MELANIN CONCENTRATING HORMONE ANALOGS: CONTRACTION OF THE CYCLIC STRUCTURE. III. CD SPECTROSCOPIC STUDY

†Ivo FRIČ[a], Michal LEBL[a] and Victor J. HRUBY[b]

[a] *Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, 166 10 Prague 6, Czechoslovakia*

[b] *Department of Chemistry, The University of Arizona, AZ 85721, U.S.A.*

Received June 10, 1991
Accepted July 30, 1991

A comparison of the CD spectra of MCH analogs differing in length but containing a heterodetic ring of the same size (compounds *I*, *IV* and *VII* or *III*, *VI* and *IX*) reveals that a conformational change occurs upon elongating the peptide chain from thirteen to seventeen amino-acid residues. The 5—17 fragments appear to prefer a β-turn conformation, whereas the 1—17 full-sequence peptides prefer α-helical conformation. Peptides containing seventeen-membered ring exhibit greater conformational adaptability (the incorporation of their cyclic moiety into an ordered conformation being easier) than those containing a twenty-six-membered ring. Spectral properties of the twenty-three-membered heterodetic ring in peptides *II* and *V* indicate that they do not possess highly ordered conformation.

A series of analogs of MCH (melanin concentrating hormone, H-Asp-Thr-Met--Arg-Cys-Met-Val-Gly-Arg-Val-Tyr-Arg-Pro-Cys-Trp-Glu-Val-OH) modified in the structure of the disulfide bridge was synthesized[1] to examine structure–activity relationships for this newly described hormone[2]. Since the biological activities of these analogs on the MCH and MSH receptors were very diverse[1,3], a study of their three dimensional structure seemed interesting. The technique chosen was CD spectroscopy, since it is extremely sensitive to conformation of the molecule, and can be applied to the study of conformation in various solvents, including those containing surface-active compounds that can simulate the interaction of hormone with biological membranes. The group of analogs studied also was suitable for the application of this technique because all its members contain the same chromophores (Trp, Tyr, disulfide) which can otherwise complicate the comparison of the spectra of different compounds. Three-dimensional structure of MCH, its shorter and linear analogs were studied recently by ^{1}H NMR and theoretical calculations[4,5].

H-Asp-Thr-Met-Arg-Cys[5]-Met-Val-Gly-Arg-Val[10]-Tyr-Arg-Pro-Cys-
-Trp[15]-Glu-Val-OH

MCH

I	$[Cys^{10}]MCH_{10-17}$	*VI*	$[Ala^5, Cys^7]MCH_{5-17}$
II	$[Cys^8]MCH_{8-17}$	*VII*	$[Ala^5, Cys^{10}]MCH_{1-17}$
III	$[Cys^7]MCH_{7-17}$	*VIII*	$[Ala^5, Cys^8]MCH_{1-17}$
IV	$[Ala^5, Cys^{10}]MCH_{5-17}$	*IX*	$[Ala^5, Cys^7]MCH_{1-17}$
V	$[Ala^5, Cys^8]MCH_{5-17}$		

METHODS

CD spectra were obtained on a Jobin–Yvon Dichrographe Mark V equipped with a data processor. The software used was DICHROSOFT version A written by Dr P. Maloň from the Laboratory of Peptide Synthesis, Institute of Organic Chemistry and Biochemistry, Prague. Spectra were recorded in cells of optical path of 1 and 0·05 cm at room temperature and peptide concentrations of about 0·3 mg/ml. The CD data of the side chain chromophores in the spectral region above 240 nm are expressed in terms of total molar ellipticity, the data of the amide bands in the region below 240 nm are expressed as mean residue ellipticities (both in degree $cm^2\ dmol^{-1}$). Solvents used were 0·05M phosphate buffer pH 7·3, 2,2,2-trifluoroethanol of spectral grade (Merck) and a 11·1 mM solution of sodium dodecylsulfate (SDS) in the above mentioned buffer. SDS was a product of Pierce Chemical Company, sequanal grade, and was used without further purification.

