	R	B	B	B	B	B	B	10.2	3.4	17.6
		16				B	B	R	R	B	B	B	B	B	B	12.0	3.3	19.9
	VI	17				B	R	R	L	R	R	B	B	B	B	10.8	9.0	13.2
III	I	1	B	B	B	R	B	R	R	B	R	B	B	R	B	0.0	4.8	3.6
	II	2	B	B	B	R	B	R	R	B	B	B	B	R	B	5.9	3.5	17.4
		3	B	B	B	R	B	R	R	B	B	B	B	B	B	8.9	3.3	17.9
	III	4	B	B	B	B	B	R	R	R	B	B	B	B	B	7.6	4.0	18.1
	IV	5	B	B	B	B	R	R	L	R	R	B	B	B	B	9.5	9.3	13.2

The underlined structures are described in greater detail in table 2

Table 2

Hypothetical bioactive conformations of α-melanotropin

Residue	Angle	Compound no. of structure in table 1								
		Structure 1 (RRBR for α-MSH_{6-9})				Structure 2 (RRRB for α-MSH_{6-9})				
		α-MSH 13	I 7	II 8	III 1	α-MSH 7	I 8	II 9	II 10	III 4
Met	φ	−127	−115	−115	−106	−121	−145	−145	−145	−145
(Cys)	ψ	141	−44	−44	−45	110	162	165	165	165
Glu	φ	65	−138	−138	−139	−152	−122	−121	−122	−121
	ψ	133	154	156	156	144	118	117	116	117
His	φ	−104	−83	−82	−84	−71	−71	−73	−74	−73
	ψ	−28	−36	−36	−35	−22	−22	−21	−21	−21
Phe	φ	−37	−76	−76	−75	−53	−70	−67	−67	−67
	ψ	−44	−39	−39	−40	−35	−43	−42	−41	−42
Arg	φ	−123	−129	−129	−129	−92	−96	−94	−93	−94
	ψ	140	148	147	148	−53	−78	−79	−80	−79
Trp	φ	−117	−52	−53	−51	−154	−158	−161	−161	−161
	ψ	−49	−54	−55	−55	144	139	140	133	140
Gly	φ	−82	−161	−161	−161	−45	−145	−145	−154	−145
(Cys)	ψ	76	140	145	146	103	141	141	141	141
Lys	φ	−143	–	−127	−127	−135	–	−131	−138	−131
	ψ	133	–	140	139	136	–	122	130	122
Pro	ψ	−40	–	−40	−41	−30	–	118	−36	118
Val	φ	−117	–	−130	−130	−120	–	−142	−138	−142
	ψ	129	–	140	139	137	–	152	150	152

Internal rotation angle values are expressed in degrees

Reduced conformational mobility observed for the Lys residue in compounds II and III may be, in principle, related to the dramatic increase in their biological activity in the case when the side chain of Lys interacts directly with the receptor. However, more important in this respect is apparently the marked restriction of the set of possible backbone conformations for the 'specific' region α-MSH_{6-9} (11, 6 and 4 structures for compounds I, II and III, respectively; see table 1). At the same time, to analyse a possible relationship between the prolonged action of compounds II and III and their conformational properties the spatial structures of the [Nle^4, D-Phe^7]-α-MSH analogue should be additionally investigated which exhibits, as shown in [7], still greater prolongation of melanotropic effect.

Our calculation results agree fairly well with the existence of a chain-reversal structure in α-MSH_{6-9} postulated in [1] for the biologically active α-MSH conformation and provide some insight as to the possible structures of this region (table 2). Experimental verification of biologically active structures described for α-MSH in table 2 can be possibly obtained by the synthesis and biological testing of compounds possessing still greater rigidity of the α-MSH_{6-9} region, e.g. as has been performed for the tuftsin molecule [8].

