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Circular Dichroism of Active Fragments of α-Melanocyte-stimulating Hormone

Kiyoshi Ikeda[1a], Wataru Urano,[1b] Kozo Hamaguchi,[1a] Kazuo Kubo and Haruaki Yajima[1c]

Department of Biology, Faculty of Science, Osaka University[1a] *and Faculty of Pharmaceutical Sciences, Kyoto University*[1c]

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The circular dichroism of a number of active fragments of α-melanocyte-stimulating hormone was studied. It was suggested that the peptides studied have no distinct secondary structure.

A number of pentapeptide stereoisomers of L-histidyl-L-phenylalanyl-L-arginyl-L-tryptophyl-glycine (I), an active fragment of α-melanocyte-stimulating hormone[2] has been prepared, including the optical antipode of I (II),[3] to determine the effect on melanotropic activity of changing the configuration of the constituent amino acid residues.[4–6] Within this systematic framework, certain trend was seen in the relationship between structure and activity as shown in Table I. The *in vitro* assay system exhibited a stereospecificity with regard to the ionizable residues of the pentapeptide, *i.e.*, the basic histidyl and arginyl residues. The D-histidine (III) and D-arginine peptides (IV) were both practically inert at the dose level that the all-L-pentapeptide (I) exhibited observable response. On the other hand, peptides containing D-isomers of the nonionized aromatic residues phenylalanine (V) and tryptophan (VI) were more active than the all-L-analogue, while the optical antipode of I was completely devoid of the MSH activity and rather exhibited an anti-MSH activity. These results suggest that there is subtle relationship between stereochemical properties of this pentapeptide and its biological function when an MSH-receptor is considered.

Table I. MSH Activity of Synthetic Pentapeptide Isomers

Peptides		His-Phe-Arg-Trp-Gly	MSH activity, U/g
I	all-L	L L L L	3×10^4
II	all-D	D D D D	inhibitory
III	D-His	D L L L	practically inactive
IV	D-Arg	L L D L	practically inactive
V	D-Phe	L D L L	1×10^6
VI	D-Trp	L L L D	1×10^5

In order to understand the relation between conformation and the MSH activities of the stereoisomers of the active pentapeptides and the chainlength-dependent activities of the longer peptides related to α-MSH, we measured the circular dichroism (CD) of the various kinds of synthetic peptides described above.

1) *a*) Location: *Toyonaka, Osaka*; *b*) Present address: *CIBA Products Co., Osaka*; *c*) Location: *Sakyo-ku, Kyoto.*
2) J.I. Harris and A.B. Lerner, *Nature*, **179**, 1346 (1957).
3) H. Yajima and K. Kubo, *J. Am. Chem. Soc.*, **87**, 2039 (1965).
4) H. Yajima and K. Kubo, *Biochim. Biophys. Acta*, **97**, 596 (1965).
5) H. Yajima, K. Kubo, Y. Kinomura and S. Lande, *Biochim. Biophys. Acta*, **127**, 545 (1966).
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Fig. 1 shows the CD spectra of the pentapeptide stereoisomers of L-histidyl-L-phenylalanyl-L-arginyl-L-tryptophylglycine (I). The all-L-pentapeptide (I) exhibited a negative maximum at about 205 mμ and a positive maximum at 222 mμ. The ellipticity per residue, $[\theta]_R$, at 222 mμ was +7100. The CD spectra of the optical antipode of I (II) was practically a mirror image of the CD spectrum of the all-L-peptide (I). The CD spectra of the D-histidine (III) and D-arginine (IV) peptides had a positive maximum at about 223 mμ. These spectra were very similar in shape to that of the peptide I, while the ellipticities were smaller than that of the latter. The CD spectra of the peptides containing the D-isomers of the aromatic amino acid residues, phenylalanine (V) and tryptophan (VI) were very different from those of the all-L (I), D-histidine (III) and D-arginine (IV) peptides. The D-tryptophan peptide (VI) exhibited a small but distinct negative maximum at 237 mμ and a very large positive maximum at about 217 mμ ($[\theta]_R$=+12100). The D-phenylalanine peptide (V) showed a large negative maximum at 215 mμ ($[\theta]_R$=−8000) and a negative maximum at 206 mμ.