RESULTS AND DISCUSSION

MCH *Fragments Consisting of a Heterodetic Ring and a C-Terminal Tripeptide*

The long-wavelength portion (240 – 300 nm) of the CD spectra of all the peptides examined resulted from the dichroic bands of the disulfide group and of the side chain aromatic chromophores of the tyrosine-11 and tryptophan-15 residues. Due to an extensive overlap, it is impossible to specifically identify all of the contributing bands. There is a readily observable negative double band of the B_{2u} transition of tryptophan at about 282 and 290 nm and a positive disulfide band at 250 – 255 nm (ref.[6]) appears also as a shoulder or negative minimum) (Figs 1, 3 and 5). The positive maximum between 265 and 270 nm which is found in the spectra of peptides *I*, *IV* and *VII* (Figs 1, 3 and 5) probably belongs to another tryptophan band[6]. The B_{2u} band of tyrosine at 278 nm and the long-wavelength band of the disulfide group at about 270 – 300 nm, if present, are weak and obscured by the tryptophan bands in our analogs.

The long-wavelength CD spectra of cyclic fragments *I* – *III* in a neutral aqueous solution differ only slightly depending on the size and structure of the ring (Fig. 1a).

The intensity of the bands and their dependence on the ring structure increases upon transfer of the peptide into trifluoroethanol (Fig. 1b), indicating that the peptides assume a more rigid conformation in this solvent. The two peptides containing a twenty-three-membered and twenty-six-membered ring (*II* and *III*) show markedly different spectra (Fig. 1b, curves 1 and 2), suggesting that they differ in conformation of both the disulfide group and the tryptophan side chain.

The positive 230 nm band is rather intense in the spectra of peptide *I* both in water and trifluoroethanol solution (Fig. 2a,b). A similar CD band is also observed for oxytocin[7], where it arises from the B_{1u} transition of a tyrosine residue which is positioned next to an N-terminal half-cystine residue of a peptide ring of a similar size. Apparently the side chain moiety of tyrosine residue in peptide *I* may assume a conformation similar to that found in oxytocin[7], namely with the aromatic group above the disufide-containing ring and pointed towards the disulfide bridge. This assumption is supported by the observation that in peptides *II* and *III* (Figs 2a and 2b) where the tyrosine residue is remote from the Cys-residue, the 230 nm band is much weaker.

As regards the amide CD bands in peptides *I*–*III*, the only marked feature observed in buffer solution is a negative π–π^* band at about 200 nm along with a very weak n–π^* band (a narrow positive band, probably of aromatic origin, appears to be superimposed on the amide band in the spectrum of peptide *I*) (Fig. 2a). This type of spectra generally is interpreted as indicating an unordered conformational state of the solute[8–10]. In the case of small peptides, this would suggest the presence of energetically related conformers which are not stabilized by intramolecular hydrogen bonds. A markedly stronger negative n–π^* band is observed for peptide *III*

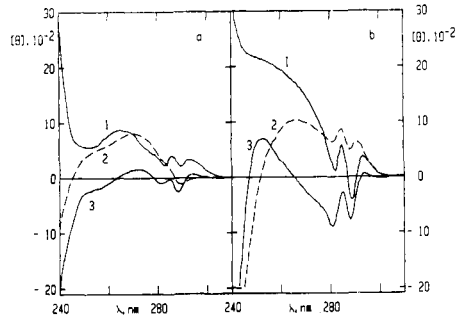

FIG. 1

Long-wavelength CD spectra, in total molar elipticity, of *I* (1), *II* (2) and *III* (3). Solvent: phosphate buffer pH 7·3 (a) and 2,2,2-trifluoroethanol (b)

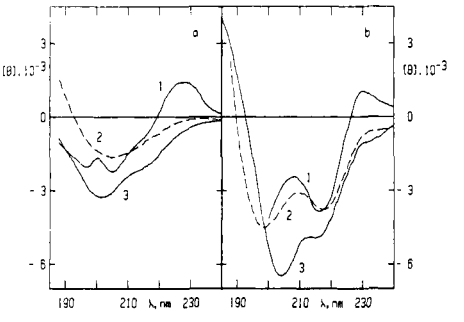

FIG. 2

Short-wavelength CD spectra, in mean residue elipticity, of *I* (1), *II* (2) and *III* (3). Solvent: phosphate buffer pH 7·3 (a) and 2,2,2-trifluoroethanol (b)

(Fig. 2a), suggesting the presence of a more ordered conformation in the twenty--six-membered ring moiety[9].