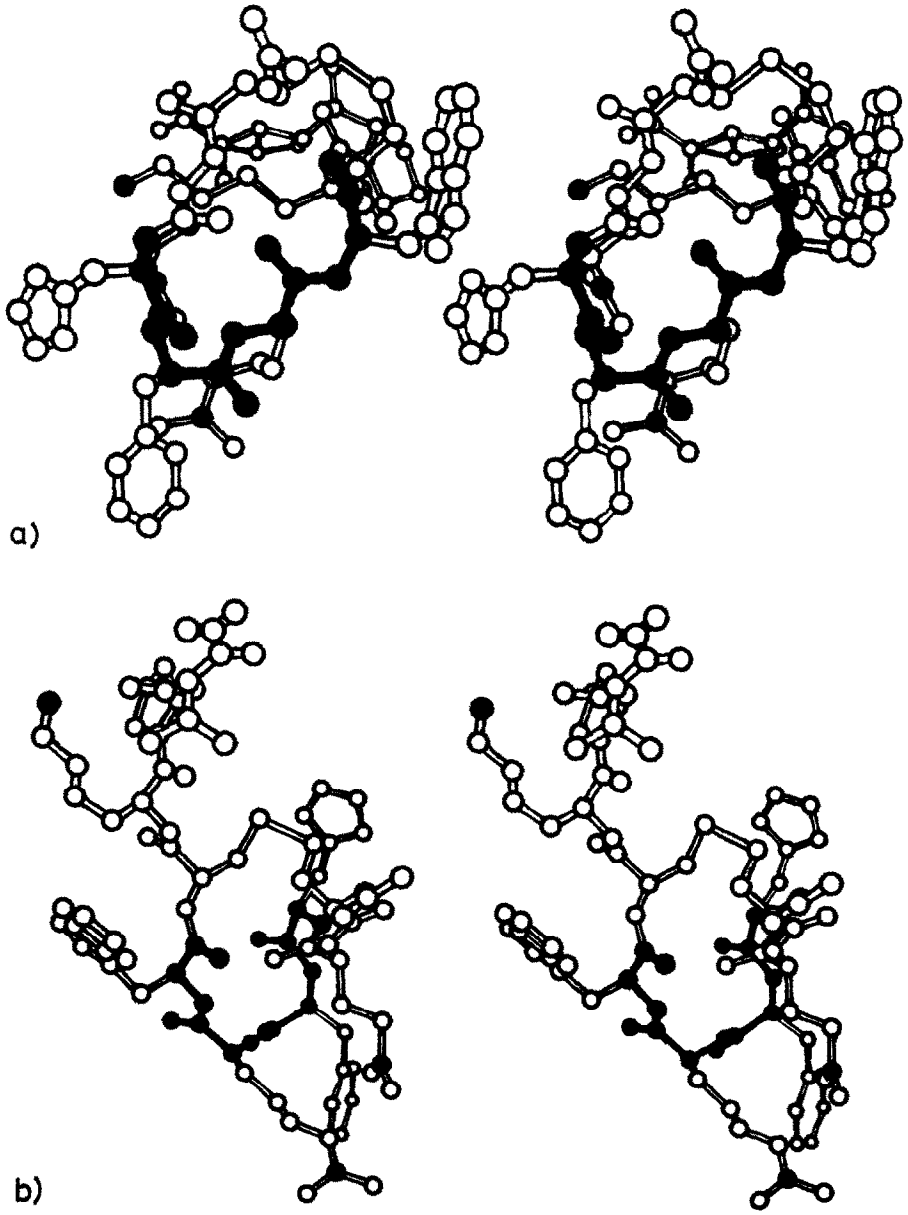

Fig.1. Stereo views of hypothetical bioactive conformations of α-MSH (Ac-[Cys^4,Cys^{10}]-α-MSH$_{4-13}$-NH$_2$ cyclo-analogue). Hydrogen atoms except for those of the C-terminal amide have been omitted. Indicated in black are the α-MSH$_{6-9}$ fragment backbone and the atoms whose interatomic distances are given in table 1. (a) Structure 8, (b) structure 9 from table 1.

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