As shown in Table I, the configuration of the amino acid residues, except D-tryptophan and glycyl residues, of the peptide VI is different from that of the peptide II. If we assume that the both peptides have no secondary structure or there is no difference in the conformation between them, a half of the algebraic sum of the CD intensities of the two peptides may correspond to the contribution from only the D-tryptophyl residue. Similarly a half of the algebraic sum of the CD ellipticities of the peptides II and V, II and III, and II and IV may

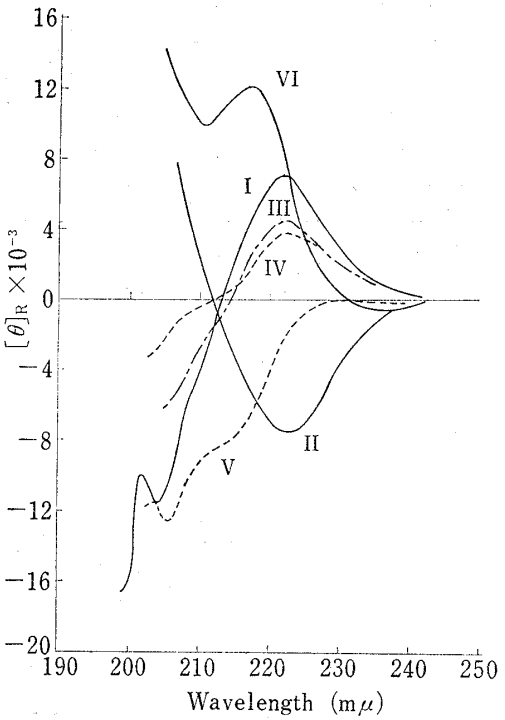

Fig. 1. CD Spectra of Pentapeptide Stereoisomers of L-Histidyl-L-Phenylalanyl-L-Arginyl-L-Tryptophylglycine in Water (see TableI)

The measurements were done at about 0.05—0.01% of the peptides with 0.5—0.02 cm-cells at room temperature.

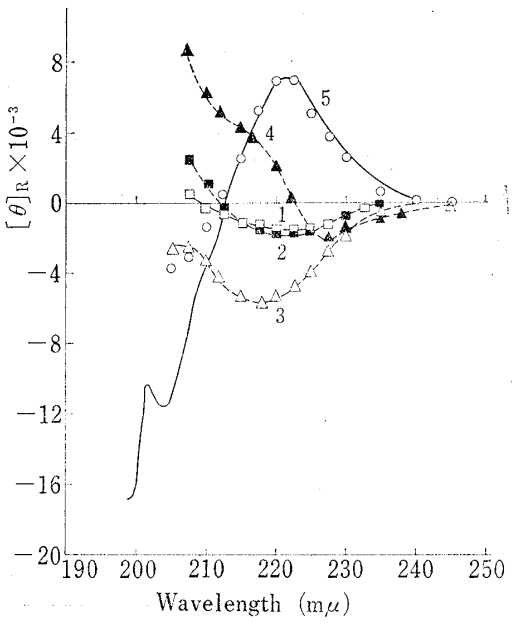

Fig. 2. A Half of the Algebraic Sum of the CD Ellipticities of the Peptides II and III (□), the Peptides II and IV (■), the Peptides II and V (△), the Peptides II and VI (▲), and the Peptides III, IV, V, and VI (○)

The solid curve 5 indicates the experimental CD spectrum of the peptide I.

reflect the CD of D-phenylalanyl, D-histidyl, and D-arginyl residue, respectively. Fig. 2 shows the results of these calculations. The CD spectrum shown by curve 4 thus calculated from those of the peptides II and VI had a negative maximum at 227 mμ with a value of $[\theta]_R$=−2000 and a shoulder at 214 mμ ($[\theta]_R$=+4600). The shape of this spectrum is very similar