CD spectra of peptides *I*–*III* in trifluoroethanol (Fig. 2b) exhibit negative n–π^* bands of considerably higher intensity than seen in aqueous solution, with enhanced intensity of π–π^* bands. This change suggests a shift toward a higher degree of conformational stabilization. The curves 1 and 2 in Fig. 2b resemble each other indicating that peptides *I* and *II* have similar overall conformations. The negative n–π^* band and the negative minimum of a positive π–π^* band at about 209 nm indicate the presence of a certain amount of a β-turn conformation (see below). The twenty-six-membered ring in peptide *III* differs in conformation from the two previous ones, as suggested by the opposite sign of the 200 nm amide band. Its spectral parameters, i.e. a relatively intense n–π^* band and a couple of oppositely signed π–π^* bands, indicates that the peptide is arranged to some extent in the α-helical conformation[6].

The (5–17) *Fragments of* MCH *Containing Rings of Different Size*

The lengthening of the peptide chain on the N-terminus by two amino-acid residues to give *VI*, brings about only a small change in the long-wavelength CD spectrum in trifluoroethanol solution (Fig. 3, curve 1). In the case of peptide *IV* the attachement of a linear pentapeptide to peptide *I* causes some quantitative changes: an enhancement of the 265 nm tryptophan band (Fig. 3, curve 1) and an attenuation of the positive 230 nm band of tyrosine (Fig. 4, curve 1) which corresponds to some alterations in the conformation of both aromatic amino acids. Furthermore, the spectrum

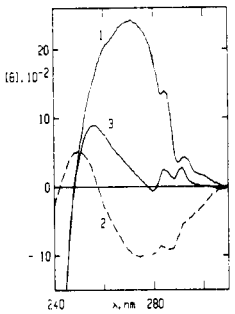

FIG. 3

Long-wavelength CD spectra, in total molar elipticity, of *IV* (1), *V* (2) and *VI* (3), in 2,2, 2-trifluoroethanol

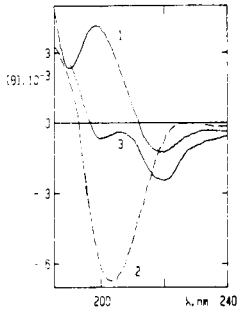

FIG. 4

Short-wavelength CD spectra, in mean residue elipticity, of *IV* (1), *V* (2) and *VI* (3), in 2,2,2-trifluoroethanol

of the twenty-three-membered ring containing peptide *V* differs substantionally from that of the shorter peptide *II*. In this case, the main 265 nm band changes sign and a positive disulfide band appears at 250 nm (Fig. 3a, curve 2). These particular properties of *V* have their counterpart in the amide band region.

The lengthening of the N-terminal peptide chain in analogs *I*, *II*, and *III* results in considerable changes of the amide CD spectra in trifluoroethanol for peptides *IV*–*VI*. Compounds *IV* and *VI* show closely related spectra (Fig. 4, curves 1 and 3) with a negative n–π^* band at 219·5 nm and a positive π–π^* band at 198·5 and 206 nm, respectively. The spectrum of *IV* (curve 1), disregarding the residual positive band at the shortest wavelength, corresponds well, both in position and intensity of the bands, to a type A CD spectrum predicted for a type I β-turn by Woody[11]. However, it is difficult to assign with certainty the actual type of β-turn from CD alone because of existing disagreements between theoretical and experimental spectra (e.g. refs[8–10,12–14]) and because of apparent extreme sensitivity of CD to minor changes of this conformational type[6]. Whatever the particular type of β-turn present, peptide *IV* appears to be largely in this conformation, perhaps in a form of a multiple β-turn. From comparison of Figs 2b and 4 it is obvious that, in trifluoroethanol, peptides *I* (*II*), *V* and *IV* represent a series of compounds which are able to take up the β-turn conformation of one type to an increasing extent. It is interesting that in the case of the twenty-six-membered ring containing compounds the attachment of only two amino-acid residues to *III* to give *IV* is able to cause such a significant conformational change (compare curves 3 in Figs 2b and 4). It may be suggested that this is an effect of cooperation (as it is also the case with peptide *IV*) of the amino-acid residues both outside and inside the ring moiety.

Unexpected CD properties are observed with peptide *V* which exhibits only one strong negative amide band at 203·5 nm. This spectral type indicates that an ordered conformation is not present to any appreciable extent, and that the ability of the twenty-three-membered heterodetic ring of given structure to assume an ordered conformation is seriously hampered.

Full Sequence Cyclic Analogs of MCH

In this series compound *VIII* was not available in an amount sufficient for CD measurements. The long-wavelength spectra of full sequence peptides *VII* and *IX* (Fig. 5) retain the main CD features observed for the corresponding (5–17) fragments. However, in the Cys^{10}-Cys^{14} bridged cyclic peptide *VII*, the main positive 265 nm band increases in intensity, whereas the CD bands of the Cys^{7}–Cys^{14} cyclic peptide *IX* showed a tendency to decrease in intensity in both TFE and SDS containing solutions. These spectral differences imply that there are considerable conformational differences at least for the disulfide group and the tryptophan side chains.

At short wavelengths, in neutral buffer solution, both peptides *VII* and *IX* exhibit the spectrum of an unordered conformation (Fig. 6a) (for *VII* the intensity of the negative band at 199 nm has a molar elipticity −9 700 units). Peptide *VII* retains the positive 230 nm band of tyrosine also observed in the short peptide *I*. Hence, the conformation of the ring moiety in *VII* does not appear to be greatly affected by the attachment of a long linear peptide chain. Peptide *IX*, which consists of a larger cyclic moiety and a shorter linear chain than peptide *VII*, displays a somewhat greater tendency to form (in water solution) an ordered, perhaps α-helical conformation suggested by its less intense π–π^* and more intense n–π^* amide bands transitions (Fig. 6a). This phenomenon, also observed for the parent shorter peptides *I* and *III*, probably is due to the effect of the disulfide bridge reducing the flexibility of the peptide chain included in the larger ring.

In trifluoroethanol both peptides *VII* and *IX* exhibit CD spectra very different from those of their shorter analogs *IV* and *VI* (Fig. 6b). Their CD spectra which possess an intense negative n–π^* band at 220 nm and two π–π^* bands, one negative at 207 nm and the other positive at 190 nm may be ascribed with considerable confidence to the α-helix[6] which apparently is the primary conformation of both peptides. The intensity of the bands corresponds to short helical segments[15]. Peptide *VII* exhibits amide bands of higher intensity than *IX* (particularly the negative n–π^* band) and therefore contains a higher amount of ordered conformation. Apparently, the greater separation of the half-cystine residues in peptide *IX* relative to *VII*

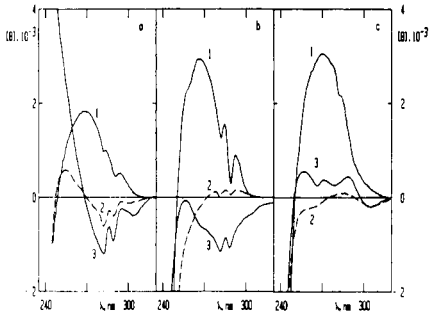

FIG. 5

Long-wavelength CD spectra, in total molar elipticity, of *VII* (1), *IX* (2), and MCH (3). Solvent: phosphate buffer pH 7·3 (a), 2,2,2-trifluoroethanol (b) and phosphate buffer pH 7·3 with 11·1 mM sodium dodecyl sulfate (c)