to that of a mirror image of that of the CD spectrum of L-tryptophan derivatives, although the absolute values of the ellipticities at 227 mμ and 214 mμ multiplied by the number of the constituent amino acid residues (five) was smaller and larger, respectively, than those of the molecular ellipticities ($[\theta]_M$=+16900 at 227 mμ and −6100 at 212 mμ) of N-acetyl-tryptophan amide in water.[7] The CD spectrum (Fig. 2) obtained from those of the peptides II and V, which corresponds to D-phenylalanyl residue, had a negative maximum at 217 mμ with a value of $[\theta]_R$=−5600. This CD spectrum is very similar to a mirror image of the spectrum of L-phenylalanine derivatives, although the absolute value of the ellipticity at 217 mμ multiplied by 5 was larger than the molecular ellipticity of N-acetyl-L-phenylalanine amide in water ($[\theta]_M$=+14800 at 220 mμ)[7] but comparable to that of N-acetyl-L-phenylalanine methyl ester in trimethyl phosphate ($[\theta]_M$=+24000 at 218 mμ).[8] Curve 1 obtained from the spectra of peptides II and III, which corresponds to D-histidyl residue, is similar to a mirror image of the CD spectrum of L-histidine in water.[8] Although the wavelength of the CD maximum, 220 mμ, was longer than that of L-histidine (214 mμ), the absolute ellipticity of this band multiplied by 5 is fair agreement with the molecular ellipticity of the amino acid ($[\theta]_M$=+9000 214 mμ).[8] In order to certify the accuracy of the above calculation, we summed algebraically the four CD spectra (curves 1 to 4) in Fig. 2 and obtained a CD spectrum which coincides with that of the all-D peptide (II).

If we assume that each of the five peptides, I, III, IV, V, and VI has no definite secondary structure or that there is no difference in the secondary structure among them, a half of the algebraic sum of the CD spectra of the peptides III, IV, V, and VI may give the CD spectrum of the all–L-peptide (I). The open circles on curve 5 shown in Fig. 2 are the values thus calculated, which are in good agreement with the experimental values of the all–L pentapetide (I).

These facts suggest that none of the pentapeptide stereoisomers studied here has any distinct secondary structure and that the difference in the activity shown in Table I is originated from only the difference in the configuration of the constituent amino acid residues of the pentapeptides.

Fig. 3 shows the CD spectra of the synthetic α-MSH and its related active fragments containing the pentapeptide I (Table II). The ellipticities are expressed on the basis of residue weight. The CD spectrum of the octapeptide (VII) having the amino acid sequence of N^{ε}-formyllysyl prolyl-valine amide (a) in the N-termianl side of the pentapeptide (I) was very similar to that of the pentapeptide, although the positive ellipticity at 222 mμ of the former was smaller than that of the latter. The CD spectrum of the decapeptide (VIII) having the sequence of N^{α}-acetyl-seryltyrosylserylmethyonylglutamic acid (b) in the C-terminal side of the pentapeptide (I) was similar to that of the pentapeptide. The CD maximum of the decapeptide, however, was at 225 mμ and its intensity was smaller than that at 222 mμ of the octapeptide (VII). The CD spectrum of N^{ε}-formyllysine tridecapeptide (IX) having the sequence of a and b in the both sides of the pentapeptide was very similar to that of the decapeptide (VIII). There was a negative maximum at 236 mμ and a positive maximum at 225 mμ. The intensity at 225 mμ was slightly smaller than that of the decapeptide. The synthetic MSH (X) derived from its formyl derivative (IX) showed no positive maximum in this spectral region. These changes in the CD spectrum with elongation of the peptide chain seem to be in the order of the changes in the MSH activity.

Fig. 4 shows the CD spectra of the peptides shown in Fig. 3 in terms of molecular ellipticity, $[\theta]_M$. We calculated CD spectrum of the peptide (IX) by summing the molecular ellipticities of the peptides VII and VIII and then subtracting the molecular ellipticity of the pentapeptide (I). As shown by open circles in Fig. 4, the calculated values of $[\theta]_M$ are in good agreement

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Table II. MSH Activity of Peptides related to α-Melanocyte-stimulating Hormone

	Peptides	MSH activity, U/g
I	H-His-Phe-Arg-Trp-Gly-OH	3.0×10^4
VII	H-His-Phe-Arg-Trp-Gly-Lys-Pro-Val-NH_2 (I); Formyl (a)	2.0×10^4, 8.0×10^6
VIII	Ac-Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-OH (b) (I)	7.0×10^4
IX	Ac-Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val-NH_2 (b) (I); Formyl (a)	2.5×10^9, 2.0×10^{10}
X	Ac-Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val-NH_2	2.3—5.4×10^{12}

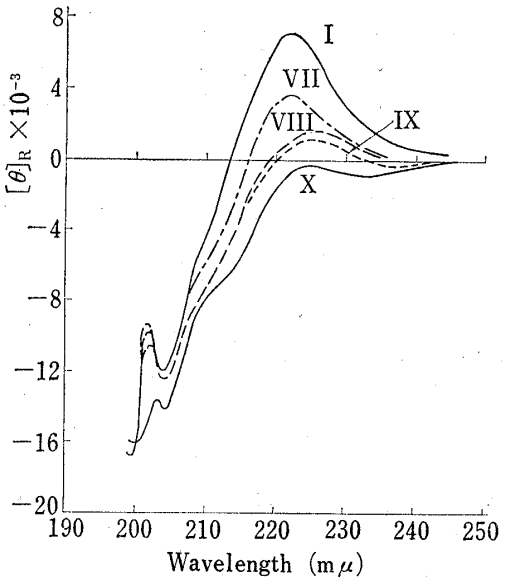

Fig. 3. CD Spectra of α-MSH and Its Related Active Fragments Containing the Pentapeptide I in Water (see Table II)

The measurements were done at about 0.05% of the peptides with 0.5—0.02 cm-cell at room temperature.

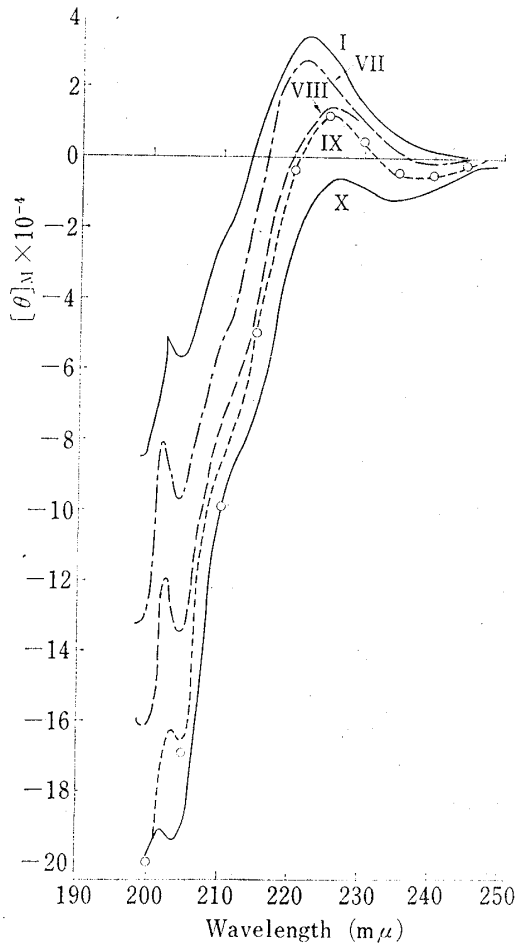

Fig. 4. CD Spectra of Peptides shown in Fig. 3, expressed in Terms of Molecular Ellipticity, $[\theta]_M$

The open circles indicate the calculated CD spectrum of the peptide IX, obtained by summing up the molecular ellipticities of the peptides VII and VIII and then subtracting the molecular ellipticities of the pentapeptide I.

with the experimental values for the tridecapeptide (IX). This additivity may indicate at least that there is no change in the conformation of each part (the pentapeptide, a, and b) whether it is contained or not in the peptides VII, VIII, and IX. However, it is uncertain whether a secondary structure exists or not in the peptide and part a and b. On the other hand, the CD spectrum was changed and the positive maximum was disappeared by the removal of the formyl group at the ε-amino group of the lysyl residue of the tridecapeptide. This may suggest the formation of a certain conformation which enables to exert full activity.

Experimental

All the peptides used in the present experiments were prepared as described in previous papers.[3,6,9-11]

Circular dichroism measurements were carried out with a Jasco spectropolarimeter, model ORD/UV-5. The instrument was standardized by reproducing the reported CD spectra of camphor and poly-L-glutamic acid. Molecular ellipticity, $[\theta]$, was obtained from the equation,

$$[\theta]=3300(\varepsilon_L-\varepsilon_R),$$

where $(\varepsilon_L-\varepsilon_R)$ is the difference between the molar extinction coefficients for left and right circularly polarized light. In the figure presented above, $[\theta]_R$ and $[\theta]_M$ represented the molecular ellipticity based on the average residue weight and the molecular weight, respectively.

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