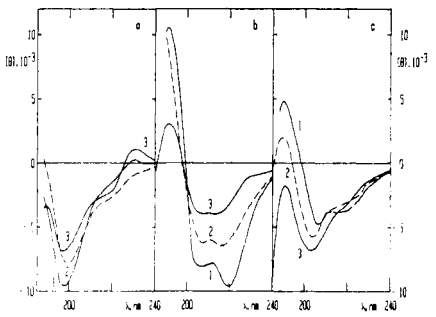

FIG. 6

Short-wavelength CD spectra, in mean residue elipticity, of *VII* (1), *IX* (2), and MCH (3). Solvent: phosphate buffer pH 7·3 (a), 2,2,2-trifluoroethanol (b) and phosphate buffer pH 7·3 with 11·1 mM sodium dodecyl sulfate (c)

somewhat hinders a full incorporation of the residues inside the cyclic structure into the helix in a helix-forming solvent such as trifluoroethanol.

Similar spectral features also are observed for *VII* and *IX* in a neutral buffer solution containing 11·1 mM SDS (i.e. at a concentration above the critical micelle concentration) (Fig. 6c). The spectra of the two peptides are related in the same way as they are in trifluoroethanol: the α-helical conformation is less populated for peptide *IX* based on the blue-shifted negative π–π^* band and the less intense n–π^* band. There is a considerable quantitative difference between the spectra obtained in trifluoroethanol and in a surfactant containing solvent (Figs 6b and 6c), the intensity of all the bands being higher in trifluoroethanol. This difference might be explained either by a lower helical content in SDS or by a contribution of other ordered structures in trifluoroethanol.

This work was supported in part by U.S. Public Health Service Grant DK 17420 and by a grant from the National Science Foundation.

REFERENCES

1. Lebl M., Hruby V. J., Castrucci A. M. L., Visconti M. A., Hadley M. E.: J. Med. Chem. *31*, 949 (1988).
2. Kawauchi H., Kawazoe I., Tsubokawa M., Kishida M., Baker B. I.: Nature *305*, 321 (1983).
3. Lebl M., Hruby V. J., Castrucci A. M. L., Hadley M. E.: Life Sci. *44*, 451 (1989).
4. Brown D. W., Campbell M. M., Kinsman R. G., White P. D., Moss C. A., Osguthorpe D. J., Paul P. K. C., Baker B. I.: Biopolymers *29*, 609 (1990).
5. Paul P. K. C., Dauber-Osguthorpe P., Campbell M. M., Brown D. W., Kinsman R. G., Moss C. A., Osguthorpe D. J.: Biopolymers *29*, 609 (1990).
6. Woody R. W. in: *The Peptides*, Vol. 7, *Conformation in Biology and Drug Design* (V. J. Hruby, Ed.), p. 15. Academic Press, New York 1985.
7. Frič I., Kodíček M., Jošt K., Bláha K.: Collect. Czech. Chem. Commun. *39*, 1271 (1974).
8. Crisma M., Fasman G. D., Balaram H., Balaram P.: Int. J. Pept. Protein Res. *23*, 411 (1984).
9. Hollosi M., Kawai M., Fasman G. D.: Biopolymers *24*, 211 (1985).
10. Hollosi M., Kover K. E., Holly S., Fasman G. D.: *Proceedings of the International Conference on Circular Dichroism*, p. 356. Publishing House of the Bulgarian Academy of Sciences, Sofia 1985.
11. Woody R. W. in: *Peptides, Polypeptides and Proteins* (E. R. Blout, F. A. Bovey, M. Goodman and N. Lotan, Eds), p. 338. Wiley Interscience, New York 1975.
12. Gierasch L. M., Deber C. M., Madison V., Niu Chien-Hua, Blout E. R.: Biochemistry *20*, 4730 (1981).
13. Bandekar J., Evans D. J., Krimm S., Leach S. J., Lee S., McQuie J. R., Minasian E., Nemethy G., Pottle M. S., Scheraga H. A., Stimson E. R., Woody R. W.: Int. J. Pept. Protein Res. *19*, 187 (1982).
14. Campbell B. E., Easwaran K. R. K., Zanotti G. C., Staples M. A., Fossel E. T., Blout E. R.: Biopolymers *25*, S47 (1986).
15. Chen Y.-H., Yang J. T., Chan K. H.: Biochemistry *13*, 3350 (1974).